# Indian medicinal plant extracts and its green synthesized metal nanoparticles: a new genre for combating human pathogens

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Some common plants in India, which are also considered as weeds, proved to confer strong antimicrobial activity against many human pathogens. They are rich in a wide variety of secondary metabolites, viz. tannins, terpenoids, alkaloids, flavonoids, phenols and quinines. In this study, some urinary tract infecting human pathogenic strains and their non - pathogenic laboratory strains collected from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India, were subjected to some plant leaf extracts. Study revealed significant results. We chose clinical isolates of pathogenic *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonae*. We also selected non pathogenic *Escherechia coli* K 12 and *Pseudomonas syringae* (MTCC) strain as test organisms. We prepared ethanolic and 70 % methanolic plant extracts of *Lippia nodiflora L., Lippia alba (Mill)., Clerodendrum infortunatum (Linn) and Aegle marmelos (Linn) Corr* and followed Kirby – Bauer's disc diffusion method for the experiment. This study revealed both ethanolic and methanolic extracts of all the plants produced significant results against the test organisms. The ethanolic extracts of *L. nodiflora* being most effective against pathogenic clinical isolate of *E.coli* with a zone diameter of 12 mm.

An attempt was made to evaluate the antioxidant properties of these plant extracts, following standard methods and correlate the findings with their respective antimicrobial properties. DPPH scavenging activity assay showed ethanolic extracts of *A. marmelos* to produce highest percentage of scavenging activity with a percentage of 96.1. Total phenolic estimation revealed both *A.marmelos* and *L.alba* to contain significant amount of phenolics, whereas ethanolic extracts of *C. infortunatum* to have highest amount of flavonoid contents.

Silver and gold nanoparticles were prepared using these plant extracts as the sole reducing agents. The nanoparticles thus prepared were assayed for antimicrobial potency against the chosen test organisms and significant results were observed.

The above study can be designed further, to formulate drug or remodel drugs in such a way so that the activity of the drugs are optimized. The antimicrobial activity produced by these plant extracts can be compared with the pathogenic and non- pathogenic strains of same organism, and the resistance development of the organisms can also be controlled studying their genetic makeup. This study will definitely be of medical and social importance as it will provide a guideline in treating uropathogens in a more promising way.

**Keywords:** antimicrobial; pathogens; antioxidant activity; green-synthesized nanoparticles

## 1. Introduction

Antibiotic misuse is one of the main reasons of generating drug resistant pathogens. Patients who are non-compliant or who do not complete the course of antibiotic therapy also cause an increase in antibiotic-resistant bacteria. Urinary tract infection (UTI), is one of the most common health care associated and community acquired infections, throughout the world. A worldwide, estimation of patients suffering from UTI is around 150 million annually, which may rise to 75% in the female population by the age of 24, and 15–25% of this group will suffer from a relapse of this disease [1-5]. Among the infants, uncircumcised male infants less than 3 months of age and females less than 12 months of age had the highest baseline prevalence of UTI [6].

Multi drug resistant urinary tract infecting human pathogens are a challenge for the clinicians across the globe. Plant materials used as herbal or ayurvedic drugs and raw materials for the pharmaceutical industries can be a promising alternative for chemical antibiotics due to quality, safety and efficacy. The pharmacological investigations of plants are being carried out to find novel drugs or templates for the development of new therapeutic agents to combat drug resistant pathogens [*7*].

Plant extracts has been demonstrated to be high in antioxidant activity and is effective in the prevention of atherosclerosis, coronary heart disease, cancer and a number of other diseases [8, 9]. There are various reports about the presence of secondary metabolites like tannins, alkaloids, glycosides, flavonoids and phenolic compounds as antioxidant factors in different plant materials. Again, overproduction of free radicals in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA, and proteins [10]and thus leads to a range of chronic diseases, such as cardiovascular disease, neuronal disease, cataracts, and several forms of cancer [11]. Silver and gold nanoparticles synthesized from plant extracts have important applications in biology such as antibacterial agents and DNA sequencing. Antimicrobial property of silver nanoparticles against *Staphyloccocus aureus, Pseudomonas aeruginosa* and *Escherichia coli* has been investigated by Rai *et al*. [12]. It was

 

also shown that the antibacterial activity of silver nanoparticles was size dependent. Silver nanoparticles mainly in the range of 1 -10 nm attach to the surface of cell membrane of the microorganism and drastically disturb its proper function like respiration and permeability [13].

