INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Formation And Characterization Of Silver Nanostructures From *Limonia acidissima* Bark Extract

Jatin Agarwal*, Dr. Ashok Kumar Rajput, Akshanshu Patel, Sudhanshu Sagar, Shubham Rana

Department of Pharmaceutics, Moradabad Educational Trust Group of Institution Faculty of Pharmacy, Moradabad, Uttar Pradesh, India.

ARTICLE INFO

Received: 13 June 2024
Accepted: 25 June 2024
Published: 27 June 2024

Keywords:
Limonia Acidissima, silver nanoparticles, bark Extraction through Soxhlet.

DOI:
10.5281/zenodo.12570397

ABSTRACT

In this study, simple approach was applied for synthesis of silver nanoparticles using Limonia acidissimia aqueous and hydro alcoholic bark extract. Controllable silver nanoparticles were developed by green approach using extract of bark of Limonia acidissimia tree. Due to presence of pytochemical compounds such as saponins, phenolic compounds, phyto sterols and quinines present in extract act as reducing agent, hence silver nanoparticles were easily produced under mild conditions. The formations of silver nanoparticles were verified by UV- visible spectroscopy. Highly stable silver nanoparticles were produced using bark extract. UV – visible spectrophotometer showed absorbance peak in range of 400-500 nm range. Only 15 minutes were required for the conversion of silver ions into silver nanoparticles at room temperature without the involvement of any hazardous chemical.

INTRODUCTION

Limonia Acidissima is the simplest species inside the monotypic genus Limmonia. Common names for the species in English consist of wood-apple and elephant-apple.[1] Limonia acidissima is a large tree growing to 9 metres (30 toes) tall, with tough, spiny bark. The leavesare pinnate, with 5-7 leaflets, every leaflet 25–35 mm long and 10–20 mm extensive, with a citrus-fragrance while overwhelmed. The flora are white and feature five petals. The fruit is a berry 5–9 cm diameter, and may be candy or sour. It has a completely hard rind which can be hard to crack open, it appears greenish-brown in shade from out of doors and incorporates sticky brown pulp and small white seeds. The fruit appears comparable in look to the Beal fruit (Aegle marmelos). It contains wide spread amount of protein, carbohydrate, iron, fat, calcium, Vit-B & C and many others. One hundred

***Corresponding Author:** Jatin Agarwal
Address: *Department of Pharmaceutics, Moradabad Educational Trust Group of Institution Faculty of Pharmacy, Moradabad, Uttar Pradesh, India.*
Email ✉: jatinagarwal0591@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

g of ripefruit pulp contains up to forty nine Kcal. Deciduous bushes, to twenty m excessive, bark dark-gray or black, deeply cracked longitudinally; thorns straight, to 2.5 cm, axillary. Leaves imparipinnate, alternate,1-3 in a cluster, estipulate; rachis 60-eighty mm long, stout, glabrous regularly narrowly winged; leafletsfour-7, contrary, sessile, estipellate; lamina 1. Three-three.8 × 1.3 cm, obovate, base cuneate or acute, apex obtuse, margin complete, glabrous, pellucid-punctate, coriaceous; lateral nerves pinnate, obscure, intercostae obscure. Flowers polygamous, dull crimson, 1.3 cm across, in axillary cymes; calyx small, flat,5- toothed, pubescent without, deciduous; petals five, unfastened, spreading; stamens 10-12, inserted round the disc; filaments dilated under, villous on face and margins; anthers linear-rectangular; disc thick, annular, pubescent; pistillode short; ovary superior, oblong, five-6-celled, at duration 1-celled, ovules many; stigma oblong, fusiform. Fruit a berry, 5-7.6 cm across, globose, whitish-brown, rind hard and woody; seeds many.[2]

Distribution:

Limonia acidissima is local to India (together with the Andaman Islands), Bangladesh, and Sri Lanka. The species has additionally been delivered to Indochina and Malaysia. [3] [4]

Scientific classification:

- Kingdom – Plantae
- Order- Sapindale
- Family – Rutaceae
- Genus- limonia
- Species – L. Acidissimia

Phyto Chemicals:

The initial phytochemical evaluation of Limmonia acidissima plant elements showed the presence of alkaloids, flavonoids, phenols, terpenoids, tannins, fats steroids, saponins, glycosides, gum, mucilage and glued oils15-18. The unripe end result contains stigmasterol. Fruit pulp incorporates massive quantity of citric acid and other fruit acids, mucilage and minerals. Alkaloids, coumarins, fatty acids and sterols were detected inside the pericarp. It also contains umbelliferon, dictamnine, xanthotoxol, scopa one, xanthotoxin, isopimpinellin, is oimperatorin and marmin. [5] Leaves incorporate stigma sterol, psoralen, bergapten, orient in, vitedin, saponarin, tannins and an essential oil. [6] Marmes in, feronolide and feronone were isolated from the bark. Seeds incorporate fixed oil, carbohydrates, proteins andamino acids. Roots include Feronia lactone, geranylumbelliferone, bergapten, osthol, isopimpinellin, marmesin and marmin. [7]

Fig No – 1 Bark Powder of limmonia acidissima plant

Fig No – 2 limmonia acidissima bark Extraction through Soxhlet

Nanoparticles

Nanoparticles are defined as particulate dispersions or solid particles with a length within the variety of10-1000nm. The drug is dissolved, entrapped, encapsulated or connected to a nanoparticle matrix. Depending upon the method of training, nanoparticles, Nano spheres or Nano

capsules can be received. Nano capsules are systems wherein the drug is restricted to a hollow space surrounded by using a completely unique polymer membrane, even as Nano spheres are matrix structures wherein the drug is bodily and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, specifically those covered with hydrophilic polymer along with poly (ethylene glycol) (PEG) called long-circulating particles, had been used as potential drug transport devices because of their ability to flow into for a extended length time goal a particular organ, as companies of DNA in gene therapy, and their potential to supply proteins, peptides and genes.[8][9][10][11]

