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(Research Article)

Characterization of *Tridax procumbens* amines with a potential to synthesize silver nanoparticles

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Abstract

Our previous findings showed that amines from *Tridax procumbens* (Family: Asteraceae) were responsible for the synthesis of silver nanoparticles. *T. procumbens* is a weed plant with rich source of medicinal compounds. In the current work, we studied the initial characterization of bioactive compounds by LC-MS, an essential tool for the characterization and identification of low molecular compounds. Further the isolated compounds were investigated for silver nanoparticle synthesis. The leaf extracts examined revealed many novel amine derivative compounds reported for the first time mainly belonging to the group of free amines: n-Pentylhydrazine hydrochloride, 2-Nitrobenzenesulfonyl chloride, 1,7-Dichlorooctamethyltetrasiloxane, 5-Chloro-1,2,4-trihydroxybenzene, 3-Chloro-2-hydroxy-5-(trifluoromethyl)pyridine and 2-(Diphenylphosphino) ethyltriethoxysilane and conjugated amines: 5-Methoxy-diisopropyltryptamine, 4-Chlorophenoxyacetic acid, Diphenhydramine, Erucamide, n(4-((2-(2,5-dimethoxybenzylidene)hydrazino)carbonyl)ph)-4-me-benzenesulfonamide and 1,3,5-Trithia-2,4,6-tristannacyclohexane, 2,2,4,4,6,6-hexamethyl. Characterization by UV-Vis spectra, XRD, EDX and TEM revealed well separated spherical shaped AgNPs size ranging 8-50 nm. These findings suggest that further work could be extended to isolate and elucidate the structures of the identified molecules using powerful instruments such as HPLC-MS, HPLC-NMR and high resolution-MS (HR-MS). Further biotechnological approaches towards synthesis of novel metals will be enhanced to promote green chemistry technology for the synthesis of nanoparticles.

Keywords: Silver nanoparticles; LC-MS; *Tridax procumbens*; Amines

1. Introduction

In this research we report for the first time, characterization of amines using LC-MS in *Tridax procumbens*. The plant is a native to tropical America, recognized for its countless pharmaceutical applications including ethno-medical practitioners as a remedy against various disease and its antimicrobial activity [1-2]. Different biomolecules from *T. procumbens* have been well characterized [3-9]. Other uses of this plant vindicated in literature include bio-adsorbent, mosquito repellency, larvicides, nanoparticle synthesis and others [10-12]. Plant amines have been associated with many cell processes, including cell division and differentiation, synthesis of nucleic acids and proteins, membrane stability, pH and thermic or osmotic stress responses and delay in senescence, also may function as allelochemical compounds and as components of the chemical and physical defences against herbivores as reviewed by Bouchereau and co-authors [13]. It has been hypothesized that the accumulation of both common and uncommon higher polyamines may serve specific protection roles in plants adapted to extreme environments [13]. In our previous studies involving nanomaterial synthesis, amines were the major compounds shown to reduce ion precursors for nanomaterial formation

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[11]. Thus, the current work postulated to isolate and characterize these amines using LC-MS and further examine whether they have the potential to synthesize the silver nanoparticles.

2. Materials and methods

2.1. Aqueous filtrate

The aqueous filtrate used was prepared as described previously [11]. It was dried in vacuo at 30°C. The resultant pellet was used further for isolation of free and conjugated amine contents according to Hennion and Martin-Tanguy [14].

2.2. Extraction and isolation of free amines

Dried aqueous filtrate was taken and mixed with 1M HCl and kept in ice bath for 1 h, pelleted at 48, 000 × g for 20 min and the supernatant at -20°C was stored until further use.

2.3. Extraction and isolation of conjugated amines

Dried aqueous filtrate was taken and mixed with 80 % aqueous methanol, centrifuged at 10000 × g for 15 min. The supernatant was evaporated in vacuo at 30°C, diluted with 10 ml deionized water and further treated with: petroleum ether (removes most of the chlorophylls from the fraction), benzene (removes aromatic amine conjugates), chloroform (removes neutral putrescine conjugates) and ethyl acetate (removes neutral spermidine conjugates). The sample was stored at -20°C until further use.