The fluorescent bacteria were used to investigate the antibacterial properties of silver nanoparticles by Gogoi *et al*. [14]. The green fluorescent proteins (GFPs) were used in these studies. The general understanding is that silver nanoparticles get attached to sulfur-containing proteins of bacteria cell causes the death of the bacteria. The fluorescent measurements of the cell-free supernatant reflected the effect of silver on recombination of bacteria.

Shahverdi *et al*. [15] studied the high synergistic activity of silver nanoparticles and antibiotics with erythromycin against *Staphyloccocus aureus*. Kong and Jang in 2008 [16] reported the antibacterial properties of the biosynthesized silver nanoparticles when incorporated on textile fabric were investigated.

Nanoparticles have numerous other benefits as well. Green synthesized silver nanoparticles were also used for impregnation of polymeric medical devices to increase their antibacterial activity. Silver impregnated medical devices like surgical masks and implantable devices showed significant antimicrobial efficiency [17].

There are reports that explored use of silver ions or metallic silver as well as silver nanoparticles in medicine for burn treatment, dental materials, coating stainless steel materials, textile fabrics, water treatment, sunscreen lotions, etc. [18].

Gold nanoparticles (AuNP) and silver nanoparticles (AgNP) synthesized from plants have extensive applications in fields like catalysis, sensors and drug delivery system. Moreover, silver nanoparticles have exceptional biocompatibiliy and low toxicity [19].

Silver ions can catalyze luminal-$H_2O_2$ systems. It was observed, when the Ag colloid was injected, chemiluminiscence emission from the luminal- $H_2O_2$ system was greatly enhanced [20, 21]. AuNP catalysts are highly active for the oxidation of many compounds, particularly CO and trimethylamine. Gas sensors based on Au nanoparticles have been developed for detecting a number of gases, like CO and NOx [22].

These nanoparticles also have the advantage of being very good optical biosensors and chemosensors. The biological binding signal between antigen and antibody using the triangular Ag-nanoparticles was measured in a research and reported by Zhu *et al*. [23].

Polymer-gold nanoparticles composites even possess interesting electrical properties [24]. The nanocomposites composed of Au and biopolymers are employed as a biosensor. Nanoparticles and quantum dots are conjugated with biospecific molecules such as antibodies, DNA, or enzymes for biological applications. Most of these applications are based on the specific optical properties of gold or silver [25]. The interaction of gold nanoparticles with light can be used for the visualization of particles [26]. In this way, the therapeutic application of metallic nanoparticles is also possible. The metallic structures can be used for hyperthermia therapy. The plasmonic photothermal therapy is a minimally-invasive oncological treatment strategy [27,28]. Generally, gold nanoparticles provide non-toxic routes to drug and gene delivery application. Gold nanoparticles are capable of delivering large biomolecules (peptides, proteins, or nucleic acids like DNA or RNA) [29]. Gold nanoparticles can be applied to amplify the biorecognition of the anticancer drug [30].

The present study was undertaken to investigate the antibacterial potentiality (*in vitro)* of dried leaves of *Lippia nodiflora L., Lippia alba (Mill)., Clerodendrum infortunatum (Linn) and Aegle marmelos (Linn) Corr,* - most of them are considered to be common Indian weeds against two drug resistant urinary tract infecting common human pathogens. The antioxidant content, total flavonoids, total phenols and DPPH scavenging activity was also assayed of these selected plant species. Synergistic study of plant extracts and some common resistant antibiotics was also explored against the drug resistant uropathogens.

## 2. Materials and methods

### 2.1 Plant material and extract preparation

Fresh leaves of the four plants were collected from northern parts of Kolkata, West Bengal, India. Fresh leaves were cleaned and washed in tap water and dried in shade for 3 weeks. Dried leaves were weighed and crushed in motorized grinder and stored in an airtight container and maintained at 4°C for further use.

Solvent system used for extraction were ethanol and 70% methanol. Dried plant dust of 200 mg was mixed individually with 5ml ethanol and 70 % methanol. The mixture is kept in an incubator shaker at 35 °C in continuous shaking for 24 hours. After 24 hours the mixture is filtered through Whattman filter paper and the filtrate is allowed to evaporate to concentrate the plant extracts. This procedure is repeated for 3 consecutive days, and the pooled filtrate is evaporated to get a final volume of 2 ml. Thus plant extract with a concentration of 100mg/ml is prepared. The extract is stored in an air tight glass container until use and maintained at 4 °C.