The essential goals in designing nanoparticles as a shipping device are to manipulate particle size, surface homes and release of pharmacologically energetic agents a good way to acquire the web site-unique action of the drug at the therapeutically top-rated price and dose regimen. Though liposomes had been used as ability companies with unique advantages which include defensive drugs from degradation, focused on to website online of movement and discount toxicity or side effects, their applications are restricted because of inherent issues such as low encapsulation performance, speedy leakage of water-soluble drug inside the presence of blood components and poor garage stability. On the alternative hand, polymeric nanoparticles provide a few unique blessings over liposomes. For example, they help to growth the stableness of medication/proteins and possess beneficial controlled release houses.[12][13]

Advantages

The benefits of using nanoparticles as a drug shipping system consist of the following:

1. Particle size and floor characteristics of nanoparticles can be easily manipulated to gain both passive and active drug concentrated on after parenteral management.
2. They manipulate and maintain launch of the drug at some stage in the transportation and on the website of localization, altering organ distribution of the drug and subsequent clearance of the drug to be able to gain growth in drug healing efficacy and discount in side effects.
3. Controlled release and particle degradation traits can be without difficulty modulated by using the selection of matrix ingredients. Drug loading is fairly excessive and drugs can be incorporated in to the systems with none chemical response; that is an vital issue for maintaining the drug pastime.
4. Site-particular focused on may be achieved by attaching targeting ligands to surface of debris or use of magnetic guidance.
5. The device can be used for numerous routes of administration such as oral, nasal, parenteral, intra-ocular and many others.

Limitations

Nanoparticles do have some limitations. For instance, their small length and huge surface vicinity can result in particle particle aggregation, making bodily dealing with of nanoparticle shard in liquid and dry bureaucracy. In addition, small particles length and massive surface vicinity easily bring about restrained drug loading and burst launch. These sensible problems ought to be conquer earlier than nanoparticles can be used clinically or made commercially to be had. The present evaluate information the contemporary improvement of nano particulate drug transport structures, floor change issues, drug loading strategies, launch manipulate and ability programs of nanoparticles.

Silver Nanoparticle

Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. These include optical, electrical, and thermal, high electrical conductivity, and biological properties.[14][15][16]

Due to their odd properties, they had been used for several applications, which include as antibacterial retailers, in business, household, and healthcare-associated merchandise, in purchaser products, medical tool coatings, optical sensors, and cosmetics, inside the pharmaceutical enterprise, the food enterprise, in diagnostics, orthopaedics, drug transport, as anticancer agents, and have in the end stronger the tumor-killing effects of anticancer drugs.[17]

Nano sized steel particles are precise and might notably exchange physical, chemical, and biological houses because of their floor-to-quantity ratio; therefore, these nanoparticles had been exploited for various purposes. [18][19]

Among several synthetic strategies for AgNPs, organic strategies seem to be easy, fast, non-toxic, reliable, and inexperienced techniques which can produce properly-described size and morphology below optimized conditions for translational research. In the cease, a in experienced chemistry method for the synthesis of AgNPs suggests much promise.

After synthesis, precise particle characterization is necessary, due to the fact the physicochemical properties of a particle could have a giant impact on their biological houses. In order to cope with the safety issue to use the total potential of any Nano material in the purpose of human welfare, in Nano medicines, or in the fitness care enterprise, etc., it is vital to signify the organized nanoparticles earlier than application.[20][21]

The biological pastime of AgNPs depends on elements along with surface chemistry, length, size distribution, form, particle morphology, particle composition, coating/capping, agglomeration, and dissolution rate, particle reactivity in answer, efficiency of ion release, and mobile type, and the sort of lowering retailers used for the synthesis of AgNPs are a critical component for the dedication of cytotoxicity. [22]

Mechanism

Although the exact mechanism of silver nanoparticles' antibacterial effects has no longer been entirely clarified, various antibacterial moves had been proposed in Figure 3. Silver nanoparticles can usually release silver ions, which may be taken into consideration the mechanism of killing microbes. [23]

Owing to electrostatic appeal and affinity to sulphur proteins, silver ions can adhere to the mobile wall and cytoplasmic membrane. The adhered ions can beautify the permeability of the cytoplasmic membrane and lead to disruption of the bacterial envelope. [24]

After the uptake of loose silver ions into cells, respiration enzymes may be deactivated, generating reactive oxygen species but interrupting adenosine triphosphate manufacturing.[25]

Reactive oxygen species can be a foremost agent in the provocation of cell membrane disruption and deoxyribonucleic acid (DNA) modification. As sulphur and phosphorus are critical components of DNA, the interaction of silver ions with the sulphur and phosphorus of DNA can purpose problems in DNA replication, cellular reproduction, or even result in termination of the microorganisms. Moreover, silver ions can inhibit the synthesis of proteins by denaturing ribosomes within the cytoplasm. [26]

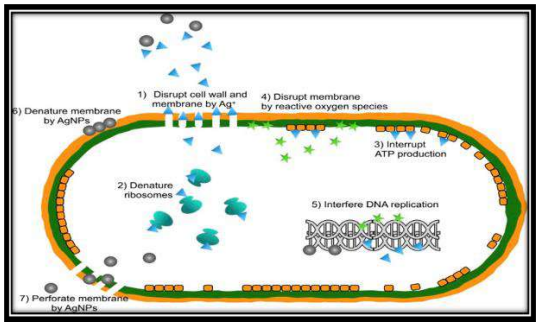


Fig No – 3 Mechanism of Action

The antibacterial moves of silver nanoparticles (AgNPs).