2.4. Thin Layer Chromatograph (TLC)

Prior, different ratios of hexane: ethyl acetate was run where (2:8) led to the formation of single spots after running a small sample placed on commercially available TLC plate 254 nm in iodine chamber to reveal presence of bioactive molecule isolates.

2.5. LC-MS and compound identification

Shimadzu LC-MS 2020 liquid chromatographic system equipped with a DGL-20A vacuum degasser, a dual pump, and a SIL-10AD VP auto sampler (Shimadzu, Japan) was used. Detection was performed on an API 4000 QTRAP mass spectrometer equipped with Turbo Ion Spray (ESI) Interface (Applied Biosystems, Concord, Ontario, Canada). Analyst 1.5 software packages (Applied Biosystems) were used to control the LC-MS system, as well as for data acquisition and processing. The analytical column used was a Thermo kromasil (Switzerland) C18 (5 µm, 250×4.6 mm). The chromatographic conditions were as follows: mobile phase gradient 80% A (10 mM NH_4Ac); 15% B (0.1% formic acid in a mixture of acetonitrile/methanol [50/50]) to 80% A; 30% B for 0–2 min to 50% A; 50% B for 2–5 min to 10% A, flow rate 0.1 ml·min−1, column temperature 90°C, detection wavelength 232 nm. Online ChemBioFinder free software available at (http://chembiofinder.cambridgesoft.com/chembiofinder/) was deployed to confirm the compounds based on their molecular weight.

2.6. Confirmation of the isolated compounds for NP synthesis

Synthesis of silver nanoparticles and characterization was done as previously described in our work [11].

3. Results and discussion

3.1. TLC and LC-MS analysis

Formation of dark and deep single spot (Figs 1 A & D) suggests presence of active compound isolates for free and conjugated amines respectively. Mass spectrometers work by ionizing molecules, sorting these ions and identifying them according to their mass-to-charge (m/z) ratios. Two key components in this process are the ion source, which generates the ions, and the mass analyzer, which sorts the ions. Full scan chromatograms for both free and conjugated amines (Figs 1 B & C, E & F) respectively indicate the major fragment ions existed at m/z 139, 222, 274, 352, 161, 198 and 377 for free amines and 274, 502, 540, 663, 187, 255, 338, 355, 389, 454 and 543 for conjugated amines. All the structurally compounds related to amines as identified by LC-MS is given in Table 1. It is worth noting that compound with ion peak mass of 274 was dominant in both the free amines and conjugated amine samples. The compound identified as 5-Methoxy-diisopropyltryptamine has been reported as psychedelic drug [15]. All the amines identified in this current study are reported for the first time. These findings suggest that further work could be extended to isolate and elucidate the structure of the identified molecules using powerful hyphenated instruments, such as HPLC-MS, HPLC-

NMR and high resolution-MS (HR-MS) for pyscho-therapy and also implicate that the compound could be a potential marker in regard to Asteraceae family.