### 2.2 Test organisms

Three common drug resistant uropathogens of West Bengal, namely *Klebsiella pneumonae* and *Escherichia coli and Pseudomonas aeruginosa* were selected as the test organisms. The clinical isolates from urinary tract infected patients were collected from a tertiary hospital, Kolkata. Working cultures were prepared by inoculating one loopful culture of both the organisms in 10 ml nutrient broth and incubated for 24 h at 37°C in a shaker. The stock cultures were maintained in nutrient agar (NA) slant at 4°C and sub-cultured monthly. Two common non pathogenic strains, *Escherechia coli* K 12 and *Pseudomonas syringae* (MTCC strain) were also assayed.

### 2.3 Antibiogram of the test organisms

The antibiogram of the organisms were done by following Kirby Bauer's Disc diffusion assay. Fifteen standard antibiotics which are commonly prescribed by the clinicians are assayed against these organisms. The antibiotics chosen were Levofloxacin, Meropenem, Moxifloxacin, Gatifloxacin, Ticarcillin, Chloramphenicol, Gentamycin, Linazoid, Colistin, Ceftriaxone, Tobramycin, Amikacin, Amoxycillin Ciprofloxacinand Ceftazidime. All antibiotic discs are procured from HIMEDIA, India. Mueller Hinton agar (MHA) plates were swabbed with the test organisms and incubated at room temperature for the inoculum absorption. The discs of the chosen antibiotics were impregnated on pre seeded MHA plates and incubated at 37ºC for 24 hours.

### 2.4 Screening for antibacterial activity by Kirby Bauer's method of Disc diffusion

Five mm of sterile discs were put in 100 µl of plant extracts (10 mg/disk). The discs were completely saturated with the extract and allowed to dry. MHA plates were seeded with test clinical isolates and incubated at room temperature for 30 minutes for complete absorption of the inoculum. Plant extract discs, one standard positive control disc of streptomycin and another control disc of ethanol for ethanolic plant extract and 70% methanol for methanolic plant extracts respectively were placed on the pre seeded MHA plate. All the plates were incubated overnight at 37°C.

### 2.5 Assessment of synergistic effect of plant extracts with antibiotics

Each of ethanolic extracts of the plant leaves and five common broad spectrum antibiotics, Azithromycin, Ceftriaxone, Ceftazidime, Levofloxacin and Streptomycin were mixed individually, in equal proportion. Sterile discs dipped in these solutions were impregnated on pre inoculated MHA plates and assayed for their antibacterial activity. Control discs containing each antibiotic, only plant extract and ethanol were also impregnated to assay their activity.

### 2.6 Antioxidant activity

#### 2.6.1 Total phenolic content

Total Phenolic Content was determined by using Folin-Ciocalteu method with gallic acid as standard [31]. A volume of 0.1 ml of each sample extract was diluted to 3 ml with distilled water and to them 0.5 ml of Folin-Ciocalteu reagent was added. After 3min, 2 ml of 20% sodium carbonate was added and the contents were thoroughly mixed. The colour developed and its absorbance was measured at 650nm in nano drops spectrophotometer (Eppendorf) after 60min. Different concentrations of gallic acid solutions (100mg/ ml, 500mg/ml, 1000mg/ml, 1500mg/ml and 2000mg/ml) were used to plot the standard calibration curve. Results were expressed as gallic acid equivalent /100mg of dry weight of plant material.

#### 2.6.2 Total flavonoid estimation

Total Flavanoid Content was determined by aluminum chloride assay [32]. One ml aliquot of plant extract and a solutions of gallic acid in different concentration (100mg/ ml, 500mg/ml, 1000mg/ml, 1500mg/ml and 2000mg/ml) were added to 10ml conical flask containing 4ml of distilled water, separately. To this 0.3 ml 5% $NaNO_2$ was added and allowed to stand for 5minutes. Following, 0.3 ml of 10% $AlCl_3$ was added to those aliquots. After 6min, 2 ml of 1M NaOH was added and the total volume was made up to 10 ml with distilled water. Absorbance was read at 510nm in nano drops spectrophotometer (Eppendorf).

#### 2.6.3 DPPH scavenging activity

The scavenging activity for DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals was measured [32]. An aliquot of 3 ml of 0.004% DPPH solution in 95% ethanol and 0.1 ml of plant extract and ascorbic acid at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of each constituents and double distilled water in the place of plant extract or ascorbic acid. The percentage

 

inhibition of DPPH radicals by the plant extracts was determined by comparing the absorbance values of the control and the experimental tubes. The lesser the absorbance, higher is the ability of the test sample to scavenge DPPH.