1) Disruption of cellular wall and cytoplasmic membrane: silver ions (Ag+) released with the aid of silver nanoparticles adhere to or bypass thru cellular wall and cytoplasmic membrane.
2) Denaturation of ribosomes: silver ions denature ribosomes and inhibit protein synthesis.
3) Interruption of adenosine triphosphate (ATP) manufacturing: ATP manufacturing is terminated due to the fact silver ions deactivate breathing enzyme on cytoplasmic membrane.
4) Membrane disruption by way of reactive oxygen species: reactive oxygen species produced by the damaged electron delivery chain can reason membrane disruption.
5) Interference of deoxyribonucleic acid (DNA) replication: silver and reactive oxygen species bind to deoxyribonucleic acid and prevent its replication and cellular multiplication.
6) Denaturation of membrane: silver nanoparticles collect within the pits of cell wall and reason membrane denaturation.
7) Perforation of membrane: silver nanoparticles without delay pass across cytoplasmic membrane, which could launch organelles from mobile.
In addition to being capable of launch silver ions, silver nanoparticles can themselves kill bacteria. Silver nanoparticles can gather inside the pits that shape on the cell wall once they anchor to the cellular surface.[27]

Characterization

UV- Visible Spectra Analysis was done by using Shimadzu UV- visible spectrophotometer. UV-visible absorption spectrophotometer with a resolution of 1nm between 200-800nm was used. 1ml of the sample (Silver nanoparticles containing aqueous and Hydro alcoholic extract of L.acidissima) was pipette out in Quartz cuvette and subsequently analysed at room temperature and their absorbance was measured.
The different concentration samples of silver nanoparticles was prepared and send to IIT Roorkee for analysing Zeta Potential, Surface Charge, PDI (Poly dispersibility Index).

Fig No – 4 UV- Visible Spectra Analysis

Material& Method

Limmonia acidissima a plant Material (bark) was collected in the month of May 2022, from area of railway colony Moradabad, Uttar Pradesh India. The stem bark was washed with double distilled water upto 3-4 times to remove the dust particle that adhered to the surface and allowed for Shed drying at room temperature. It was pulverized to coarse powder with the help of grinder the coarse powder was passed through sieve no.24 to maintain uniformity and stored in a cool and dry place.

Method of Extraction of Plant Material

A) Preparation of Hydro alcoholic Extract
Preparation of Hydro alcoholic extract of Limmonia Accidisima, Linn (Rutaceae) , powdered bark is done successively in a continuous Soxhlet apparatus with the following solvents (Methanol and water) 50:50The Extract was stored in Round Bottom Flask in at -5 to -20^{o}C.
B) Preparation of Aqueous Extract
Preparation of Aqueous extract of Limmonia Accidisima Linn (Rutaceae), was done in a Soxhlet apparatus by using the solvent double distilled water. The extract was Filtered and then stored in a Round Bottom flask in deep freezer at -4to-5^{o}C.

Procedure

1) Qualitative Phytochemical Analysis

S. No	Phytochemical Constituents	Test Name / Test Procedure	Observation	Result
1	Tannins (Aqueous extract)	Add 3ml of stock solution in test tube and then dilute it with 1ml of chloroform, add 1ml of acetic anhydride in it and add 1 ml of sulfuric acid in it	Green color observed	Negative (-)
	(Hydro alcoholic extract)	Add 3ml of stock solution in test tube and then dilute it with 1ml of chloroform, add 1ml of acetic anhydride in it and add 1 ml of sulfuric acid in it	Green color observed	Negative (-)
2	Fixed oils (Aqueous extract)	Fixed oil+equal volume of ethanol on cooling at 0^{O}C, clear solution is obtained after 3hrs	Clear solution observed	Negative (-)
	(Hydro alcoholic solution)	Fixed oil+equal volume of ethanol on cooling at 0^{O}C, clear solution is obtained after 3hrs	Clear solution observed	Mild Positive (+)
3	Volatile oils (Aqueous extract)	1ml of drug + add alcoholic solution of Sudan Red III	1ml of drug + add alcoholic solution of Sudan Red III	Moderately positive (++)
	(Hydro alcoholic solution)	1ml of drug + add alcoholic solution of Sudan Red III	1ml of drug + add alcoholic solution of Sudan Red III	Mild positive (+)
4	Quinones (Aqueous extract)	Take few ml of extract and treat it with concentrated HCl	Yellow ppt obtained	Highly positive (+++)
	(Hydro alcoholic extract)	Take few ml of extract and treat it with concentrated HCl	Yellow ppt obtained	Mild positive (+)
5 (a)	Alkaloids (Aqueous extract)	Dragendorff's Reagent (Potassium Bismuth Iodide)	Reddish Brown ppt	Highly positive (+++)
	(Hydro alcoholic extract)	Dragendorff's Reagent (Potassium Bismuth Iodide)	Reddish Brown ppt	Highly positive (+++)
(b)	(Aqueous extract)	Wager's Reagent (IodinePotassium bismuth iodide)	Reddish Brown ppt	Mild Positive (+)
	(Hydroalcoholic Extract)	Wager's Reagent (IodinePotassium bismuth iodide)	Reddish Brown ppt	Mild Positive (+)
(c)	(Aqueous extract)	Hager's Reagent (Saturated soln of Picric acid)	Yellow ppt	Highly positive (+++)
	(Hydroalcoholic Extract)	Hager's Reagent (Saturated sol of Picric acid)	Yellow ppt	Highly positive (+++)
6	Coumarins (Aqueous Extract)	3 ml of 10% NaOH + 2 ml of aqueous extract is added in it	Yellow color observed	Highly positive (+++)
	(Hydroalcoholic extract)	3 ml of 10% NaOH + 2 ml of aqueous extract is added in it	Yellow color observed	Moderately positive (++)

