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Screening Of Anti-Microbial Activity Of Some Common Weeds By *In-Vitro* Green Synthesis Of Silver Nano Particles

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Abstract

From time immemorial mankind has been using plants for food, shelter, health care, cosmetics and recently they are exploiting to such an extent that no plant on this earth is left without any application to mankind. Plants are definitely a boon to all the living organisms on this planet. But there are certain plants which are of less commercial value, difficult to control its growth, less aesthetic and ornamental value. Such plants are being labelled as weeds which are also even problematic to the other commercially important crops. They also affect the other main crops, depleting their nutrient supply and reduce the serene beauty of that place. Hence when any commercial applications are discovered using these weeds it would solve the problem of controlling them and the label "Weed" can be removed. One promising application in health field is their anti-microbial properties hence this study was carried out to check their anti-microbial activities with respect to pathogenic bacteria. Though all the plants in this earth produce certain secondary metabolites for their defence during a stress condition, these metabolites or phytochemicals are having the capacity to combat the micro-organisms and prevent their growth. This strategy is used to exploit their natural anti-microbial properties for the welfare of mankind. This anti-microbial effect can be enhanced by the addition of nano-particles. It has been proven that nano-particles are extensively used in drug discovery, drug-delivery and many more pharmaceutical areas due to its nano size. And of all the nano particles silver plays a wide role in enhancing the anti-microbial activity. Hence in this research work using different plant extracts silver nitrate was reduced to nano-particles and then its activity was checked against certain pathogenic bacteria's. Once the preliminary study proves the presence of anti-microbial compounds later using molecular tools these useful compounds can be targeted at genetic level in such a way that their production can be enhanced and are more useful for the mankind.

Keywords: Anti-microbial activity, Green synthesis, Silver Nanoparticles, Weeds, Plant extracts.

Introduction

Nanoscience is a new branch of interdisciplinary science which can be described as the fundamental properties, synthesis and application of nano size objects [1]. The prefix 'Nano' means one billionth or 10^{-9} units. Therefore, nanoparticles are clusters of atoms in the size range of 1-100 nm [2]. Metal nanoparticles can be prepared by two methods- The Physical and the Chemical method. The physical method includes methods like evaporation or condensation and laser ablation. The chemical method includes metal ions in solution being reduced in conditions favouring the formation of small metal clusters or aggregates [3]. However physical and chemical methods are extremely expensive and involve the use of various harmful chemicals that harm our environment. Hence scientists have come up with a third method of production- that is Biological synthesis. This includes synthesis of nanoparticles using bacteria, fungi and plant extracts. The main advantage of using plant extract is that they are easily available, nontoxic and contain several metabolites like terpenoids, flavonoids, ketones, aldehydes, amides, and carboxylic acids that reduce silver ions. The exact mechanism for each plant varies as the involved phytochemicals also vary from plant to plant however the major mechanism includes reduction of ions [4]. Also, plant extract itself display antimicrobial activity owing to their phytochemical constituents like phenolic compounds. Recently nanotechnology has been applied for the development of new antimicrobials to manage the pathogenic bacteria that causes havoc for agricultural crops, humans and animals. Recently there have been several new developments in nanomaterial synthesis like polymeric carbon based and metallic nanoparticles that can be applied in managing

