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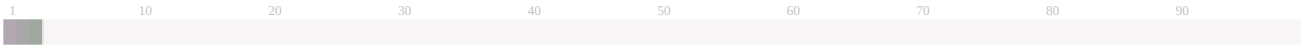

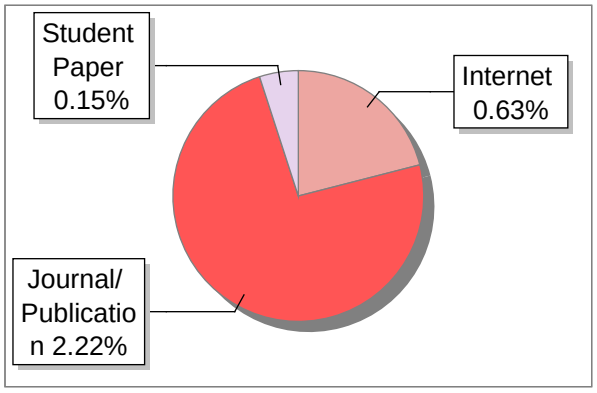

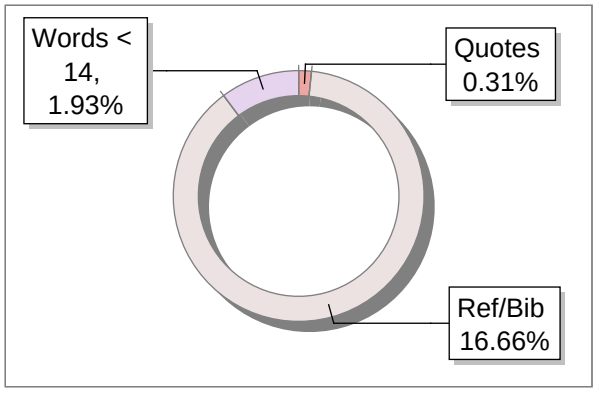

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Studies on Major Medicinal Plants of Jamnagar Geographical Locationto Extract Useful Bioactive Compounds

THESIS

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IN

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BY

Nishant Saxena

UNDER THE ADMINISTRATION OF

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1.0

INTRODUCTION

Medicinal plants represent a vast array of plant species distributed globally, and each has the potential to yield valuable medicinal substances. From petite perennial herbs flourishing in Elevated Mountain regions to plants adapted to diverse climates, these botanical entities play a critical role in meeting pharmaceutical needs. The World Health Organization (WHO) underscores the significance of therapeutic plants, reporting that significant world's population (around 80%) trusts predominantly on old-style and herbal medication for their prime healthcare requirements. This reliance underscores the cultural, historical, and practical importance of medicinal plants in addressing the healthcare needs of a considerablepercentage of the international populace.Higher plants remain untapped reservoirs for drug development, presenting promising possibilities for medicinal purposes. Their potential to contribute to humanity in the 21st century is rooted in a rich historical background. Nevertheless, a recent global initiative spearheaded by the WHO aims to integrate substituteprescriptions and old-fashionedmedications into mainstream healthcare amenities. This constitute a distinct system encompassing Chinese, Ayurvedic, Unani, and many different types of original treatments. The widespread use of complementary or alternative medicines has been notably prevalent in developing countries and is increasingly gaining popularity in developed nations. These medicines are making positive strides in the healthcare system, demonstrating efficacy in the dealing of disorders such as tumor and other serious illnesses (Naczk, et. al: 2006).

Plants own the capacity to provide anextensive array of diverse biologically active complexes. Medicinal plants, in particular, yield numerous antioxidant composites such as phenolic amalgams, carotenoids, anthocyanins, and tocopherols (Jakubowski, et. al; 1997). Medicinal plants tend to accumulate high concentrations of phytochemicals, which have the ability to safeguardfromessentialinjury (Suffredini et. al; 2004). These beneficial phytochemicals serve as natural antioxidants, supplementing the body's needs (Boots et. al; 2008).

Several studies have highlighted that numerous plants are abundant sources of vitamins A, C, and E (Suffredini et. al; 2004). The primary role of antioxidants is to regulate and diminish oxidative destruction in humans by inhibiting corrosioninstigated by responsive oxygen species (ROS), thereby enhancing life span of self (Ames, et. al; 1993). Energeticcharacters in droppingswelling, retarding elderly, and avertingpositive cancers are played by beta-carotene, antioxidants, and various phenolic compounds (Duthie, et. al; 1996). The popularity of indigenous medicines utilizing plant components has spread globally.

Global Scenario

The utilization of traditional/indigenous and complementary medicine is widespread globally, with varying degrees of adoption observed in different regions. In China, traditional herbal preparations constitute a significant portion, making up approximately 25-45% of entiretherapeuticingesting. In North America and commercial parts of Europe, above 55% of the populace has experimented with harmonizing or substitutemedication at minimumon one occasion. Cities such as London, San Francisco, and South Africa demonstrate even higher usage, with 75% of the population incorporating Old-fashionedharmonizingSubstitutemedications. Canada sees about 70% of its population exploring complementary medicine, while in Germany, an astonishing 90% of the populace has turned to natural remedies at some point. This trend extends to medical professionals, with the various doctors receiving specialized exercise in naturally prepared remedy nearly doubling every five years. In the United States, a substantial 158 million adults engage in complementary medicines, leading to an annual expenditure of approximately US $17 billion on old-fashionedmedications, permitting to the USA Commission for Alternative and Complementary Medicines. The United Kingdom follows suit with an annual spending of approximately US $230 million on alternative medicine. This reflects a widespread interest in non-conventional healthcare approaches, with individuals exploring these options for various

reasons, including addressing specific health concerns or promoting overall well-being. It emphasizes the need for informed decision-making and consultation with healthcare professionals when considering complementary or alternative treatments.

Indian Picture:

The usage of naturally produced medicationsare deeply ingrained in the tradition of India, which is also the principalmanufacturer of therapeuticbasils globally. Often mentioned to as the BotanicOrchard of thisSphere, India boasts a well-established knowledge base of herbal medicine. With its vast size and diverse ecosystems, India harbors opulentnaturalvariety, housing approximately 45,500 species, out of this around 15,100 are advancedvegetation. Over the course of around 4,500 years, India has been harnessing and applying its abundant biodiversity in the healthcare sector.The country possesses the eldest, richest, and greatestvariedsocialcivilizations, encompassing nature conservation practices intertwined by the usage of therapeuticshrubberies. This heritage is not only evident in ancient fiction and socialintegritywhile this is also reflected in the constitution, policies, legislation, and various industrialization dedicated to preserving and utilizing India's botanical wealth for healthcare purposes.

Selected Plant Information:

Historically, experts and vaids within the field of Ayurveda and its development have described the past glory of Gujarat land. It’s a section of 1,96,025 km2 and a shoreline of about 1610 km - the lengthiestshoreline in INDIA. it's extension lead to 4 mainmountvarieties of the country viz Aravallis, Vindhyas, Satpudas and Sahyadris (Western Ghats) positioned within the same order from north to south. This state has 4 major rivers viz Narmada, Tapi (Tapti), Mahi and Sabarmati. The Tropic of Cancer traverses Gujarat, imparting a typical sub-tropical climate to the region. The state exhibits diverse agro-climatic zones characterized by different types of vegetation. The climate spectrum ranges from extremely arid conditions in Kachchh to highly humid environments in Valsad district. Kachchh

experiences a high arid climate, while Rajkot, Jamnagar, and Amreli districts have a medium semi-arid climate. Junagadh, Bhavnagar, Ahmedabad, and Kheda districts fall under the semi-arid category. Bharuch is characterized by a sub-humid climate, Surat has a humid climate, and Valsad district experiences a very humid climate. This climatic diversity contributes to Gujarat's varied ecological and agricultural landscape.

The north-western region, particularly Gandhinagar, in the State experiences arid conditions with an annual rainfall of less than 500 mm. Moving towards the central part, represented by Ahmedabad, the annual rainfall increases to more than 700 mm. In the southern part of Gujarat, particularly in Surat, the average annual rainfall is substantially higher, reaching around 2000 mm. The temperature in Gujarat exhibits variation throughout the year. During the winter, temperatures range between 12° and 27°C, with occasional instances of freezing levels being recorded. In contrast, summer temperatures are notably higher, averaging between 25°C and 43°C. It is not uncommon for temperatures to soar as high as 50°C during the summer months.The spermatophyte species were documented from Gujarat are about 2200, which is around 12.5%. In which, 916 plant species are recognized as therapeuticallysignificant plants. Gujarat is way forwardas compared to rest of the states of India per species of Ayurveda plants available. Some respectedtherapeutic plants of unusual existence are found in Gujarat. The objective of currentinvestigation is to matureaeconomically viable methodology for phytochemical studies focused on extracting, measuring, and identifying beneficial bioactive compounds present in medicinal plants found or cultivated in the Jamnagar geographical region.

Practice of using plant-based drugs has contributed largely to the well-being of humankind from ancient times up to the 21st century. Plant drugs being natural have certain advantages such as low toxicity, minimum side effects and boost immunity of the body. A vast array of humankind across the

world including in developing countries, is more and more trusting and relying on plant based conventional medicines. As per the WHO, herb-based medicine is defined as any herb/shrub/treecontaining biologically active components in all of theirshares, which can be utilized for remedial and beneficialresolutions (Doughari JH, 2009). Morphologically, *C. procera* is plant which is a pulpy-wooden, perennial and evergreen shrub. This plant has few numbers of stems, branches and leaves. Its bark is bounty, slightly greyish in colour and wrinkled in appearance. Every time when cuts are made on leaves, branch or stem, an ample amount of white latex will flow out from the cut point site. It has a very taproot type or root system and with a few numbers of branches, which is established very deep inside the soil (B. M. Sharma. 1968).

Scientists have underscored the remedial and medicinal belongings of C. *procera*, utilizing various fractions of plant for therapeutic purposes (Silva MCC et. al; 2010). The latex of C. *procera* contains bioactive phytochemicals, including steroids, alkaloids, and cardiac glycosides, which hold significant medicinal importance and exhibit various pharmacological activities (Mohamed NH et. al; 2015). In indigenous medicine systems, C. *procera* has found extensive use in treating a multitude of ailments. It alsoengaged as a blood purifier, stomachic, anthelmintic, digestive, purgative, sedative, and antidote for snakebite poison. Additionally, it is applied in the treatment of conditions such as piles, dysentery, asthma, ulcers, tumors, liver diseases, boils, spleen disorders, leprosy, and eczema (Kirtikar, K.R et. al; 1935 and Nadkarni, A.K et. al; 1960). Diverseportions of the C. *procera* plant, including latex, flowers, roots, stems, leaves, and bark, have been identified to contain numerous pharmacologically active phytochemicals. These components exhibit anti-microbial, anti-inflammatory, larvicidal, anthelmintic, anti-cancerous, insecticidal, and antidiarrheal activities (Ahmed KK et. al; 2005). The primary object of current work is to deliver a compiled information and highlight the phytochemistry and pharmacological actions associated with the plant C. *procera.*

Presently the market of plant-based therapeutics is developing prominently (E. L. Cooper 2009). Therefore, it is strongly encouraged to generate and disseminate knowledge from scientific research and clinical trials on traditional and herbal medicines. The non-toxic nature and cost-effectiveness of therapeuticmaterials have workedas a pivotal role in advancing the development of plant-based drugs and fostering research in pharmacology. This becomes particularly significant when considering the application of active constituents as therapeutic agents which are extracted from plants (Masood E, 1997).Herbal and traditional medications are considered to be safer and effective and have an extended history of their usage. The significance of the approach endorsed by indigenous medicine has been retained, enabling drug discovery based on natural products as well as the treatment of various chronic diseases (B. Patwardhan, et. al; 2044). In many cases, the blends of plant-based active constituents have been favorable and effective along with the combination of synthetic drugs to treat various diseases (S. L. Badole, et. al; 2008). More than 50 % of all modern and synthetic drugs have origin from naturally available products and they are crucial constituents of pharmaceutical industries as well as drug development agendas (Baker JT, et. al; 1995. The WHO has made a prediction that about 80% of the total biospherepopulace will be dependent on native medicines for their chiefpharmaceutical requirements in the near future. Therefore, the screening of some plant metabolites based on medicinal properties is considered a crucial step to support pharmacological studies (Raj JY, et. al; 2012).

Several important plants, reported to possess medicinal properties and used for treating various diseases, are available. Considered as a valuable source for bio-degradable, novel, non-toxic, and renewable drugs, an outline of the medicinal properties of C. *auriculata* is provided in the current review article. Fitting to the Caesalpiniaceae family, it is usuallyidentified as tanner's cassia (Nandan A, et. al; 2011). C. auriculata goes by diverse names in different languages, such as pitapuspa, manojyana, pitakalika, carmaranga, avartaki, pitakala in Sanskrit, tarwar in Hindi, avaram, avarai in

Tamil, tanner's tea, mataran tea in Malayalee, and tanners Cassia in English (Nwangwa EK. 2012). This plant is originate in various portions of India and many regions of Asia, characterized by its yellow-colored flowers and its status as an evergreen plant (Pari L, et. al; 2002). Scientific literature has detailed several medicinal properties of C. auriculata, encompassing anti-inflammatory, antibacterial, antidiabetic, anti-microbial, anticancer, antioxidant, anti-hyperlipidemic, hepatoprotective, and nephroprotective activities (Pari L, et. al; 2002, Purushotham KN, et. al; 2014, Maneemegalai S, et. al; 2010, Dhanasekaran JJ. et. al; 2011, Gaikwad K, et. al; 2012, Kanchana A, et. al; 2011, Manogaran S, et. al; 2004, Raja DK, et al; 2013, Devi PU, et. al; 2006).

Natural goods derived from micro-organisms, animals and plants have been used as traditional remedies to cure many types of diseases (Yuan H, et. al; 2016). The wide-ranging usage of the plant based herbal products are known for the remedial and health supplements acquired from commonly used natural therapeutic plants and herbs (Sultana, S.et. al; 2014). Presently, medicinal plants can be a substantial substitute of the existing synthetic medications for the novel drug discovery program (Sarker, P., et. al; 2019). Remarkably, one-fourth portion of the present medicine market is plant based, and that is derived from only 5 to 15% of the total medicinal plants. This fact clearly indicates a considerable gap in the plant based drug discovery (Gurnani, N.et. al; 2014). Some of the examples are captured here where plants were used for the remedial purposes/treatments of several diseases and illnesses around the globe. Plants with antidiabetic properties in Indian indigenous medicine. Plants to cure infectious diseases and immune booster potentials in African indigenous medicine. Plants with antimalarial activities in Thai indigenous medicine. Herbs for stroke remedy in Korean indigenous medicine. Plants with cognitive function and enhancement of memory of an individual in Chinese indigenous medicine (Anisa G. et. al; 2016).

Globally, plants have functioned as a cost-effective reservoir of unique and novel compounds for various drugs, significantly contributing to the well-being and fitness of humanity. Approximately more than 75% populace of the globe benefits from indigenous medicines derived from plant-based extracts, as per the WHO Indigenous Medicine report in 2002. Various countries have harnessed different plants for medicinal and clinical purposes, recognizing them as potent and effective drug sources (Silva NCC and Junior A.F. 2010). Plants having therapeutic potential play a crucial and vigorouscharacter in human routinely, serving as valuable sources of diverse compounds for treating various diseases. In India, the plant-based medicine system relies on the information of medicationsderivative from plants.

The utilization of higher plants for medicinal purposes has been an enduring tradition, tracing its roots back to ancient times, due to their richness in medicinally active compounds. Natural products and those derived from plants hold considerable significance in the pharmaceutical industry, with over 50% of existing medical drugs having their origins in natural compounds. In India, traditional medicine has long relied on plant-derived compounds for the treatment of various ailments. Researchers worldwide are actively involved in identifying and assessing numerous plant-based drugs, acknowledging their high therapeutic potential. Pharmacognostical studies serve as a crucial criterion for the identification of these drugs. The biologically active compounds synthesized during secondary metabolic activities in plants contribute to their organic and natural properties (Mahesh B., and Satish S. 2008).

Plants belonging to the Bignoniaceae family, such as *Tecoma stans*, have been widely utilized in traditional medicinal practices across various countries. Traditional healers and ethnic medical practitioners rely on several plant species to address diverse health issues. *T. stans*, characterized by its appealing yellow flowers and pinnate foliage, thrives in warmer climates globally. Extracts derived from the plant's bark, leaves, and roots contain a range of biologically active constituents integral to

traditional medicines. These include alkaloids, flavonoids, glycosides, phenols, saponins, and terpenoids (Mann A. et al.; 2008).

The presence of phyto-constituents like flavonoids, glycosides, phytosterols, phenols, saponins, tannins, and triterpenes, either individually or in combination, suggests potential wound-healing effects. Virtually all parts of the *T. stans* plant harbor various therapeutic compounds. The leaves exhibit antibacterial, anticancer, anthelmintic, wound-healing, and antispasmodic activities (Kumanan R et al.; 2010). Terrestrial components of the plant display antioxidant and wound-healing activities, while the roots demonstrate antibacterial properties. Additionally, the flowers display anticancer and antidiabetic activities (Sugavanam K. et al.; 2012).

Extraction Techniques

In the pharmaceutical context, the process of separating the medically active components ofany natural tissues from the any element is referred to as extraction. This separation is achieved with the help of specific solvents within established extraction methods. The resultant products extracted from plants are typically impure liquids, semisolids, or powders designed for oral or external use. These products fall into various categories, containingfluidextracts, decoctions, tinctures,infusions, pilular (semisolid) extracts, and powdered extracts. Traditionally, these types of preparations have been referred to as galenicals, a term derived from the name of Galen, the Greek physician from the second century.

The main goals of standardized extraction procedures for crude drugs involve isolating therapeutically active components and removing inert materials. This process is carried out using a selective solvent referred to as a menstruum.This ensures that the resulting pharmaceutical preparations are therapeutically effective and meet the required quality standards.The extract obtained through the extraction process serves various purposes in the field of medicine. It can be directly used as a therapeuticrepresentative in the form of distillates and fluid extracts. Alternatively, it may undergo

further processing to be integrated into various dosage forms like tablets or capsules. In some cases, the extract may also undergo fractionation to isolate individual chemical entities, leading to the development of modern drugs. Examples of such modern drugs include hyoscine and vincristine.

2.0

LITERATURE REVIEW

A Reviewon*Calotropis procera*

Scientific Classification

As per the scientific classification, this plant come under Asclepiadaceae family and which is famous as milkweed family commonly worldwide. Order, class, division, subkingdom and kingdom, which are gentianales, magnoliopsida, magnoliophyte, tracheobionta and plantae respectively, follow scientific classification of Calotropis (Sharma K, et. al; 2011).

Geographical Distribution

C. *procera* is innate plant for the tropical and subtropical region of Africa and Asia. It is inborn to India, Saudi Arabia, United Arab Emirates, Nepal, Afghanistan, Nigeria, Iraq, Kuwait, Niger, Yemen, Israel, Zimbabwe, Iran, Algeria, Oman and Pakistan. This species is acclimatized and grows naturally in a wide range of topographicalarea, which includesAustralia, the Seychelles, Thailand, Central and South America (Brazil), many Pacific Islands, including Hawaii and Mexico. This plant is highly tolerant to high salt concentration in soil and has elevated degree to the drought-resistance as well. Seeds of this plant are distributed with the help of the wind and animals. Some species can easily be established as a weed to the sideways of roads and lagoon edges. It favours growing in areas of little rainfall and in sandy soils (M.F. Azhar et. al; 2014).

Insecticidal Properties

Insecticidal ingredients have been used to eradicate various types of insects and its mode of action generally comprises of larvicidal and ovicidal activities which used to kill insects' larva and eggs respectively. The root bark extract of C. *procera*, prepared by petroleum ether, chloroform and methanol have shown toxic effect to adult insects and insecticidal actions. Particularly, larva forms are more vulnerable to the toxic effects compared to adult forms. The root extract effect was examined, which revealed that, it has moderate type of repellent effect on T. *castanem*. Among all three extracts, methanol extract reflected more toxic effect to both stages such as larval and adult stages of insects as compared to others (M.A. Alam et. al; 2009). Larvicidal activity has been reported in Musca domestica and Anopheles stefensi at very low latex concentration of C. *procera*. The sub lethal quantity of the latex also gave negative impact on the survival of pupae and larvae by inhibiting their growth. It also decreases larval weigh significantly. Structural deformities in several organs were the results of the latex components including flavonoids, toxins, proteins, triterpenes and acetogenins that act at the cellular level. Furthermore, the effects mentioned above were inhibitory to the growth and development of insects and were of an irreversible nature. The insecticidal activity of different types of proteases and endogenous soluble proteins from the laticifer fluid of C. *procera* has been identified (R. Upadhyay 2014). The extract of C. *procera* leaves has been shown to have adverse effects on the reduced survival of instar larvae of the desert locust Schistocerca gregaria. In adult Schistocerca gregaria, effects such as the non-appearance of sexual maturity and the seizure of ovarian growth have been reported (Abbassi, K et. al; 2004). C. *procera* has been found to be effective against Sarcophaga haemorrhoidales (flesh fly) (Moursy, L.E. 1997) and is used to control Poecilocerus pictus (painted grasshopper) (Chandra, H et. al; 1993) and Henosepilachna elaterii (ladybird beetle) (Ahmed, U.A.M et. al; 2016). Insecticidal activities were found against the flour beetle Triboleum confusum by the root bark of C. *procera* (Jahan, S. et. al; 1991) and against the red flour beetle Tribolium castaneum by the leaf extract of C.

procera (Abbasi, A.B. et. al; 2012). High larvicidal activity was found from C. *procera* against larvae of the Anopheles labranchiae mosquito, with larvicidal activity having an LC50 (24 h) ranging from 28 to 325 ppm (Quazi S. et. al; 2013). Multiple fractions of the latex portion of C. *procera* were evaluated against the larval growth and development, as well as the egg hatching of the Aedes aegypti mosquito, and were found to be significantly inhibitory in nature (Gupta S. et. al; 2012).

Anti-cancerous Activity

Cancer is one of the major diseases that involve uncontrolled and abnormal growth of the cells inside body. Both, developing and developed countries are struggling to control this disease since, it has a potential to spread and invade other body parts. Therefore, to prevent the carcinogenic development in the body, it is important to check anti-cancerous activity of several biological and chemical agents that are available naturally. Several attempts have been made to review anti-cancerous activity of C. *procera* by multiple scientists. Use of herbal and indigenous medicines is becoming the most attractive alternative to cure cancer since chemotherapy have a number of side effects. Mathur et. al: (2009) has been reported Cytotoxic effect on COLO 320 tumour cells by the extract of *Calotropis* sp. Cell death triggering activity was also observed because of the presence of the cardio-tonic steroid UNBS1450 which serves as an effective inhibitor of the sodium pump (R. Mathur et. al; 2009). Anti-cancer study was witnessed in the HepG2 cancer cells with the various root extracts of C. *procera* in multiple solvents such as extract of methanol (CM), hexane (CH), ethyl acetate (CE), and aqueous (CW). The cell proliferation activities were assessed using the Tetrazolium bromide (MTT) calorimetry technique. Various amounts provided information about CH, CE and CM having cytotoxic activity, while CW did not show any such activity. Process of apoptosis in HepG2 cells was initiated by the plant extract when cells were in the S phase and by preventing them to enter into the G2/M phase. Several studies have reported that the root bark portion of C. *procera* has demonstrated high in-vivo tolerance of tumor

growth and prolonged survival in human xenograft models of nude mice (Van Quaquebeke et. al; 2005).

Analgesic and Anti-nociceptive Activity

Condition of analgesia is achieved by use of any analgesic group of drugs or chemicals. In medical terminology, analgesic or anti-nociceptive activity refers to pain relief or the process of blocking the exposure of injury stimulus by central nervous systems. A substance exhibiting this activity has the great prospective to act on various peripheral and central nervous system pathways.These substances have either potential to eliminate sensation temporarily or permanent. All fragments of C. *procera* such as root, leaves, latex, and other parts have been analysed and evaluated to check the analgesic activity by Basu et. al;(1991). The analgesic activity was assessed using the acetic acid-induced writhing model against the aqueous solution of the dried latex, ethanolic extract of aerial portions of the plant, and chloroform extract of the root portion of the plant. These demonstrated highly significant analgesic activity, with the ethanolic extract of the flowers showing a lower amount of analgesic activity (Basu, A., et. al; 1991). In another study, the dry latex part of C. *procera* was evaluated for analgesic activity and was found to be more effective against acetic acid-induced writhing at a dose of 415 mg/kg compared to the oral dose of 100 mg/kg aspirin. In the tail flick model, the 830 mg/kg dose of dry latex showed marginal analgesia compared to aspirin (Meena AK. et. al; 2010).

Anti-inflammatory Activity

Any biological or synthetic substance that has anti-inflammatory activity, is used to treat the inflammation or swelling in any parts of body. It also has property similar to an analgesic, which

includes pain remedy by lowering inflammation. Constituents of C. *procera* have been investigated for their potential of anti-inflammatory activity. According to Vaiyapuri et al. (2015), effective anti-inflammatory activity is retained by the crude part of the dry latex of C. *procera*. It was found to be effective at a significant level against the acute inflammatory response by a single dose of the aqueous suspension of the dry latex of the plant (Vaiyapuri PS, et al; 2015). A substantial amount of anti-inflammatory activity was found in rats by the chloroform-soluble fraction of the root of this plant. The potency of the anti-inflammatory activity of crude dry latex was tested in the carrageenan-induced rat paw edema model. Acetone, petroleum ether, methanol, and aqueous extracts of the dry latex of C. *procera* were evaluated, and the anti-inflammatory activity found was quite significant, particularly the aqueous and acetone extracts, which were found to have the greatest anti-inflammatory activity (Majumder, P.K. et al; 1997).