7	Flavonoid Glycoside (Aqueous Extract)	Take 1 ml stock solution +few drops of dilute NaOH Intense yellow color appeared It becomes colorless on adding few drops of dilute acids	Colorless	Moderately positive (++)
	(Hydroalcoholic Extract)	Take 1 ml stock solution +few drops of dilute NaOH Intense yellow color appeared It becomes colorless on adding few drops of dilute acids	Colorless	Highly positive (+++)
8	Saponins (Aqueous Extract)	Take 1 ml of stock solution +20 ml of distilled water, shake by hand for 15 min.	A Foam layer was obtained	Negative (-)
	(Hydroalcoholic Extract)	Take 1 ml of stock solution +20 ml of distilled water, shake by hand for 15 min.	A Foam layer was obtained	Negative (-)
9	Steroids (Aqueous Extract)	The extract was taken in a test tube take 10 ml of chloroform and add 10 ml of concentrated sulfuric acid)	The upper layer in the test tube was turned into red and sulfuric acid layer showed yellow color	Moderately positive (+++)
	(Hydroalcoholic Extract)	The extract was taken in a test tube take 10 ml of chloroform and add 10 ml of concentrated sulfuric acid)	The upper layer in the test tube was turned into red and sulfuric acid layer showed yellow color	Negative (-)
10	Borntrager's Test for Glycoside (Aqueous Extract)	Borntrager's test (3ml of extract +dil.H2SO4 was added,sol was boiled and filtered and add equal volume of benzene was added,solwas shaken well and the organic layer was separated, add equal volume of dilute ammonia sol to the organic layer	Ammonia layer turned into pink color	Moderately positive (++)
	(Hydroalcoholic Extract)	Borntrager's test (3ml of extract +dil.H2SO4 was added, sol was boiled and filtered and add equal volume of benzene was added,sol was shaken well and the organic layer was separated, add equal volume of dilute ammonia sol to the organic layer	Ammonia layer turned into pink color	Moderately positive (++)
11	Polyphenols Test (Aqueous Extract) (Hydroalcoholic Extract)	Add aqueous FeCl3 solto Aqueous extract Add aqueous FeCl3 sol to Aqueous extract	It gives blue, violet, purple, green, red or brown color It gives blue, violet, purple, green, red or brown color	Highly positive (+++) Highlypositive (+++)
12	Gums (Aqueous Extract) (Hydroalcoholic Extract)	Aqueous sol of gums+ Ruthenium red dye- No pink color observed- Aqueous sol of gums+ Ruthenium red dye No pink color observed	No pink color No pink color	Negative (-) Negative (-)

The crude powder of stem was subjected to qualitative phytochemical analysis. The phytochemical analyzed constituents were alkaloids, flavonoids, Glycosides, tannins, steroids, polyphenols saponins, quinines, lignans, fixed oils, volatile oil, gum, mucilage. Identification test for these phytochemical constituents are to be carried out from which the alkaloids, saponins, glycosides, quinines, coumarins, flavonoid glycosides, polyphenols are found to be highly positive.

Method Of Preperation Of Silver Nano Particles-

Silver nitrate used as such (purchased Merck, India) 1Mm solution of silver nitrate was prepared in conical flask, then 1,2,3,4and 5 ml; of plant extract (both aqueous and hydroalcoholic0 was added separately to 10 ml of silver nitrate solution keeping its concentration at 1Mm.

Silver Nanoparticles were also synthesized by varying the concentration of AgNO3 (1mM-5mM) keeping the extract concentration constant (1ml). This setup was incubated in a dark chamber to minimize the photo oxidation of silver nitrate at room temperature.

Procedure:

Silver nanoparticles used as such (purchased from Merck, India). 100ml, 1mM solution of silver nitrate was prepared in a conical flask then 1, 2,3,4,5 ml of plant extract(both aqueous and hydro alcoholic)was added separately to 10 ml of silver nitrate solution keeping its concentration at 1mM. Silver nanoparticles were also synthesized by varying concentration of AgNO3(1mM-5mM)keeping extract concentration constant (both aqueous and hydro alcoholic) 1ml. This setup was initiated in dark chamber to minimize photo oxidation of silver nitrate at room temperature.

Different concentrations of both aqueous and hydro alcoholic plant extracts containing silver nitrate solution were prepared by naming them as:

SNP_HA1, SNP_HA2, SNP-HA3, SNP-HA4, SNP-HA5 – For 1mM silver nitrate solution containing 1, 2,3,4,5 ml of hydro alcoholic extract SNP_AE1, SNP_AE2, SNP_HA3, SNP_HA4, SNP_HA5 – For 1mM silver nitrate solution containing 1, 2,3,4,5 ml aqueous extract

HA_SNP_Ag1 , HA_SNP_Ag2, HA_SNP_Ag3, HA_SNP_Ag4 , HA_SNP_Ag5 – for solution containing 1ml hydro alcoholic extract and different concentrations of silver nitrate solution i.e. 1,2,3,4,5mM

AE_SNP_Ag1,AE_SNP_Ag2, AE_SNP_Ag3,AE_SNP_Ag4,AE_SNP_Ag5- for solution containing 1 ml of aqueous plant extract and different concentration of silver nitrate solution i.e. 1,2,3,4,5mM.