### 2.7 Nanoparticle synthesis

Aqueous solution (1mM) of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. A volume of 3ml of extract was added to 40 ml of 1mM $AgNO_3$ solution. The effect of temperature on the synthesis of silver nanoparticles was carried out at 60 °C. Small aliquot of solution was used for the UV–VIS spectroscopy and absorbance was read at 424 nm [33].

For the synthesis of AuNPs, 10 ml plant extract was added to 90 ml of 1mM Gold (III) chloride solution with continuous stirring at room temperature. The absorbance was taken at 600 nm wavelengths. After 3 hours, the mixture was centrifuged at 10,000 rpm for 20 min at room temperature. The resulting pellet was washed with sterilized double distilled water twice and allowed to air dry. This dried powder thus formed is the gold nanoparticle [34].

#### 2.7.1 Effect of Nanoparticles on test organisms

Antimicrobial properties of the gold and silver nanoparticles were screened by disc diffusion method against the microorganisms as stated above. The 24 hrs broth cultures were swabbed against the MHA plates by sterile cotton swabs. Sterile filter paper discs of 5mm diameter were dipped into nanoparticle solution and placed on the plates with Silver nitrate and Chloroauric acid as control. Then plates were incubated for 24 h at $37^0$C and zones of diameter were measured.

## 3. Results and discussions

Common antibiotics belonging to broad-spectrum penicillin, third generation Cephalosporin, fourth generation Cephalosporin, Quinolones, Tetracycline, Macrolides, Aminoglycosides, and Sulfonamides were tested against the three clinically isolated uropathogens. Only the pathogenic strains are included in this study, to check their drug resistant pattern. The antibiotics selected for this experiment was Pipercillin/Tazobactum (PT), Doxycycline (Do), Amikacin (Ak), Ciprofloxacin (Cip), Ofloxacin (O), Levofloxacin (Le), Linozoid (Lz), Amoxyclav (Amc), Gentamycin (Gen), Azithromycin (Az), Ceftriaxone (Ctr), Ceftazidime (Caz), Tobramycin (To), Gatifloxacin (G), Meropenem (Mr), Colistin (Cl), Moxyfloxacin (Mo), Ticarcillin (Ti) and Chloramphenicol (C). The antibiotics and their mean inhibition zone were tabulated in table 1.

The antibiogram in table 1 illustrated the sensitivity, intermediate and resistance pattern of the test organisms. *K. pneumonae* showed complete resistance against maximum antibiotics. Only Chloramphenicol and Doxycycline produced clear zone of diameter 17mm and 11mm respectively.

*E.coli* showed variable resistance pattern against all the antibiotics. Linozoid showed highest sensitivity with a zone diameter of 26 mm. *P. aeruginosa* showed highest sensitivity against Meropenem.

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The antibacterial activity of the four leaf extracts both in ethanol and 70 % methanol were examined independently against two clinical isolates causing acute and recurring urinary tract infection in human. The antibacterial activity of both the solvent extracts exhibited varying magnitudes of inhibition patterns with standard positive control with antibiotic streptomycin (30µg/ml), depending on the susceptibility of the tested microorganisms. The mean inhibitory zone of four plant leaf extracts in two different solvents against the three uropathogens is summarized in Table 2, 3 and 4.

The analysis of ethanol and methanol extracts against the uropathogens showed a significant level of inhibition against all the species. Table 2 depicted, that the ethanolic extract of *L. alba* showed maximum zone of inhibition with a zone diameter of 17mm against *K. pneumonae* but the ethanolic extract of *C. infortunatum* failed to produce any inhibition zone. It was also found that, *K.pneumonae* was totally resistant against the methanolic extracts of all the four plant species.

From Table 3, it can be stated that, although all the plant extracts of both the solvents were active against *E.coli*, except the ethanolic extract of *L.nodiflora*, no other extract could produce significant result. The inhibition zone of *L.nodiflora* ethanol extract against *E.coli* is 12 mm.

Table 4 revealed that ethanolic extract of *A.marmelos* is most active against *P.aeruginosa*. Ethanolic extracts of all the four plant leaves showed moderate activity against both the non clinical strains, i.e. *E.coli* K 12 and *P. syringae*. Since the ethanolic extracts of *L. nodiflora* and *L. alba* showed highest activity against all the uropathogens, the ethanolic extracts of these plant leaves were mixed in equal proportions with some common antibiotic solutions.

The concentration of the antibiotic solutions were maintained as per the concentration of the commercial discs used in the antibiogram experiment. The antibiotics selected for this experiment were either totally resistant or intermediate against these pathogens, they are- Levofloxacin, Azithromycin, Ceftazidime, Ceftriaxone and Streptomycin. The mean inhibitory zone of the leaf extracts and antibiotics against three urinary tract infecting pathogens is presented in Table 5. All the non-pathogenic strains are excluded, as they showed sensitivity against all the antibiotics used.