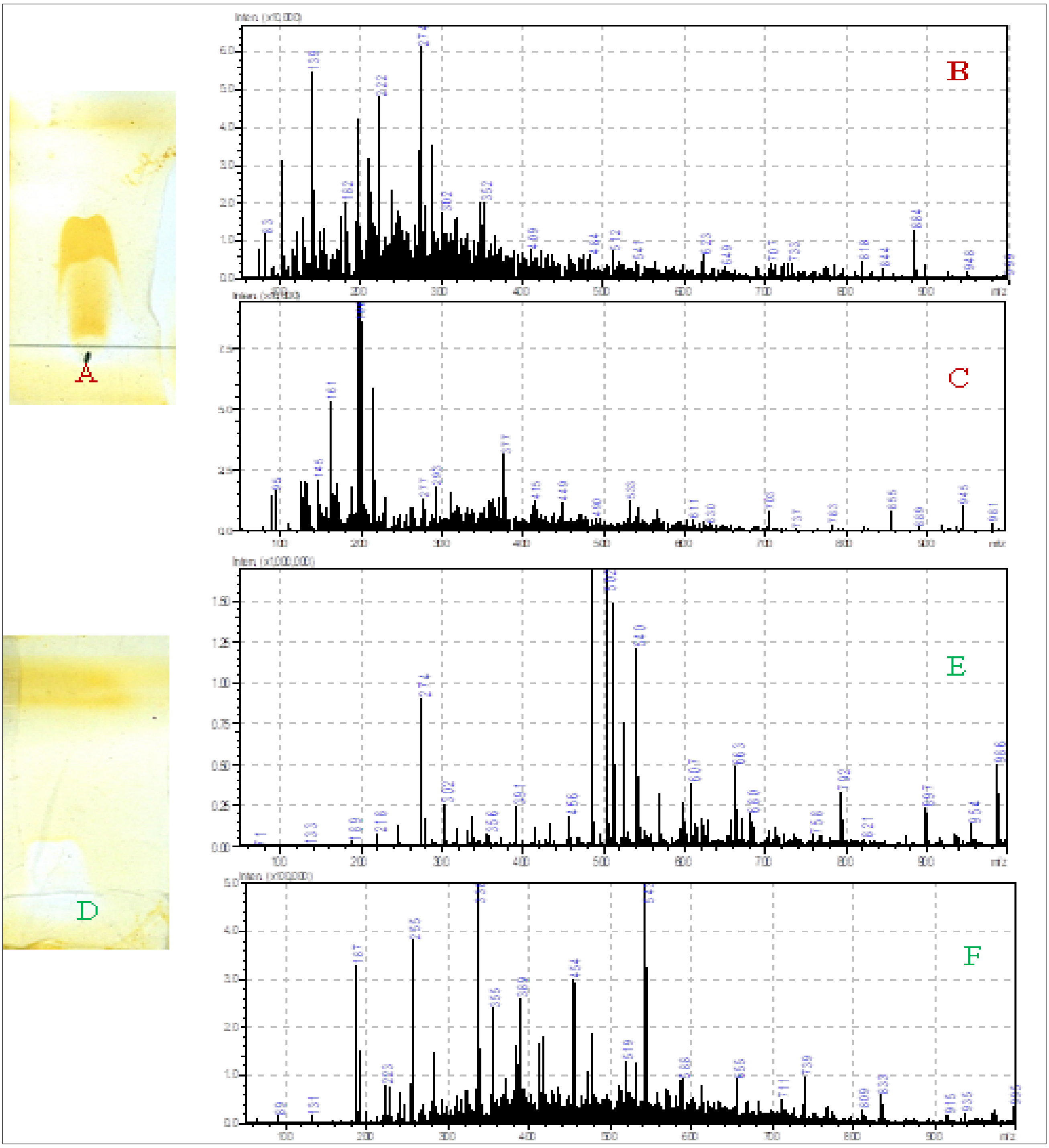


Figure 1 (A) TLC plate for free amines (B) Positive (C) Negative for Free amines, (D) TLC plate for conjugated amines (E) Positive (F) Negative for conjugate amines at 50-1000 nm.

3.2. Characterization of silver of silver nanoparticles synthesized by amines

Fig 2 depicts synthesis of biogenic AgNPs mediated by free amine and conjugated amine in combination. It was observed that, synthesis was achieved as earlier as 20 min. This suggests that if amines alone could be adopted for AgNP synthesis, the process could be robust. Formation of brown colour (Fig 2 A) was evidence for the AgNPs, which was further confirmed by UV-Vis spectrum and peak wavelength detected at 412.5 nm (Fig 2 inset), which concur to previous reports for characteristic for Ag^0. TEM analyses depicted well separated spherical shaped AgNPs measuring 8-50 nm with an average of 25.5 nm (Fig 3) which collaborates with XRD results (Fig 4), whereas inset diagram shows EDX indicating Ag which are in agreement with previous research conducted [10, 12].

