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Evidence of Capping Effect in Saponin-Conjugated Mono and Bimetallic Nanoparticles: HPTLC Fingerprinting

Mukti Sharma[1,2*] and Shalini Srivastava [2]

[1]Department of Chemistry, Faculty of Science, J.S. University, Shikohabad, 283135, India
[2]Department of Chemistry, Faculty of Science, Dayalbagh Educational Institute, Agra, 282005, India

*E-mail: muktisharma90@gmail.com

Abstract- Phyto-fabrication of metal nanoparticles is grounded on the use of synergistic redox potential of plant secondary metabolites altering metal valance states. The resulting nano state occupies incredible changed optical properties with improved activities. During plant-mediated fabrication, medicinally important bioactive secondary metabolite also form capping on freshly generated metal nanoparticles and becomes a source for additional enhancement of bio-efficacy. The capping effect of therapeutically significant secondary metabolites of the plants on the freshly produced metal nanoparticles is one of the governing factors of green nanotechnological enhancement in pharmacological activities. So far, any proof based on analytical evidences of capping effect has not been reported. The present short communication warrants confirmatory HPTLC fingerprinting evidence of capping effect of extracted saponin from the seed of the plant *Madhuca longifolia* on the freshly phytofabricated mono (Au, Ag) and bimetallic (Au-Ag) nanoparticles.

Keywords: saponin, capping effect, mono and bimetallic nanoparticles, HPTLC fingerprinting.

INTRODUCTION

The plant secondary metabolites have been extensively utilized for the fabrication of mono and bimetallic nanoparticles and are found effective to enhance the pharmacological properties [1-4]. The overall enhancement in the bio-efficacies is attributed to the nanosizing effect involving astonishing surface plasmon resonance, high surface area as well as the capping effect of the medicinally important plant secondary metabolites [5-7]. The plant-mediated nanoparticles loaded with secondary metabolites (native plant extract as such or isolated phytoactive principle) have revolutionized the treatment of various medical ailments [8, 9]. The nano sizing effects have been extensively studied using various analytical techniques [10]. However, the capping effect of organic layer has not been proven technically except faded layer appearance around nanoparticles visible in Transmission Electron Microscopy [11-16].

High-Performance Thin-Layer Chromatography (HPTLC) seems to be an ideal technique for the identification of organic compounds in phytofabricated nanoparticles allowing excellent resolution, speed of separation, high sensitivity and good reproducibility. The present piece of research warrants our recent investigation on the authentication of the capping effect of plant secondary metabolites (saponin) isolated from the seeds of *Madhuca longifolia* using HPTLC fingerprinting and related techniques. *Madhuca longifolia* (Mahua; Sapotaceae family), a deciduous indigenous tree and its seeds have an abundant amount of saponins, and explored for various bio-efficacies [17,18].

EXPERIMENTAL

Extraction, characterization of saponin, phytofabrication of mono and bimetallic nanoparticles and its characterization

The seeds of the tree *Madhuca longifolia* were gathered from the village of Rajaborari (Madhya Pradesh, India) and were recognized by Department of Botany, Dayalbagh Educational Institute Agra, India. The seeds were washed, dried and powdered in a grinder. The seeds were subjected to soxhlation with petroleum ether (40-60^{0}C) for 36 h. The defatted *Madhuca longifolia* seed powder (100 g) was subjected in aqueous ethanol (300 ml) and exposed 3 min for microwave irradiation (Voltage: 1150 W ; frequency : 2.45 GHz). The sample was sonicated at room temperature for 40 min. The seed extract was subjected to a scheme for the isolation of saponins [17]. Seed extract (25 g) was treated with diethyl ether (80 ml) and shifted to a separating funnel. The aqueous layer was treated with an additional solvent mixture (6:1) n-butanol: 5% sodium chloride and ether layer had rejected. The organic layer (n-butanol) layer was dried in a crucible and crude saponin content was obtained. The crude saponin content was used for phytofabrication for mono and bimetallic nanoparticles.

High resolution mass spectrometry

The extracted crude mixture of saponin was characterized using a high-resolution spectrometer (Micro Mass ESI-TOF MS). The spectra (sample: 2 µl; flow rate: 1-10 µl min^{-1}) was recorded in [M-H$^-$] negative mode.