From the above table 5, representing synergistic effect of leaf extracts and some antibiotics, it can be stated that all the solutions of plant extracts and antibiotics were active against both the pathogens. Although *K. pneumonae* was resistant to all selected antibiotics, except streptomycin, the synergistic effect of all the antibiotics with the plant extracts were very significant. The *L. nodiflora* extract with levofloxacin and ceftazidime produced satisfying results, with a zone diameter of 23mm and 19 mm respectively. *K. pneumonae* showed to be sensitive against the extract of *L. alba* and all the antibiotics. The combination of levofloxacin and *L.alba* against *K. pneumonae* proved to be highly significant, with a zone diameter of 29 mm.

The *E. coli* isolate showed sensitivity against Levofloxacin and Streptomycin but showed intermediate resistance pattern against Azithromycin, Ceftazidime and Ceftriaxonae. The synergistic effect of the antibiotics with *L. nodiflora* showed a high sensitivity against the combination, levofloxacin and *L. nodiflora* and Steptomycin and *L.nodiflora* with a zone diameter of 24mm.

The extract of *L. alba* with all the antibiotic solutions showed sensitivity against *E. coli*, with a highest zone diameter of 24mm in case of levofloxacin and *L. alba* combination.

The leaf extracts with the antibiotics failed to produce any synergistically significant result against *P. aeuginosa*. Only the *A. marmelos* extract with levofloxacin, showed to be synergistically active against this uropathogen.

The amount of total phenolic content (TPC) is expressed as mg gallic acid equivalent /100mg of dry plant leaves (Table 6). *L. alba* showed the maximum and *L. nodiflora* showed the minimum phenolic content among the selected extracts. Content varied widely among the samples and ranged from 0.30 mg gallic acid equivalent/100mg of dry leaves weight to 1.46mg gallic acid equivalent/100mg of dry leaves weight. *L. alba* showed high phenolic content while *C. infortunatum* and *A. marmelos* also exhibited comparable phenolic content contributing to their antimicrobial property.

*C. infortunatum* showed maximum and *L. nodiflora* showed the minimum flavonoid content among the extracts (Table 6). The total flavonoid content varied widely among the samples and ranged from 1.44 mg gallic acid/100mg to 19.68mg gallic acid/100mg of dry leaves weight.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical which readily undergoes reduction in presence of an antioxidant. It has a maximum absorption at 517 mm. The ease and convenience of this reaction contributes to its widespread use in the free radical-scavenging activity assessment [35].DPPH scavenging ability of all the plant leaves were screened in ethanol solvent system was shown (figure). The ethanolic extract of *A. marmelos* depicted highest scavenging activity with a percentage of 96.1%. The lowest activity was shown by *C. infortunatum* with a scavenging activity of 47.9%. *L.alba* and *L. nodiflora* also showed moderate DPPH activity with a scavenging activity of 69.9% and 57.4%.

### 3.1 Green synthesized nanoparticles

Ecofriendly plant extracts contain biomolecules, which act as both reducing and capping agents that can be utilized to synthesize stable and shape-controlled nanoparticles. Most of the plant parts like leaves, roots, latex, bark, stem, and seeds are being used for nanoparticle synthesis. The benefits of green synthesized nanoparticles are that, the plants are easily available and safe to handle and possess a large variety of active agents that can promote the reduction of metal ions.

The active agent contained in these plant parts makes the reduction and stabilization of the nanoparticles possible. Main compounds which affect the reduction and the capping of the nanoparticles are biomolecules such as phenolics, terpenoids, polysaccharides, flavones, alkaloids, proteins, enzymes, amino acids, and alcoholic compounds. However, quinol and chlorophyll pigments, linalool, methyl chavicol, eugenol, caffeine, theophylline, ascorbic acid, and other vitamins have also been reported [36-44]. The nontoxic phytochemicals including afore mentioned flavonoids and phenols have unique chemical power to reduce and also effectively wrap nanoparticles, thus preventing their agglomeration. Phenolic compounds possess hydroxyl and carboxyl groups, which are also able to bind to metals [45].

The nanoparticles thus synthesized were subjected to assay against the pathogens and non pathogenic stains to study their antimicrobial potency (Table 6 and 7). From this study, it can be stated that AgNP of *L. alba* and *A.marmelos* plant extracts are most effective against *K. pneumonae* (Table 7). From Table 8, it is seen that the AuNP showed highest sensitivity against *L.alba* plant extract against *E.coli*. All the non pathogenic strains showed significant sensitivity against all the nanopparticles.