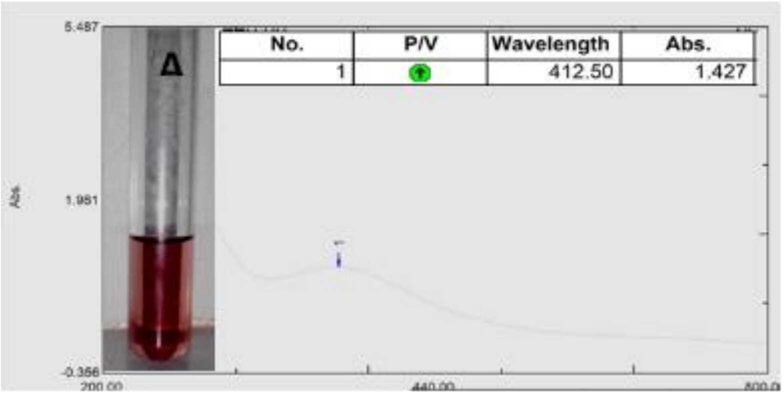


Figure 2 Confirmation of biogenic AgNPs synthesis by UV-Vis spectrum using free and conjugated amines at absorbance wavelength peak at 412.5 nm, inset (A) shows brown colour indicating formation of AgNPs

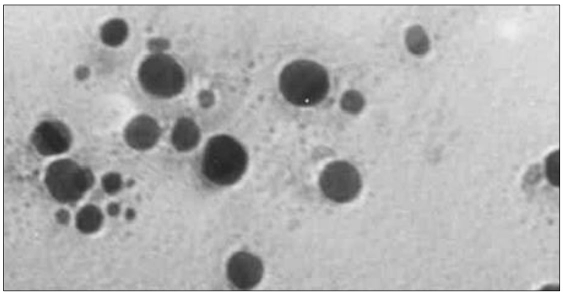


Figure 3 TEM analyses of AgNPs synthesized and stabilized by amines indicating spherical shaped measuring 8-50nm with an average of 25.5 nm.