Anti-microbial Activity

To maintain disease free condition, several anti-microbial agents are used. Anti-microbial activity denotes to the procedure of inhibiting or completely killing of disease causing microorganisms. It has been evaluated that various parts of C. *procera* have the potential for anti-microbial activities (anti-bacterial and anti-fungal). According to the available literatures, ethanol, chloroform, and water extracts of the latex and leaf of C. *procera* were tested for the anti-microbial activity against bacteria i.e. Streptococcus pneumonia, Staphylococcus albus, Escherichia coli, Streptococcus pyogenes and S. aureus by paper disk and agar well diffusion methods. The biggest inhibition zone against E. coli was witnessed with the ethanolic extract of latex in both the disc diffusion method and the well diffusion method (Khairnar AK, et. al; 2012). The anti-bacterial activity of fractions from leaf extract, including 14B-dihydroxy-5-card-20(22) enolide (proceragenin), new cardenolide, and 7B of C. *procera*, was

evaluated against the test organism Pseudomonas pseudomallei, the causal agent of melioidosis. All fractions of the leaf extract completely inhibited the growth of the tested bacteria (18). The anti-fungal activity of the crude aqueous extract of the stem bark portion of C. *procera* against the test organisms Tricophyton gypseum and Epidermophyton flocosum was evaluated using the agar plate diffusion method. The results indicated that the aqueous extract exhibited a minimum fungicidal concentration of 4.0 mg/ml and 2.0 mg/ml, as well as a minimum inhibitory concentration of 0.9 mg/ml and 0.5 mg/ml, respectively (Kuta, F.A. 2008).

Anti-diarrheal Effect

Any substance or medicine which is used to treat diarrheal condition in body is termed anti-diarrheal in nature. The mode of action of these kinds of substances is lowering the gut movement caused by sudden diarrhoea. In-depth assessment was carried out by Kumar et: al, in 1994 to check anti-diarrheal effect of dry latex of C. *procera* against castor oil treated rats by evaluating factors such as fluid accumulation induced by castor oil, transit time in the intestine and concentration of an electrolyte in the intestinal fluid. Equivalent to atropine and phenylbutazone, dry latex of this plant also exhibits a noticeable decrease in severity and frequency of excretion in rats treated with castor oil. A decrease in intestinal transit of up to 27 to 37% was observed in the dry latex when comparing normal rats to castor oil treated rats (Kumar VL, et. al; 1994).

LesionTherapeuticActivity

Lesion Therapeutic activity of any substance refers to replacement of damaged tissue by newly produced tissue. The process of wound healing is very fragile and complex process and it is also susceptible to interruptions. To evaluate wound healing capacity of C. *procera* latex was selected by

various scientists. Guinea pig was used, and excision was performed on the back side of animal. For seven days, latex was applied under sterile condition for two times a day. Positive results were observed in wounded area like increase in DNA, protein synthesis and more accumulation in amount of collagen fibers in latex treated animals (Rasik AM, et. al; 1999).

Anti-ulcer Activity

Lesions on the surface of the skin or a mucous membrane, characterized by a superficial loss of tissue, are known as this disease.Mode of action of any naturally occurring or synthetic anti-ulcerative compound involves decrease or inhibition of gastric acid secretion and production, acid neutralization and gastrointestinal mucosa surface protection from acid secretion. The anti-ulcer activity of the C. *procera* plant was assessed using various in-vivo ulcer models. The result revealed that substantial amount of defence was detected in guinea pigs having histamine induced duodenal ulcers. Gastric ulcer induced by absolute alcohol, serotonin, reserpine and aspirin in rats was also significantly inhibited by the plant (Quazi S et. al; 2013).

Hepato-protective Activity

Several chemically and biologically active compounds have a hepato-protective activity which includes the ability to prevent damage to the liver. C. *procera* plant is full of phyto-constituents that can also have hepato-protective activity. The evaluation of this activity was carried out using the 70% ethanolic aqueous extract of the flower against paracetamol-induced hepatitis in rats. Variations in the levels of biochemical parameters indicative of hepatic damage, such as GSH, cholesterol, bilirubin, SGOT, SGPT, and HDL serum, were observed in both treated and untreated groups of rats. The concentration of 2000 mg/kg paracetamol was able to increase bilirubin, SGOT, cholesterol, and SGPT, on the other

hand, GSH and HDL serum levels were decreased. When rats were given doses of ethanolic aqueous extract of the plant flower, altered levels of biochemical parameters were established to a normal range (S.R. Setty, et. al; 2007).

Anti-fertility Activity

Compounds having the ability to either reduce or completely suppress the fertility of any organism are known as anti-fertility agents. The anti-fertility effect and changes in hormonal levels were assessed in albino rats using the ethanolic extract of the root of C. *procera*.100% inhibition or strong anti-implantation, as well as heterotrophic activity, have been seen at 250 mg/kg dose. There were no signs of anti-estrogenic activity was observed.

Immuno-modulatory Activity

Immuno-modulator compounds can regulate and normalize the immune response in the body. This kind of constituents acts either to stimulate or to suppress the immune system parts to fight against any disease or infection inside the body. The immune-modulatory activity of the ethanolic extract of the root bark of C. *procera* was assessed through the haematological profile, which included parameters such as total leukocyte count, total red blood cell count, and percentages of lymphocytes and neutrophils.It was also assessed as vascular permeability, humoral mediated antibody titter, and immunological tests in mice, peritoneal macrophage count, cyclophosphamide-induced myelo-suppression, and delayed-type hypersensitivity, at 50, 100, and 200 mg/kg dose levels. Findings have shown that the ethanolic extract was able to stimulate the protective system by controlling and modifying several immunological parameters (Ramos V, et. al; 2007).

Bio-activeConstituents and Phytochemical Compounds

C. *procera* has many bio-active constituents and phytochemicals and they can have beneficial as well as toxicological properties for humans and other organisms. These naturally occurring bio-active constituents can be found and pulled out from the various parts of plant-like flower, latex, leaves, roots, bark, and seeds. These compounds have different roles in biochemical and physiological reactions such as inhibitors for the enzymatic reactions, co-factors for enzymatic reactions, and also able to remove reactive toxic compounds. The phytochemical evaluation revealed that there are several bioactive constituents such as sterol, resins, cardenolides, terpenes, flavonoids, proteolytic enzymes, triterpenoids, and tannins. Chemically, C. *procera* possesses numerous medicinal properties and is an aromatic plant. The latex of C. *procera* contains active compounds such as calcium oxalate, resinols as esters of steam-volatile fatty acids, and cardiac poisons (Y. Murti, et. al; 2010).Compounds like 1-pentadecene (9.5%), 1-heptadecene (8.2%) and tyranton (54.4%) have been presented in the leaf oil while isobutyl nonane (13.7%) and Z-13-docosenamide (31.8%) have been presented in the stem oil. Mundarol isovalerate, calotropterpenyl and rutinoside are present in the root bark of this plant. C. *procera* flower is a source of chemical constituents like lupeol, L-rhamnose, calactin, giganteol, calotropin, gigantin, retinoside, glucose, lactucery acetate and calotoxin (A. GUPTA, et. al; 2003). Several anti-bacterial, cytotoxic, insecticidal, and larvicidal compounds have been isolated from this plant. It is also a source of complex organic carbonates, flavonoids, sterols, cardenolides, and alkaloids. A maximum amount of calcium is available in the leaves, while the latex is a rich source of manganese. Metals like nickel, copper, lead, cadmium, iron, and zinc are also present inside the leaves and latex portions of the plant (V.N. Verma.2014). The latex from leaves and stem has constituents like calotropains FI, F11, D1 and D 11, gigatin, calotoxin, nitrogen, sulphur, protease enzyme, calactin, and

ascorbic acid. Glycosides like amyrins, alpha and beta types of calotropeols have been present in the root and stem barks of this plant. Different fatty acids, waxes, and glycolipids are also constituents of leaves whereas, roots contain calotropin, a type of cardiac glycosides. Sitosterol is also isolated from the plant. The chemical components like calotoxin, uzarigenin, calotropin, proceroside, trypsin, and uscharin have been present in latex and flower contain terpenes and cyclisadol constituents. The plant leaves have calotropin, calotropagenin, amyrin, and amyrin acetate as their components. Seeds of this plant contain frugoside, calotropin, coroglaucigenin and corotoxigenin as their constituents (S. Sarkar, et. al; 2014).

Phyto-chemistry

Several phytochemicals have been presented in the plants and this group of compounds has protective and disease preventive properties. Several scientific findings prove that phytochemicals can also advantageous to safeguard humans against several diseases. The plant of C. *procera* contains giganteol, gigantin and wax. The latex portion of this plant contains calortopin, a non-toxic enzyme and calactin which is a toxic glycoside. Leaves contain mudarine as their active compound and other resins, acids, and toxic glycosides like uscharin, calotoxin, and calotropin. Compounds like anthocyanins, calotropoleanyl ester, cylotoxin and cardiac glycosides are also present in the plant (S. Quazi, et. al; 2013). The stalk and leaves have calotropin, latex contains an ester of terpenol and flowers contain calotropenyl acetate. This plat also has benzoylinesolone and proceragenin as their constituents (A.K. Meena, et. al; 2011). The root bark portion contains four novel types of triterpenes, namely alotroprocerone A, calotroprocerol A, calotroproceryl acetate A, and calotroproceryl acetate B (Sabrin RM, et. al; 2012).

This plant also contains phytochemicals like alkaloids, tannins, flavonoids, saponins, sterols, triterpenes, hydrocarbons, and coumarins. Compounds like fatty acids, amino acids, minerals, proteases, and resins have been present in this plant. Latex of this plant contains 9.7%, stem contains 4.8%, flowers contain 7.6%, roots contain 1.7%, and leaves 5.0% of rutin (quercetin-3-rutinoside), which is a major flavonoid in this plant (Tiwari KP, et. al; 1978). Phytochemicals like cyaindin-3-rhamnoglucoside, cyclosadol, 25-diol, α-and β-amyrins, quercetin-3- rutinoside, β-sitosterol, multiflorenol, triterpene calotropenyl acetate, procestrol, cyanidin-3-rhamnoglucose, β-sitost-4en-3one, alkaline phosphate, stigmasterol, β-sitost-4en-3one and cycloart-23-en-3β have been present in the flowers of this plant (Carruthers B, et. al; 1984).

The objective of this effort was to illustrate the biological properties and phyto-chemistry of Calotropis *procera*. This plant acts as an undesirable weed, widely spread alongside the geographical area of the world, has countless economical and medicinal potentials. Many side effects and more dependence on synthetic drugs need to have an alternative path to overcome several challenges. The considerable and consistent biological activities of this plant can inspire several pharmaceutical industries and scientific communities across the world to perform further research. Ultimately, this is likely to lead to promising developments in the field of natural and plant-based novel drugs with fewer or no side effects, making them safer for human consumption. Therefore, further scientific research on this plant could be conducted to explore its potential in curing various diseases.

A Reviewon*Cassia auriculata*

Taxonomic Classification

Kingdom	:	Plantae
Phylum	:	Tracheophyta
Class	:	Magnoliopsida
Order	:	Fabales
Family	:	Fabaceae
Genus	:	*Cassia*
Species	:	*Cassia auriculata*

Plant Description

The leaves of C. auriculata are stipulate, closely arranged, alternate, numerous, pubescent, narrowly furrowed, paripinnate compound, with a straight linear gland between the leaflets of each pair, and very shortly stalked (2-2.5 cm long, 1-1.3 cm broad). According to Kirtikar and Basu (1935), the leaflets are dull green, reniform-rotund, and slightly overlapping. The flowers of this plant are bisexual, irregular, glabrous, bright yellow in color, and about 5 cm long. It has five sepals which are imbricate, membranous, distinct, unequal, and glabrous with inner ones are smaller than the two outer ones. Five

petals per plant are bright yellow veined, imbricate, and crisped along the edge. The flower has about 10 anthers and a superior type of ovary with outlaying ovules. The fruits are a pale green or brown and small legume, flat, oblong, papery, obtuse, pilose, thin, about 1.5 cm broad, and 7.5–11 cm long. About 12 to 20 seeds are present inside each fruit in their isolated cavity (Dassanayake, M.D.& Fosberg, F.R.1981). Figure 1 clearly illustrates the physical representation of whole plant and its flower, fruit, and leaf.

Figure 1: A picture of Cassia auriculata- flowers (1A), fruit (1B) & leaf (1C).

Plant phyto-chemistry:

Different parts of C. auriculata have several types of phytochemical constituents those can be isolated in different types of solvent system. Figure 2 illustrates the graphical representation of phytochemical analysis of ethanolic extracts of plant's seed, flowers and leaves (Kalaivani et. al;2008,Subramanian S. et. al;2011).

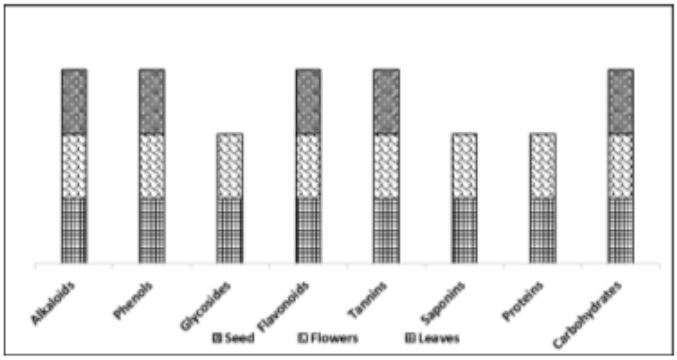


Figure 2: Data Showing Phytochemical analysis of *Cassia auriculata*

Different parts of plant are having various therapeutic potential for the healing of several sicknesses (Table: 1).

Sr.	***Plant parts***	***Therapeutic values***
1	*Leaf*	*Extract has soothing effect and effective against oxidative stress induced by alcohol (22)*
2	*Flower*	*Used to treat throat irritation, nocturnal emissions, diabetes and urinary discharges (24)*
3	*Bark*	*Used to check haemorrhage, effective against disordered processes of nutrition (25)*
4	*Seed*	*Used in conditions like diarrhoea, dysentery, chronic purulent conjunctivitis, leprosy, swellings, worm infestations, abdominal disorders and skin diseases (26)*
5	*Root*	*Used in conditions like asthma, skin diseases, tumours and leprosy (26)*

Table 1: Therapeutic properties of different plant parts

Antimicrobic Activity:

The inhibition of bacterial growth, prevention of the formation of microbial colonies, and potential destruction of microorganisms are encompassed by the term "anti-microbial agents." This plant has anti-bacterial activity and researchers had checked this property using the agar disc diffusion method.

During this study, methanol, ethanol, and aqueous extracts of the dry flower as well as methanol, ethanol, and acetone extracts of fresh flower were tried on various microorganisms such as *Pseudomonas aeruginosa*, *Proteus mirabilis, Salmonella typhi, Enterococcus faecalis, Vibrio cholera,Staphylococcus aureus*, *Shigella dysentrae*, *Escherichia coli*,*Salmonella paratyphi* A and *Bacillussubtilis*. The anti-microbial activity was found maximum against all the microorganisms except *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Scientist also characterized above mentioned extracts and observed existence of multiple Phyto-constituents such as cardiac glycosides, tannins, flavonoids, terpenoids, steroids, and saponins, which are responsible for anti-microbial activity (Maneemegalai S, et. al; 2010).

Antidiabetic Activity:

The reduction of blood glucose levels is known to be associated with this activity. The antidiabetic activity of medicinal plants is primarily attributed to the presence of alkaloids, phenolic compounds, flavonoids, and terpenoids. It has been observed by researchers that the reduction in blood glucose levels and an increase in plasma insulin can be achieved through the oral administration of flower aqueous extract at a concentration of 0.45 g/kg of body weight for 30 days on a regular basis. In contrast, concentrations of 0.15 and 0.30 g/kg were found to be non-significant in controlling blood glucose levels. This is attributed to the presence of diasulin in this plant, which is one of the essential constituents of a poly-herbal formulation and exhibits antidiabetic properties at a concentration of 40 mg/dl (Kirtikar and Basu, 1935).

Antipyretic Activity:

This activity is known to reduce the elevated body temperature, and responsible agents are identified as antipyretics. Antipyretic properties of C. auriculata was evaluated using serial extracts of plant leaves and flowers. The antipyretic activity was calculated after some modifications in well-established Brewer's method using rats with some modifications. Initially, the basal rectal temperature of each rat was measured with the help of clinical digital thermometer. During the study, 0.45 g/kg bodyweight oral administration of flower aqueous extract for one day has been shown significantly higher result for the antipyretic activity (Pari L, et. al;2002).

Antioxidant Activity:

This activity is an excellent example which proves the functional benefit of plant extracts. Compounds responsible for this activity is known as antioxidants. These variety of natural antioxidants are present in plant extracts that protect and preserve their physical and metabolic integrity. Methanol and ethanol extracts of C. auriculata flowers were assessed for their antioxidant activity. This activity was analyzed by an updated assay based on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and decolonization of the radical mono-cation of' 2,2 -azinobis-(3-ethylbenzothiazoline-6- sulfonic acid) (ABTS). Both the assays had shown that methanol and ethanol extracts of flowers from plants possess respectable antioxidant activity (Kumaran, A., Joel Karunakaran, R. 2007).

Anti-inflammatory and Analgesic Activity:

The primary response of the body towards any infection or injury, critical for both innate and adaptive immunity, is famous as inflammation. The anti-inflammatory agents are responsible for inhibition of Cyclooxegenase enzymes which generally converts Arachidonic acid to prostaglandins. The results of membrane stabilization assays, proteinase inhibitory activity, and albumin denaturation assay have

been found that C. auriculata flower extract in acetone possesses anti-inflammatory activity (Rani AA, et. al; 2014). In another study, the anti-inflammatory and analgesic activities of the ethyl acetate and petroleum ether fractions of this plant were scanned through several experimental models of inflammation and pain. It was observed that the ethyl acetate fraction was more efficient compared to the petroleum ether fraction (Mali AA, et. al; 2013). Additionally, anti-inflammatory and analgesic activity were observed in the C. auriculata leaf methanol extract, utilizing methods such as the hot plate method, tail immersion, carrageenan-induced rat paw edema, and cotton pellet-induced chronic granulomatous methods (Mali AA, et. al; 2013).).

Anthelmintic Property:

The anthelmintic property is attributed to anthelmintics, which constitute a group of antiparasitic drugs capable of expelling parasitic worms (helminths) and other internal parasites from the body without causing significant damage to the host.Leaf extract of chloroform, methanol, and petroleum ether of C. auriculata was tested for their anthelmintic property against earthworms. Out of these three extracts, the extract of methanol showed significant anthelmintic activity against earthworms (Chaudhary S, Kumar A. 2014). Another study reflected that, the leaf aqueous extract of this plant is also a better option for the anthelmintic property against tapeworms (Raillietina spiralis), roundworms (Ascardia galli), and earthworms (Eicinia faeteda). Parameters including time of death and time of paralysis of these worms were recorded using different concentrations ranging between 10 to 50 mg/ml of the aqueous plant extract. Results proved that a substantial amount of anthelmintic activity was exhibited by all the extracts and 50 mg/ml concentration was found to have the most effective among all. Distilled water was used as a control and 10 mg/ml Piperazine citrate was used as a reference in this study (Satish B. Kosalge*, and Ravindra A. Fursule.,2009). Doses more than 50 mg/ml can also be tried in future to optimize the dosage for maximum anthelmintic activity.

Anti-cancer Activity:

The anticancer activity is the effect of being reversed, suppressed, or prevented in carcinogenic progression by natural, synthetic, biological, and chemical agents. Multiple studies have proved that the C. auriculata plant possesses several compounds that can act as chemo-preventative agent. Some compounds isolated from this plant are also observed supportive in cancer prevention against the HCT15 (a type of colon cancer cell line). Leaf extract of this plant is an inducer of apoptosis that can be useful in treating larynx cancer, breast cancer, and other cell lines by an in-vitro method. Particularly, inhibition of growth of mfc-7 and hepG-2 cells was observed by the induction of apoptosis process with the help of leaf extract of this plant (Prasanna R, et. al; 3009).

Anti-ulcer Activity:

Antiulcer activity in the upper gastrointestinal tract is identified to be associated with prostaglandins of the E series (misoprostol, enprostil). These prostaglandins inhibit gastric acid secretion and provide mucosal protection against multiple noxious agents. According to the reported study, a decreased amount of ulcer formation was detected in pyloric ligated rats when they were treated with the methanolic extract of leaves from this plant. Parameters such as ulcer index and percentage of ulcer incidence were used to assess the antiulcer activity. The results demonstrated a decrease in the ulcer index compared to the control group (Ahmed MF, et. al; 2010).

Anti-arthritic Property:

Rheumatoid arthritis, a disease of unknown causes, is a rheumatic and autoimmune pathology recognized for its increasing frequency and adverse consequences. A disease typically occurs in

individuals aged between 50 and 60 years, with women being more commonly affected than men are. A substantial amount of phytoconstituents is present in this plant, and the potency to cure arthritis is found in the ethyl acetate extract of this plant. The anti-arthritic activity of the leaf of C. auriculata was evaluated using the Freund's complete adjuvant-induced model for arthritis (Bandawane DD, et. al; 2014).

Hepatoprotective Activity:

The potential of methanolic leaf extract against liver damage caused by carbon tetrachloride on Wistar albino rats was assessed. Liver-protecting properties were establish in this study. Many herbal preparations for the liver disorders contain C. auriculata as a key component. Good hepatoprotective activity was showed by leaf extract of this plant against liver damage caused by alcohol. The mode of action was the protection against the free radical facilitated oxidative stress (Rajagopal SK, 2003). Methanolic extract of this plant root had proven hepatoprotective against the anti-tubercular and ethanol induced hepato-toxicity (Jaydeokar AV, et. al; 2014). Acetone extract of leaves of this plant also has hepatoprotective activity in mice model against d-galactosamine induced cytotoxicity (Nakamura S, et. al; 2014).

Based on scientific studies, it is quite evident that C. auriculata plant contains several types of phyto-constituents for the therapeutic uses to cure numerous diseases and adverse body conditions of human being such as, anti-ulcer, anti-diabetic, hepatoprotective, anti-oxidant, anti-cancer, anti-microbial, anti-pyretic, anti-inflammatory, anthelminthic and anti-arthritic. This is because of plant possesses several therapeutic activities, can play a vital role towards disease free human life by using plant based pharmaceutical products. Further studies need to be performed on mechanism of actions as well as

principles, and yield of the bioactive compounds from this plant which could provide lead for the field of drug discovery for several diseases and other therapeutic activities.

A Reviewon*Bougainvillea spectabilis*

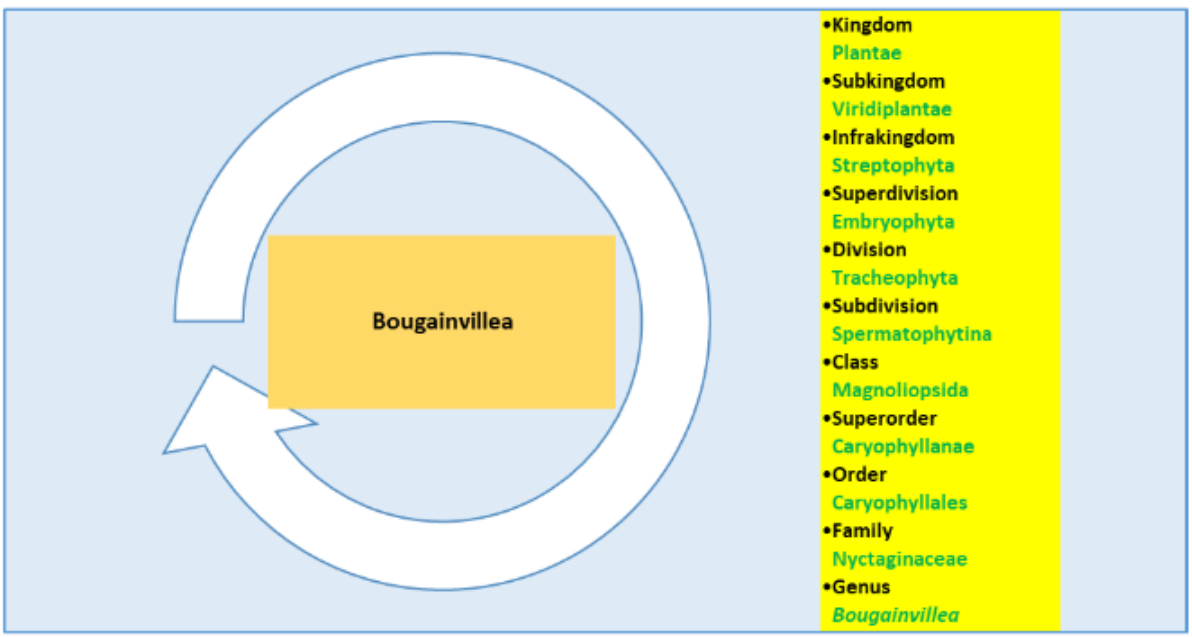
Morphological Description

B. spectabilis is a shrub type ornamental climber plant. It is ainborn of tropical region of South America and can grow around many parts of the world (Aruna Kumari T., and Saravana Kumar A. 2017). It has thin and papery bracts. The magenta and purple colour is commonly seen, but it may also range from orange to white in colour (Warren W. 2013). The plant stem can be spread up to 2-4 m, with large clumping and multi trunked, woody perennial vines. Stiff arched thorns are present that helps the shrub to climb up by growing out slender arching sticks. The stem colour becomes dull green brown from mid green during the growth. The bark is corky and soft. The rounded and oval shaped leafs are 2-6 cm wide and 5-10 cm long. Morphologically, leaves have hairy growth in lower portion and leathery texture on upper portion, and profoundly green in colour. Flowers arise in a cluster of three from the leaf axils. They are slender, small with hairy tubes and enclosed by showy. The colourful bracts are oval in shape, wrinkled, fairly big and have colours such as magenta, rose, purple and rusty

red. It has 1-2 cm long fruits that are elongated and has five lobed achene. It is rather ordinary, not attractive, hard and dry fruit cover (Kobayashi KD, et. al; 2007).

Scientific Classification

Following details illustrates the taxonomical classification of *Bougainvillea* (Integrated Taxonomic Information System-ITIS).