 

### 3.2 Characterization of green synthesized nano particles

One of the first approaches of using plants as a source for the synthesis of metallic nanoparticles was with alfalfa sprouts [46], which was the first report on the formation of AgNPs using a living plant system. Alfalfa roots have the capability of absorbing Ag from agar medium and transferring them into the shoots of the plant in the same oxidation state. In the shoots, these Ag atoms arranged themselves to form nanoparticles by joining themselves and forming larger arrangements. In comparison to bacteria and fungi, green synthesis using plants appears to be faster and the first investigations demonstrate that synthesis procedures are able to produce quite rapidly AgNPs. Shankar *et al*. [47] showed that using Geranium leaf takes around nine hours reaching 90% reaction compared to the 24 to 124 hours necessary for other reactions reported earlier Therefore, the use of plant extracts in green synthesis has spurred numerous investigations and studies up till now. It was demonstrated that the production of metal nanoparticles using plant extracts could be completed in the metal salt solution within minutes at room temperature, depending on the nature of the plant extract. After the choice of the plant extract, the main affecting parameters are the concentration of the extract, the metal salt, the temperature, the pH, and the contact time [48].

In green synthesis of AgNPs using plant extracts, various constituents may contribute in reduction process of silver ions. Therefore, changing the chemical state (e.g., ionization) of these constituents can be affected on performance and rate of reduction process [49]. Hyderi and Rashidipour [49] investigated the effect of extract pH on the synthesis of AgNPs in the range of 2–11 by using UV-Vis spectrophotometer. The results show that the rate of AgNPs synthesis increases with increasing pH up to pH= 9 and then decrease. There as on for this behavior may be due to the ionization of phenolic compounds and tannins in the extract of oak [50-54]. Synthesis of silver nanoparticles was performed. Synthesis of silver nanoparticles was performed at different temperatures in the range of 4 to 60∘C. Their study showed that the efficiency of silver nanoparticles synthesis was highest at 45∘C.

Hyederi and Rashidipour,[49], in their study, of silver nanoparticles synthesized from extracts of oak, fruit hall (jaft) established, that in order to complete reduction of silver ions to silver nanoparticles, different concentrations of extract (Jaft extract) were mixed with a constant volume of silver nitrate (1 mM). Their study revealed that increasing the concentration of extract led to synthesizing more AgNPs and ultimately level off at concentration of 40 g/L. The XRD patterns of synthesized AgNPs are compatible with the standard pattern. The SEM and TEM images show that the synthesized AgNPs are in spherical structures. Presence of similar peaks with small shift in both spectra in FT-IR studies reveals the synthesized AgNPs contains natural compounds from extract. Stability of AgNPs can be attributed to existence of these compounds in the shell of the synthesized nanoparticles. The TEM images of the nanoparticles are also discussed in details by Rauwel *et al*. [55] in their review article on green synthesized nanoparticles. From their TEM studies it can be established that size and shape distribution of the nanoparticles are effected by many factors including the nature of the plant extract, the pH of the solution, and the temperature of the reaction. Nevertheless, obtaining uniform size and shape distribution of Ag NPs remains a subject of investigation.

## 4. Conclusion

Ethanolic extract of *L.alba* showed highest antibacterial activity against *K.pneumonae* and ethanolic extract of *L. nodiflora* exhibited highest inhibitory potency against *E. coli*. All the methanolic extract showed minimum inhibition against *E.coli*, but none of the methanolic extracts of any plant was able to produce any inhibition against *K. pneumonae*. The phytochemical analysis of this study reveals, that the ethanolic extracts of the examined plant leaves are promising sources of potential antioxidant and may be efficient as preventive agents in some diseases and can be considered as a natural herbal source in pharmaceutical industry. Furthermore, the development of natural antimicrobials will help to decrease the negative effects of synthetic drugs.

The anti-oxidant activity of these extracts could not be explained only on the basis of their phenolic content but also requires proper characterization of the phytochemical profile. This lack of relationship is in agreement with other literature [56]. It is known that only flavanoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity [57, 58].

This study supports the study of Sengul *et al*., (2009) [59], which reported no correlation between total phenolic content and antioxidant capacities of a number of medicinal plant extracts. In this present study, the antioxidant capacity observed was not exclusively from the total phenolic contents, but could possibly for the presence of some other phytochemicals such as ascorbic acid, tocopherol and pigments, tannins saponins etc. which also contribute to the total antioxidant capacity. Also, phenolic content determined according to the Folin-Ciocalteu method is not an absolute measurement of the amount of total phenolic materials. Different types of phenolic compounds have different antioxidant activities, which is dependent on their unique structure. The extracts possibly contain different type of phenolic compounds, which have different antioxidant capacities. So, fractionation and characterization of these active compounds will be the future work to investigate these compounds extensively.