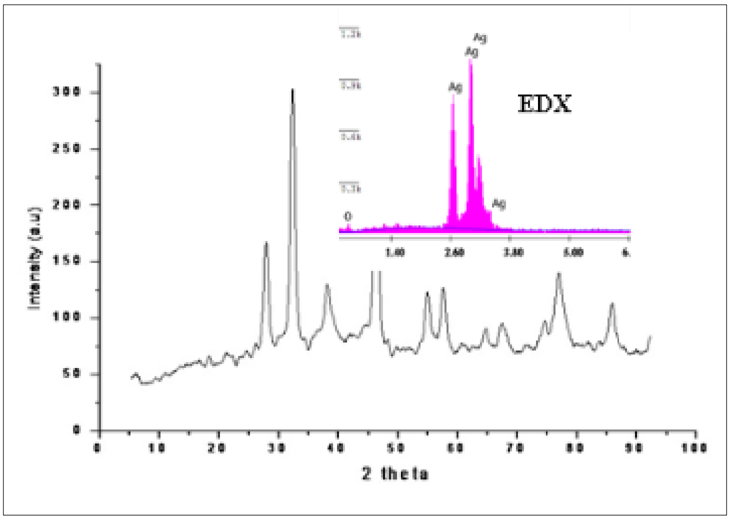
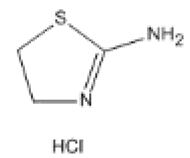
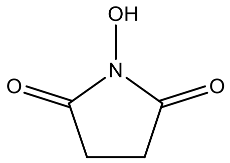
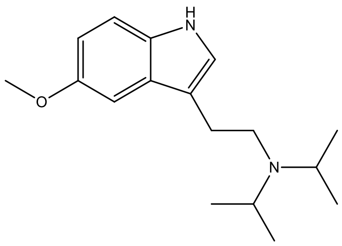
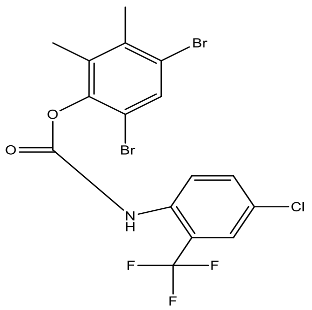
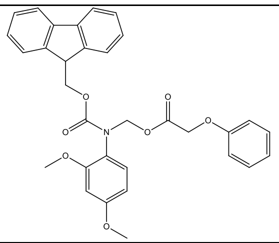
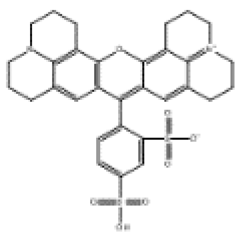
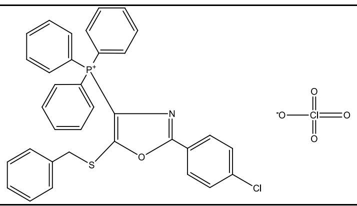
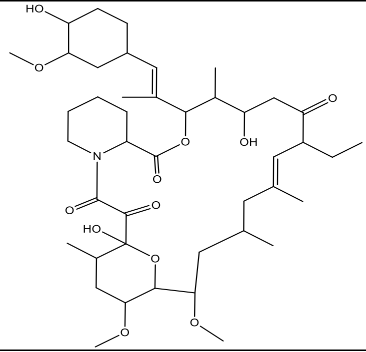
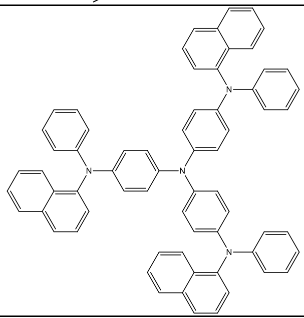
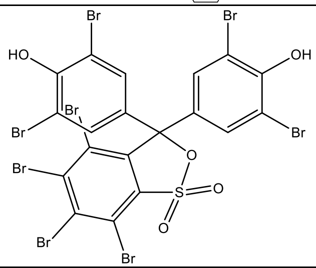
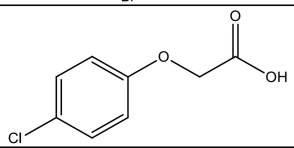
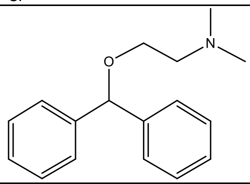
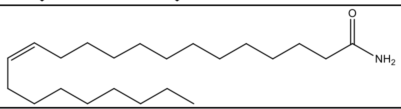
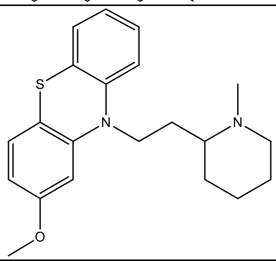
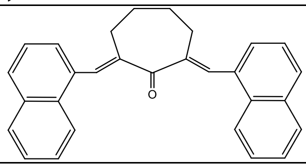
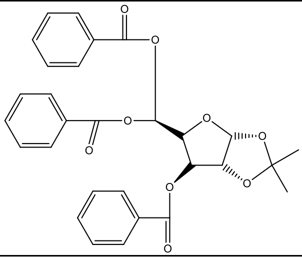
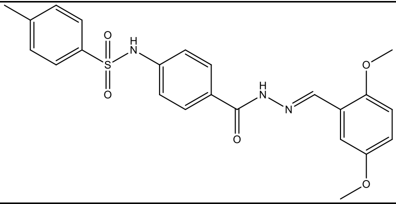
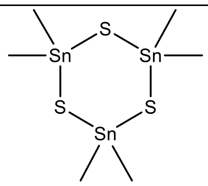
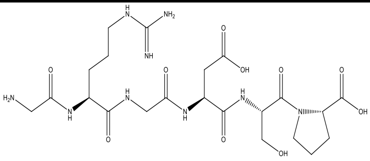
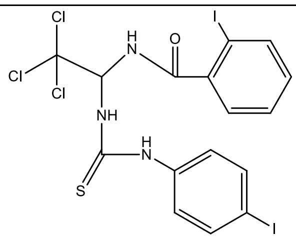
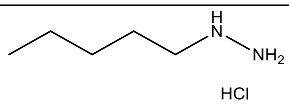
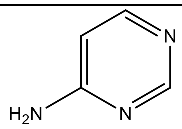
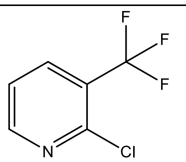
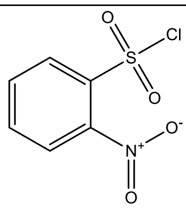