The genus *Bougainvillea* was discovered in 1786 in Brazil by the French navigator Louis Antoine de *Bougainvillea* (Fawad SA, et. al; 2012).Out of total eighteen species, three are horticulturally important, including B. glabra, B. peruviana and B. spectabilis. Other species includeB. lehmanniana, B. berberidifolia, B. infesta, B. buttiana, B. lehmannii, B. campanulata, B. herzogiana, B. malmeana, B. modesta, B. pachyphylla, B. pomacea, B.praecox, B. spinosa, B. stipitata, and B. trollii (Saikia H, Lama A. 2011).

Phytochemical Constituents

All different parts of this plant have some phytochemical substances which has high medicinal values. Following table illustrates the phytochemical constituents present in different parts of this plant.

Table-1: Phytochemical Constituents of *Bougainvillea*.

Compound		Parts used	Reference
Volatile compounds	Butyl formate	Leaves and branches	(N. Vuković, et. al; 2013)
	Butyl acetate		
	Methyl 2-methylbutanoate		
	Methyl hexadecanoate		
	Ethyl hexadecanoate		
	Propyl hexadecanoate		
	Ethyl 3-hydroxy-hexanoate		
	Methyl linolenate		
	Ethyl (E)-crotonate		
	(Z)-2-Hexenal	Leaves and branches Root	(N. Vuković, et. al; 2013) (S. Singh, et. al; 2009)
	2-Heptadecanone		
	O-xylene		
	2-Furfural		
	Terpinolene		
	Terpinen-4-ol		
	Methyl salicylate		
	Trans-dihydrocarvone		
	Verbenone		
	Pulegone		
	Dihydroedulan II		
	Theaspirane B		
	Dehydroionene		
	α-copaene		
	(E)-β-damascenone		
	2,5-Dimethyl-4-hydroxy-3(2H)-furanone		
	Aromadendrene		
	Cadina-1.4-diene		
	(Z)-3-Hexenyl salicylate		
	α-santalol		
	(Z,Z)-farnesol		
Fatty acid and fatty alcohols	2-Methylpropanoic acid		
	Dodecanoic acid		
	n-Octacos-9-enoic aci		
	1,2-Dipalmitoleoyl glycery		
	n-Hentriacontanol		
Peltogynoids and flavonoids	Bougainvinone A	Stem bark	(L.T. M.Do, et. al; 2016)
	Bougainvinone B		
	Bougainvinone C		

Compound		Parts used	Reference
	Bougainvinone D		
	Bougainvinone E		
	Bougainvinone F		
	Bougainvinone G		
	Bougainvinone H		
	Bougainvinone I		(L. T. Do, et. al; 2018)
	Bougainvinone J		
	Bougainvinone K		
	Bougainvinone L		
	Bougainvinone M		
	5,7,3',4'-Tetrahydroxy-3-methoxy-6,8-dimethylflavone		
	5,7,4'-Trihydroxy-3-methoxy-6,8-dimethylflavone		
	2'-Hydroxydemethoxymatteucinol		
Phytosterol, terpenes, and carbohydrates	Isophytol	Leaves and branches	(N. Vukovic, et. al; 2013)
	Phytol		
	β-sitosterol	Stem bark	(S. Jawla, et. al; 2013)
	Pinitol	Leaves	(C. R. Nayayanan, et. al; 1987)

Anti-bacterial Property

As per the available literatures, B. spectabilis leaf extract was prepared using multiple solvents such as methanol,acetone, petroleum ether, chloroform, and water to access anti-bacterial activity. Bacteria mainly, Escherichia coli, Klebsiella pneumoniae, Vibrio cholerae and Staphylococcus aureus were used for antibacterial study of leaf extract. K. pneumoniae exhibited maximum inhibition zone of 13.5 mm from the methanolic extract (Dhankhar S. et. al; 2013). Anti-bacterial activity was also analysed from the flower extract in multiple solvents such as aqueous, chloroform, ethanol and ethyl acetate. *Proteus*, *Bacillus*, *Pseudomonas* and *Klebsiella* were used for antibacterial study. Maximum inhibition zone was found in the aqueous and ethanolic extracts as compared to other solvents extracts. Qualitative analysis

was also carried out to check the existence of flavonoids, terpenoids, phlobatannins and alkaloids during this study (Swamy MK, et. al; 2012).

Whole plant extracts generated using multiple solvents were evaluated for the anti-bacterial activity against multiple bacteria (Hajare CN, et. al; 2015). Bacterial species including Micrococcus luteus,*Bacillussubtilis*, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhii, Serratia marcescens, Shigella flexneri, Streptococcus faecalis, Staphycococcus aureus*, and *Vibrio cholerae*were used to access the anti-bacterial activity of plant extract. Study proved that ethyl acetate, chloroform, ethanolic and methanolic extracts exhibited larger inhibition zone as compared to aqueous and diethyl ether extracts. Qualitative analysis of these extracts for the phytochemical screening revealed the availability of several phytochemicals such as alkaloids, anthroquinones, amino acids, carbohydrates, flavonoids, furanoids, glycosidal sugars, phenols, phytosterols, proteins, saponins, tannins and triterpenoids. The presence of different phytochemicals in plant extracts may be responsible for the anti-bacterial property (Umamaheswari A, et. al; 2008).

Anti-diabetic Property

The hypoglycemic activity of the plant stem bark was assessed in alloxan-induced albino diabetic rats. Orally administered for seven days, the ethanolic extract of the stem bark was given at different concentrations, including 100, 250, and 500 mg/kg/day. This study showed that extract contains substantial hypoglycemic activity i.e. up to 22.2% stronger than standard hypoglycemic drug namely glibenclamide which is also given orally as a control (Jawla S. et. al;2012). The major antidiabetic constituents present in the stem bark of the plant were β-sitosterol, pinitol, quercetin and quercetin-3-O-α-L-rhamnopyranoside.

Plant methanolic and aqueous extracts were also evaluated in diabetic mice for the glucose tolerance and considerable decrease in intestinal glucosidase activity. 100 µg concentration of these extracts were injected in mice via intra-peritoneal mode for twenty one days. Results showed notable increment in skeletal muscle glycogen and hepatic contents as well as rise in glucose-6-phosphate dehydrogenase activity. These extracts also having the capability to rise in plasm insulin due to rejuvenation of insulin producing cells (Bhat M. et. al; 2011).

Anti-fertility Property

Swiss albino male mice were administrated by plant leaves extracts at very high dose (800mg/kg/day) for 50 days to evaluate anti-fertility property of this plant extract. Significant increment was observed in the concentration of anodic protein in seminal plasma (3.74 mg/ml) as compared to control mice (2.37 mg/ml). The increase of anodic protein concentration caused additional negative charges on the surface membrane of sperms, which in turn inhibits fertilizing capacity of the sperm. Other way of this plant is increased amount of M-isozyme from LDH in treatment group (5.68 units/ml/hr) as compared to control group (3.31 units/ml/hr). Adverse effect on sperm metabolism in epididymis was observed due to tissue respiration shift from aerobic to anaerobic condition that was caused by the conversion of pyruvate to lactate (Hembrom AR. Et. al;2014). Leaves of this plant were orally administrated with the dose of 8000 mg/kg/day in male and female Swiss albino mice to access the effect for 30 days. Study proved significant reduction in the caudal epididymal count of sperms up to 0.65x106 per ml in the treatment group as compared to 5.05x106 per ml in the control group. Histological study showed hypertrophy of interstitial cells of leydig and reduction in thickness of germinal epithelial cells along with the size of the seminiferous tubules. The lumen of the tubules was found to be lacking the sperms. On the other hand, in female mice, it was clearly observed that the metaestrus phase was prolonged up

to 25.0 h in the treated group as compared to 10.6 h (control group). Other than this, hormonal levels of estrogen and testosterone were also observed considerably declined in mice (Mishra N. et. al;2009).

150, 300, 450 and 600 mg/kg/day of oral administration of the plant leave extracts were given to male rats to evaluate the effect on fertility and reproductive organs for 65 days. Outcome from this study revealed drastic reduction in the different sperm parameters such as viability, count and motility. Sperm viability was reduced from 86.6% to 63.9%, sperm count was reduced from 9.38x106 per ml to 6.76x106 per ml and sperm motility was reduced from 65.8% to 42.8%, respectively in control group as compared to treatment group (Ikpeme EV, et. al; 2015).

Anti-hyperlipidemic Property

The plant leaves has property of reduction in the concentration of serum cholesterol. Oral dosages of leaf ethanolic extract was given at multiple concentrations such as 50, 100 and 200 mg/kg/day to rats for seven days. Significant reduction in the triglyceride and total cholesterol levels were observed after ingestion of plant leaf extract. Other study also reported that compared to standard hypo-lipidaemic drug simvastatin, the plant leaf extract can considerably reduce serum lipid profile in rats. Rats were orally administrated the methanolic extract of plant leaf in different concentration such as 100 and 200 mg/kg/day for eight weeks. Result of the study proved notable reduction in triglyceride, total cholesterol, low and very low density lipoprotein levels (Adebayo JO, et. al; 2005).

Anti-inflammatory Property

The anti-inflammatory activity of the plant was studied using carrageenan and dextran, and the activity was estimated by the Freund's adjuvant-induced arthritis model. Methanolic extracts of the plant at concentrations of 20 and 50 mg/kg were used to evaluate the anti-inflammatory property. In

carrageenan induced acute inflammatory models 20.6% and 67.6% anti-inflammatory effect was observed respectively, while in dextran induced edema this effect was found to be 30.0% and 66.0% respectively. Significantly higher anti-inflammatory effect was observed in the 50 mg/kg concentration in the arthritic model that competes to standard dexamethasone drug (Mandal G, et. al; 2015).

Anti-oxidant Property

Solvents such as acetone, chloroform, methanol, petroleum ether, and water were applied to examine the anti-oxidant property of plant leaves. Methods such as nitric oxide radical scavenging activity, metal-chelating assay, and superoxide radical scavenging activity were engaged for this study.Outcome from this study proved that the aqueous extract of this plant demonstrated significantly higher anti-oxidant activity as compared to other extracts (Dhankhar S. et. al; 2013). Aqueous and methanolic extracts of this plant leaf were also evaluated for the radical scavenging activity and phytochemical content. Findings from this study suggested that methanolic extract had higher radical scavenging activity as well as high amount of phytochemicals as compared to aqueous extract (Venkatachalam RN. Et. al; 2012).

Anti-ulcer Property

The anti-ulcerative activity of the plant leaf extract was assessed in three rat models and compared with a well-known available synthetic drug. (a) Water immersion stress-induced ulcer, which was compared to omeprazole, (b) Aspirin-induced gastric ulcer, which was compared to ranitidine, and (c) Ethanol-induced ulcer, which was compared to sucralfate.Pharmacological action mechanisms such as cyto-protection, anti-secretory and proton pump hypothesis were evaluated for this study. Findings from this study showed reduction in total acidity, gastric volume and free acidity. In some cases 100% inhibition

of ulcer was also reported. Protection index was found around 72% and cyto-protective effect was found around 89.7% in water immersion stress induced ulcer (Malairajan P.et. al; 2007).

This review has made an attempt to advise B. spectabilis, to be one of the picks in the field of medicinal plants. Scientific communities are constantly discovering natural products because of a number of side effects are linked with the conventional medicinal system. Based on the current verdict, B. spectabilis, though known for its ornamental value also exhibited considerable medicinal importance. Most of the described scientific research work is executed on extracts from various plant parts, which demonstrated a diverse range of biological activities and pharmacological ingredients. Diverse combination of the active constituents after isolation and identification have potential in curing various health related disorders. Extensive studies of the phytochemical constituents and pharmacological properties and their mechanisms of action, efficacy, and safety are advisable for future research of this plant.

A Reviewon *Tecoma stans*

Taxonomic Classification:

Domain	:	Eukayiota
Kingdom	:	Plantae
Subkingdom	:	Angiosperm
Phylum	:	Tracheobionta
Subphylum	:	*Euphyllophytina*
Super division	:	Spermatophyta
Division	:	Magnoliophyta (Eudicots)
Class	:	Magnoliopsida - Dicotyledons
Subclass	:	Asteridae
Order	:	Scrophularials
Family	:	Bignoniaceae
Genus	:	*Tecoma*
Species	:	*Tecoma stans*

Plant Description:

Tecoma stans belongs to the Bignoniaceae family and is considered a promising species from the trumpet wine family. This plant is known by various common names, including esperanza, yellow bells, trumpet bush, and yellow elder. It is a perennial shrub with the ability to flower, reaching a height of 5 to 7 meters during its lifespan. The bark of the plant is pale brown to grey in color, becoming soft with age.

The leaves of the plant are compound and imparipinnate, with 2 to 5 pairs of leaflets, a larger solitary terminal leaflet, and an opposite arrangement. The plant's fruits are up to 20 cm long, narrow,

containing several winged seeds, and arranged in somewhat packed, pointed capsules. Thefruits remain on the tree in messy bunches, initially green in color during youth and turning brown upon ripening.

Flowers are produced in clusters at the ends of branches, approximately 6 cm in length. They are light to bright yellow with dark orange stripes at the throat, featuring five circular lobes and a trumpet-shaped structure. There are a total of four stamens attached at the top of the tube in uneven pairs, yellow in color, about 6 mm long, linear, and versatile (Pulipati, S., and Srinivasababu P. 2017).

Figure 1: Plant picture of *Tecoma stans*.Flowers (1A), leaf (1B), fruit (1C)

Phyto-chemical Elements:

The bark, root, and leaves of *Tecoma stans* contain various biologically active phytoconstituents, and traditional and folk medicines utilize extracts from these plant parts for treating various diseases and medical conditions. Different bioactive compounds, including alkaloids, flavonoids, glycosides, phenols, saponins, and terpenoids, have been isolated from this plant. Various solvents are used in the extraction process to screen and isolate different phytochemicals.

The bark and leaves of the plant exhibit anti-inflammatory activity. Tecomin, a key alkaloid isolated from *Tecoma stans*, is of significant therapeutic importance due to its role in hypoglycemic properties, making it a remedial agent for the treatment of type-II diabetes. Studies have revealed the biosynthesis of monoterpene alkaloids in callus tissue, along with the presence of primary and secondary metabolites such as triterpenoids (α-amyrine, oleanolic acids, and ursolic), phenolics (caffeic, chlorogenic, sinapic acids, o-ceramics, and vanillic), sugars (fructose, sucrose, glucose, and xylose), lapachol, and p-sitosterol.

Several alkaloids, including boschniakine and 5β-hydroxyskitanthine (Base C), were found to be inactive in in-vivo and in-vitro studies. Recent discoveries include the presence of indolic compounds and iridoid glycosides in the leaves of the plant. Other potential phytoconstituents, such as amino acids, flavonoids, glycosides, monoterpenes, phytosterols, quinones, saponins, tannins, and triterpenes, may also be present and require confirmation (Raju S., et al. 2011).

Medicinal Uses:

The *Tecoma stans* plant has been utilized since ancient times, and modern pharmacological studies have underscored its significance for remedial purposes. Various parts of the plant, including the bark, root, and leaves, have been employed in herbal medicine to treat numerous ailments. In Latin and South America, this plant is recognized for its role as a blood glucose level-reducing agent. The bark of the plant demonstrates mild choleretic, cardio tonic, and muscle relaxant activities. Additionally, it is employed in the treatment of digestive problems and the control of yeast infections. The roots of the plant act as potent tonics, vermifuges, and diuretics. Grinded roots, when mixed with lemon juice, are reported to be used both externally and ingested in small amounts for remedial purposes, particularly for rat and snakebites (Jadhav D. 2006).

Pharmacological Activities:

In pharmacology, the term "biological activity" or "pharmacological activity" refers to the beneficial effects of a drug on living matter. Drugs are complex chemical mixtures, and plant substances contain active ingredients or pharmacophores that can be used directly or modified by other constituents to exhibit pharmacological activity. *Tecoma stans* has been subjected to screening for various pharmacological activities, including anti-microbial, anti-oxidant, anti-diabetic, anti-proliferative, anti-inflammatory, anti-cancer, cytotoxic, and wound healing properties by multiple scientists.

Anti-microbial Activity:

The anti-microbial activity of the methanolic extract from the bark and leaves of this plant has been studied against a broad range of fungi, gram-positive and gram-negative bacteria. Results indicated that the bark extract is more effective than the leaf extract for anti-microbial activity (Naseri M.K.G et. al; 2007). Phyto-chemical evaluation also confirmed the presence of anthraquinones, alkaloids, flavonoids, phenols, saponins, steroids, and tannins. Three extract fractions exhibited the maximum content of total phenol (177-216 mg gallic acid equivalent/g), contributing to the plant's anti-microbial activity (Anand M., and Ramaiah B. 2019). Anti-microbial activity was also observed against the growth of B. subtilis and E. coli by aqueous and alcoholic extracts at different concentrations of this plant (Al-Azzawi A.M. et. al; 2012). Another study demonstrated that plant leaf extracts in water, ethanol, and methanol were effective against different bacteria, including Clavibacter michiganensis, E. coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Pseudomonas fluorescens, and Staphylococcus aureus (Naseri M.K.G et. al; 2007).

Anti-oxidant Activity:

The occurrence of tannins, which possess potent anti-oxidant activity, has been reported in the alcoholic extracts of this plant (Zhang L.L. et. al; 2008). The termination of the radical chain reaction during the oxidation of triglycerides can also occur due to flavonoids present in the plant, acting as scavenger's free radicals (Penman A.R., and Gordon M.H. 1998). The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay was used to measure the anti-oxidant activity of ethanolic and methanolic extracts of this plant's parts, against standards such as butylated hydroxytoluene and ascorbic acid. This assay demonstrated that a higher concentration of the methanolic extract of the plant has more anti-oxidant potential compared to the standard ascorbic acid at a concentration of 20 µg/ml (Robinson J.P. et. al; 2017).

Anti-diabetic Activity:

The anti-diabetic activity of *Tecoma stans* is attributed to alkaloids such as tecostanine and tecomine (Hammouda Y. et. al; 1964). Studies have demonstrated that the acute and sub-chronic administration of alkaloids, like tecomine, resulted in reduced levels of triglycerides and cholesterol (Santamaría L.A. et. al; 2009). The ethanolic extract of the plant stem, with a concentration of 200 mg/kg, exhibited statistically significant activity in decreasing blood glucose levels. The ethanolic extract demonstrated a more significant value of 147.5±4.4 mg/dl compared to the positive control group (standard value i.e., 124.6±3.9 mg/dl). The potential anti-diabetic activity of the stem's ethanolic extract of this plant is attributed to its ability to enhance insulin discharge from the pancreatic β-cells and the presence of phyto-chemicals such as alkaloids, flavonoids, and saponins in the extract (Elosh G, et. al; 2013). The aqueous extract of leaves also exhibits anti-diabetic activity by encouraging glucose intake in both

insulin-sensitive and insulin-resistant humans without any significant side effects (Castroa A.J.A. et. al; 2010).

Anti-inflammatory Activity:

The prevention of edema in 4 hours and 3 hours after carrageenan challenge by aqueous and alcoholic extracts at concentrations of 250 and 500 mg/kg, respectively, has been demonstrated in studies. This prevention might mitigate the altered appearance and inflammation due to chemical mediators. Anti-inflammatory action was also observed through the inhibition of heat-induced albumin denaturation and cell membrane stabilization of red blood cells by the ethanolic, methanolic, and aqueous extracts of this plant (Melappa G. et. al; 2011).

Anti-cancer Activity:

The anti-proliferative activity of plant bark and leaves has gained potential significance in the field of cancer treatment. Traditional uses of flower and bark in treating various types of cancers have also been reported. Studies have shown that the presence of hydroxyl groups in the aromatic rings of acteoside plays a crucial role in the anti-proliferative effect (He Z.D. et. al; 2001). Additionally, anti-proliferative activity against the MCF-7 cell line has been observed with 5-hydroxy-skytanthine hydrochloride, a monoterpene alkaloid isolated from *Tecoma stans*. Another study demonstrated that the ethanolic leaf extract of this plant at a concentration of 64.5µg/ml exhibits anti-cancer activity against the breast cancer cell line MCF-7 (Thirumal M. et. al; 2012). Furthermore, extracts from the plant's bark, stem, flower, and root were tested for their anti-proliferative activity against MCF-7 breast cancer cell lines, with the bark showing the highest anti-proliferative activity compared to other plant extracts (Marzouk M. et. al; 2006).

Cytotoxic Activity:

In various studies, researchers focused on evaluating the impact of different concentrations of aqueous and alcoholic extracts from *Tecoma stans* on mouse embryo fibroblast cell lines. The extracts were tested for their ability to induce cytotoxic effects, essentially causing cell death. Higher concentrations of these extracts showed promising results in terms of their ability to induce cytotoxicity, implying potential anti-proliferative effects on the tested cell lines.

To confirm the impact, researchers conducted morphological examinations using inverted microscopy, observing changes in the appearance of the treated cell lines, a common method to verify cell death. The cytotoxic activity of *Tecoma stans* extracts was found to be dependent on both the concentration of the extract and the duration of exposure to the cells. Additionally, the presence or absence of fetal bovine serum in the testing environment was noted to influence the observed cytotoxic effects.

For quantitative assessment, the researchers utilized the MTT assay, a widely used method to measure cell viability. The results were reported as a percentage of cell viability, providing a quantitative measure of the cytotoxic impact of *Tecoma stans* extracts on mouse embryo fibroblast cell lines (Gaitan I. et. al; 2011).

Wound Healing Activity:

The wound healing activity of *Tecoma stans* was investigated using a methanolic extract of the plant bark in albino rats. Wound healing is a complex process involving well-defined cellular and biochemical events that lead to the progressive growth and rejuvenation of the wounded tissue area. The plant's methanolic extract, when systematically administered or locally applied, demonstrated

wound healing effects in both incision and excision models. The observed wound healing activity could be attributed to the individual or combined effects of various phyto-constituents present in the plant, including flavonoids, glycosides, phenols, phytosterols, saponins, tannins, or triterpenes (Das V.N.R. et. al; 2010).

Tecoma stans has been traditionally used by indigenous medicine practitioners to treat various diseases, and modern research activities support the presence of a wide spectrum of pharmacological ingredients in different parts of the plant. Scientific studies with crude extracts have demonstrated anti-microbial, anti-oxidant, anti-diabetic, anti-proliferative, anti-inflammatory, anti-cancer, cytotoxic, and wound healing activities. The diverse phyto-constituents present in the plant extracts, such as alkaloids, amino acids, flavonoids, glycosides, phenols, phytosterols, saponins, tannins, and terpenoids, contribute to these therapeutic properties either individually or in combinations. Ongoing investigations into this plant are driven by its vital and effective pharmacological applications. This review aims to consolidate relevant evidence from various global scientific studies, highlighting the plant's exclusive remedial and therapeutic properties for supporting human health and suggesting potential future research directions.

A Review on Extraction Processes

The process of discovering novel drugs in medicinal plants involves a well-structured approach, starting with the careful collection of plant materials from their natural habitats, ensuring their preservation. The desired plant parts are then subjected to solvent extraction to capture a broad spectrum of phytochemicals present in the plant. Subsequent concentration of the extract removes excess solvent, and through a series of tests and analyses, potential bioactive compounds are identified and isolated. Molecular structure elucidation follows, utilizing various analytical techniques. These compounds are then put through pharmacological and toxicological assessments to gauge their suitability as new drugs. In some cases, these compounds may be synthesized or semi-synthesized for further research and development. This systematic and multidisciplinary process plays a vital role in the quest for novel pharmaceutical agents derived from medicinal plants, potentially leading to the creation of safer and more effective medications (Kebede et al., 2021).

Extraction plays a crucial role in natural product research, and ongoing efforts focus on enhancing and discovering more efficient and cost-effective extraction techniques. The comprehensive review delves

into various conventional and modern extraction methods, scrutinizing their optimization parameters, and conducting a comparative analysis of their advantages and disadvantages.It also delves into recent applications of these techniques. Standardizing extraction procedures is of paramount importance as it directly influences the quality and effectiveness of herbal drugs, ensuring they meet pharmaceutical standards and are suitable for medical applications. This systematic approach aids in harnessing the therapeutic potential of natural products and their incorporation into medical treatments, emphasizing the importance of research in this field.

Plant life is a remarkable realm of complex chemical processes, giving rise to a wide range of phytochemicals, or plant-derived compounds. These phytochemicals can be categorized into two main groups: primary metabolites and secondary metabolites. Primary metabolites are essential compounds that are crucial for a plant's basic growth and development. They include molecules like nucleic acids (DNA and RNA), carbohydrates, fatty acids, and proteins. These primary metabolites are universal and necessary for all plants to function and thrive. They are involved in fundamental processes such as energy storage, cellular structure, and growth regulation (Hussein and El-Anssary 2018).

Secondary metabolites, on the other hand, are a diverse group of compounds produced by plants primarily to adapt to their environment and ensure their survival. These secondary metabolites serve various functions and are often part of the plant's defense mechanisms. For instance, in environments with insect threats, plants may produce secondary metabolites with insecticidal properties. In regions rich in pathogenic fungi, plants might develop anti-fungal secondary metabolites to protect their roots. Some of these secondary metabolites can also have anti-fungal, antibacterial, or antiviral properties, which contribute to the plant's ability to fend off threats.

The world of secondary metabolites is particularly intriguing to scientists, pharmacologists, and biochemists due to the diverse roles these compounds play in plant cells. Specific secondary

metabolites are considered bioactive compounds, as they can have significant pharmacological or toxicological effects on both animals and humans. They can be classified into categories such as terpenes and terpenoids, alkaloids, and phenolic compounds, each with unique structural characteristics and functions depending on the synthesis pathways they undergo. In summary, the realm of plant chemistry is a captivating one, with plants employing a vast array of phytochemicals, both primary and secondary, to support their growth, adapt to their environments, and defend against threats. These compounds have profound implications for various fields, from botany to pharmacology, offering valuable insights into the intricate chemical interactions that occur within the plant kingdom (S.H. Taha, et al., 2019; C. Awuchi, 2019).