Fig No – 5 Different concentrations of both aqueous and hydro alcoholic plant extracts containing silver nitrate solution

All solutions are transferred to eppendorf. All solutions are filtered by passing them through MERCK MEMBRANE filter of size 0.45micrometer using 20 ml syringe. The solutions are again filtered through MERCK MEMBRANE filter so as to obtain particle free solution now using 5 ml syringe. Different concentrations of solution were transferred to eppendorf and two samples of each solution were prepared. One is send to IIT ROORKEE for analyzing Zeta potential, surface charge and PDI (poly dispersibility index).

Other sample was transferred to eppendorf for UV analysis. Different concentration samples were analyzed in SHIMADZU UV- visible spectrophotometer and their absorbance was

noted. Usually absorbance of silver nanoparticles is shown between range 200-800nm. Different absorbance graphs were analyzed and over lay were taken out.

Table no -1 Plant Profile

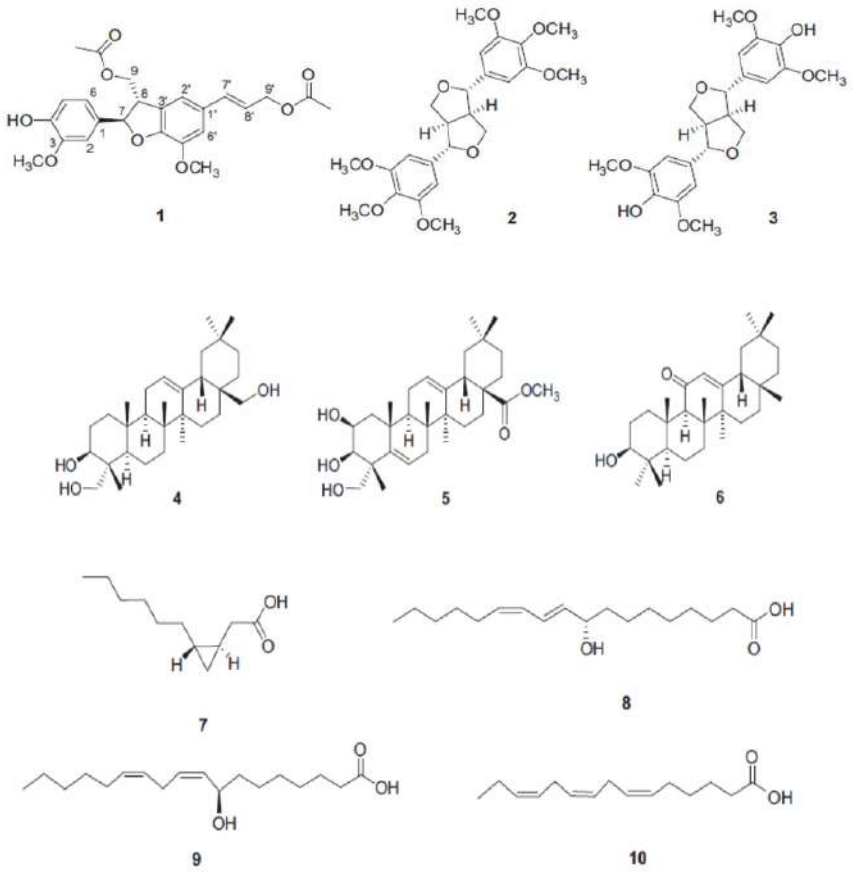
Rank	**Scientific Name and Common Name**
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Super-division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Sapindales
Family	Rutaceae – Rue family
Genus	Limonia L. – limonia
Species	Limoniaacidissima L. – Indian woodapple
Name verified on	09-Dec-1988 by ARS Systematic Botanists

Limonia acidissima linnis a large tree growing to 9m (30 ft) tall, with rough, spiny bark. The leaves are pinnate, with 5-7 leaflets, each leaflet 25–35 mm long and 10–20 mm broad, with a citrus-scent when crushed. The fruit is a berry 5– 9 cm diameter, and may be sweet or sour. The fruit looks similar in appearance to fruit of bael (Aeglemarmelos).

Wood apple is an erect, slow-growing tree with a few upward-reaching branches bending outward near the summit where they are subdivided into slender branch lets drooping at the tips. The bark is ridged, fissured and scaly and there are sharp spines 3/4 to 2 in long on some of the zigzag twigs. The deciduous, alternate leaves, 3 to 5 in long, dark-green, leathery, often minutely toothed, blunt or notched at the apex, are dotted with oil glands and slightly lemon-scented when crushed. Yellowish green flowers, tinged with red, 1/2 in across, are borne in small, loose, terminal or lateral panicles. The pulp is brown, mealy, odorous, resinous, astringent, acid or sweetish, with numerous small, white seeds scattered through it. It is native in the Indo Malaya eco zone to Bangladesh, India (Chota Nagpur, Madhya Pradesh and Maharashtra.), Pakistan, Sri Lanka, and in Indochinese ecoregion east to Java and the Malaysia eco region. Kawista grow naturally in areas of Sri Lanka, India, Myanmar and Indochina, and then spread to Malaysia and Indonesia.