## 5. Future research work

FTIR can be performed to the extract before and after addition to the silver nitrate solution to get a complete profile of the nanoparticles. XRD, TEM and SEM are included in the near future study. Further the nanoparticles can be used in drug development and drug designing to combat the drug resistant pathogenic bacteria.

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## Tables

**Table 1** Antibiogram of K. pneumonae, E. coli and P.aeruginosa

| Strains | Inhibition zone diameter in mm | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PT | Do | Ak | Cip | O | Le | Lz | Amc | Gen | Az | Ctr | Caz | To | G | Mr | Cl | Mo | Ti | C |
| *K.pneumonae* | 00 | 00 | 11 | 00 | 00 | 00 | 00 | 0 | 09 | 0 | 0 | 7 | 0 | 00 | 0 | 00 | 0 | 00 | 17 |
| *E.coli* | 25 | 20 | 15 | 20 | 18 | 18 | 26 | 8 | 15 | 19 | 14 | 0 | 10 | 13 | 21 | 00 | 19 | 00 | 25 |
| *P.aeruginosa* | 20 | 10 | 18 | 28 | 26 | 18 | 00 | 00 | 20 | 17 | 24 | 18 | 00 | 00 | 27 | 00 | 24 | 24 | 21 |

**Table 2** Antimicrobial activity of the plant extracts against *K. pneumonae*

| Leaves of medicinal plants used | Inhibition zone (in mm) | | | | |
|---|---|---|---|---|---|
| | Ethanolic extracts | Ethanol solvent (as control) | Methanolic Extracts | Methanol solvent (as control) | Positive control (antibiotic streptomycin) |
| *L. nodiflora* | 11 | 00 | 00 | 00 | 15 |
| *L. alba* | 17 | 00 | 10 | 00 | 15 |
| *A.marmelos* | 12 | 00 | 00 | 00 | 15 |
| *C.infortunatum* | 07 | 00 | 00 | 00 | 15 |

**Table 3** Antimicrobial activity of the plant extracts against *Escherechia coli*

| Name of plants used for extraction. | Inhibition zone (in mm) | | | | |
|---|---|---|---|---|---|
| | Ethanolic extracts | Ethanol solvent (as control) | Methanolic Extracts | Methanol solvent (as control) | Positive control (antibiotic streptomycin) |
| *L. nodiflora* | 06 | 00 | 00 | 00 | 00 |
| *L. alba* | 00 | 00 | 00 | 00 | 00 |
| *A.marmelos* | 09 | 00 | 06 | 00 | 00 |
| *C.infortunatum* | 07 | 00 | 06 | 00 | 00 |

**Table 4** Antimicrobial activity of the plant extracts against *P.aeruginosa*

| Leaves of medicinal plants used | Inhibition zone | | | | |
|---|---|---|---|---|---|
| | Ethanolic extracts | Ethanol solvent control | Methanolic Extracts | Methanol solvent extracts | Positive control (antibiotic streptomycin) |
| *L. nodiflora* | 12 | 00 | 00 | 00 | 21 |
| *L. alba* | 09 | Leaves of medicinal plants used | 07 | 00 | 21 |
| *A.marmelos* | 07 | 00 | 06 | 00 | 21 |
| *C.infortunatum* | 08 | 00 | 06 | 00 | 21 |

**Table 5** Synergistic effects of *L. nodiflora* and *L. alba* in ethanolic solvents with different Antibiotics

| Sl No. | Name of Ethanolic Plant extracts with Antibiotics | Zone(mm) against *K.pneumoniae* | Zone (mm) against *E.coli* |
|---|---|---|---|
| 1 | Levofloxacin (30µg/ml) | - | 20 |
| 2 | Azithromycin (15µg/ml) | - | 08 |
| 3 | Ceftazidime (30µg/ml) | - | 9 |
| 4 | Ceftriaxone (30µg/ml) | - | 6 |
| 5 | Streptomycin (30µg/ml) | 15 | 22 |
| 6 | Ethanolic extract of *L. nodiflora* | 11 | 12 |
| 7 | Ethanolic extract of *L.alba* | 16 | 08 |
| 8 | Levofloxacin-*L.nodiflora* | 23 | 24 |
| 9 | Azithromycin- *L. nodiflora* | 07 | - |
| 10 | Ceftazidime-*L.nodiflora* | 19 | 14 |
| 11 | Ceftriaxone-*L.nodiflora* | 07 | 09 |
| 12 | Streptomycin-*L. nodiflora* | 15 | 24 |
| 13 | Levofloxacin- *L. alba* | 29 | 24 |
| 14 | Azithromycin- *L. alba* | 12 | 11 |
| 15 | Ceftazidime- *L.alba* | 15 | 17 |
| 16 | Ceftriaxone- *L.alba* | 10 | 12 |
| 17 | Streptomycin- *L. alba* | 15 | 22 |