Conventional Extraction Techniques

Maceration:

Maceration is a straightforward yet effective method for extracting valuable compounds from plant materials. In this process, the plant material, which is either coarse or in powdered form, is soaked in a chosen solvent under room temperature conditions for a minimum of three days. During this time, intermittent agitation or stirring is applied to ensure efficient extraction. Once the extraction is complete, the mixture is separated by passing it through sieves or a mesh with small openings. Following this, the remaining solid residue (known as marc) is pressed to extract any remaining liquid, and the resulting liquid extract is further purified through either filtration or decantation after allowing it to settle. To minimize solvent loss due to evaporation, it is advisable to carry out maceration in a container with a stopper or lid. The goal is to prevent the premature concentration of the extract due to solvent evaporation during the extraction process. If a more concentrated product is desired, it can be achieved through vacuum evaporation after the initial extraction. Selecting the appropriate solvent is a

critical consideration in the maceration process, as it significantly influences the types of phytochemicals that will be extracted from the plant samples. The solvent choice may also impact the extraction of thermolabile phytochemicals, which are compounds sensitive to heat. In summary, maceration is a valuable technique for extracting phytochemicals from plant materials, providing a method that can be tailored to preserve specific compounds based on the choice of solvent and extraction conditions (N.N. Azwanida 2015).

The maceration extraction method, while straightforward, has certain limitations. It is generally considered less efficient and time-consuming compared to other extraction techniques. However, under optimized conditions, it can still yield significant results. For instance, in a study involving chokeberries, maceration was found to provide high yields of phenolic compounds and anthocyanins, highlighting the potential for efficiency when the method is fine-tuned (N. C′ uji c et. al; 2014).

On the contrary, research on the extraction of flavonoids from pigeon pea (Cajanus cajan) leaves showed that maceration resulted in the lowest yield when compared to other techniques such as microwave-assisted extraction, reflux, and ultrasound-assisted extraction. This discrepancy in extraction efficiency emphasizes the importance of considering the specific plant material and compounds of interest when selecting an extraction method (165. S. Jin et. al; 2011).

Various studies have used maceration to extract different phytochemicals from different plant sources. For example, flavonoids were extracted from turmeric rhizomes using nonionic surfactant Triton X-100 at a neutral pH and a temperature of 35°C. In another case, flavonoids were extracted from fruits of Arbutus unedo L. using a higher temperature of 79.6°C in a 3.7% diluted ethanol solution. Flavonoids were also obtained from the leaves of Ficus carica and Euphorbia neriifolia using 75% concentrated ethanol at room temperature. The choice of solvent in these studies was based on the polarity of the

targeted phytochemicals, with less polar solvents used for less polar compounds and more polar solvents for more polar compounds (B.R. Albuquerque et. al; 2016).

In the case of polyphenols, such as anthocyanins, they were effectively extracted from dried chokeberry fruits using a 50% ethanol solution as the solvent. These examples demonstrate the versatility of maceration as an extraction method, provided that the conditions and solvents are carefully chosen to suit the specific phytochemicals and plant materials under investigation (Sharma and Janmeda 2014).

Decoction:

Decoction, a method of extraction involving boiling plant material in water, has limitations such as extracting water-soluble impurities and being unsuitable for thermolabile or volatile components. Chemical transformations occur during decocting, as observed in the study of Danggui Buxue Tang, where flavonoid glycosides in Astragali Radix underwent hydrolysis to form calycosin and formononetin. The efficiency of hydrolysis was influenced by factors like pH, temperature, and herb quantity (Zhang et. al; 2014). In another study on Sanhuang Xiexin Tang (SXT) and Fuzi Xiexin Tang (FXT), it was found that the decoction process improved the dissolution of bioactive compounds when compared to maceration. The elevated temperature during decoction deactivated β-glucuronidase, impacting the conversion of glycosides to aglycones. The study also observed interactions between chemicals from different herbs, providing insights into the reduction of toxicity and enhancement of efficacy in Traditional Chinese Medicine formulations (Zhang et al., 2013).

Digestion:

Digestion, as an extraction method, shares similarities with maceration but involves slight warming during the extraction process. The purpose of this slight warming is to enhance the efficiency of the

extraction solvent, but it must be done with care to prevent any alterations to the bioactive phytochemicals within the plant material being extracted. The temperature typically maintained for digestion falls within the range of 35 to 40°C. However, in the case of tougher plant materials or those with poorly soluble phytochemicals, the temperature may be increased to a maximum of 50°C.

The digestion extraction process involves placing the desired plant parts into a container along with a suitable solvent that has been pre-heated to the specified temperature. This optimized temperature is then maintained for a duration that can vary from as short as half an hour to as long as 24 hours. To ensure thorough extraction, the container is periodically shaken or agitated at regular intervals. The use of digestion as an extraction method offers the advantage of improved extraction efficiency, making it particularly suitable for plant materials that may require slightly elevated temperatures to facilitate the extraction of specific phytochemicals (N.N. Azwanida 2015).

Percolation:

Infusion, characterized by its simplicity, involves immersing plant material in a boiling solvent, typically water, and allowing it to stand for around 15 minutes. This process results in a dilute solution rich in easily soluble constituents of the plant. Tea serves as a classic example of an infusion, with various brands showcasing distinct extraction profiles. Notably, caffeine extraction from tea leaves, including popular brands like alokozay, lipton, tapal, and tetley, has been explored at different brewing times and temperatures. The infusion technique extends to extracting phenolic compounds from fruits like Tilia cordata, emphasizing the versatility of this method. Some infusions even hold medicinal value, addressing health issues like diarrhea, bronchitis, and asthma. In a similar vein, percolation emerges as a more efficient alternative to maceration (R. Sharif et al., 2014; M. Cittan et al., 2018).

Percolation stands out as a widely utilized technique for crafting fluid extracts, particularly tinctures. The essence of percolation lies in the meticulous process of gradually passing a liquid, often ethyl alcohol, through plant material drop by drop. This method involves slowly saturating the solvent with phytochemicals as it courses through the plant material, steadily descending with the help of a fresh solvent introduced from the top. Prior to entering the percolator, the plant material must be delicately shredded, avoiding excessively fine particles that could complicate the separation process and result in a cloudy extract with residue settling at the bottom. To facilitate diffusion, moistening the plant matrix with the extraction solvent proves beneficial, promoting smooth extraction of phytochemicals (N.N. Azwanida 2015).

The percolation process involves pouring the extraction solvent into the percolator from the top, allowing it to percolate gradually through the plant material. The speed of percolation is influenced by the characteristics of the plant material, striking a balance between providing sufficient times for solvent penetration into plant cells and avoiding excessive consumption. An optimal solvent flow rate is typically around 5 mL per minute for 1 kg of plant material. The choice of solvent depends on the chemical properties of the secondary metabolites being extracted. Usually, a water-alcohol solvent mixture is employed for efficient extraction, with water hydrating plant walls and alcohol mirroring the chemical composition of many active components.

For example, 70% ethanol is used for extracting phenolics like epicatechin. Additionally, petroleum ether is applied to extract antioxidants such as phenols and flavonoids. Notably, hydrochloric acid in an aqueous solution has been utilized for percolation to extract alkaloids from wild fruits. Alcohol also acts as a preservative, ensuring the longevity of the extracted leachate. Once the percolation process is complete, the plant material is pressed to recover any residual solvent, and the resulting solution is

combined with the leachate. Extraction is deemed complete when a colorless liquid, free of phytochemicals, elutes from the percolator (J.E.W. Paz et al., 2017; F. Zhang et al., 2005).

Solvent extraction method:

In the current extraction process, a Universal Extraction System by Buchi is employed for solvent extraction. Here is a breakdown of the procedure:

Sample Preparation: The dried powder of various plant parts is collected and placed in glass thimbles. These thimbles serve as containers for the plant material during the extraction process.

Selection of Solvents: Different solvents are chosen based on their selectivity for specific phyto-constituents. The choice of solvents is crucial in ensuring the extraction of desired compounds.

Extraction Cycles: The extraction procedure is carried out in cycles, with each cycle involving the use of the selected solvent. Typically, ten cycles are performed for each extract. This repetitive process allows for thorough extraction and ensures that a wide range of phyto-constituents are captured.

Controlled Temperature: During the extraction, the temperature is carefully controlled. It is maintained just below the boiling point of the respective solvents. This controlled temperature ensures efficient extraction while preventing excessive evaporation of the solvent.

Filtration: After the extraction process, the resulting solvent extract is filtered. This step helps remove any solid residues or impurities from the extract, leaving behind a purified solution.

Concentration: The filtered solvent extract is then concentrated. This concentration step is typically performed in a vacuum concentrator. By reducing the volume of the extract, the phyto-constituents become more concentrated and easier to analyze.

This extraction process using the Universal Extraction System by Buchi allows for precise and efficient extraction of phyto-constituents from plant materials. The use of controlled temperature, solvent

selection, and repetitive cycles ensures a comprehensive extraction process, making it a valuable method for studying the chemical composition of plants and their potential applications in pharmaceuticals, nutraceuticals, and other industries (Harborne JB. 1973).

Modern extraction techniques

Reflux extraction:

This is known for its efficiency, the soxhlet extraction method requires less time and solvent compared to percolation or maceration methods. However, it is not suitable for extracting thermolabile natural products.In a study with Stemona collinsiae root, refluxing with 70% ethanol yielded the highest amount of the natural bio-insecticidal compound didehydrostemofoline (50, Fig. 5) at 0.515% w/w of the extract, outperforming extracts prepared using other methods such as sonication, Soxhlet, maceration, and percolation. Another study comparing extraction efficiency for a Traditional Chinese Medicine (TCM) compound composed of seven herbs found that the reflux method, using 60% ethanol as the extraction solvent, outperformed the decoction method in yielding baicalin and puerarin (Zhang, 2013).

Soxhlet extraction:

Soxhlet extraction, a continuous method for extracting phytochemicals using a hot solvent, combines the principles of reflux extraction and percolation. This automated technique is highly efficient, requiring less time and solvent compared to methods like maceration or percolation. The process involves placing ground plant material into a thimble, typically made of firm filter paper or cellulose, which is then, put in the Soxhlet apparatus. The extraction solvent, such as ethanol or methanol, is

heated, vaporizes in the sample thimble, condenses in the top condenser, and drips back, ensuring continuous extraction of phytochemicals.

Soxhlet extraction yields higher amounts of compounds compared to maceration-based techniques. For instance, it has successfully extracted fatty acids from hemp seeds and phenolic compounds from leaves. However, the higher temperatures used in Soxhlet extraction can degrade thermolabile compounds, as observed in a comparative study where polyphenols and alkaloid extracts decreased compared to maceration extraction (Chin et al., 2013).

Accelerated solvent extraction (ASE):

Accelerated Solvent Extraction (ASE) has gained prominence due to its advantages, such as low solvent demand, high output, and efficiency in a shorter period. Operating under elevated solvent temperature and pressure, ASE surpasses techniques like maceration or Soxhlet extraction. Notably, ASE has demonstrated superior performance in recovering both lipophilic and hydrophilic phytochemicals from raspberry pomace compared to Supercritical Fluid Extraction (SFE). The stainless steel extraction cell, packed with the sample, is filled with solvent and subjected to increased temperature and pressure, enhancing extraction through improved diffusion coefficient and lowered viscosity.The inert packing material prevents blockages, and the extracted compounds are collected for analysis (Rahmalia et. al; 2015). Achieving optimal phytochemical recovery yields through Accelerated Solvent Extraction (ASE) involves critical parameters like temperature, pressure, and extraction time. The robustness of ASE was demonstrated in the extraction of cocaine and benzoylecgonine from coca leaves, showcasing an optimum temperature of 80 °C, a pressure of 20 MPa, and an extraction time of 10 minutes. Notably, temperature variations impact the extraction efficiency of different bioactive

compound classes differently; higher temperatures favor phenolic acids content yield, while lower temperatures are more efficient in extracting high yields of flavonoids (Figueroa et. al; 2018).

Indeed, the critical extraction conditions can exert individual or combined influences on extraction efficiency. In cases like the recovery of carbohydrates and phenolic compounds from barley hull, temperature was the sole influential factor, rendering pressure and extraction time insignificant. Conversely, during the ASE of steviol glycosides from stevia leaves, temperature, extraction time, and the number of cycles collectively impacted phytochemical yield. Optimization, including reducing particle size, further improved glycosides recovery. Similarly, in carotenoid extraction, time and temperature played a significant role, with optimal conditions identified at 60 °C for 15 minutes in three cycles. Interestingly, increasing solvent volume beyond three cycles did not enhance carotenoid recovery (Saha et. al; 2015).Absolutely, ASE stands out for its speed, efficiency, reproducibility, environmental friendliness, and energy efficiency in comparison to various extraction methods. Its versatility is evident, but it's important to note the specificity of extraction conditions for different phytochemicals. As seen in the extraction of Beta-glucans and phenolic compounds from waxy barley, temperature played a contrasting role—favoring lower extraction yield for β-glucans but enhancing recovery yield for phenolics. The impact of temperature on molecular fragmentation could explain the observed differences, highlighting the technique's nuanced application based on the target compounds (Román et al.; 2015).

Indeed, the solubility of phytochemicals in the solvent is a pivotal factor influencing the efficiency of ASE. Generally, higher solubilities lead to increased extraction and enhanced recovery yields. For instance, nonpolar compounds like butylidene dihydrophthalide, 4–hydroxy-4-methyl-2-pentanone, and 9,12-octadecanoic acid from angelica roots showed higher yields when using n-hexane under specific conditions. However, it's crucial to match solute and solvent polarities for optimal results. Achieving a

balance, whether with polar or nonpolar combinations, is essential for maximizing extraction efficiency (Machado et al.; 2015).The use of solvent mixtures has proven effective in achieving better extraction yields. Notably, a combination of moderately polar solvents has demonstrated enhanced recovery of phenols, given the moderately polar nature of these compounds. Phenols, with their acidic nature and enhanced polarity due to the benzene ring, are readily obtained when using polar solvents. This approach optimizes both temperature and pressure, allowing for rapid extractions completed in less than an hour. For instance, flavonoids, primarily phenolic compounds, were efficiently extracted using the ASE technique at an optimal temperature of 80 °C with a solvent mixture of 60% ethanol, demonstrating the effectiveness of this method (Gomes et al.; 2016).

The versatility of the ASE technique is evident in various extraction applications. Clear and colorless pesticide extracts from alfalfa leaves and the separation of four lignans from fructus schisandrae fruits showcase its effectiveness. The technique extends to marine sponges, allowing for the extraction of compounds from these sources as well. However, it's important to note that ASE may not always outperform Soxhlet extraction in every aspect. In the case of extracting estragole, an essential oil from fennel, Soxhlet extraction exhibited enhanced robustness and higher phytochemical yields compared to ASE. Despite similar estragole concentrations, ASE was favored for its shorter extraction time and lower solvent consumption, with optimal conditions at 125 °C, 7 minutes, and 3 cycles (Solana et al.; 2014).

ASE proves advantageous in specific extraction scenarios, particularly for lipids and phenolic compounds from cereals. Unlike Soxhlet, which tends to oxidize lipids due to continuous heating, ASE provides a more suitable alternative for lipid extraction. Additionally, ASE addresses the challenge of obtaining phenolic compounds from cereals, where these compounds are often tightly bound to cell wall components. For instance, polyphenols from sorghum bran were successfully recovered using

ASE with a mixture of 50% and 70% ethanol and water at an elevated temperature of 150 °C (Barros et al.; 2013).

Cold extraction method:

The process involves drying various plant parts in an artificial environment at a low temperature, typically within the range of 50-60°C. The dried plant parts are then ground into a fine powder and used for the extraction process using different solvents. Here's a step-by-step description of the procedure:

Drying: Different plant parts are carefully dried in an artificial environment, maintaining a low temperature of 50-60°C. This drying process ensures that the plant material is adequately dehydrated and ready for further processing.

Powdering: The dried plant parts are then ground into a fine powder. This step helps in increasing the surface area of the plant material, facilitating better contact with the extracting solvent.

Extraction: The dried powder is accurately weighed and placed into a conical flask. The respective solvents, chosen for their selective extraction properties, are added to the flask. The mixture is allowed to stand at room temperature for thirty minutes, with periodic shaking after every twenty-four hours. This extended extraction period ensures that the solvent effectively captures the desired compounds from the plant material.

Filtration: After the designated extraction period, the extract is filtered to separate the liquid portion containing the extracted compounds from the solid plant material. This filtration is typically carried out using Whatman filter paper under vacuum pressure. The filter paper traps the solid residue, allowing the liquid extract to pass through.

Drying: The liquid extract, now separated from the plant material, is dried at room temperature. It is spread out in a watch glass dish for this purpose. Both the dish and the extract are weighed before and after the drying process.

Weight Calculation: The weight of the dried extract is determined by calculating the difference in weight between the dish before and after drying. This weight corresponds to the quantity of extracted compounds obtained from the plant material.

This meticulous procedure ensures the effective extraction of bioactive compounds from the plant material, which can then be used for various purposes, including pharmaceuticals, nutraceuticals, or scientific research. It also allows for the accurate quantification of the extracted compounds, a crucial step in pharmaceutical and scientific applications (Harborne JB. 1973).

Supercritical fluid extraction (SFE):

This method is harnesses the unique properties of supercritical fluids, often using carbon dioxide (CO_2), to extract compounds from various materials. SFE is a versatile technology with broad applications for extracting valuable compounds on a commercial scale. In SFE, a supercritical fluid exhibits physical properties of both gas and liquid phases, achieved at its critical point by specific temperature and pressure conditions. This technique involves solubilizing extractable chemicals, separating them from the sample, and then removing the solvent as conditions change. It's an effective method for extracting compounds from various sources, particularly from food products (Raja et al.; 2021).The process involves the following steps:

Creation of Supercritical Fluid: Gases, typically CO_2, are compressed under high pressure to transform them into a dense liquid state, known as a supercritical fluid. This supercritical CO_2 possesses properties of both a gas and a liquid.

Extraction Process: The supercritical fluid, usually CO_2, is then pumped through a cylinder or extractor vessel containing the material (such as plant matter) from which compounds need to be extracted. The supercritical fluid acts as a highly effective solvent in this state, penetrating the material and dissolving the desired compounds.

Separation and Recovery: The extract-laden supercritical fluid is then directed into a separation chamber. In this chamber, the extract is separated from the supercritical fluid. The gas (CO_2) is recovered for re-use, and the extracted compounds are collected.

One of the notable advantages of SFE is that it leaves no solvent residues in the extracted material because the supercritical CO_2 evaporates completely, leaving behind a clean and pure extract. Additionally, the solvent properties of CO_2 can be precisely manipulated by adjusting the pressure and temperature, making it a versatile and selective method for extraction. SFE is widely used in various industries, including food, pharmaceuticals, and essential oils production, as it offers an efficient and environmentally friendly means of extracting valuable compounds from raw materials without the use of traditional organic solvents (Patil PS and Shettigar R 2010).

Microwave-assisted extraction (MAE):

Microwave-assisted extraction, or microwave extraction, is an innovative method that combines the principles of microwave heating with traditional solvent extraction. This technique is particularly effective in enhancing the extraction of compounds from plant materials. MAE utilizes microwave frequencies ranging from 300 MHz to 300 GHz to improve the extraction process. By directly impacting polar molecules, microwaves heat the solvent and plant tissue, improving the kinetics of

extraction. This energy conversion involves dipolar rotations, and heating is proportional to the dielectric constant of the solvents. The viscosity of the solvent plays a crucial role, affecting the extraction process by facilitating ion dispersion and solvation. The MAE process involves the diffusion of solvents into the sample, separation of solute from functional sites, and the release of solutes into solvents. This technique is known for preserving the biological activities of extracts, such as improving antioxidant activity, total phenolic content, and color quality in green tea extraction optimization.

MAE has been successfully applied to extract various phytochemicals, including saponins from seeds, polyphenolic antioxidants from leaves, sterols from dried mushrooms, and flavonoids from leaves. Notably, MAE is efficient for extracting polar compounds like flavonoids, polyphenols, and saponins, as microwaves directly impact these compounds. Advanced MAE instruments and methods, such as solvent-free microwave-assisted extraction (SFMAE) and pressurized microwave-assisted extraction (PMAE), contribute to the progression and robustness of this extraction technique (Suhaimy et al.; 2021).

Application of Microwave Energy: In microwave-assisted extraction, a mixture of solvents and plant tissue is exposed to microwave energy. The microwave energy rapidly heats the entire mixture.

Selective Heating of Moisture: The key target for microwave heating in dried plant material is the small, microscopic traces of moisture that naturally exist within plant cells. These moisture molecules absorb the microwave energy, causing them to heat up rapidly.

Pressure Build-Up and Cell Rupture: As the moisture within the plant cells heats up, it undergoes evaporation, leading to the generation of significant pressure within the cells. This increase in pressure exerts force on the cell walls, pushing them from the inside. Ultimately, this pressure causes the cell walls to rupture.

Release of Phyto-constituents: The ruptured cell walls result in the release or exudation of the active constituents contained within the plant cells. This effect significantly increases the yield of phyto-constituents obtained during the extraction process.

MAE offers several advantages, including a higher extraction yield and reduced extraction time. The selective heating of moisture within plant cells is a key feature of this technique, as it targets the precise location where the desired compounds are stored. This method is widely used in the extraction of phytochemicals, essential oils, and bioactive compounds from a variety of plant materials and is considered an efficient and environmentally friendly alternative to traditional extraction methods.

Pressurized hot water extraction (PHWE):

Pressurized Hot Water Extraction (PHWE) is gaining attention as an environmentally friendly alternative to traditional extraction methods. This technique utilizes hot water under enhanced pressure, maintaining it in a liquid state between 100 and 250 °C. With advantages such as shorter extraction times, higher-quality extracts, and cost-effectiveness, PHWE has been employed to extract various compounds, including avoparcine, antioxidants, phenolics, saponins, and cannabinoids from Cannabis sativa seeds.Notably, PHWE has been successful in recovering vitamin C, phenolic compounds, and flavonols from Moringa oleifera leaves at temperatures of 91 °C and 100 °C (Nupia et al.; 2012).

Various compounds, including antioxidants and steviol glycosides from Bertoni leaves, have been recovered using Pressurized Hot Water Extraction (PHWE), which utilizes water as a universal solvent, dissolving both polar and nonpolar phytochemicals. The critical factor for PHWE is maintaining water in its nonpolar form, allowing it to efficiently dissolve phytochemicals. However, optimal extraction conditions vary, as seen in the recovery of polyphenols from Thymus vulgaris, where a lower temperature of 100 °C was more suitable, preventing degradation of phytochemicals and preserving

antioxidant activity. PHWE's superiority in recovering phytochemicals is evident, surpassing other solvents like aqueous methanol. Temperature modulation in PHWE impacts the makeup of phenolic compounds, influencing extraction efficiency. Furthermore, the increased yield of phenolic compounds from grape pomace at an optimum temperature of 100 °C is exemplified by the use of natural deep eutectic solvents in subcritical fluid extraction (Loarce et al.; 2020).

The efficient and environmentally friendly technique for obtaining phenolic compounds from winemaking by-products through subcritical water extraction is exemplified by the use of deep eutectic solvents derived from natural sources. The preservation of the bioactivity of extracts, particularly in terms of antioxidant activity, is notable in this process.Comparative studies have highlighted PHWE's high efficiency in extracting phytochemicals with potent antioxidant properties, as seen in the case of T. montanum aerial parts, where antioxidant activities were maintained at medium temperatures but declined at higher temperatures (above 160 °C). Additionally, the extraction of polyphenols from lotus seedpods revealed a positive correlation between the concentration of polyphenols and their antioxidant and antiproliferative activities, highlighting the potential of PHWE in producing bioactive extracts (Yan et al.; 2020).

A notable achievement of Pressurized Hot Water Extraction (PHWE) is the selective extraction of phytochemicals with specific biological capacities. In comparison to conventional ethanol extraction, superior effectiveness in selectively obtaining phenolic compounds with high antioxidant capacity from kānuka leaves has been demonstrated by PHWE. The interplay between extraction time and temperature is crucial, influencing both the preservation and yield of phytochemicals. It's worth noting that while elevated temperature and extraction time improved the recovery yield of flavanols and alkaloids from cocoa shells, prolonged extraction times and higher temperatures had a detrimental effect on the extracted phytochemicals (Okiyama et al.; 2018).

The impact of temperature on extraction yield varies across different plant materials and compounds. For instance, the yield of xylan from quinoa stalks decreases at elevated temperatures due to molecular degradation. Conversely, the extraction yield of flavonoids from Bidens pilosa shows an increase with rising temperature. A study conducted at a moderate temperature of 80 °C revealed excellent recovery of polyphenols and tannins, but a substantial decrease in polyphenol yield occurred at a higher extraction temperature of 150 °C. Additionally, there was a notable reduction in the antioxidant activity of the polyphenols recovered at the higher temperature (Pinto et al.; 2021).

The efficacy of Pressurized Hot Water Extraction (PHWE) can be notably enhanced when combined with other extraction techniques. For instance, the recovery of hesperidin and narirutin from Citrus unshiu peel by-products was improved by employing pulsed electric field in conjunction with subcritical water extraction. This technique generally offers superior products, is less time-consuming, and requires less energy compared steaming distillation. Water's unique features as a preferred extractant in PHWE stem from its eco-friendly nature, universal solvent properties, suitability for recovering thermolabile phyto-compounds, and ready availability (Hwang et al.; 2021).

Enzyme-assisted extraction (EAE):

In convinced plants, extracting phytochemicals from the network of polysaccharide and lignin can be challenging because of hydrogen bonding and hydrophobic interactions. These compounds remain dispersed in the cell cytoplasm and are not easily accessible through conventional solvent extraction. To overcome this, enzymes are employed at the time of extraction and supports to losen/break down cell walls and release bound phytochemicals at high yields. Specific enzymes, including pectinase,cellulase, and amylase, are used for this purpose. Two main approaches, enzyme-assisted aqueous extraction (EAAE) and enzyme-assisted cold pressing (EACP), utilize enzymes to enhance

phytochemical yield, with the former being employed for oil extraction from various seeds and the latter for breaking down seed cellular walls (Oliver et al.; 2021).