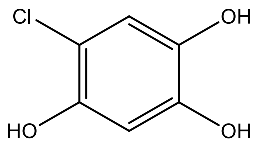
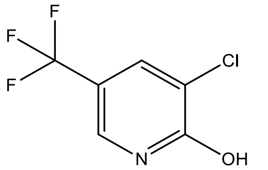
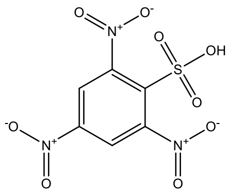
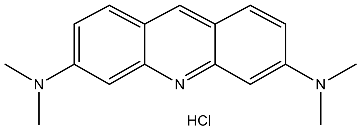
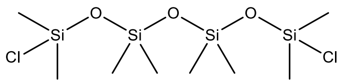
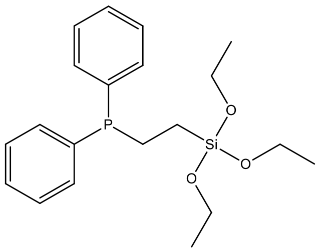
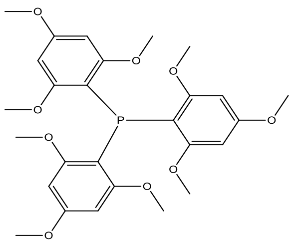
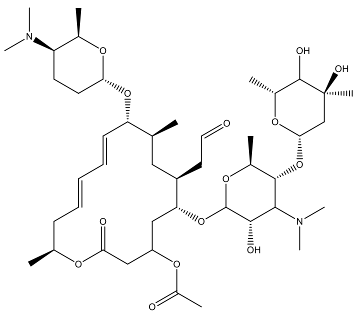
Figure 4 XRD spectrum of synthesized and stabilized AgNPs at peak 29.50º and 32.50º depict crystalline nature with average grain size of 25.5 nm. Inset is EDX spectrum showing Ag alone.

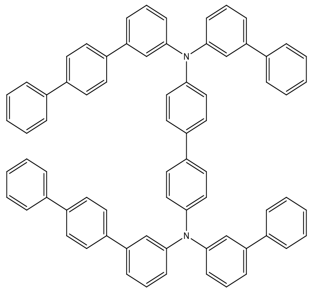
Table 1 Compounds structurally similar to amines identified by LC-MS

MW	Chemical formula	Structure	Name given
139	$C_3H_7ClN_2S$		2-Amino-2-thiazoline hydrochloride
115	$C_4H_5NO_3$		N-Hydroxysuccinimide
274	$C_{17}H_{26}N_2O$		5-Methoxy-diisopropyltryptamine
502	$C_{16}H_{11}Br_2ClF_3NO_2$		2,4-dibromo-5,6-dimethylphenyl n-(4-chloro-2-(trifluoromethyl) phenyl) carbamate
540	$C_{32}H_{29}NO_7$		4-[(R,S)-alpha-1-(9H-Fluren-9-yl)-methoxy formamido]-2,4-dimethoxybenzylphenoxyacetic acid
607	$C_{31}H_{30}N_2O_7S_2$		Sulforhodamine 101 (free acid)
663#	$C_{34}H_{26}Cl_2NO_5PS$		(5-Benzylsulfanyl-2-(4-chlorophenyl)-oxazol-4-yl)-triphenyl-phosphonium, perchlorate