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bacterial plant diseases [5].A barrier that prevents the easy entry of external agents into plant cells is indeed the plant cell wall. The pore diameter of the cell wall varies from 5 to 20 nm. Therefore, only nanoparticles or nanoparticle aggregates can cross the cell wall to enter the plasma membrane with a diameter lower than the pore diameter. They can also enter through stomatal openings or leaf bases of trichomes when applied to leaves and are translocated from there to different tissues [6].Some experiments have shown that nanoparticles are not benign and can affect cellular, subcellular and protein levels. Nanoparticles migrate across the body and deposit in target organs, infiltrate cell membranes, lodge in mitochondria, and cause injurious reactions[7]. Silver nanoparticles (AgNPs) are the most widely used in today's research. Silver ions are known for their potent inhibitory and bactericidal effects. Silver is also known to have been successful against burns, chronic osteomyelitis urinary tract infections etc. Several proposals exist to explain the antimicrobial effects of silver ions. The most accepted proposal is that the heavy metals react with proteins by combining with the SH (thiol) groups which inactivates the proteins [8]. Due to these properties silver is widely used to make nanoparticles. Also, the antibacterial activity is closely related to the size of the particles; that is smaller the silver nuclei higher is the antibacterial activity. For effective utilisation of Silver Nanoparticles (AgNPs) it is necessary for them to be available at cheaper rates. As antibacterial agents AgNPs have a wide range of applications in disinfecting medical devices and water treatment [9]. Recently AgNPs were also used in the textile industry to produce silver nanocomposite fibres that incorporated silver nanoparticles inside the fabric and exhibited high antimicrobial activity against E. *coli* [10].Micromolar doses of silver ions are enough to kill bacteria but has no harmful effect on humans while silver at very high concentrations may be toxic to mammals, freshwater and marine organisms and disrupt the biological functions of cells [6]. Shape of nanoparticles also affects their activity. According to a study conducted truncated triangular nanoparticles show bacterial inhibition at concentration of 1µg while spherical nanoparticles require a concentration of 12.5µg. Thus, silver nanoparticles with different shapes have different effects on bacterial cells [11]. Hence control of size and size distribution is considered important which can be controlled depending on the chosen method of synthesis and the reducing agents [10]. The surface plasmon resonance plays an important in determination optical spectra of metal nanoparticles which shifts to a longer wavelength with the increase in particle size. The small size of the nanoparticle means that it has large surface area to meet bacterial cells and has a higher percentage of interaction when compared to larger particles [12]. New multi drug resistant strains of bacteria have become a serious problem in public health. These resistant bacteria and high price antimicrobial drugs have led scientists and researchers to find drugs which are broadly applicable and are economically viable. Since silver has broad antimicrobial activity against both gram positive and gram-negative bacteria, AgNPs with high surface/volume fraction are being extensively used. Therefore, silver nanoparticle impregnated medical devices like masks and implantable devices that exhibit antimicrobial activity has gained a lot of popularity [13].Nanoparticle infused creams dressings and gel extensively reduce bacterial infections in chronic wounds [14]. However according to research conducted AgNPs are less effective against gram positive bacteria when compared to gram negative ones due to thicker peptidoglycan in the former case [15]. In this project silver nanoparticles are synthesised using extracts of commonly available weeds around Bangalore and they were tested for antimicrobial properties against human pathogenic bacteria- *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia* obtained from Apollo Hospital Bannerghatta road, Bengaluru.

Bacterial samples:

Three pure pathogenic bacteria samples obtained from Apollo hospitals were used in this project, there characteristics are briefly described below:

a) Escherichia coli:

E.coli are gram negative and rod shaped bacteria. The gastrointestinal tract of warm-blooded animals is colonized by *E. coli* within a couple of hours or a few days after birth. But *E. coli* is more than a harmless intestinal inhabitant; it can also be a highly versatile, and frequently deadly, pathogen. It is responsible for several intestinal and extraintestinal diseases. ExPEC (Extraintestinal Pathogenic *Escherichia coli*) strains are the cause of a diverse spectrum of invasive human and animal infections, often leading to septicemia. Uropathogenic *E. coli* (UPEC) can cause urinary tract infections. EIEC (Enteroinvasive *E. coli*) causes a broad spectrum of human's diseases like invasive inflammatory colitis but may also elicit a watery diarrhoea syndrome [16].*E.coli* are usually sensitive to ciprofloxacin, Bactrim or Gentamycin. In most cases treating with antibiotics can triple the risk of developing hemolytic uremic syndrome leading to kidney failure. Hence use of AgNP may help prevent such situations.

b) Klebsiella pneumonia:

Klebsiella pneumoniae is an enteric bacillus with a prominent capsule and a gram-negative, lactose fermenter. This bacterium was linked with both Old World and New World primate with peritonitis, septicemia, pneumonia and meningitis. Prominent polysaccharide capsules typically grow which increase virulence through phagocytosis defence of bacteria and the prevent the destruction by serum bactericidal factor. Any of *Klebsiella pneumoniae* superbug strains are now particularly resistant to certain antibiotics such as Carbapenems, which are considered last resort medicines. However, there is a susceptibility to ciprofloxacin or ofloxacin in certain strains[17].

c) Staphylococcus aureus:

Staphylococci are gram-positive bacteria of 0.5 to 1.5 µm diameters distinguished by individual cocci, separated into clusters with grape-like in more than one plane. ng They are non-motile, non-spore forming facultative anaerobes. S. aureus is a significant pathogen that infects both hospitalized and stable immunological patients. It may cause local skin, nose, urethra, vagina and gastrointestinal tract infections, which are often mild and do not endanger lives. Enterotoxin ingestion from S. Aureus can cause food poisoning in contaminated food[18]. Staphylococci are strongly resistant to the most widely used antibiotics, such as erythromycin [19], ampicillin b and tetracycline. Vancomycin is inhibiting *S. aureus* production in some cases[20].