Through EAAE, non-extractable polyphenols were successfully obtained from sweet cherry fruits at an optimal temperature of 55 °C. This technique has also been applied to extract phenolics from citrus peels at temperatures ranging from 20 to 60 °C, carotenoids from sunflower petals at 40 °C, lycopene from tomato peels at 30 °C, flavonoids from grapefruit peels at 50 °C, and anthocyanins from Cacahuacintle Maize. EAAE is often combined with other extraction methods, leveraging enzymes to render non-extractable phytochemicals accessible to solvents and thus facilitating their extraction. For instance, enzymes used during microwave processing improved the extractability of phenolic compounds from olive pomace at higher extraction temperatures and faster heating rates compared to conventional solvent extraction with water.Additionally, higher pectin yields were obtained from sisal waste through sequential treatment with enzymes followed by ultrasound compared to other extraction methods that did not involve enzymes (Yang et al.; 2017).

The concentration of enzymes and the pH of the environment are crucial factors that depend on the nature and action of the enzymes employed. To achieve the maximum extract yield, carbohydrases, such as Viscozyme L, a multi-enzyme complex containing arabinase, cellulase, β-glucanase, hemicellulase, and xylanase, are often used at an optimal acidic pH of 4.5 and a temperature of 50 °C in a 0.1 M acetate buffer. The concentration of enzymes has a direct impact on the extract yield; for instance, higher amounts of cellulase have been shown to enhance licorice extraction yields. After pretreating the plant material, the enzymes detach the phytochemicals that were tightly bound to the plant material, making them more accessible for extraction. In a particular instance, researchers acquired flavonoids from pomelo peels by subjecting the peels to 4.5% pectinase treatment for different

incubation durations. Subsequently, they utilized an ultrasound-assisted extraction method at a lower optimal temperature of 30 °C (Anh et al.; 2021).

The use of enzymes to pretreat plant material has proven effective in enhancing the extraction of valuable compounds. As an illustration, the most favorable conditions for extracting rosemary leaves involved a 1-hour pretreatment with pectinolytic enzymes followed by a 24-hour solid-liquid conventional extraction using a 50% hydroethanolic solvent. This method produced an extract exhibiting superior radical scavenging activity of antioxidants compared to an extract obtained without enzyme pretreatment. Likewise, enzymatic protein hydrolysis has proven to be an effective approach for optimizing the extraction of phenolic compounds from wine lees, leading to extracts with enhanced functionalities (Sobrino et al.; 2021).

Optimizing enzyme-assisted extraction involves considering various parameters. In a study on green yerba mate, polyphenol extraction was optimized using response surface methodology. Independent variables included enzyme concentration, temperature, pH and reaction time. Carbohydrases significantly increased polyphenol extraction from 38.67% to 52.08%. The research revealed that all independent variables demonstrated significance at the linear level, whereas pH and temperature did not exhibit significance at the quadratic level. Noteworthy combinations of significance comprised enzyme and reaction time, pH and temperature, as well as enzyme and pH (Heemann et al.; 2019).

The ability of enzyme-assisted extraction (EAE) to improve the extraction of diverse compounds is intriguing. Notably, EAE led to oil extracts with higher fatty acid content compared to conventional techniques. The extraction of polysaccharides with glucose oxidase resulted in more than double the yield compared to the enzyme-free method. Moreover, the use of pectinase and cellulose increased the yields of lycopene and carotenoids from tomatoes.EAAE's use of water instead of other potentially harmful chemicals is a positive environmental aspect. Understanding the plant material's composition

and morphology is crucial, as it should align with the selected enzyme's catalytic properties and operational conditions. The combination of enzymes with modern extraction techniques, such as ultrasonic, microwave, and supercritical fluid extractions, presents a promising approach to enhance phytochemical yields efficiently (Bhattacharjee et al.; 2006).

Ultrasound-assisted extraction, UAE (sonication extraction):

Ultrasound pertains to electromagnetic waves with frequencies higher than those audible to the human ear, typically falling within the range of 20 kHz to 2000 kHz. As it traverses through a medium, ultrasound induces expansions and contractions following wave patterns. The mechanical impact of acoustic cavitation, prompted by ultrasound, facilitates increased surface area contact between solvents and plant samples, thereby enhancing cell wall permeability. The crucial process of cavitation involves the formation, growth, and collapse of bubbles. Studies indicate that adjusting the frequency of ultrasound can modify and positively influence the extraction of compounds from the sample (Machado et al.; 2019).

Interestingly, a study observed that lower frequencies, specifically 40 kHz, resulted in higher yields of phenolics compared to 120 kHz. This highlights the significance of simultaneously assessing ultrasonic parameters to improve and optimize extraction efficiency. Another study employing UAE reported increased phenol yields from rhizomes with 75.3% ethyl alcohol over a 40-minute extraction time compared to a solvent-only context. The ultrasonic-assisted extraction technique operates by utilizing the mechanical action of ultrasound on plant cell walls, enhancing surface contact between solvent molecules and the plant sample matrix. As a result, ultrasound alters and disrupts the physical and chemical characteristics of plant materials, expedites the release of phytochemicals, and reinforces the mass movement of the solvent system into plant cells (Altemimi et al.; 2016).

The ability to expedite the extraction process, reduce energy consumption, and enhance the recovery of phytochemicals from annatto seeds was established by the UAE technique. In this study on annatto, the extracted phytochemicals were efficiently preserved by UAE, favoring their functional activities. The wide range of applications of UAE makes it a crucial extraction technique for obtaining bioactive compounds. Successful extraction of phenolic compounds from strawberries and oranges has been succeeded using this technique. Additionally, extracting phenolic derivatives and anthocyanins from grape peels using UAE has been reported. The efficiency of UAE is further emphasized by a study reporting significantly shorter extraction times of 30 minutes and superior yields compared to the maceration method, which took 120 minutes and resulted in lower yields (Zhang et al.; 2009).

Furthermore, the extraction process is markedly expedited by the UAE technique, requiring a smaller quantity of solvent and establishing it as a preferred method. Triterpenoid saponins were efficiently extracted from edible seeds at an optimal sonication output amplitude of 60%, phenolic compounds from pumpkin slices at a reduced ultrasonic power of 44.60%, and anthocyanins from red cabbage leaves using an ultrasonic output power of 100 W. One significant aspect of UAE is its capacity to preserve the integrity of compounds vulnerable to degradation at high temperatures. For instance, carotenoids, phenolics, and vitamin C have been successfully extracted from spices like ginger, garlic, and turmeric without altering their chemical structure (Thakker et al.; 2018).

Moreover, effectiveness has been demonstrated by UAE when combined with other extraction techniques to reduce overall extraction time. Ultrasound-assisted hydrotropic extraction, for instance, proved to be a more superior and sustainable alternative than hydrotropic extraction alone, attributed to a shortened extraction duration and reduced hydrotrope concentration. Additionally, the efficacy of UAE is influenced by the choice of solvent. The use of ionic liquids instead of conventional organic solvents, coupled with ultrasound extraction, has shown improvements in the process. For instance, the

extraction yield of carotenoids from orange peels increased fourfold, from 7.88 ± 0.59 µg/g using acetone solvent to 32.08 ± 2.05 µg/g when employing the ionic liquid 1-n–butyl–3-methylimidazolium tetra-fluoroborate (Murador et al.; 2019).

The utilization of eutectic solvents in conjunction with UAE has been proven to enhance the extraction yield of phytocompounds. For instance, when coupled with UAE, deep eutectic solvents efficiently yielded more than 97% flavonoids from common buckwheat sprouts. Similarly, in the extraction of anthocyanins from wine lees, eutectic solvents demonstrated greater effectiveness and efficiency compared to acidified ethyl alcohol. The reported extractions highlight ultrasound-assisted extraction as an environmentally friendly, green, and cost-effective technique compared to conventional methods for extracting phytochemicals. UAE offers shortened extraction times, low energy requirements, and utilizes smaller amounts of solvents. A distinctive feature of UAE is its ability to recover green extracts in a concentrated form without residual solvents, impurities, or defects. The extraction potential is significantly enhanced through the utilization of ionic liquids as solvents. The reduced time of process and temperatures are crucial for extracting thermolabile phytochemicals, like phenolics, making this technique even more attractive (Mansur et al.; 2019).

Pulsed electric field (PEF) extraction:

The effectiveness of PEF extraction has been well-established, significantly increasing extraction yield while reducing extraction time. This efficiency is attributed to its ability to enhance mass transfer during extraction by disrupting membrane structures. The success of PEF treatment depends on various parameters, including field strength, specific energy input, pulse number, and treatment temperature. Notably, PEF extraction is a non-thermal method, minimizing the degradation of thermolabile compounds. In a specific study focusing on ginsenosides, the highest yield (12.69 mg/g) was achieved

through PEF under conditions of a 20 kV/cm electric field intensity, 6000 Hz frequency, 70% ethanol–water solution, and 150 l/h velocity.Exceeding the yields obtained through methods like microwave-assisted extraction (MAE), heat reflux extraction, ultrasound-assisted extraction (UAE), and pressurized liquid extraction (PLE), the PEF extraction method proved highly effective. Additionally, the entire PEF extraction process, which took less than 1 second, was significantly shorter than the durations required by the other tested methods.

Another study focused on antioxidants extracted from Norway spruce bark highlighted notable results with PEF treatment. Treated samples exhibited much higher phenolic content (eight times) and antioxidant activity (30 times) compared to untreated samples, underscoring the potency of PEF in enhancing phytochemical extraction (Bouras et al., 2016).

Parameters to Select theMost Suitable Extraction Method:

i) The elimination of any foreign matter is imperative during the authentication of plant material before initiating any extraction process.

ii) Ensure the utilization of the correct plant part, and for quality control, document essential information such as the age of the plant, and the time, season, and location of collection.

iii) The drying conditions for plant material should be tailored to the nature of its chemical constituents. Typically, using a hot or cold blowing airflow is preferred for drying plant material. If a crude drug with high moisture content is intended for extraction, appropriate weight corrections should be applied.

iv) Specify grinding methods, and it is advisable to avoid techniques that generate excessive heat during the grinding process.

v) Ensure that powdered plant material maintains a uniform size.

vi) Consider the nature of constituents:

a) When extracting therapeutic compounds with non-polar characteristics, it is recommended to use a non-polar solvent. For instance, in the extraction of lupeol, the active constituent of Crataeva nurvala, hexane is commonly employed. Likewise, for plants like Bacopa monnieri and Centella asiatica, whose active constituents are glycosides, a polar solvent like aqueous methanol may be used.

b) For thermolabile constituents, it is advisable to opt for extraction methods such as cold maceration, percolation, and CCE (continuous extraction). In contrast, for thermostable constituents, Soxhlet extraction is recommended when nonaqueous solvents are used, while decoction is suitable when water serves as the menstruum.

c) Suitable precautions should be taken when dealing with constituents that degrade while being kept in organic solvents, e.g., flavonoids and phenylpropanoids.

d) When conducting hot extraction, it is crucial to avoid temperatures higher than necessary, as extended exposure to elevated temperatures may lead to the breakdown of glycosides. This is particularly important to prevent the degradation of thermolabile compounds during the extraction process.

e) Standardization of the time of extraction is important, as:

Insufficient time means incomplete extraction.

Optimizing the extraction time is essential as prolonged durations can result in the extraction of unwanted constituents. For instance, extended boiling of tea can lead to the extraction of tannins, contributing to the astringency of the final preparation. Therefore, careful attention to extraction time is necessary to achieve the desired composition in the extract.

f) Ensuring thorough extraction relies not just on the duration of each extraction but also on the number of extractions performed. Paying proper attention to both aspects is essential to comprehensively extract desired constituents from the plant material.

vii) It is imperative to specify and control the quality of water or menstruum used.

viii) The concentration and drying procedures significantly contribute to maintaining the safety and stability of active constituents acquired during the extraction process. While drying under reduced pressure, such as with a Rotavapor, is a commonly used method, lyophilization, despite being more expensive, is increasingly employed to safeguard the integrity of active compounds in the final extract.

ix) When choosing an extractor for the extraction process, thoughtful consideration should be given to the design and material of fabrication. These factors play a pivotal role in ensuring the efficiency and success of the extraction, influencing the quality and yield of the final extract.

3.0

RESEARCH

WORK

Anti-bacterial and Anti-fungal Properties of *Bougainvillea spectabilis* and *Tecoma stans* Plant Extracts against Human Pathogens

Introduction

Medicinal plants are defined by the WHO as plants that possess properties or compounds suitable for therapeutic purposes or the synthesis of metabolites to create valuable drugs. In India, these plants are widely distributed and form a crucial component of the country's flora. Conducting pharmacological evaluations of metabolites from plants serves as an established method to identify lead compounds, which can be instrumental in developing novel and safe medicinal agents. The significance of medicinal plants and traditional health systems is increasingly gaining attention as potential solutions to global healthcare challenges. This resurgence of interest has led to substantial growth in international research on medicinal plants. Similarly, traditional medical practices have become an integral part of the culture in many developing countries (Chopra et al., 1992; Ghani 2003).

In the annals of history, the roots of medicinal concoctions traced back to the embrace of nature's bounty, predominantly stemming from flora be it the unadorned essence of raw botanical specimens or the distilled essence found within primitive extracts and intricate blends. In our contemporary understanding, it is surmised that myriad plant species, reaching into the thousands, have been celebrated across diverse cultures for their curative properties.

Species under study *Bougainvillea spectabilis* commonly known as *bougainvillea* is linked with the Nyctaginaceae family (Hannah Joyce R. Caliling, 2020). The plant is sometimes referred to as “Paper Flower”, the reason being its bracts are thin and papery (Jiraungkoorskul, 2017). The floral arrangement in this plant is at leaf axile and in clusters of three. The propagation of the plant *Bougainvillea* is done by cutting, layering, and budding. (Singh, 2015) The leaf of this thorny woody perennial vine, contains various active components such as furanoids, saponins, flavonoids, quinones, phenols, sterols, triterpenoids, glycosides, tannins, and small quantities of sugars (Pratibha Chauhan, 2016). The chemical constituent which largely contributes to its medicinal significance is 3-o-

methylchironinositol (D-pinitol) isolated from the leaves of *Bougainvillea spectabilis* (Sikandar Khan Sherwani, 2013),the vital constituents which contribute to the therapeutic properties are bougainvinones, pinitol, quercetagetin, quercetin, and terpinolene (Jiraungkoorskul, 2017). Apart from its medicinal properties the plant species also has been recently studied as an alternative to Wright stain in blood smear preparation. Extract of the bracts of the plant was macerated with 100% methanol for about 72 hours before carrying out the staining procedure to achieve the crude extract (Hannah Joyce R. Caliling, 2020). The plant additionally serves as a phytomonitor and helps in quantifying foliar dust fall. The active monitoring of dust fall at critical locations helps keep an account of the air pollution (Nitesh C.J., 2017)

A perennial shrub and a member of Bignoniaceae family, *Tecoma stans* is an evergreen shrub that grows rapidly and is commonly known as Yellow-Elder due to its bell-shaped yellow flowers. The leaf showcases opposite or subopposite phyllotaxy and the appearance of the leaf is odd-pinnately compound (Watson, 1993). The plant species act as invaders in a natural grassland ecosystem and they modify the biodiversity of the region by abolishing natural resources. Its drought resistance nature and easy maintenance makes it a preferred plant for ornamental gardens (Bhat, 2019). Yellow-Elder is native to Northern America and extends up to Mexico and Central America. The plant species was later introduced to several other parts of the world like southern Africa, India, and Hawaii. *Tecoma stans* has monoterpene alkaloids as constituents specifically Tecomine 1 and Tecostanine 2 which largely contribute to the hypoglycaemic behavior of the plant extract (Luca Costantino, 2003) Other than this phytosterols, triterpenes, flavonoids, phenols, saponins and iridoid glycosides were also identified from the leaves and roots of this plant (Sunitha Katta, 2016).

The present study examined the antibacterial and anti-fungal activities of plant extracts (*Bougainvillea spectabilis* and *Tecoma stans*) against selected clinical pathogenic strains of bacteria and fungi. The

extraction procedure for both plant's extracts are same where methanol, water, hexane, and ethyl acetate solvents were used and evaluated on the basis of antibacterial and anti-fungal activity.

Materials and Methods

Gathering and Identification of Research Plant Materials:

*Bougainvillea spectabilis*and *Tecoma stans*plant were used during this study were collected during 2019 to 2021 from Jamnagar district Geographical region of Gujarat. Identification of these plants were done visually by comparing plant parts with available pictures on public database.

Preparation of Crude Extracts:

All plant materials were collected and then sun dried for 3 days. After completion of sun drying, material was grinded by mixer grinder (Make: Phillips) and fine power was made. These powder of both plants were analysed for water content, ash content, carbon, hydrogen, nitrogen and sulphur content. This fine powder was used for extract preparation using multiple solvents. 20 gm fresh plant material was used for extraction with 60 ml solvents. Extraction was carried out in Soxhlet Extractor with 4 different solvents which are water, methanol, hexane and ethyl acetate. The filtrate underwent evaporation to dryness under reduced pressure employing a rotary vacuum evaporator (BUCHI).The concentrated crude extracts obtained from evaporator was dried inside oven (Thermo) at 60℃ for 6 hrs and dried extract powder was stored at 4℃ (Dieudonné Lemuh et: al; 2015).

Estimation Process of moisture content:

This method includes measuring the mass of water in a known mass of the sample. The determination of moisture content is carried out by comparing the mass of the sample before and after the evaporation of water.

% moisture = {(W1-W2)/W1} X 100

In this procedure, W1 represents the initial weight, and W2 is the dried weight of the sample before and after drying, respectively. The fundamental principle of this method is because water has a lower boiling point than other major components present in the samples, such as lipids, proteins, carbohydrates, and minerals.

To summarize, the process involves weighing the petri dish and taking 3 g of the sample, placing this sample in the petri dish. Subsequently, the petri dish with the sample is subjected to drying in a hot air oven at 105℃ for 6 hours. After the completion of the 6-hour drying period, the petri dish is removed from the hot air oven, allowed to cool to room temperature, and then reweighed. This cycle is repeated every hour until the weight becomes constant. Once a constant weight is achieved, the moisture content is measured in duplicate using the formula mentioned above (Anju Paul et al., 2018).

Estimation Process of ash content:

Ash refers to the residue that remains after the heating of organic waste or organic matter in the presence of oxidizing agents. It serves as an indicator of the total ash content in a sample. Analytical techniques used to determine the total ash rely on the principle that ash can be distinguished from other components in a sample in a measurable manner.

The ash content of the different plant biomass was determined by a muffle furnace according to ISO 1171-1981.Weigh the crucible at first step. Then take around 3 g of sample and put it in crucible and then it is heat for 2 hours at 550℃ in muffle furnace. Then it is taken out cooled and weighed. After

getting final weight ash content was calculated in duplicate as per below mentioned formula (Anju Paul et: al; 2018).

% Ash= (W4/W3) X 100

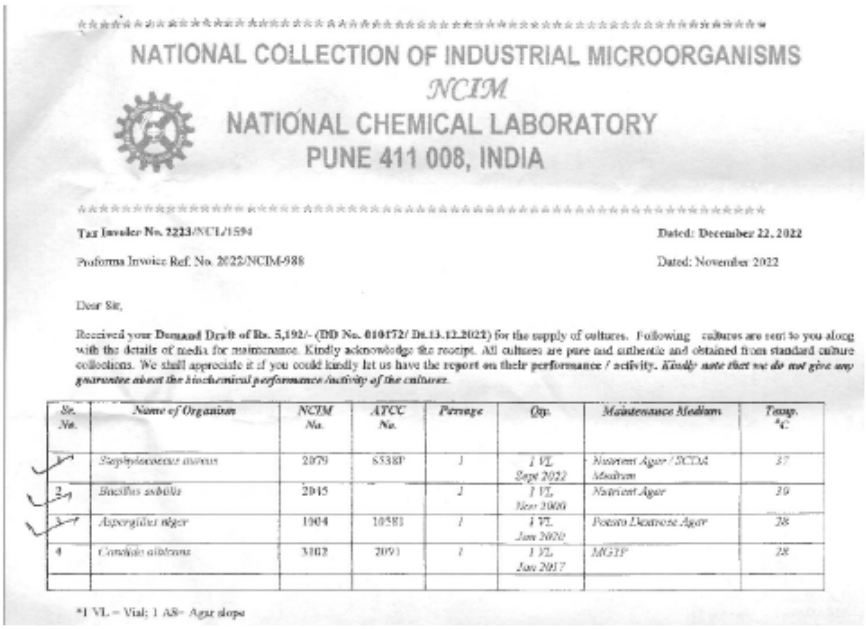
Here, W3 is initial weight and W4 is weight after ashing of sample.

Analytical Method for Elemental Analysis:

The elemental analyser uses a combustion method to convert carbon (C), hydrogen (H), nitrogen (N), sulphur (S) and oxygen (O) elements into simple gases. The measurement of these gases was conducted using a thermal conductivity detector (TCD). 10 mg of plant biomass samples were weighed on aluminium boat, placed into elemental furnace, and burnt in a pure oxygen environment at 1150°C. The elemental composition (CHNS) of dried microalgal biomass was determined using vario MACRO cube CHNS/O analyser (Elementar) while O was calculated on deduction basis. Weight percent of each element was analyzed and calculated in duplicate (Nishant Saxena et: al; 2020).

Analytical Method for Anti-microbial Activity (Disc Diffusion Method):

The anti-microbial activity of extracts obtained from the plant biomass of *Bougainvillea* spectabilis and *Tecoma stans* was assessed using the disc diffusion method. Extracts at a concentration of 5% were subjected to testing against pathogenic organisms, including *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus*niger, and *Candida albicans*. These strains were sourced from the National Collection of Industrial Microorganism (NCIM) in Pune, with specific strain codes 2079, 2045, 1004, and 3102, respectively. (Fig.1). All these strains were inoculated, preserved and revived as per the shared procedure by NCIM using respective media/broth for the anti-microbial study (Anju Paul et: al; 2018).



NATIONAL COLLECTION OF INDUSTRIAL MICROORGANISMS
NCIM
NATIONAL CHEMICAL LABORATORY
PUNE 411 008, INDIA

Tax Invoice No. 2223/NCL/1594 Dated: December 22, 2022

Proforma Invoice Ref. No. 2022/NCIM-988 Dated: November 2022

Dear Sir,

Received your Demand Draft of Rs. 5,192/- (DD No. 010172/ Dt.13.12.2022) for the supply of cultures. Following cultures are sent to you along with the details of media for maintenance. Kindly acknowledge the receipt. All cultures are pure and authentic and obtained from standard culture collections. We shall appreciate it if you could kindly let us have the report on their performance / activity. *Kindly note that we do not give any guarantee about the biochemical performance /activity of the cultures.*

Sr. No.	Name of Organism	NCIM No.	ATCC No.	Passage	Qty.	Maintenance Medium	Temp. °C
1	*Staphylococcus aureus*	2079	6538P	3	1 VL Sept 2022	Nutrient Agar / SCDA Medium	37
2	*Bacillus subtilis*	2015		3	1 VL Nov 2006	Nutrient Agar	30
3	*Aspergillus niger*	1904	10581	1	1 VL Jan 2020	Potato Dextrose Agar	28
4	*Candida albicans*	3102	2091	1	1 VL Jan 2017	MGYP	28

*1 VL = Vial; 1 AS= Agar slops

Fig.- 1: Strain Identification and Procurement Details

Media Preparations

Nutrient Agar (NA): Weighed 28 g of Nutrient agar using calibrated weighing balance and added 1000 ml of distilled water in NA. After addition mixed well for 15-20 minutes using magnetic stirrer. After mixing placed this solution inside the water bath for proper dissolution of agar.

Potato Dextrose Agar (PDA): Weighed 39 g of potato dextrose agar using calibrated weighing balance and added 1000 ml of distilled water in the PDA. Mixed the solution very well and after completion of mixing placed the solution in water bath until agar is dissolved completely.

Malt Glucose Yeast Peptone Agar (MGYP): Weighed 41.4 g of malt glucose yeast peptone agar using calibrated weighing balance and added 1000 ml of distilled water in the MGYP. Mixed the solution very well and after completion of mixing placedthe solution in water bath until agar is dissolved completely.

All required materials used in anti-microbial activity (i.e. Petri dish, test tubes, filter paper dishes, distilled water and pipette) and media (i.e. NA, PDA and MGYP) were autoclaved at 121℃ (15 psi) for 15 minutes.

Preparation of 5% Extract Solution: Weighed accurately 5.0 gm of each extract and diluted with 100 ml of distilled water. 4 extracts of *Bougainvillea spectabilis* and *Tecoma stans* were prepared using water, methanol, hexane and ethyl acetate which were nominated as BW, BM, BH, BE, TW, TM, TH & TE respectively.

All materials used in this disc diffusion assay and all types of culture media were sterilized by standard autoclave cycle at 121℃ & 15 lbs for 30 minutes.

Serial Dilution: All the strains were grown in respective media and fresh dilutions were prepared for this study.Mix each strain thoroughly in sterilized distilled water, take 2 scoopfuls of each strain, and pipette out 1 ml from this mixture. Add the pipetted solution to another test tube containing 9 ml of sterilized distilled water. Repeat this process for up to 10^5 dilutions, and use the final diluted solution to assess anti-microbial activity.Extract specific solvent was used as a negative control (-ve) and cefpodoxime proxetil (200 mg) antibiotic was used as positive control (+ve) during antibacterial study. In case of anti-fungal activity, negative controls were same while Fluconazole (400 mg) was used as a positive control.