High performance thin-layer chromatography fingerprinting

HPTLC system (CAMAG, Muttenz, Switzerland) was containing twin trough chamber with lid (10×10 cm^2), sample applicator (Linomat 5), UV-Visible cabinet with dual-wavelength (254/366 nm) and photo documentation. Each sample [saponin loaded gold (S@AuNp$_s$): S_2, silver (S@AgNp$_s$): S_3 and bimetallic (S@Au-AgNp$_s$) nanoparticles: S_4] was monitored against extracted saponin mixture as standard and applied as the band (6 mm) with a 6 µL volume with syringe (Hamilton, Bonaduz, Switzerland) by a sample applicator (CAMAG Linomat 5) on the pre-coated silica gel aluminium plate (60 F254), size (10 × 10 cm^2) of 250 µm of the thickness (E. MERCK, Darmstadt, Germany).

Before applying bands, the plate was pretreated with methanol and activated (100^{0}C; 5 min). The twin trough glass chamber (10 x 10 cm^2) was earlier saturated with triphasic mobile phase [1-butanol: acetic acid: DDW: (5:1:4)] for 15 min before development of chromatogram. HPTLC plate was kept in a hot air oven for dry. The scanning speed (20 mm/sec) and slit dimensions (5×0.45 mm) were retained during the analysis. Visualization of the bands was carried out using a densitometer under UV light (254 nm). A separate similarly loaded HPTLC sheet was also developed using the same mobile phase and derivatized with anisaldehyde reagent specific for clear saponin visualization (visible light) and marked as (S_1 $^{\backprime}$) WINCATS software (1.4.10) was used for recording R_f values and fingerprint data.

Energy dispersive X-ray spectroscopy and Transmission electron microscopy (EDX and TEM) analyses

EDX and TEM (Tecnai G2 T 20 ST, Germany) were used to record the elemental composition (highlighting the presence of Au, Ag in the phytofabricated nanoparticles) and capping effect of saponins on nanoparticles.

RESULTS AND DISCUSSION

In phyto-mediated nanoparticles, the plant secondary metabolites do not provide the redox potential only but also forms a coating on freshly produced shield metal nanoparticles. This capping effect of saponin is reflected valuable for preserve them in a nano form by shielding them from aggregations. The effect has also been characterized for enhancing bio-efficacy owing to their medicinal properties. There is a lack of authentic shreds of evidence confirming this effect except the appearance of a faded layer around nanoparticles visible in Transmission Electron Microscopy. As reported in the literature [11-16], the TEM images of saponin loaded mono and bimetallic nanoparticles in our case also report the faint thin layer around the spherical-shaped nanoparticles (Fig. 1).

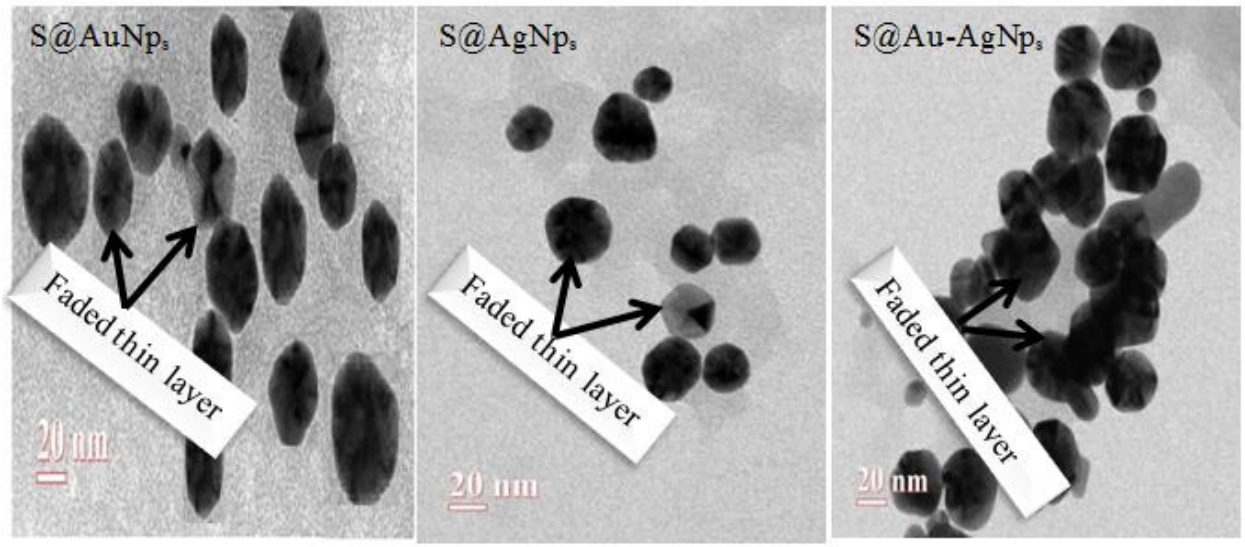

Fig. 1 TEM images of S@AuNp$_s$, S@AgNp$_s$ and S@Au-AgNp$_s$ nanoparticles exhibiting faded layer around nanoparticles