Plant samples:

There are approximately 250,000 species of plants throughout the world and it is estimated that about 8,000 of these species are considered as weed plants.A weed is considered as a plant in the wrong place, or the plants that grows and reproduces aggressively outside its native habitat [21]**.** Commonly these plants are unwanted in human controlled areas such as farm fields, gardens, lawns, and parks. A plant that is weed in one context is not a weed when growing in a situation where it is wanted. Sometimes plants become dominant when introduced into new environment as there is no animals in that environment that feed on them, hence these plants grow abundantly and become weed in that particular area. In some conditions these weeds produce allelopathic chemicals which is indigenous to plants and they are not yet adapted to it, these chemicals may limit the growth of plants,germination of plants and growth of seeds and seedlings [22]**.**A number of native and non-native plants are considered as unwanted for number of reasons [23]. They interfere with the food and fibre production in agriculture hence they must be controlled in order to prevent loss of crop yield and also, they interfere with other cosmetic, decorative or recreational goals such as in lawns, landscape architecture, playing fields and golf courses[24]. The commonly available wees used for this research are

Lantana sp:

It is a rough textured and usually prickly shrub weed with oppositely arranged leaves belonging to Verbenaceae family. It's a genus consisting of about 150 species. It comes from tropical Americas and African countries, but occurs in various areas as an invasive plant. They are generally referred to as lantana, popular lantana, Indian curse, Camara lantana. Thorny shrub species Lantanas include upright shrub, half or more or less suspended shrub species that exceed 2-3 metres in height [25,26]. The stems are angular, with bent spines. The leaves are simple, opposite, with rough lamina, oval, dented frequently with an acute apex. The inflorescence consists of a hemispheric head axillary or terminal, of several tiny tubular flowers, of purple, pink or orange. The fruits are fleshy drupes, which range in colours from blue to black around 3 mm in diameter. The ethanolic extracts of *L. camara* and *L. montvidensis* have the antibacterial activity against common and multi-resistant bacteria isolated from clinical material are presented against gram positive and gram negative strains.The in vitro antimicrobial activity of ether soluble fraction of upper ground parts of Lantana species and its Petroleum ether insoluble and soluble portions and sub fractions were deliberated against various Gram positive and Gram-negative bacteria and fungi while inactive against fungi tested.With the persistent manifestation of opposing pathogenic microorganisms, there is an emergent inquisitiveness in the discovery of new antimicrobial agents. The use of plant extracts can be great significance in therapeutic treatments with known antimicrobial properties[27]**.**The antimicrobial properties of plant have been examined by a numerous researcher worldwide [28]**.** The extracts from the leaves exhibit antimicrobial, fungicidal, insecticidal, nematicidal, biocidal activity. Plant extracts are used as medicine for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, and high blood pressure.

Leucas aspera:

Leucas aspera is a species in leucas genera and lamiaceae family. They are commonly known as 'Thumbai or Dronpushpi [29]**.**It contains about 200 species; these species are widespread over Africa and Southern and Eastern Asia. *Leucas aspera* in India, Philippines as well as the plains of Mauritius and Java is considered as a very common weed [30]. These are found in dry, open, sandy soil and abundantly found in waste areas. It is an annual herb or under shrub grows to a height of 15-60 mm[31]. It has a well-developed tap root system, the stem is quadrangular, much branched, and leaves are opposite, subsessile, liner-lanceolate, entire obtuse, narrowed at the base. Leaves can reach to a length of 8mm and 1.25mm broad and petiole length is 2.5-6mm long and verticillaster type of inflorescence; flowers are white, small and directly attached to the base of the stalk of the flower. Flowers are complete, bisexual, irregular, zygomorphic, hypogynous, and pentamerous. Fruit is 2.5mm long and it is a nutlet[30]**.** This plant flowers during cold season and fruiting occurs during hot season[32].The phytochemical properties of both leaves and root extracts showed the presence of bioactive compounds like alkaloids, flavonoids, phenols, tannins, saponins, sterols and pigments.[33]Chloroform and Ether extracts from *L. Aspera* showed the existence of antifungal action against *Trichophyton* and *Microsporum gypsum.* The minimum inhibitory concentration was calculated to be 5 mg / mL. *Leucas aspera* has both fungistatic and fungicidal activities[34]. The smoke of the *L. aspera* leaves is more harmful to the filarial vector mosquitoes, *Culex quinquefasciatus* greater than synthetic mosquito pads, as it contains 4 percent d-allethrin[35].The methanol extract from *L. Aspera* flowers display the presence of alkaloid residues and the flower juice has strong antibacterial activity[36]**.**The essential oils from *L.*

aspera showed bacteriostatic activity on different microbes *Staphylococcus aureus, Vibrio cholerae, Salmonella typhi, Klebsiella aerogenes, Escherichia coli, Proteus vulgaris, Pseudomonas pyocyanea* and *Dys. Flexneri*[37]**.**