All positive controls tablets were dissolved in 10 mL & 20 mL respectively to make final solution concertation of 2% with respective diluent and then 0.1 mL volume of both positive controls were used. One glass petridish was marked in 4 different areas for each extract where herbal extract was in duplicate, while positive and negative control was in singlet form. 4 plates of this same type were organized for anti-bacterial activity of one plant and similarly 4 separate plates were organized for anti-fungal activity of the same plant. Likewise, separate set of plates was prepared for each activity of each

plant using 0.1ml of dilution liquid containing microorganism, 0.1 ml of 5% herbal extract, and 15 ml nutrient agar (Table -1). All the plates were positioned in incubator (at 38°C) for 48 hours after the agar is solidified, it generally take around 15-30 minutes. After completion of 48 hours, all plates were taken out from incubator tocalculate the results of anti-microbial activities of experimental Herbal extract. Incubation conditions for bacterial strains wasfollowed at 37.0±2.5°C for 48 hrs and for fungal strains,it was at 25.0±2.5°C for 5 days. Inhibition zone was measured strictly only after completion of respective incubation conditions as mentioned above. All estimations were done in duplicate and mean values were reported with STDEV.

Table-1 Details of Antibacterial &Anti-fungal activity of Plant's Extracts

Plant Name	Activity Name	Extract Name
Bougainvillea spectabilis	Anti-bacterial activity against *Bacillus substilis* (2045)	*Bougainvillea* water extract (BW)
		Bougainvillea methanol extract (BM)
		Bougainvillea hexane extract (BH)
		Bougainvillea ethyl acetate extract (BE)
Tecoma stans	Anti-bacterial activity against *Staphylococcus aureus* (2079)	*Tecoma* water extract (TW)
		Tecoma methanol extract (TM)
		Tecoma hexane extract (TH)
		Tecoma ethyl acetate extract (TE)

Bougainvillea spectabilis	Anti-fungal activity against *Aspergillus niger* (1004)	*Bougainvillea* water extract (BW)
		Bougainvillea methanol extract (BM)
		Bougainvillea hexane extract (BH)
		Bougainvillea ethyl acetate extract (BE)
Tecoma stans	Anti-fungal activity against *Candida albicans* (3102)	*Tecoma* water extract (TW)
		Tecoma methanol extract (TM)
		Tecoma hexane extract (TH)
		Tecoma ethyl acetate extract (TE)

Results & Discussion:

Fine powder of both plants (*Bougainvillea* and *Tecoma*) were made as per the process mentioned in material method section. This was the raw material for this study hence its quality evaluation was done by analyzing key parameters such as moisture, ash, carbon, hydrogen, nitrogen and sulphur content.

Table-2 Quality Evaluation of Raw Material (Plant's Powder)

Analytical	***Bougainvilleaspectabilis***	***Tecoma stans***

Parameters		
N %	1.48	0.08
STDEV	0.01	0
C %	43.85	41.34
STDEV	0.03	0.2
H %	6.11	6.61
STDEV	0.05	0.05
S %	0.05	0
STDEV	0	0
O %	41.3	48.45
STDEV	0.07	0.13
Moisture %	6.71	7.73
STDEV	0.30	0.16
Ash %	7.21	3.52
STDEV	0.21	0.06

Moisture content was observed 6.71 % in *Bougainvillea* plant and 7.73% in *Tecoma* while ash content was witnessed 7.21% and 3.52% (on dried basis) respectively. More than double ash content was found in *Bougainvillea* plant as compared to *Tecoma.* Similarly, sulphur content also found more in *Bougainvillea* as compared to *Tecoma* which is 0.05%. Sulphur and ash contents are directly proportional to each other since sulphur contributes to ash content. CHNO content of *Bougainvillea* was observed 43.9%. 6.1%, 1.38% and 41.3% respectively while in case of *Tecoma* 41.3%, 6.61%, 0.08 and 48.5% respectively. All contents were observed more in *Bougainvillea* as compared to

Tecoma except moisture, hydrogen and oxygen contents. Hydrogen and oxygen are directly proportional to moisture content which has been proved with this data (Table-2).

Measurement of anti-bacterial activity:

The all four extracts of *Bougainvillea* (BW, BM, BH& BE) and *Tecoma* (TW, TM, TH & TE) were tested against two pathogenic strains of bacteria. As presented in figure 3 and 5, the clear inhibition zone of BW, BM, BH & BE were visible against clinical pathogenic bacterial strains and similar observations were reported in case of TW, TM, TH& TE as well which is clearly visible in figure 7 and 9. Out of these BM and TM were the most effective in-front of the *S. aureus, B. substilis* with a range of inhibition zones (20.5 –25 mm and 20-21.5 mm respectively) which are Gram-positive bacteria:.

All *Bougainvillea plant* extracts BW, BM, BH and BE showed inhibition zone against*Bacillus substilis* which were 2.5, 25, 3.5 and 1.5 mm respectively (Fig.2). Extremeinhibition zone was witnessed in methanol extract of *Bougainvillea*, which were around 78% of positive control. No zone was visible in any negative control, which concludes these solvents do not have any anti-bacterial activity.

Similarly, against *Staphylococcus aureus,*inhibition zones were observed 2.0, 20.5, 2.5 and 2.0 mm correspondingly (Fig.4). Here also methanol extract showed maximum inhibition zone, which were around 75% of positive control. Full bacterial growth was visible in all negative controls. This data clearly concluded that *Bougainvillea* plant grown in Jamnagar district region has antibacterial compounds which can be extracted by methanol.

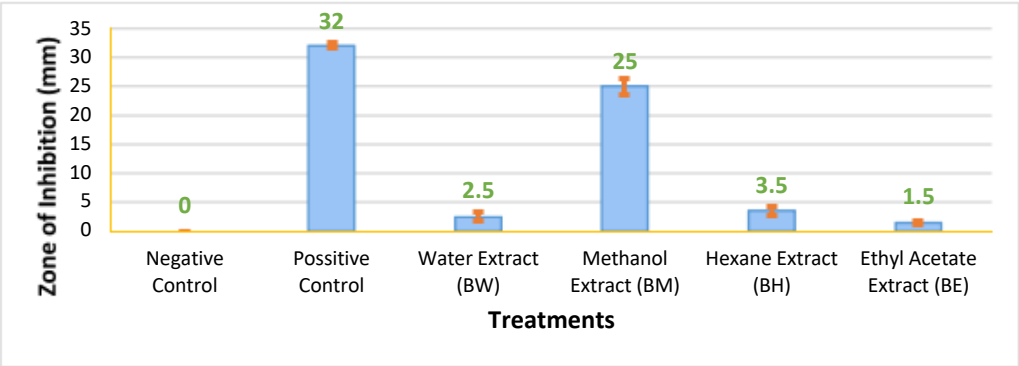


Fig.- 2: Anti-bacterial activity of *Bougainvellea* Extracts on *Bacillus substilis*

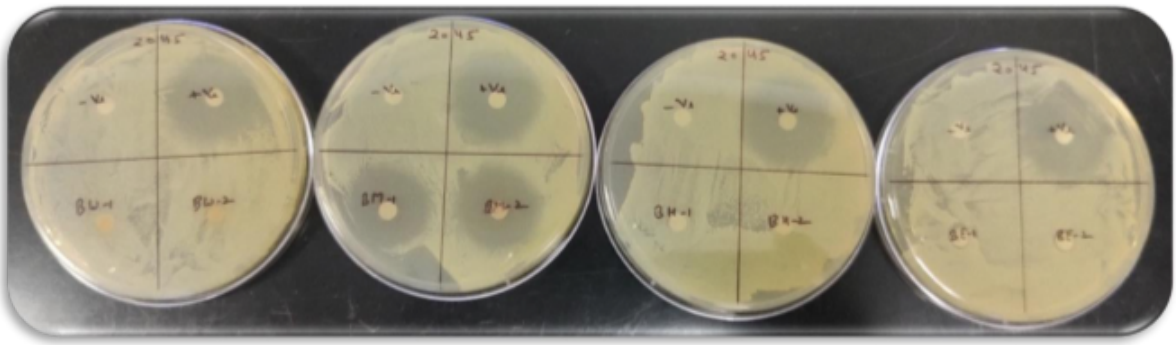


Fig.- 3: Picture Showing Anti-bacterial activityof BW, BM, BH & BEagainst *Bacillus substilis*

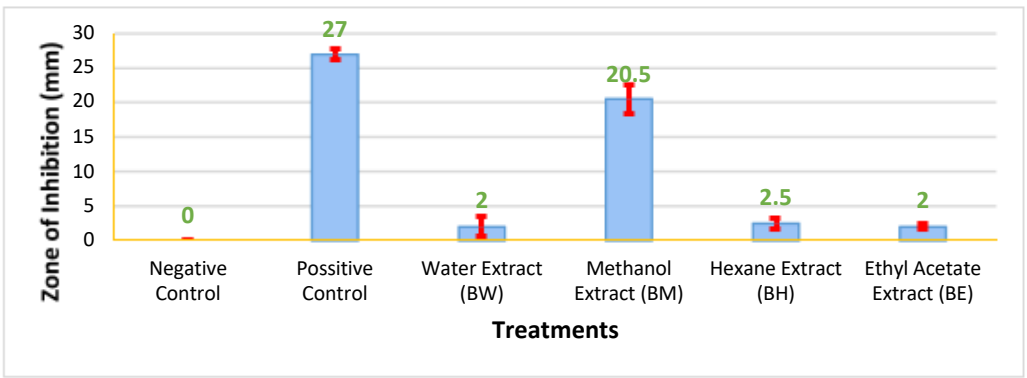


Fig.- 4: Anti-bacterial activity of *Bougainvellea* Extracts on *Staphylococcusaureus*

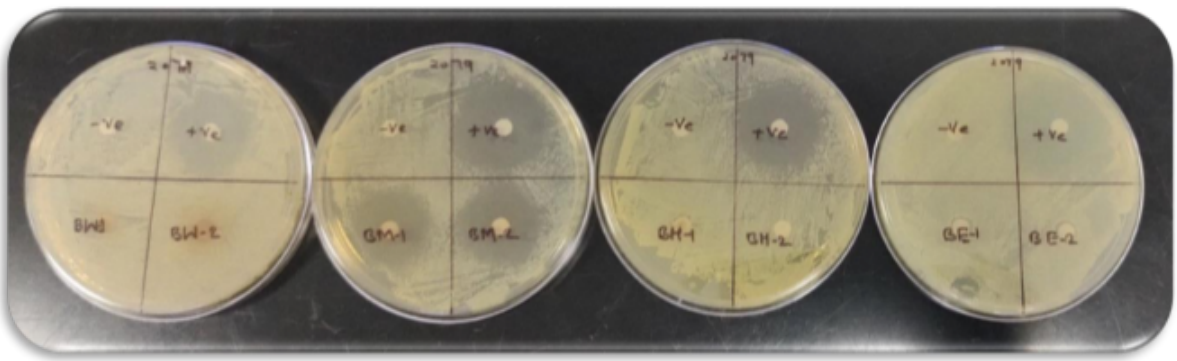


Fig.- 5: Picture Showing Anti-bacterial activity of BW, BM, BH & BE against *Staphylococcus aureus*

All *Tecoma* plantextracts TW, TM, TH and TE showed inhibition zone against*Bacillus substilis*, which were 7, 21.5, 1.5 and 1.0 mm respectively (Fig.6). Maximum inhibition zone was observed in methanol extract of *Tecoma, which* were around 79% of positive control. No zone was visible in any negative control, which concludes these solvents do not have any anti-bacterial activity.

While, against *Staphylococcus aureus,* TM and TH did not display any inhibition zone only TM and TE indicated some inhibition zone, which were observed 20 and 1.5 mm respectively (Fig.8). Here also methanol extract showed maximum inhibition zone, which were around 76% of positive control. Full bacterial growth was visible in all negative controls. This data clearly concluded that *Tecoma* plant grown in Jamnagar district region has antibacterial compounds which can be extracted by methanol.

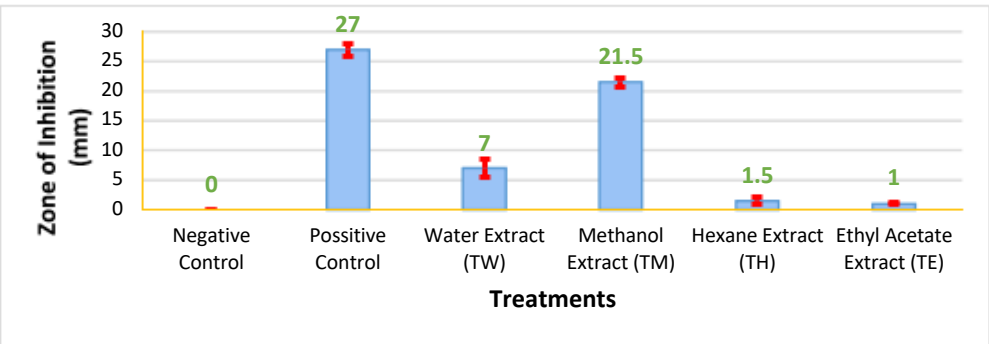


Fig.- 6: Anti-bacterial activity of *Tecoma* Extracts on *Bacillus substilis*

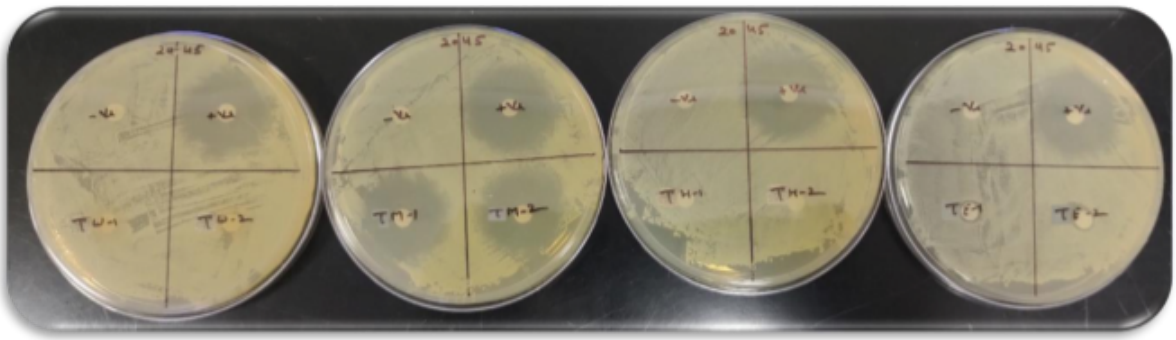


Fig.- 7: Picture Showing Anti-bacterial activityof TW, TM, TH & TEagainst *Bacillus substilis*

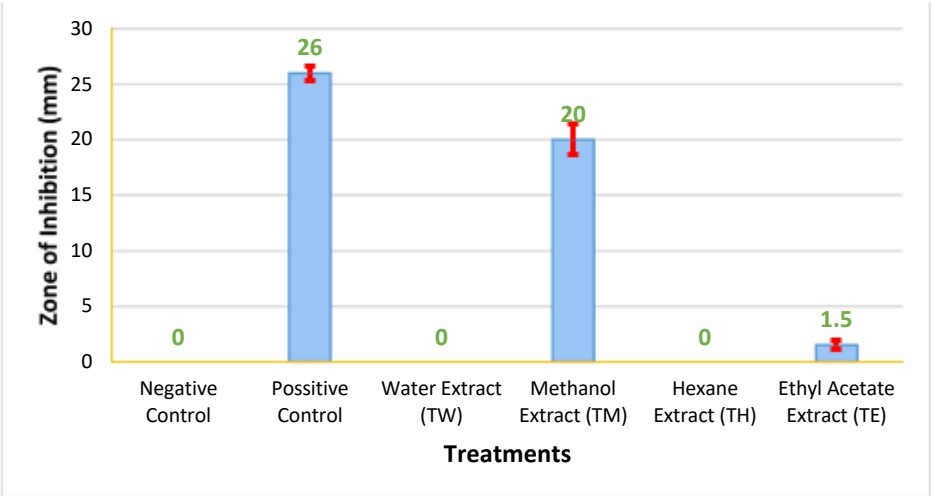


Fig.- 8: Anti-bacterial activity of *Tecoma* Extracts on *Staphylococcus aureus*

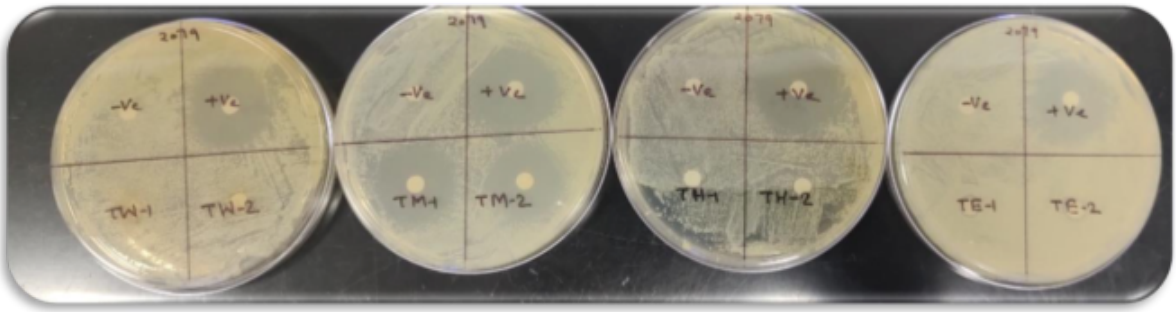


Fig.- 9: Picture Showing Anti-bacterial activity of TW, TM, TH & TE against *Staphylococcus aureus*

Measurement of anti-fungal activity:

The all four extracts of *Bougainvillea* (BW, BM, BH& BE) and *Tecoma* (TW, TM, TH & TE) were tested against both the organisms (*Aspergillus niger*&*Candida albicans*). Very small inhibition zone was observed in methanol extract of *Bougainvillea* and *Tecoma* plant against both organism.

All *Bougainvillea* plantextracts BW, BM, BH and BE didn't show any inhibition zone against *Aspergillus niger* except BM. Maximum inhibition zone was observed 2.5 mm only in methanol extract of *Bougainvillea* which were around only 10% of positive control (Fig. 10). No zone was visible in any negative control, which concludes these solvents do not have anti-fungal activity.

Similarly, against *Candida albicans,*inhibition zone was observed 1.5 mm (Fig. 11) in TM extract. Here also methanol extract reflected maximum inhibition zone that were around 7 % of positive control. Full growth was visible in all negative controls. This data clearly concluded that anti-fungal compounds can't be extracted by water, methanol, hexane and ethyl acetate from *Bougainvillea* plant grown in Jamnagar district region.

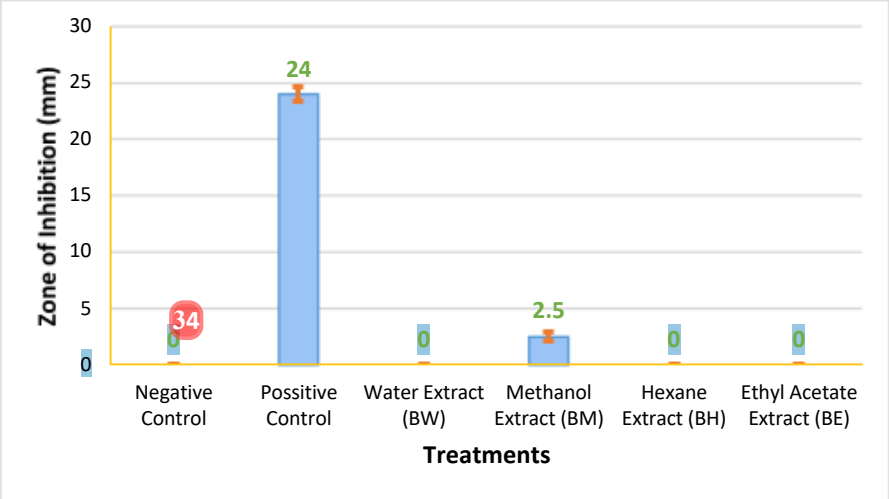


Fig.- 10: Anti-fungal activity of *Bougainvellea* Extracts on *Aspergillus niger*

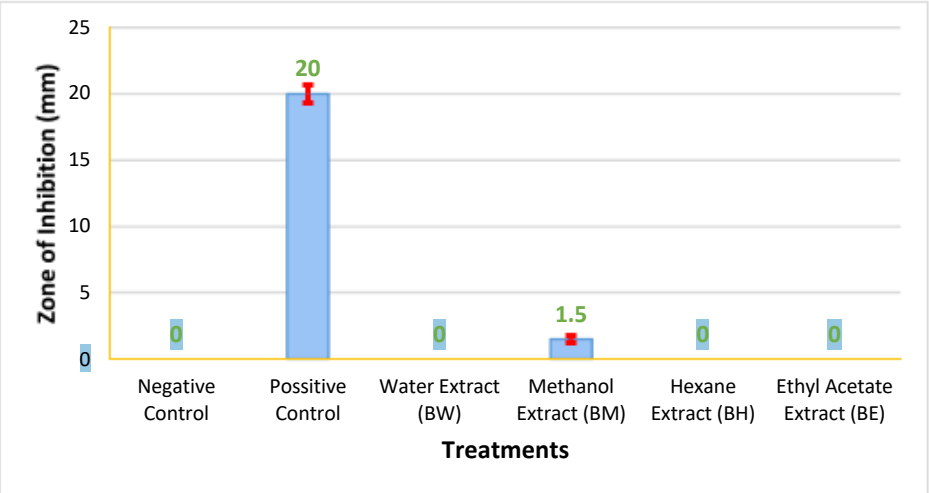


Fig.- 11: Anti-fungal activity of *Bougainvillea* Extracts on *Candida albicans*

All *Tecoma* plantextracts TW, TH and TE did not show any inhibition zone against*Aspergillus niger* except TM. Maximum inhibition zone was observed 2.0 mm only in methanol extract of *Tecoma* that was around only 8 % of positive control. While positive control showed significant inhibition zone of 23 mm against *Aspergillus niger*(Fig. 12). No zone was visible in any negative control, which concludes these solvents do not have anti-fungal activity.

Similarly, against *Candida albicans,*inhibition zone was observed 2.5 mm in TM extract. Here also methanol extract showed maximum inhibition zone, which were around 10 % of positive control. While positive control indicated significant inhibition zone of 25 mm against *Candida albicans* (Fig. 13). Full growth was visible in all negative controls. This data clearly concluded that anti-fungal compounds could not be extracted by water, hexane and ethyl acetate from *Tecoma* plant grown in Jamnagar district region.

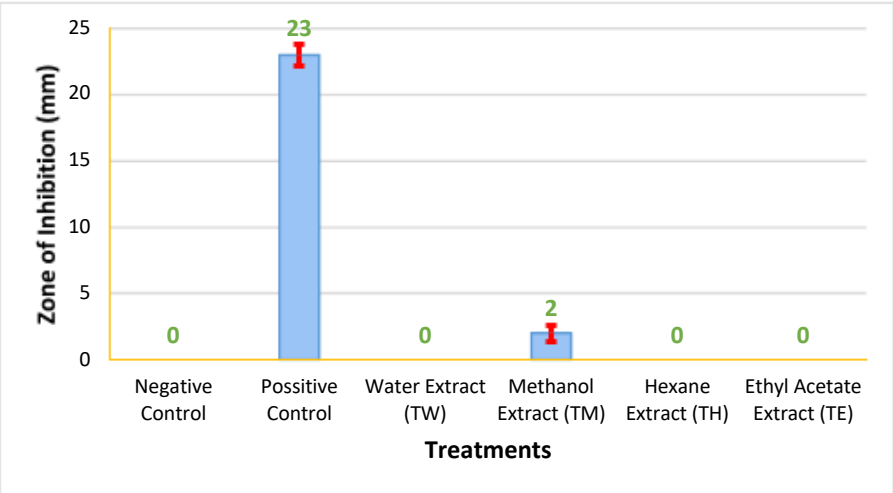


Fig.- 12: Anti-fungal activity of *Tecoma* Extracts on *Aspergillus niger*

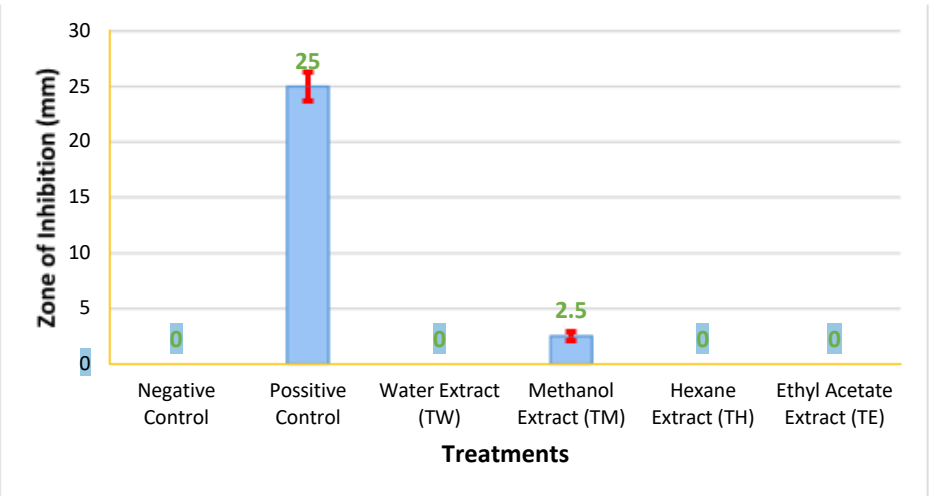


Fig.- 13: Anti-fungal activity of *Tecoma* Extracts on *Candida albicans*

The anti-bacterial activity of the extract and its dose-dependent nature are indicated by this study. Results from the anti-bacterial assay reveal that the crude extracts from *Bougainvillea* and *Tecoma* are effective against the tested bacterial strains but show inactivity against fungal strains. Among all the tested extracts, the methanol extract demonstrated the highest effectiveness. Many scientists have observed that plants serve as an essential source of pharmacophores and can function as new

chemotherapeutic agents. The initial step in developing a chemotherapeutic agent from plants involves conducting in vitro assays for antibacterial and anti-fungal activity. The active extracts can be utilized to isolate the specific compounds responsible for the antibacterial activities in the plants.Multi drug resistance has seen in pathogenic bacteria in recent years. This issue has developed interest to search new antibacterial agents from any natural sources. Many studies also reported that multi drug resistance has been displayed by gram negative bacteria *P. aeruginosa* to many antibiotics. However, the extracts, particularly the polar ones, exhibit significant activity against P. aeruginosa. Antibacterial agents derived from natural sources also offer the advantage of minimizing the side effects associated with synthetic or semi-synthetic antibacterial agents. The antibacterial activity of extracts from natural sources varies across different organisms. The zones of inhibition observed ranged from 10 mm to 27 mm. The results from this study indicate that methanol has the ability to extract more antibacterial compounds compared to other solvents. Hence, methanol extracts of *Bougainvillea* and *Tecoma* possesses supremeanti-bacterial activity against *Staphylococcus aureus* and *Bacillus substilis* in comparison withother solvents. Similarly, Dieu-Hien Truong at; al: (2019) concluded that methanol extracted maximum antibacterial compounds from plant material. The highest extraction yield (33.2%) was obtained with methanol, followed by distilled water (27.0%), ethanol (12.2%), acetone (8.6%), chloroform (7.2%), and dichloromethane (4.9%), among the tested solvents. (Dieu-Hien Truong at; al: 2019).