Sr. No.	**Part of Plant**	**Medicinal Properties**
1	**Leaf**	Astringent and Carminative; good for vomiting, indigestions, hiccup and dysentery (Yusuf *et al.* 2009) diuretics
2	**Fruit**	Anticancer, refrigerant, stomachic, stimulant, astringent, aphrodisiac, diuretic, cardio tonic, tonic to the liver and lungs; cures cough, hiccup and dysentery; good for asthma, consumption, tumors, ophthalmic and leucorrhoea. Diarrhea, dysentery, stomatitis and sore throat
3	**Seed**	Seeds are used in heart diseases
4	**Root and stem bark**	Antifungal and insecticidal activities.

Phyto constituents



Structure no.	Name
1	neolignan
2	(+)-yangambin
3	(+)-syringaresinol
4	hederatriol
5	bassic acid methyl ester
6	3β-hydroxyolean-12-en-11-one
7	cascarillic acid
8	(+)-α-dimorphecolic acid
9	8(*R*) hydroxylinoleic acid
10	(6*Z*,9*Z*,12*Z*)-pentadecatrienoic acid

Compound	Stem oil	Seed oil	Entire Plant oil	Anise oil	Kaith leaf oil
â-pinene	3.1	6.3	4,8	--	--
â-pinene	0.1	trace	0.1	--	1.9
â-phellanderene	1.0	trace	2,7	--	--
limonene	18.9	4,0	10.0	--	0.5
1,8-cineole	3.1	0.5	6.3	--	---
Ã-terpinene	0.9	trace	1.5	--	---
terpinolene	0.5	trace	2.3	--	—
fenchone	0.1	0.1	0.3	--	0.6
linalool	0.1	trace	0.6	--	----
methyl chavicol	3.9	0.8	3.9	--	2.2
cis-anethole	0.4	0.1	0.5	--	---
trans-anethole	65.6	92.1	55.5	--	44.3
anisic aldehyde	trace	0.1	trace	--	6.9
anisic ketone	trace	trace	trace	--	—

Chemical constituents in different parts:

Fruit Pulp: citric acid and other fruit acids, mucilage and minerals, Alkaloids, coumarins, fatty acids and sterols have been detected in the pericarp

Leaves: Stigmasterol, psoralen, bergapten, orientin, vitedin, saponarin, tannins and an essential oil. It also contains umbelliferone, dictamnine, xanthotoxol, scoparone, xanthotoxin, *iso*pimpinellin, *iso*-imperatorin and marmin

Seeds fixed oil, carbohydrates, proteins and amino acids.

Roots:feronialactone, geranylumbelliferone, bargapten, osthol, *iso*pimpinellin, marmesin and marmin.

Stem bark:Coumarins, steroids, triterpenoids, benzoquinones, and tyraminederivatives

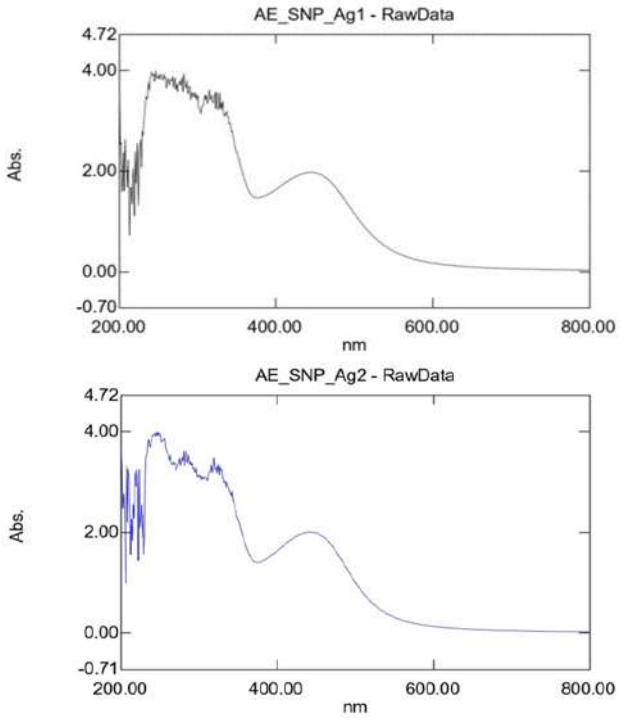
andMarmesin, feronolide and feronone have been isolated from different parts of this natural source. Ursolic acid and a new flavanone glycoside-7-O-methylporiol-4′-β-xylopyranosyl-D-glucopyranoside (I) have been isolated from heartwood.

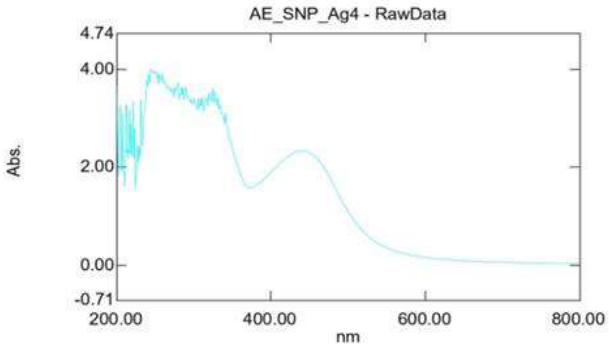
RESULT AND DISCUSSION

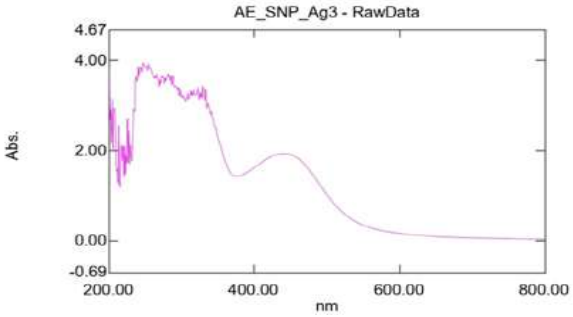
Visual observation and UV-Visible spectroscopy