**Table 6** Total Phenol Content and Total Flavanoid Content

| Name plant extracts | Total phenol content (mg gallic acid equivalent/ 100mg dried plant leaves) | Total flavanoid content (mg gallic acid equivalent/ 100mg dried plant leaves) |
|---|---|---|
| *L.nodiflora* | 0.30 | 1.44 |
| *L.alba* | 1.46 | 10.80 |
| *C. infortunatum* | 1.43 | 19.68 |
| *A.marmelos* | 0.74 | 9.76 |

**Table 7** Effect of Ag nanoparticles on the uropathogens

| Pathogens tested | Inhibition zone ( mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | *L.nodiflora*_ AgNP | *L.alba*-AgNP | *A.marmelos*-AgNP | *C. infortunatum*-AgNP | Control (antibiotic streptomycin) | Control (ethnol solvent) | Control ($AgNO_3$) |
| *E.coli* | 00 | 09 | 08 | 0 | 21 | 00 | 00 |
| *K. pneumonae* | 07 | 06 | 00 | 0 | 15 | 00 | 00 |
| *P. aeruinosa* | 07 | 06 | 00 | 0 | 00 | 00 | 00 |

**Table 8** Effect of Au nanoparticles on the uropathogns

| Pathogens tested | Inhibition zone (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | *L.nodiflora*-AuNP | *L.alba*-AuNP | *A.marmelos*-AuNP | *C. infortunatum*-AuNP | Control (antibiotic streptomycin) | Control (ethnol solvent) | Control ($AgNO_3$) |
| *E.coli* | 09 | 10 | 0 | 0 | 20 | 0 | 00 |
| *K. pneumonae* | 09 | 11 | 11 | 0 | 14 | 0 | 00 |
| *P. aeruinosa* | 06 | 06 | 06 | 0 | 00 | 0 | 00 |

## Figure

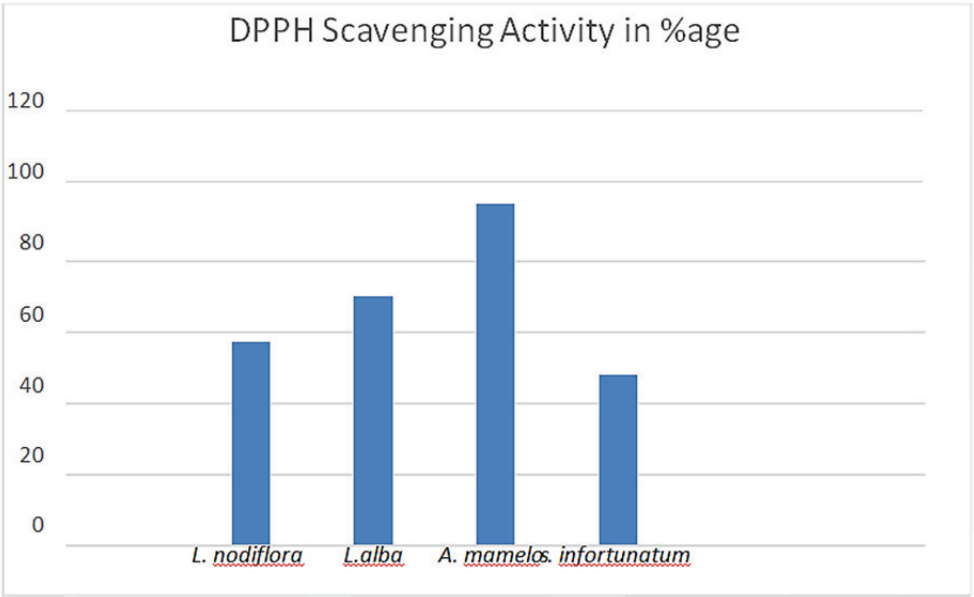


**Fig. 1** Bar diagram representation of DPPH scavenging activity of the plant extracts.