The anti-bacterial activity varies with solvents of different polarity, as the solubility of these compounds may differ in various solvents. Therefore, the results indicate that anti-bacterial properties depend on the extracting solvent, with the methanol extract showing a significant inhibition zone while other extracts showed negligible effects. The efficacy of these extracts is also contingent upon their aptitude to disperse and permeate within the assay's chosen medium. Even though these extracts aren't

pristine compounds, the outcomes gleaned undeniably underline their formidable potential. Thus, these extracts can be further purified to generate anti-bacterial compounds, which has the capability to create a huge opportunity to develop phytomedicine against these microbes. The emphasis is on the inherent safety and lower susceptibility to drug resistance in bacteria associated with these natural extracts. The pathogens mentioned in the text contribute to life-threatening diseases, and while synthetic antibiotics are commonly used to manage these conditions, they come with their set of side effects. The statement suggests that natural extracts could serve as a viable alternative due to their perceived safety and potential effectiveness against these pathogens. Given the escalating challenges related to drug resistance in bacteria and the higher costs linked to synthetic anti-bacterial agents, pharmaceutical companies are urged to explore and adopt alternative strategies. This shift toward natural extracts aligns with the growing recognition of their potential in addressing healthcare challenges and providing sustainable alternatives to conventional pharmaceutical approaches.

In case anti-fungal activity no inhibition zone was visible in aqueous, hexane and ethyl acetate extract of *Bougainvillea* and *Tecoma*. Only methanolic extract showed very small inhibition zone against *Aspergillus niger* and *Candida albicans*. Saeed Ahmad and Muhammad Akram also performed similar study. They prepared plants extracts using similar solvents such as methanol, aqueous, hexane and ethyl acetate and evaluated their anti-fungal activity against *Aspergillus niger&candida albicans* (Saeed Ahmad and Muhammad Akram 2019).

CONCLUSION

The inhibition zone assay, a common method for evaluating anti-microbial activity, provided insights into the effectiveness of plant extracts against microorganisms. The absence of inhibition zones around some discs indicated a lack of inhibitory activity, while clear inhibition zones suggested the ability of

the tested plant extracts to hinder bacterial growth. In this study, extracts from *Bougainvillea* and *Tecoma*, particularly those obtained using methanol as a solvent, demonstrated significant anti-bacterial activity against the tested organisms. This implies that these plant extracts have compounds capable of inhibiting or killing bacteria. However, when it came to anti-fungal activity, none of the solvents used were effective in extracting compounds with inhibitory effects from *Bougainvillea* and *Tecoma* grown in the Jamnagar location.

These findings validate the traditional knowledge held by local communities regarding the anti-microbial properties of these plants. The results also propose that the plant extracts, especially those obtained with methanol, contain bioactive compounds with antibacterial properties. Such compounds could be explored for their potential in the development of new drugs for treating various infectious diseases. The presence of polyphenolic compounds in the methanol fractions from *Bougainvillea* and *Tecoma* is highlighted as a possible contributing factor to their antibacterial activities. Polyphenols are known for their antioxidant and anti-microbial properties. Overall, this study provides valuable preliminary pharmacological evidence supporting the traditional medicinal usage of *Bougainvillea* and *Tecoma* and suggests avenues for further research in the development of therapeutic applications.

Anti-bacterial and Anti-fungal Properties of *Calotropisprocera*and*Cassia auriculata*Plant Extracts against Human Pathogens

Introduction

Medicinal plants, as defined by the WHO, encompass those remarkable botanical entities that house properties or compounds capable of serving therapeutic purposes or synthesizing metabolites essential for the creation of valuable pharmaceuticals. India, with its rich biodiversity, is generously endowed with these medicinal treasures, which constitute a vital segment of its floral diversity. The scientific evaluation of metabolites derived from plants represents an established methodology for identifying promising lead compounds, subsequently fostering the improvement of groundbreaking and safe agents for remedialuse. The global recognition of the significance of medicinal plants and traditional healthcare systems has been steadily increasing, positioning them as pivotal resources in addressing the world's healthcare challenges. This resurgence of interest in medicinal flora has ignited a remarkable upsurge in international research endeavors. Simultaneously, traditional medical practices have evolved to become integral facets of the cultural tapestry in many developing nations (Chopra et al., 1992; Ghani, 2003). Delving into history, one finds that all medicinal concoctions, whether in their rudimentary form as raw botanical materials or in their refined incarnation as crude extracts and intricate mixtures, were fundamentally derived from plants. Recent estimates vividly illustrate that across various cultures, a multitude of plant species, numbering in the thousands, have been acknowledged for their invaluable medicinal applications.

An erect perennial shrub belonging to Asclepiadaceae family which is also referred to as “Akra” and “milk weed” is *Calotropis procera*. This exotic shrub is xerophytic and is an immortal perennial plant, which generally found in dry and semi-dry habitats (Verma, 2016). The species name of the soft-

wooded shrub "*procera*" is of Latin origin and stands for the cuticular wax present on the plant's leaves and stem. As the plant shows flexible adaptations to various regions it also has been used for fodder,fiber, and timber purposes. The leaves showcase opposite phyllotaxy and the inflorescence is solid and multi flowered umbellate cyme. Propagation of the plant takes place via seeds, root suckers and regeneration through broken/cut stems and roots (Amarpreet Kaur, 2021). On phytochemical analysis of the crude plant extract several secondary bioactive chemical metabolites like terpenoids, flavonoids, saponins, steroids and cardiac glycosides were revealed (Ghais Uddin, 2012). The two major cardenolides that contribute to increased cardiac contractile force in patients suffering from heart failure and cardiac arrhythmias is ischarin and ischaridin (Zarga, 2015).

In areas like West Africa and Asia traditionally the shrub has been utilized for its curative behavior against bronchitis, pain, asthma and tumors. Secretion of latex by *Calotropis procera* is considered to be extremely useful as anti-diarrheas, wound healing agent, anti-rheumatism agent and anti-inflammatory, (Muzammal, 2014). The toxic nature of the plant extends from dermatitis, iridocyclites to poisonous effect of the milky latex when in contact with eyes which could cause temporary blindness (Muzammal, 2014). It has also been taken into consideration as andevelopingnatural fiber source. The fiber offered by the plant is renewable with low density, natural, high strength, with crude oil absorption capacity and hydrophobic- oleophilic characteristics (Amarpreet Kaur, 2021)

Use of medicinal concoctions for treating a variety of diseases has been in practice for long. One such prominent medicinal plant belonging to Caesalpiniaceae family is *Cassia auriculata.*(Verma, 2016). It is commonly referred to as Tanner's *Cassia* or Tanners Senna or Avaram tree and used all over Asia for its medicinal properties. It has been widely found to be useful in tonic form, as an astringent and has also been an active part of anti-diabetic research. Not only this, it has shown curative action against conjunctivitis and ophthalmia (J.Meenupriya, 2014) In India the plant is scattered throughout hot

deciduous forests. *Cassia auriculata* is an inhabits wild, dry regions of Madhya Pradesh, Tamil Nadu, Rajasthan and other parts of India. The phyllotaxy of the plant is alternate, stipulate, paripinnate compound with numerous leaves placed close to each other. Inflorescence displayed by the plant is terminal and the flowers are irregular, bisexual and bright yellow in color (Guruprasad C.Nille, 2015). The bark of the shrub is utilized as an astringent, leaves and fruits of the plant have proven to be anthelminthic whereas the seeds and roots have been used to resolve eye troubles and skin diseases respectively (Ivvala Anand Shaker, 2012). The plant extracts on phytochemical preliminary screening indicated flavonoids, tannins, saponins, phenolic compounds, proteins, glycosides as constituents. These compounds extracted using ethyl acetate has shown anti-oxidant abilities (R. Radha, 2013) On the other hand when the root of the plant underwent phytochemical analysis it resulted in the isolation of glycoside also known as 7,4- dihydroxy flavone-5-O-beta-D- galactopyranoside (Vandana Meena, 2019).

In this current study, we delved into the realm of antibacterial and anti-fungal activities by exploring the potential of plant extracts from *Calotropis procera* and *Cassia auriculata*. The extraction process for both of these plant extracts followed similar procedure, employing a range of solvents, including methanol, water, hexane, and ethyl acetate. The efficacy of these extracts was then assessed based on their antibacterial and anti-fungal properties against specific clinical strains of pathogenic bacteria and fungi.

Materials and Methods

Gathering and Identification of Research Plant Materials:

The collection and identification of plant materials, specifically *Calotropis procera* and *Cassia auriculata*, for this study were conducted in the Jamnagar district of Gujarat during PhD program. The

identification process involved a visual assessment, where plant parts were compared to images available in public databases.

Preparation of Crude Extracts:

For the preparation of crude extracts, the collected plant materials underwent a series of steps. Firstly, they were sun-dried over a period of three days. Once the sun-drying was completed, the dried materials were finely ground using a mixer grinder (Phillips), resulting in a fine powder. This powder from both plants was then analyzed to determine ash content, water content, carbon, hydrogen, nitrogen, and sulfur content. The fine powder served as the starting material for extract preparation, utilizing various solvents. Specifically, 20 grams of fresh plant material were employed for extraction, with each batch using 60 ml of solvent. Extraction procedures were carried out using a Soxhlet Extractor and involved four different solvents: water, methanol, hexane, and ethyl acetate. The resulting filtrate was subjected to evaporation under reduced pressure, accomplished using a rotary vacuum evaporator (BUCHI). The concentrated crude extracts obtained from the evaporator were further dried in an oven (Thermo) at a temperature of 60℃ for a duration of 6 hours. The resultant dried extract powder was then stored at 4℃ for subsequent use, following the methodology detailed by Dieudonné Lemuh et al. in 2015.

Estimation Process of Moisture Content:

This method includes measuring the mass of water in a known mass of the sample. The determination of moisture content is carried out by comparing the mass of the sample before and after the evaporation of water.The formula used to calculate the percentage of moisture is:

% moisture = [(W1 - W2) / W1] x 100

In this procedure, W1 represents the initial weight, and W2 is the dried weight of the sample before and after drying, respectively. The fundamental principle of this method is because water has a lower

boiling point than other major components present in the samples, such as lipids, proteins, carbohydrates, and minerals.The process for determining the moisture content of a sample involves several steps. Initially, a petri dish is weighed, and 3 grams of the sample are added to it. Subsequently, the petri dish with the sample is placed in a hot air oven set at 105℃ and allowed to dry for 6 hours. After the 6-hour drying period, the petri dish is removed from the oven, cooled to room temperature, and weighed again. This weighing process is repeated at hourly intervals until a constant weight is achieved, signifying that the sample has reached a consistent level of dryness. Once this constant weight is obtained, the moisture content is calculated using a specific formula, and measurements are taken in duplicate to ensure accuracy. This method is commonly employed in various industries, such as agriculture and food processing, to assess the moisture content of samples, a critical parameter for quality control and product development, guaranteeing that products meet the required moisture standards or specifications. This method provides a reliable means of determining the moisture content of a sample by measuring the mass loss during drying, thus allowing for precise quantification of water content (Anju Paul et: al; 2018).

Estimation Process of Ash Content:

Ash refers to the residue that remains after the heating of organic waste or organic matter in the presence of oxidizing agents. It serves as an indicator of the total ash content in a sample. Analytical techniques used to determine the total ash rely on the principle that ash can be distinguished from other components in a sample in a measurable manner.

The determination of the ash content in various plant biomass samples follows a standardized procedure based on ISO 1171-1981. This method comprises several steps: firstly, a crucible is weighed to establish its initial weight. Approximately 3 grams of the sample are then placed into the crucible. Subsequently, the crucible with the sample is subjected to heating in a muffle furnace set at 550℃ for a

duration of 2 hours. Once the heating process is completed, the crucible with its residual ash is removed from the muffle furnace and allowed to cool down to room temperature. Finally, the crucible, now containing the residual ash, is weighed to obtain its final weight. This method is widely used for the precise determination of ash content in plant biomass samples and is critical in various industries, particularly in agriculture and environmental analysis, where knowledge of ash content is essential for quality control and compliance with regulatory standards. Once the final weight is determined, the ash content can be calculated using the following formula (Anju Paul et: al; 2018).

% Ash = (W4 / W3) x 100

Where:

Here, W3 is initial weight and W4 is weight after ashing of sample. This method allows for the precise quantification of the ash content in a sample and is typically performed in duplicate to ensure accuracy, as indicated by Anju Paul et al. in 2018.

Estimation Process of Elemental Analysis:

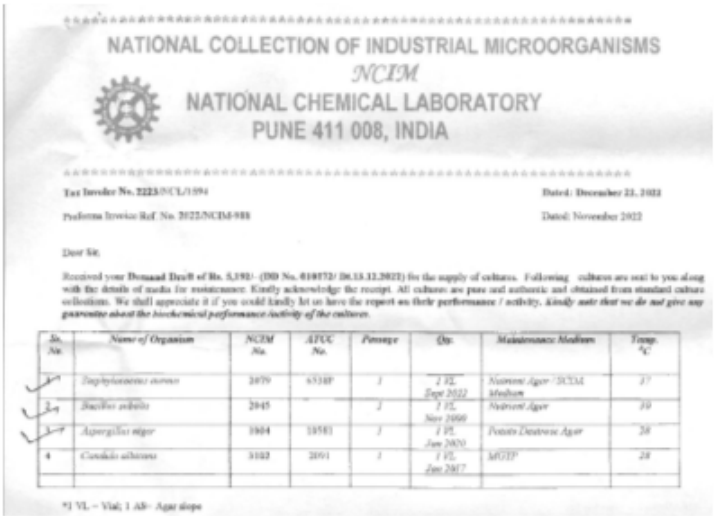
The elemental analyzer employed in this process utilizes a combustion method to convert elements like carbon (C), hydrogen (H), nitrogen (N), sulfur (S), and oxygen (O) within the plant biomass samples into simple form of gases like CO_2. NO_2, and SO_2. These gases are subsequently measured using a thermal conductivity detector (TCD).The vario MACRO cube CHNS/O analyzer, manufactured by Elementar, is operated to conclude the elemental arrangement (CHNS) of the dried plant material. The oxygen content (O) is calculated deductively.

The procedure for determining the elemental composition (CHNS) of dried microalgal biomass comprises several key steps. Initially, approximately 10 milligrams (mg) of the plant biomass sample is weighed and loaded onto an aluminum boat. This boat, with the sample, is then placed into the elemental furnace. The next phase involves initiating the combustion process within a pure oxygen

environment, subjecting the sample to high temperatures, specifically at 1150℃. During this combustion, the elements Carbon (C), Hydrogen (H), Nitrogen (N), Sulfur (S), and Oxygen (O) present in the sample are converted into gaseous form. These gases are subsequently collected, measured, and quantified using a thermal conductivity detector (TCD). This method is widely employed in scientific research, particularly in the fields of chemistry and biology, to precisely determine the elemental composition of plant powder, providing valuable insights into its chemical makeup for various applications. The weight percentage of each element (C, H, N, S, and calculated O) is analyzed and calculated in duplicate to ensure accuracy and reliability of the results. This analytical technique deliversvaluedevidence about the elemental arrangement of the plant biomass samples, aiding in various scientific and research applications (Nishant Saxena et: al; 2020).

Analytical Method for Anti-microbial Activity (Disc Diffusion Method):

The anti-microbial activity of extracts derived from the plant biomass of *Calotropis procera* and *Cassia auriculata*was assessed using the disc diffusion method. These extracts, prepared at a concentration of 5%, were subjected to testing against pathogenic microorganisms, namely *Staphylococcus aureus, Bacillus subtilis*, *Aspergillus niger*, and *Candida albicans*. The specific strains used for this study were obtained from the National Collection of Industrial Microorganisms (NCIM) in Pune and were identified by their respective strain codes, which are 2079, 2045, 1004, and 3102, as shown in Figure 1. To ensure consistency and reliability in the anti-microbial study, all these microbial strains were acquired, preserved, and revived in accordance with the procedures provided by NCIM. The appropriate media or broth were used for their inoculation and maintenance. The methodology, as described by Anju Paul et al. in 2018, enabled the rigorous assessment of the anti-microbial properties of the plant extracts against these selected pathogenic organisms. (Anju Paul et: al; 2018).



NATIONAL COLLECTION OF INDUSTRIAL MICROORGANISMS
NCIM
NATIONAL CHEMICAL LABORATORY
PUNE 411 008, INDIA

Tax Invoice No. 2223/NCL/1594 Dated: December 23, 2022

Proforma Invoice Ref. No. 2022/NCIM-988 Dated: November 2022

Dear Sir,

Received your Demand Draft of Rs. 5,192/- (DD No. 418572/ Dt.13.12.2022) for the supply of cultures. Following cultures are sent to you along with the details of media for maintenance. Kindly acknowledge the receipt. All cultures are pure and authentic and obtained from standard culture collections. We shall appreciate it if you could kindly let us have the report on their performance / activity. *Kindly note that we do not give any guarantee about the biochemical performance/activity of the cultures.*

Sr. No.	Name of Organism	NCIM No.	ATCC No.	Passage	Qty.	Maintenance Medium	Temp. °C
1	*Staphylococcus aureus*	2079	6538P	3	2 VL Sept 2022	Nutrient Agar / NCIM Medium	37
2	*Bacillus subtilis*	2645		3	1 VL Nov 2000	Nutrient Agar	30
3	*Aspergillus niger*	1804	10581	3	1 VL Jan 2020	Potato Dextrose Agar	28
4	*Candida albicans*	3102	2091	3	1 VL Jan 2017	MGYP	28

*1 VL – Vial; 1 AS– Agar slope

Fig.- 1: Strain Identification and Procurement Details

Media Preparations:

The preparation of various agar media and the 5% extract solution for the anti-microbial activity study followed a systematic procedure. For Nutrient Agar (NA), 28 grams of Nutrient agar were weighed and added distilled water (1000 ml)with the NA. The mixture was placed in a water bath until the agar was completely dissolved. Similarly, for Potato Dextrose Agar (PDA), 39 grams of potato dextrose agar were weighed and added distilled water (1000 ml)with the PDA, and the mixture was placed in a water bath until the agar was completely dissolved. For Malt Glucose Yeast Peptone Agar (MGYP), 41.4 grams of malt glucose yeast peptone agar were weighed and added distilled water (1000 ml)with the MGYPand complete dissolution was done in a water bath.

Additionally, the preparation of the 5% Extract Solution involved accurately weighing 5.0 grams of each extract and diluting each extract with 100 ml of distilled water. Four extracts were prepared from *Calotropis procera* and *Cassia auriculata* using multiple solvents (water, methanol, hexane, and ethyl acetate), and these extracts were labelled as CW, CM, CH, CE, KW, KM, KH, and KE. This method

ensures the consistent and precise preparation of agar media and extract solutions for anti-microbial activity studies, a crucial process in microbiological research and analysis.

Sterilization:

All materials required for the anti-microbial activity study, including Petri dishes, test tubes, filter paper dishes, distilled water, and pipettes, as well as the prepared media (NA, PDA, and MGYP), were autoclaved at 121℃ and 15 pounds per square inch (psi) for 30 minutes. These meticulous preparations ensured the sterility and consistency of the materials and media used in the anti-microbial activity study, allowing for accurate and reliable results. The process involved in serial dilution and evaluating anti-microbial activity can be summarized as follows:

Serial Dilution:

The preparation of microbial strains and dilutions for the study followed a systematic procedure. Initially, the microbial strains were grown in their respective media. Fresh dilutions were then prepared for the study. Two scoops of each microbial strain were mixed thoroughly in sterilized distilled water. From this mixture, 1 ml was pipetted and added to additional test tube, which already contains 9 ml of disinfected distilled water. This process was repeated iteratively until 105 dilutions were achieved. The final diluted solution was subsequently used for evaluating anti-microbial activity. To serve as controls in the study, a negative control (-ve) consisting of the extract-specific solvent was employed. Additionally, a positive control (+ve) was utilized, involving the dissolution of 200 mg of cefpodoxime proxetil antibiotic in 10 ml of the respective diluent. For anti-fungal activity testing, the negative controls remained consistent, while a 2% solution was created by dissolving 400 mg of Fluconazole in 20 ml with the respective diluent. This method ensures the proper preparation of microbial strains and dilutions, with the use of negative and positive controls, for the calculation of anti-microbial and anti-fungal activity, a crucial process in microbiological and pharmaceutical research.

Anti-microbial Activity Testing:

The evaluation of anti-microbial activities involved specific procedures. For anti-bacterial activity, 0.1 ml of the 5% herbal extract, 0.1 ml of the dilution liquid containing microorganisms, and 15 ml of nutrient agar plates were used. Glass petri dishes were marked into four different areas for each extract, with the herbal extract in duplicate and positive and negative controls in singlet form. Four separate plates were prepared for anti-bacterial activity for each plant, and the same process was repeated for anti-fungal activity. Each set of plates was prepared for each activity of each plant. All the plates were located in an incubator at 38℃ for 48 hours for bacterial strains and at 25℃ for 5 days for fungal strains after complete solidification of media. Following the incubation period, the inhibition zone was measured. All estimations were conducted in duplicate, and reported standard deviation and mean values, to determine the anti-microbial activities of the herbal extracts. This rigorous methodology ensures the accurate assessment of the anti-microbial properties of the herbal extracts, a crucial step in understanding their potential applications in medicine and healthcare. This comprehensive approach ensured a thorough assessment of the anti-microbial properties of the herbal extracts against both bacterial and fungal strains, providing reliable and informative results.

Table-1 Details of Plant's Extracts

Plant Name	Extract Name
Calotropis procera	*Calotropis* water extract (CW)
	Calotropis methanol extract (CM)
	Calotropis hexane extract (CH)
	Calotropis ethyl acetate extract (CE)
	Cassia water extract (KW)
	Cassia methanol extract (KM)

Cassia auriculata	*Cassia* hexane extract (KH)
	Cassia ethyl acetate extract (KE)

Results & Discussion:

The initial material for current study, which consisted of fine powder derived from both *Calotropis procera* and *Cassia auriculata*, underwent a quality evaluation process. This evaluation involved the analysis of several key parameters to ensure the quality of the raw material. The parameters assessed included moisture content, ash content, carbon content, hydrogen content, nitrogen content, sulphur content: These quality evaluations help ensure that the raw material used in the study meets specific standards and provides accurate and reliable results. Understanding the composition of the material is essential for the success of the research problem.

Table-2 Quality Evaluation of Raw Material (Plant's Powder)

Analytical Parameters	***Calotropis procera***	***Cassia auriculata***
N %	0.41	0.29
STDEV	0	0
C %	41.14	42.51
STDEV	0.04	0.11
H %	6.7	6.74
STDEV	0.01	0
S %	0.06	0
STDEV	0.01	0

O %	44.48	46.94
STDEV	0.07	0.13
Moisture %	6.53	7.49
STDEV	0.21	0.13
Ash %	7.11	6.43
STDEV	0.17	0.08

Moisture content was observed 6.53 % in *Calotropis* plant and 7.49% in *Cassia* while ash content was witnessed 7.11% and 6.43% (on dried basis) respectively. Higher ash content was found in *Calotropis* plant as compared to *Cassia* whereas, less moisture content found in *Calotropis* as compared to *Cassia*. The higher as content and lower moisture content in *Calotropis* suggest that it has more water and inorganic content as ash directly contribute to inorganic content.Likewise sulphur content also found more in *Calotropis* as compared to *Cassia* which is 0.06%. Sulphur and ash contents are directly proportional to each other since sulphur contributes to ash content. CHNO content of *Calotropis* was observed 41.1%. 6.7%, 0.41% and 44.5% respectively while in case of *Cassia* 42.5%, 6.74%, 0.29 and 46.9% respectively (Table-2). Both plants have varying carbon, hydrogen, nitrogen, and oxygen content. *Calotropis* generally has higher nitrogen content and less carbon and oxygen content as compared to *Cassia*. Hydrogen content is relatively similar between the two plants. The data suggests a direct relationship between moisture, hydrogen and oxygen content. When moisture content increases, hydrogen and oxygen content also tends to increase. These quality evaluation findings delivervaluedunderstandings into the chemical composition and characteristics of the raw materials, which can be crucial for the resolve research problem and data interpretation.

Measurement of anti-bacterial activity:

The study involving the four extracts of *Calotropis* (CW, CM, CH, and CE) and *Cassia* (KW, KM, KH, and KE) revealed their effectiveness against two pathogenic bacterial strains. As shown in figures 3 and 5, CM, exhibited clear zones of inhibition against both pathogenic bacterial strains, and similar results were observed for KM as evident in figures 7 and 9. Among all extracts, CM and KM displayed the highest efficacy against Gram-positive bacteria such as *S. aureus* and *B. subtilis*, with inhibition zones ranging from 21.5 to 23.5 mm and 21 to 24 mm, respectively. However, *Calotropis* plant extracts CW and CH did not exhibit any inhibition zone, whereas CM and CE showed zones of inhibition against *Bacillus subtilis* measuring 23.5 mm and 12 mm, respectively (Fig. 2). The methanol extract of *Calotropis* demonstrated the maximum inhibition zone, approximately 87% of the positive control. No inhibition zone was observed in any of the negative controls, indicating that these solvents did not possess any anti-bacterial activity.