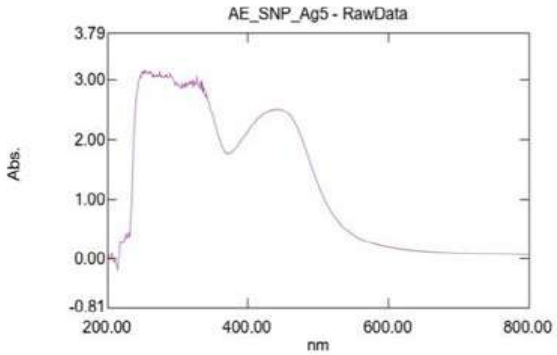
In all experiments, addition of plant extract of A. indica into the beakers containing aqueous solution of silver nitrate led to the change in the colour of the solution to yellowish to reddish brown within reaction duration due to excitation of surface plasmon vibrations in silver nanoparticles . On addition of different concentration (1e5 mL) of leaf extracts to aqueous silver nitrate solution keeping its concentration 10 mL (1 mM) constant, the colour of the solution changed from faint light to yellowish brown and finally to colloidal brown indicating formation of silver nanoparticles. Different parameters were optimized including concentration of silver nitrate and A. indica leaf extract, and time which had been identified as factors affecting the yields of silver nanoparticles. Silver nanoparticles were synthesized at different concentrations of leaf extract such as 1e5 mL using 1 mM of silver nitrate were analysed by UV spectra of Plasmon resonance band observed at 436 to 446 nm similar to those reported in literature . If we increase the leaf extract concentration to 4 mL, there is increase in wavelength. The slight variations in the values of absorbance signifies that the changes are the particle size On increasing concentration of extract there is increase in intensity of absorption. It is generally recognize that UV-Visible spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions.

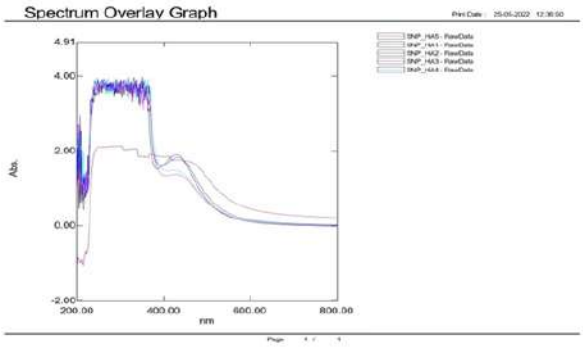
Parallel changes in colour have been observed when different concentrations (1 mMe5 mM) of silver nitrate was used by keeping plant extract (1 mL) constant. The appearance of the brown colour was due to the excitation of the Surface Plasmon Resonance (SPR), typical of silver nanoparticles having absorbance values which were reported earlier in the visible range of 446e448 nm . There is increase in intensity of absorption peaks after regular intervals of time and the colour intensity increased with the duration of incubation. It was also observed from Fig. 2b that the intensity of absorption peaks increases with increase in the concentration of the silver nitrate salt. All the results are very close already reported in literature showing absorbance at 445 nm of silver nanoparticles synthesized by Cochlospermumreligiosum extract. The UV-visible spectra recorded, implied that most rapid bioreduction was achieved using A. indica leaf extract as reducing agent. The UV-visible spectra and visual observation revealed that formation of silver nanoparticles occurred rapidly within 15 min.











CONCLUSION

A simple one-pot green synthesis of stable silver nanoparticles using A. indica leaf extract at room temperature was reported in this study. Synthesis was found to be efficient in terms of reaction time as well as stability of the synthesized nanoparticles which exclude external stabilizers/reducing agents. It proves to be an eco-friendly, rapid green approach for the synthesis providing a cost effective and an efficient way for the synthesis of silver nanoparticles. Therefore, this reaction pathway satisfies all the conditions of a 100% green chemical process. The synthesised silver nanoparticles showed efficient antimicrobial activities against both E. coli and S. aureus. Benefits of using plant extract for synthesis is that it is energy efficient, cost effective, protecting human health and environment leading to lesser waste and safer products. This eco-friendly method could be a competitive alternative to the conventional physical/chemical methods used for synthesis of silver nanoparticle and thus has a potential to use in biomedical applications and will play an important role in opto-electronics and medical devices in near future.

REFERENCE

1. Germplasm Resources Information Network (GRIN). Agricultural Research Service (ARS), United States Department of Agriculture (USDA). Retrieved 2016-04-03.
2. Four. Dr. N Sasidharan (Dr. B P Pal Fellow), Kerala Forest Research Institute, Peechi
3. "Limoniaacidissima L." Plants of the World Online. Royal Botanic Gardens, Kew. Retrieved 2018-03-25.
4. Smith, Albert C. (1985). Flora Vitiensis nova: a brandnew Flora of Fiji (spermatophytes handiest). Vol. Three. Lawaii, Hawaii: Pacific Tropical Botanical Garden. Pp. 526–527. Retrieved 2018-03-25 – via Biodiversity Heritage Library, digitized by means of Smithsonian Libraries.
5. Eight. Chakroborty DP. Chemical exam of Feroniaele phantom Corr. J Sci. Industr. Res., 1959; 18B: 90-91
6. Patra A, Misra SK, Chaudhury SK. Constituents of Limoniaacidissima software of -dimensional NMR spectroscopy in shape elucidation. J. Indian. Chem. So, 1988; sixty five: 205-208.