High resolution mass spectrogram of extracted saponin

The Micro Mass ESI-TOF MS spectrogram [19] along with few unassigned peaks depicted the presence of four saponins in crude saponin mixture: *Mi-saponin A (1)*; m/z 1221.5963, *Mi-saponin B (2)*; m/z 1353.6344, *Madhucoside A (3);* m/z 1483.6742 and *Madhucoside B (4);* m/z 1515.6863 (Fig.2).

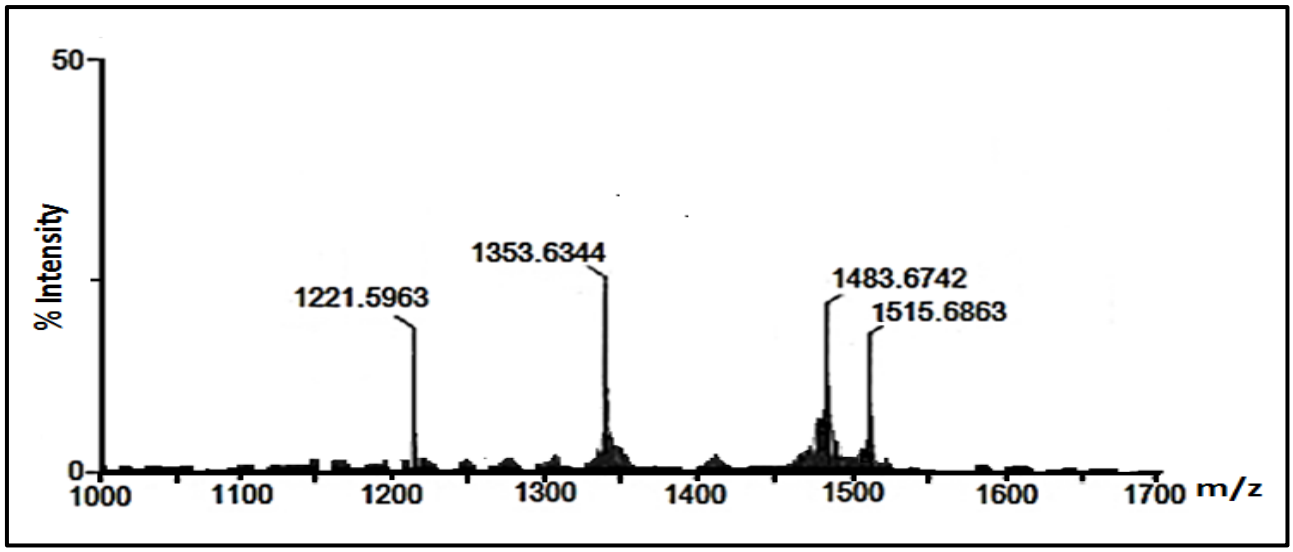

Fig. 2 HRMS spectrogram of crude saponins

High performance thin-layer chromatography

The presence of identified four saponins has been confirmed in the saponin loaded mono and bimetallic nanoparticles based on the appearance of the same set of HPTLC fingerprinting in them. The bands of four identified saponins were visualized in UV 254 nm (S_1). These four bands on a separate HPTLC plate were also visualized more clearly after its derivatzation with anisaldehyde and marked ($S_1^{'}$). The standards of all the saponins are not commercially available. Therefore, the saponin mixture extracted from the seeds of the plant *Madhuca longifolia* after their characterization (HRMS), considered as standard. 3-D densitogram confirms the capping effect of saponin in saponin loaded gold (S@AuNp$_s$), silver (S@AgNp$_s$) and bimetallic (S@Au-AgNp$_s$) nanoparticles in terms of similar R_f values (Fig. 3a,b).