Similarly, against *Staphylococcus aureus*, CW and CH did not show any inhibition zone, while CM and CE displayed zones of inhibition measuring 21.5 mm and 11.5 mm, respectively (Fig. 4). Once again, the methanol extract exhibited the maximum inhibition zone, accounting for approximately 82% of the positive control. All negative controls displayed full bacterial growth. This data strongly suggests that *Calotropis* plants grown in the Jamnagar district region contain antibacterial compounds that can be effectively extracted using methanol.

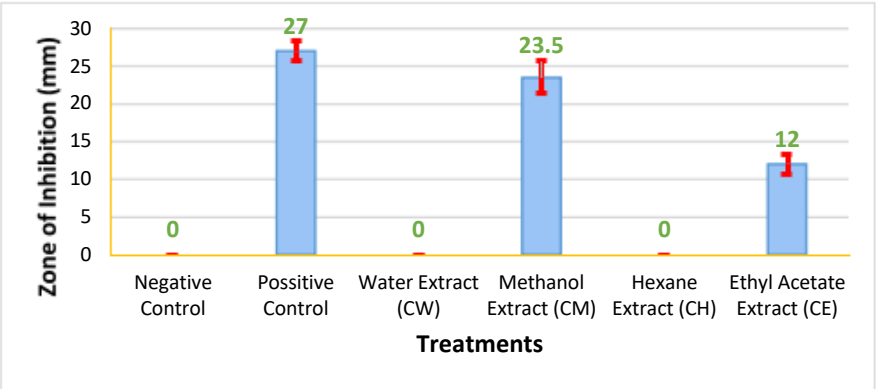


Fig.- 2: Anti-bacterial activity of *Calotropis* Extracts on *Bacillus substilis*

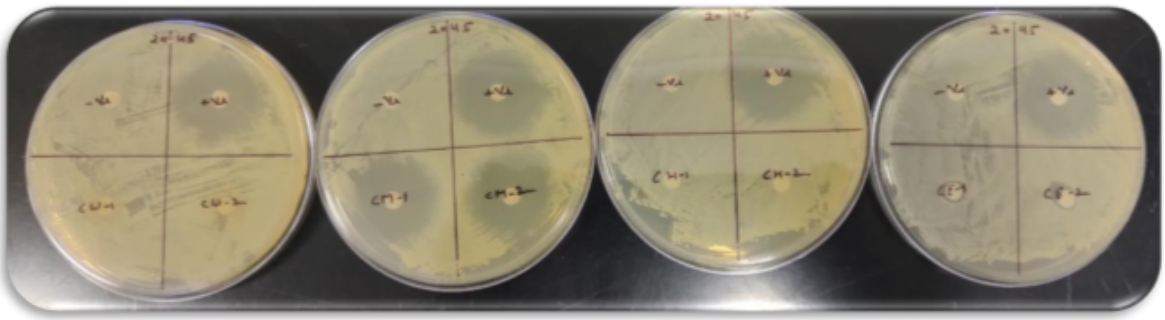


Fig.- 3: Picture Showing Anti-bacterial activityof CW, CM, CH & CEagainst *Bacillus substilis*

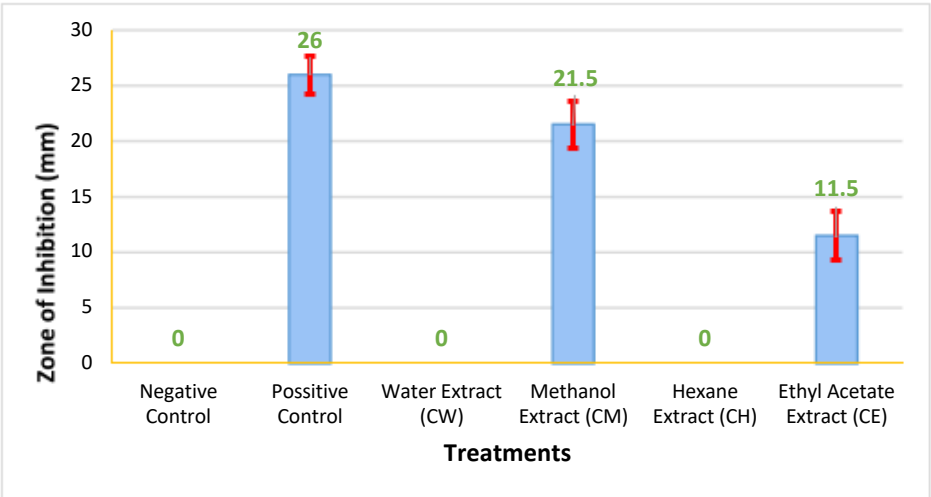


Fig.- 4: Anti-bacterial activity of *Calotropis* Extracts on *Staphylococcus aureus*

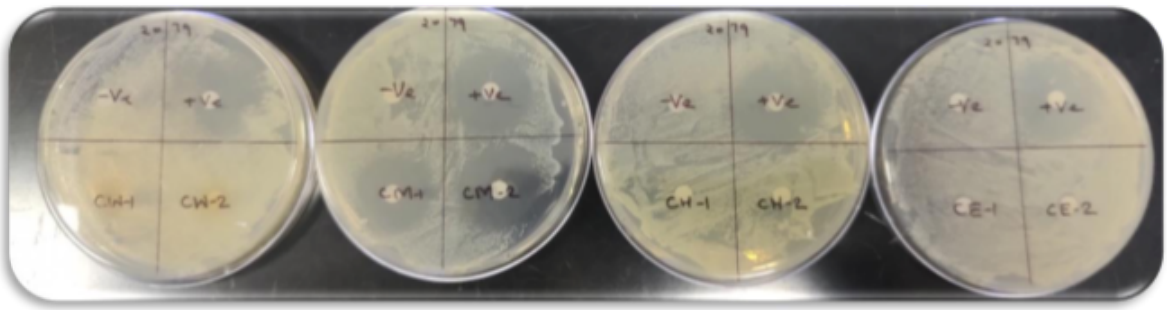


Fig.- 5: Picture Showing Anti-bacterial activity of CW, CM, CH & CE against *Staphylococcus aureus*

The extracts from *Cassia* plants, KW, and KH, did not exhibit any inhibition zone, while KM and KE showed clear inhibition zone against*Bacillus subtilis* measuring 24 mm and 10 mm, respectively (Fig. 6). The methanol extract of *Cassia* displayed the maximum inhibition zone, approximately 77% of the positive control. No inhibition zone were observed in any of the negative controls, indicating that these solvents lacked anti-bacterial activity. Against *Staphylococcus aureus*, KW and KH did not show any zones of inhibition, while KM and KE displayed inhibition zone measuring 21 mm and 11.5 mm, respectively (Fig. 8). Once again, the methanol extract exhibited the maximum inhibition zone, accounting for around 77% of the positive control. Full bacterial growth was observed in all negative controls. This data strongly suggests that both *Calotropis* and *Cassia* plants grown in the Jamnagar district region contain antibacterial compounds that can be effectively extracted using methanol.

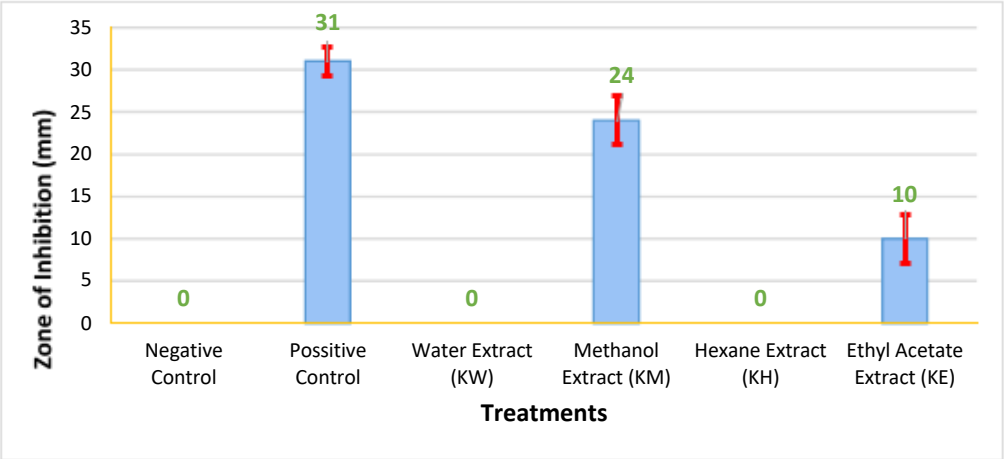


Fig.- 6: Anti-bacterial activity of *Cassia* Extracts on *Bacillus substilis*

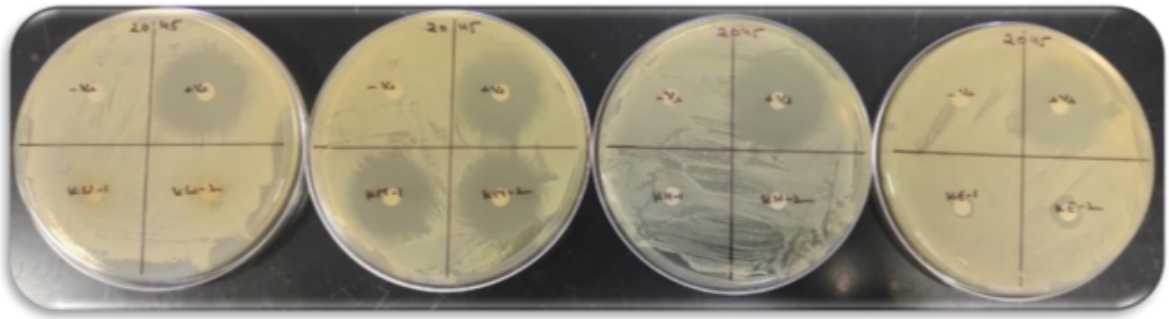


Fig.- 7: Picture Showing Anti-bacterial activityof KW, KM, KH & KEagainst *Bacillus substilis*

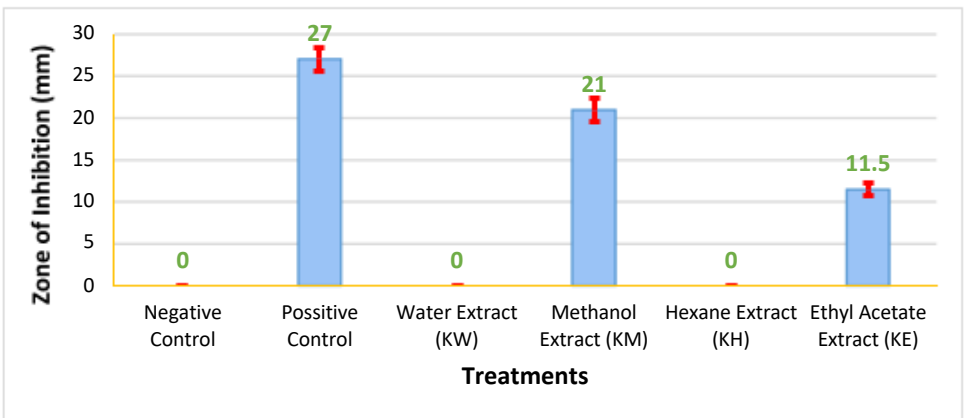


Fig.- 8: Anti-bacterial activity of *Cassia* Extracts on *Staphylococcus aureus*

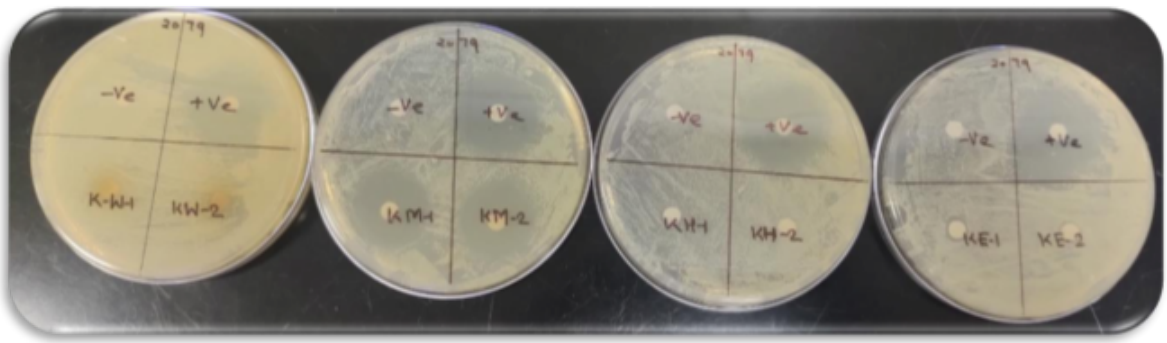


Fig.- 9: Picture Showing Anti-bacterial activity of KW, KM, KH & KE against *Staphylococcus aureus*

Measurement of anti-fungal activity:

All four extracts of *Calotropis* (CW, CM, CH& CE) and *Cassia* (KW, KM, KH & KE) were tested against both *Aspergillus niger* and *Candida albicans*. However, very small inhibition zone was observed in the methanol extract of *Calotropis* and *Cassia* plants against both organisms. *Calotropis* plant extracts, including CW, CH, and CE, did not show any inhibition zone against *Aspergillus niger*, except for CM. The maximum inhibition zone observed was only 2.5 mm in the methanol extract of *Calotropis*, which accounted for only about 10% of the positive control (Fig. 10). No inhibition zone was visible in any of the negative controls, indicating that these solvents lacked anti-fungal activity.

Similarly, against *Candida albicans*, ainhibition zone measuring 2.0 mm (Fig. 11) was observed in the CM extract. Once again, the methanol extract displayed the maximum inhibition zone, which was around 8% of the positive control. Full growth was visible in all negative controls. This data clearly indicates that anti-fungal compounds cannot be effectively extracted from *Calotropis* plants grown in the Jamnagar district region using water, methanol, hexane, or ethyl acetate or *Calotropis* does not have any compound with anti-fungal activity.

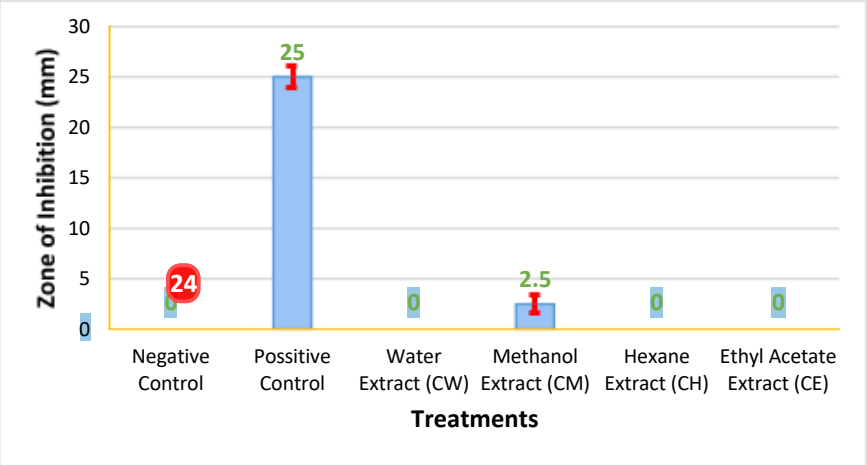


Fig.- 10: Anti-fungal activity of *Calotropis* Extracts on *Aspergillus niger*

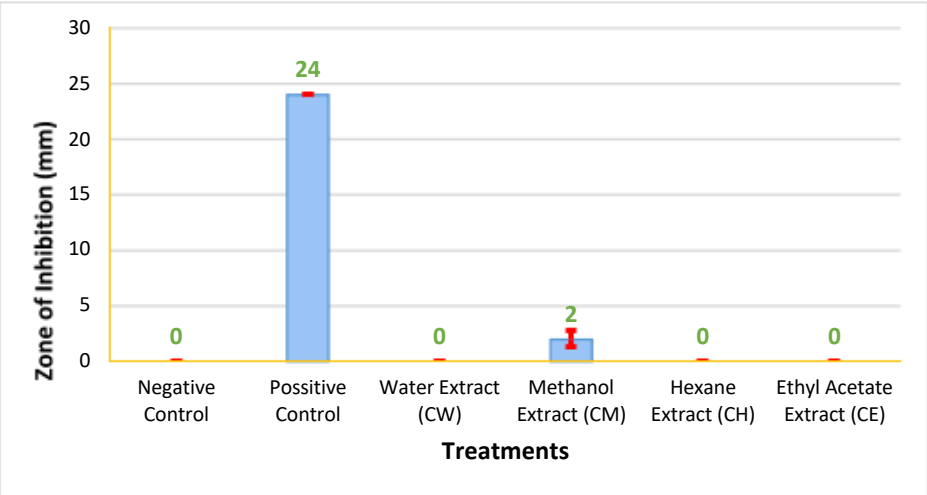


Fig.- 11: Anti-fungal activity of *Calotropis* Extracts on *Candida albicans*

The *Cassia* plant extracts, including KW, KH, and KE, did not show any inhibition zone against *Aspergillus niger*, except for KM. The supremeinhibition zone observed was only 1.5 mm in the methanol extract of *Cassia*, which accounted for only about 6% of the positive control. In contrast, the positive control exhibited a substantialinhibition zone of 24 mm against *Aspergillus niger* (Fig. 12). No inhibition zone was visible in any of the negative controls, indicating that these solvents lacked anti-fungal activity.

Similarly, against *Candida albicans*, ainhibition zone measuring 2.5 mm was observed in the KM extract. Once again, the methanol extract displayed the maximum inhibition zone, which was around 9% of the positive control. The positive control showed a significant inhibition zone of 27 mm against *Candida albicans* (Fig. 13). Full growth was visible in all negative controls. This data clearly concludes that anti-fungal compounds cannot be effectively extracted from *Cassia* plants grown in the Jamnagar district region using water, hexane, or ethyl acetate or *Cassia* does not have any compound with anti-fungal activity.

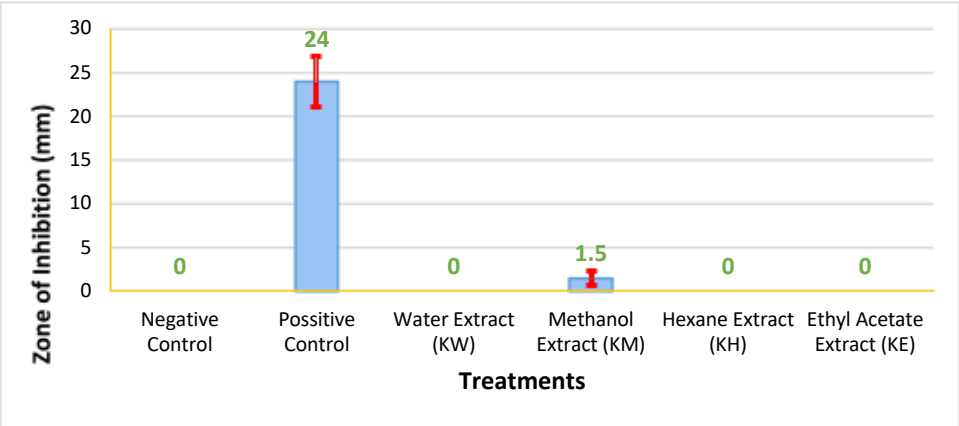


Fig.- 12: Anti-fungal activity of *Cassia* Extracts on *Aspergillus niger*

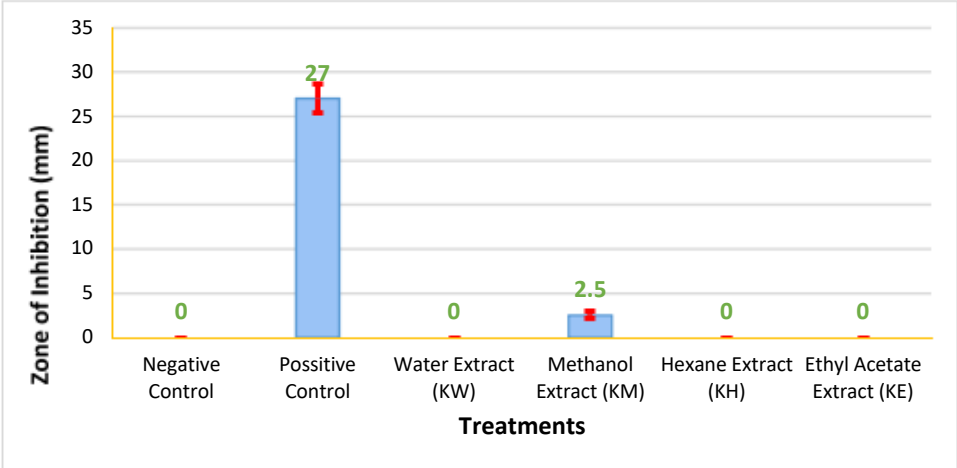


Fig.- 13: Anti-fungal activity of *Cassia* Extracts on *Candida albicans*

The pointer of extract's anti-bacterial activity and the dose requirement of the activity are imperative factors to consider in appraising the effectiveness of plant extracts. The effects of the antibacterial assay shows that the crude extracts of *Calotropis* and *Cassia* are livelyin front of the tested bacterial strains while being inactive against fungal strains. Among all the extracts evaluated, the methanol extract showed to be the maximum effective. It has been widely recognized by scientists that plants are important sources of pharmacophores and can serve as potential new chemotherapeutic agents. The initial step in developing a chemotherapeutic agent from plants is to assay there in vitro antibacterial and anti-fungal activity. Active extracts can then be used to recognize the specific lively compounds accountable for the antibacterial activities within the plants.

In current years, there has been aupward concern around multi-drug resistance displayed by pathogenic bacteria. Many studies have reported the appearance of multi-drug resistance, predominantly in gram-negative bacteria like *Pseudomonas aeruginosa*, which has shown resistance too many antibiotics. However, the extracts, specifically the polar ones, demonstrated decentaction against P. *aeruginosa.* Antibacterial agents derived from natural sources offer a promising alternative to synthetic or semi-synthetic antibacterial agents and can help mitigate their potential side effects. While the extracts are not cleancomplexes, the responses strongly advise the potential of these extracts as sources of antibacterial agents. Further purification of these extracts could lead to the purification of specific antibacterial compounds, opening up opportunities for the development of phytomedicine against these pathogenic microbes. It is essential that the inhibitory activities of the crude extracts are equivalent to or greater than those of commonly used standards.

It'svery imperative to note that the anti-bacterial activity of natural extracts can vary across different organisms, as indicated by the observed inhibition zone ranging from 10 mm to 27 mm in the study. Methanol was found to be particularly effective in extracting antibacterial compounds in comparison to

other solvents. Therefore, methanol extracts of *Calotropis* and *Cassia* demonstrated supremeanti-bacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* compared to other solvents. This aligns with the observations of a study by Dieu-Hien Truong et al. (2019), which also concluded that methanol extraction yielded the highest antibacterial compounds from plant materials. The variation in anti-bacterial activity observed when using solvents of varying polarity is likely due to differences in the solubility of antibacterial compounds in these solvents. The results indicate that the antibacterial properties of the plant extracts depend on the choice of extracting solvents, with methanol extract showing the most significant inhibition zone, while other extracts exhibited negligible activity. The ability of these compounds to dissolve and diffuse in the assay media also plays a significant role in their activity. In the case of anti-fungal activity, no inhibition zone was observed in the aqueous, hexane, and ethyl acetate extracts of *Calotropis* and *Cassia*. Only the methanolic extract showed a very small inhibition zone against *Aspergillus niger* and *Candida albicans*. Similar studies conducted by Saeed Ahmad and Muhammad Akram also used solvents like methanol, water, hexane, and ethyl acetate to prepare plant extracts and evaluated their anti-fungal activity against *Aspergillus niger* and *Candida albicans*.

In summary, the study suggests that *Calotropis* and *Cassia* plants from the Jamnagar district region possess antibacterial compounds that can be effectively extracted using methanol, making them potential sources of natural antibacterial agents. These findings hold promise for further research into identifying and developing new chemotherapeutic agents from these plant extracts.

One significant advantage of using natural extracts for anti-bacterial activity is that they are generally harmless and fewer prone to the expansion of medicine resistance in bacteria, as these compounds occur naturally. Given the cumulative challenges linked with drug-resistant bacteria and the high costs of artificial antibacterial agents, alternative solutions like phytomedicine are needed, especially for

pharmaceutical companies. Generally, these discoveries highlight the prospective of plant extracts as sources of antibacterial and anti-fungal compounds, with their activity influenced by the choice of solvent for extraction. Further research and purification of these compounds could lead to the development of effective phytomedicines against bacterial and fungal pathogens.

CONCLUSION

The inhibition zone assay conducted in the study yielded two primary types of observations: discs without any surrounding clear or inhibition zones, indicating the absenteeism of inhibitory activity, and clear inhibition zones, indicating bactericidal action of the tested plant extract. The study found that the method of extracts evaluated in the study exhibited anti-bacterial activity against the tested organisms. It suggests that methanol could be a potential solvent for extracting antibacterial compounds from *Calotropis* and *Cassia*. However, none of the tested solvents was suitable for extracting anti-fungal compounds from *Calotropis* and *Cassia* grown in the Jamnagar region. These findings align with traditional knowledge held by local users who have historically selected these plants for their anti-microbial properties. The outcomes of the learningsustenance the therapeutic use of these plants and propose that their extracts contain compounds with antibacterial properties, making them potential candidates for the development of new drugs to treat infectious diseases. Observeddata from this study can be established that the methanol fractions obtained from *Calotropis* and *Cassia* contain certain components that pay to their antibacterial activities. Typically, the presence of polyphenolic compounds in the fraction is associated with antibacterial capacity, providing preliminary pharmacological support for their therapeutic use.

4.0

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