7. Rahman Mohd. Mukhlesur, Gray Alexander I. Antimicrobial components from the stem bark of Feronia limonia. Phytochem, 2002; 59: seventy three.
8. Langer R. Biomaterials in drug delivery and tissue engineering: one laboratory's experience. Acc Chem Res 2000; 33: 94-101
9. Bhadra D, Bhadra S, Jain P, Jain NK. Pegnology: a review of PEG-ylated systems. Pharmazie 2002; 57: 5-29.
10. Kommareddy S, Tiwari SB, Amiji MM. Long-circulating polymeric nanovectors for tumor-selective gene delivery. Technol Cancer Res Treat 2005;
11. 615- 25. 4. Lee M, Kim SW. Polyethylene glycol-conjugated copolymers for plasmid DNA delivery. Pharm Res 2005; 22: 1-10.
12. Vila A, Sanchez A, Tobio M, Calvo P, Alonso MJ. Design of biodegradable particles for protein delivery. J Control Release 2002; 78: 15-24.
13. Mu L, Feng SS. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol(R)): PLGA nanoparticles containing vitamin E TPGS. J Control Release 2003; 86: 33-48.
14. Gurunathan S., Park J.H., Han J.W., Kim J.H. Comparative assessment of the apoptotic potential of silver nanoparticles synthesized by Bacillus tequilensis and Calocybe indica in MDA-MB-231 human breast cancer cells: Targeting p53 for anticancer therapy. Int. J. anomed. 2015;10:4203–4222. doi: 10.2147/IJN.S83953. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
15. Li W.R., Xie X.B., Shi Q.S., Zeng H.Y., Ou-Yang Y.S., Chen Y.B. Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli. Appl. Microbiol. Biotechnol. 2010;8:1115–1122. doi: 10.1007/s00253-009-21595. [PubMed] [CrossRef] [Google Scholar]
16. Mukherjee P., Ahmad A., Mandal D., Senapati S., Sainkar S.R., Khan M.I.,Renu P., Ajaykumar P.V., Alam M., Kumar R., et al. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis. Nano Lett. 2001;1:515–519. doi: 10.1021/nl0155274. [CrossRef] [Google Scholar]
17. Chernousova S., Epple M. Silver as antibacterial agent: Ion, nanoparticle, and metal. Angew. Chem. Int. Ed. 2013;52:1636–1653. doi: 10.1002/anie.201205923. [PubMed] [CrossRef] [Google Scholar]
18. Li L., Hu J., Yang W., Alivisatos A.P. Band gap variation of size- and shape- controlled colloidal CdSe quantum rods. Nano Lett. 2001;1:349–351. doi: 10.1021/nl015559r. [CrossRef] [Google Scholar]
19. Sharma V.K., Yngard R.A., Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv. Colloid Interface. 2009;145:83–96. doi: 10.1016/j.cis.2008.09.002. [PubMed] [CrossRef] [Google Scholar]
20. Lin P.C., Lin S., Wang P.C., Sridhar R. Techniques for physicochemical characterization of nanomaterials. Biotechnol. Adv. 2014;32:711–726. doi: 10.1016/j.biotechadv.2013.11.006. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
21. Pleus R. Nanotechnologies-Guidance on Physicochemical Characterization of Engineered Nanoscale Materials for Toxicologic Assessment. ISO; Geneva, Switzerland: 2012. [Google Scholar]
22. Carlson C., Hussain S.M., Schrand A.M., Braydich-Stolle L.K., Hess K.L., Jones R.L., Schlager J.J. Unique cellular interaction of silver nanoparticles: Size-dependent generation of reactive oxygen species. J. Phys.

Chem. B. 2008;112:13608–13619. doi: 10.1021/jp712087m. [PubMed] [CrossRef] [Google Scholar]

23. Bapat RA, Chaubal TV, Joshi CP, et al. An overview of application of silver nanoparticles for biomaterials in dentistry. Mater Sci Eng C. 2018;91:881– 898. doi: 10.1016/j.msec.2018.05.069 [PubMed] [CrossRef] [Google Scholar]
24. Khorrami S, Zarrabi A, Khaleghi M, Danaei M, Mozafari M. Selective cytotoxicity of green synthesized silver nanoparticles against the MCF-7 tumor cell line and their enhanced antioxidant and antimicrobial properties. Int J Nanomedicine. 2018;13:8013–8024. doi: 10.2147/IJN.S189295 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
25. Ramkumar VS, Pugazhendhi A, Gopalakrishnan K, et al. Biofabrication and characterization of silver nanoparticles using aqueous extract of seaweed Enteromorpha compressa and its biomedical properties. Biotechnol Rep. 2017;14:1–7. doi: 10.1016/j.btre.2017.02.001 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
26. Durán N, Nakazato G, Seabra A. Antimicrobial activity of biogenic silver nanoparticles, and silver chloride nanoparticles: an overview and comments. ApplMicrobiolBiotechnol. 2016;100(15):6555–6570. doi: 10.1007/s00253-016-7657-7 [PubMed] [CrossRef] [Google Scholar]
27. Liao C, Li Y, Tjong SC. Bactericidal and cytotoxic properties of silver nanoparticles. Int J Mol Sci. 2019;20(2):449. doi: 10.3390/ijms20020449 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

HOW TO CITE: Jatin Agarwal*, Dr. Ashok Kumar Rajput, Akshanshu Patel Sudhanshu Sagar, Shubham Rana, Formation And Characterization Of Silver Nanostructures From Limonia Acidissima Bark Extract, Int. J. of Pharm. Sci., 2024, Vol 2, Issue 6, 1219-1232. https://doi.org/ 10.5281/zenodo.12570397