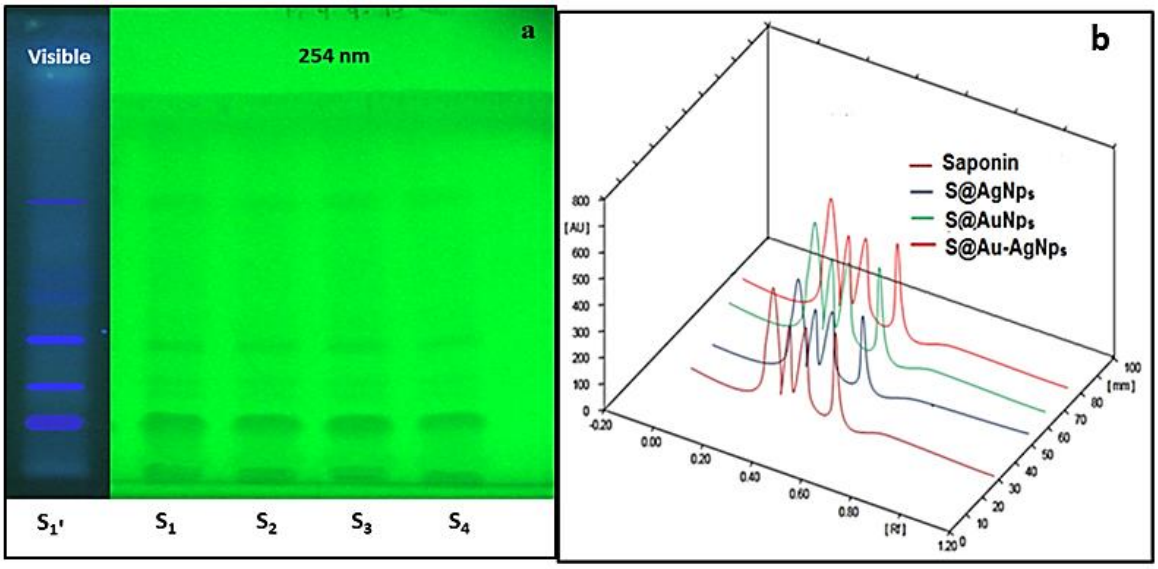

Fig. 3 (a) HPTLC fingerprinting of standard saponin (S_1' at visible light), standard saponin (S_1 at UV 254 nm), S@AuNp$_s$ (S_2), S@AgNp$_s$ (S_3) and S@Au-AgNp$_s$ (S_4) (b) 3-D densitogram of saponin and its loaded mono and bimetallic nanoparticles

The R_f values of the standard saponin were obtained and matched with their respective saponin loaded mono and bimetallic nanoparticles. HPTLC fingerprinting profile of standard and saponin loaded all the nanoparticles in terms of R_f value and % area has been summarized (Table 1).

Table 1. HPTLC profile of standard saponin, mono and bimetallic nanoparticles

Band	Std. Saponin (S_1)		S@AuNp$_s$ (S_2)		S@AgNp$_s$ (S_3)		S@Au-AgNp$_s$ (S_4)	
	Max R_f	% Area	Max R_f	% Area	Max R_f	% Area	Max R_f	% Area
I	0.21	3.66	0.21	3.20	0.21	3.49	0.20	3.00
II	0.33	6.50	0.33	5.80	0.33	5.20	0.32	4.70
III	0.40	8.26	0.40	7.80	0.40	7.40	0.40	6.80
IV	0.51	9.26	0.52	9.01	0.51	9.10	0.51	8.80

The presence of metals (Au, Ag) in the phytofabricated nanoparticles could not be performed on the HPTLC silica gel plate as the interaction between the gold and silver with silica gel (stationary phase) seems to be much stronger than mobile phase (1-butanol: acetic acid: water 5:1:4). The strong metallic interaction with silica gel prevents their movement [20, 21]. There is a lack of authentic shreds of evidence confirming this effect except for the appearance of a faded layer around nanoparticles visible in Transmission Electron Microscopy. The TEM images of mono (S@AuNp$_s$, S@AgNp$_s$) and bimetallic (S@Au-AgNp$_s$) nanoparticles were also

recorded. It is evident that the edges of the spherical-shaped nanoparticles are surrounded by light shade impressions.

The appearance of the peak of Cu (0.8 KeV) is associated to the Copper grid at which the fabricated nanoparticle was coated. EDX spectra (Fig. 4) also supports the observed fact that additional peaks of C (0.2 KeV) and O (0.5 KeV) might have originated from the adhered saponin on the surface of nanoparticles.