792	$C_{43}H_{69}NO_{12}$		Ascomycin
897	$C_{66}H_{48}N_4$		4,4',4''-Tris(N-(1-naphthyl)-N-phenylamino)triphenylamine
986	$C_{19}H_6Br_8O_5S$		Tetrabromophenol Blue
187*	$C_8H_7ClO_3$		4-Chlorophenoxyacetic acid
255*	$C_{17}H_{21}NO$		Diphenhydramine
338*	$C_{22}H_{43}NO$		Erucamide
55#	$C_{21}H_{26}N_2OS$		Oxyridazine
389#	$C_{29}H_{24}O$		2,7-Bis-naphthalen-1-ylmethylene-cycloheptanone

519	$C_{29}H_{26}O_9$		1,2-O-Isopropylidene-3,5,6-tri-O-benzoyl-alpha-D-glucofuranoside
454*	$C_{23}H_{23}N_3O_5S$		n(4-((2-(2,5-dimethoxybenzylidene)hydrazino)carbonyl)ph)-4-me-benzenesulfonamide
543*	$C_6H_{18}S_3Sn_3$		1,3,5-Trithia-2,4,6-tristannacyclohexane, 2,2,4,4,6,6-hexamethyl-
588	$C_{22}H_{37}N_9O_{10}$		H-Gly-Arg-Gly-Asp-Ser-Pro-OH
655	$C_{16}H_{12}Cl_3I_2N_3OS$		2-iodo-n-(2,2,2-trichloro-1-(3-(4-iodo-phenyl)-thioureido)-ethyl)-benzamide
139*	$C_5H_{15}ClN_2$		n-Pentylhydrazine hydrochloride
95	$C_4H_5N_3$		4-Pyrimidineamine
182	$C_6H_3ClF_3N$		2-Chloro-3-(trifluoromethyl)pyridine
222*	$C_6H_4ClNO_4S$		2-Nitrobenzenesulfonyl chloride

161*	$C_6H_5ClO_3$		5-Chloro-1,2,4-trihydroxybenzene
198*	$C_6H_3ClF_3NO$		3-Chloro-2-hydroxy-5-(trifluoromethyl)pyridine
293	$C_6H_3N_3O_9S$		2,4,6-Trinitrobenzenesulfonic acid
302	$C_{17}H_{20}ClN_3$		3,6-Acridinediamine, N,N,N',N'-tetramethyl-, monohydrochloride
352#	$C_8H_{24}Cl_2O_3Si_4$		1,7-Dichlorooctamethyltetrasiloxane
377*	$C_{20}H_{29}O_3PSi$		2-(Diphenylphosphino) ethyltriethoxysilane
33	$C_{27}H_{33}O_9P$		Tris(2,4,6-trimethoxyphenyl)phosphine
855	$C_{44}H_{74}N_2O_{14}$		Acetylspiramycin

945	$C_{72}H_{52}N_2$		N,N'-Bis(3-biphenylyl)-N,N'-bis(p-terphenyl-3-yl)benzidine

Abbreviations used: MW – molecular weight, * Significant, # Not very significant

4. Conclusion

Thus, the question whether these amines, have potential to synthesize and stabilize AgNPs was true, suggesting that amines could potentially be involved in the synthesis of silver nanoparticles. Based on these revelations, advanced characterization techniques using powerful instruments such as HPLC-MS, HPLC-NMR and high resolution-MS (HR-MS). could be studied as well as biotechnological advancements including plant tissue culture can be explored in the future, to enable adoption of green nano-technologies for large scale production of biomaterials for their various applications.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors submit that they don't any competing interests

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