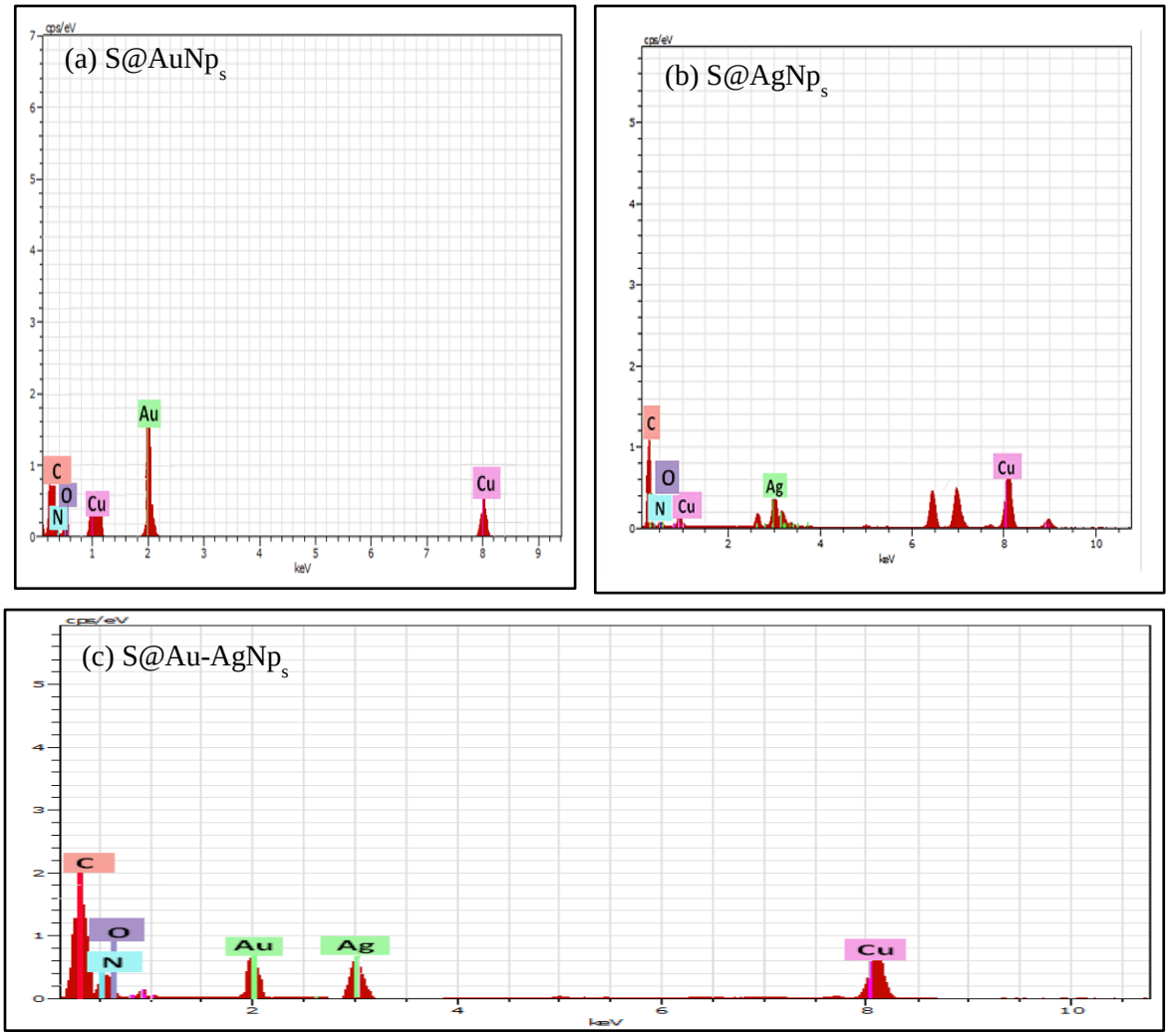

Fig. 4 EDX spectra conforming Au, Ag and both metals in saponin loaded (a) S@AuNp$_s$, (b) S@AgNp$_s$ (c) S@Au-AgNp$_s$ nanoparticles

CONCLUSIONS

HPTLC fingerprinting being a versatile technique for the identification of compounds at trace level has been innovatively used to establish the capping effect of saponins in the phytofabricated mono and bimetallic nanoparticles. The strong metallic (Au, Ag) interaction with silica gel prevents their movement on the HPTLC plate. Therefore, the presence of Ag and Au metals in the phytofabricated nanoparticles has been ascertained by recording its EDX

crystallography which simultaneously supports the capping phenomenon of the presence of saponins on the freshly generated mono and bimetallic nanoparticles.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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