Oxalis sp:

Oxalis is a largegenus of the flowering plants in the family Oxalidaceae comprising about 570 species[38]**.**The genus is present throughout the world, and the diversity of species is especially abundant in tropical Brazil, Mexico and South Africa. Many of the species are known as wood sorrels because they have an acidic flavour. Other plants are referred to as yellow sorrels or pink sorrels because of the hue of their flowers. Most plants are known colloquially as false shamrocks, and others are called sour grasses. It's a herbaceous weed. The species has many weedy traits, such as being readily self-pollinated, producing many seeds in a short period, and being able to grow quickly in open fields, which is why it is considered a cosmopolitan weed as it appears in most of the world's tropical and temperature zones. It is found in parks, lawns, arable land and pastures. It is a perennial herb with a thin main root. The stems are prostrate to sub erect, about 40 mm long, slender, weak, branched, frequently rooted at nodes, covered with flexible hairs. Leaves of three leaflets, alternate in colour, green or purple. Petiole is very short, 1-7 mm long, normally 2-3 mm long, wide, free, truncated apex, glabrous to densely covered with long hair. Lamina of leaflets equal, narrowly obcordate, glabrous or sparsely hairy, with densely hairy midrib below; narrow or wide laminate sinus, 1/2 laminate length; 1-5-flowered inflorescence; 1-4 mm long peduncle. Bracts 2-3, linear or linear-lanceolate, sepals 3-4, elliptical-ovate to elliptical-obovate, 5-10 petals, oblong-obovate, golden, glabrous. Stamens at 2 levels; filaments glabrous, connate to the nucleus. Styles shorter than, equivalent to or marginally longer than stamens, 10-18 mm long capsule, 1-3 mm seed, wide-ellipsoid[39]. Oxalis produces chemicals that are normally harmful to bacteria. Traditionally, the powdered stem is used for the treatment of rheumatic knee pain and malaria and is also used as an expectorant. The bark is used to treat snakebite and bronchitis. Leaf juice is used as a digestive agent, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic. Hepatoprotective, antileishmanial, antiviral and anti-fungal activity and analgesic, antipyretic and ulcerative activity have also been documented. The plant also has anti-allergic anti-helminthic activity and newly documented hepatoprotective activity[40]**.**

Parthenium sp:

Parthenium is a flowering plant genus of the family Asteraceae. It is endemic to the tropics of America. Popular names include white top marijuana, famine weed, Santa-Maria, Santa Maria feverfew [41]. It is locally referred to in India as carrot grass, congress grass or Gajar Ghans. In India, Australia and parts of Africa, it is a common invasive species [42]. *Parthenium* is a native of the Gulf of Mexico region, Central America, South North America, the West Indies and Central South America. Mostly these are cosmopolitan in distribution. *Parthenium* is an annual herb that is increasingly maturing, erect and very branched. It has a dense tap root system that is erect and very branched, reaching up to 2 m in height. When the plant matures into a hardy bush, the stem is hairy, octangular, longitudinally grooved and becomes robust and woody.Leavesare simple, alternating, pinnately or bipinnately dissected, growing smaller towards the apex of the branches. Four kinds of glandular and non-glandular, multicellular white trichomes are covered by the stem and leaf surface. The flowers are creamy white, growing from the leaf forks, about 4 mm long. A large amount of anemophilous pollen grains are made. With thin white scales, each flower produces four to five black wedge-shaped seeds that are 2 mm long. [43] The antiviral, antifungal, antibacterial, antihelmintic, antimolluscal, and anti-inflammatory effects of parthenium have been recorded in so many investigations. In *Escherichia coli, Bacillus subtilis, Enterococcus sp., Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Enterobactor aerogenes, Aspergillus niger, Candida albicans, Aspergillus flavus,* various researchers have reported antimicrobial efficacy. In parthenium the antimicrobial efficacy is due to the presence of five terpenoids, volatile oils and flavonoids as well as amino acid, sugars and phenolic derivatives. Parthenium showed good activity against pathogenic microorganisms where modern antibiotic therapy has failed. The crude extracts obtained from the parthenium may be used to treat the infections caused by *Bacillus subtilis, S. aureus, P. aeruginosa* and *Saccharomyces cerevisiae*[44]**.**

Tridax sp.

As a common weed and pest herb of the Compositae family (Asteraceae), it is best knownas the coat buttons or tridax daisy. It is endemic to the tropical Americas, but has been spread worldwide to tropical, subtropical and mild temperate areas. *Tridax* is an annual, often perennial, ascending herb prostrate with a flowering axis of up to 50 mm long. It is coated in hair that is rigid and upright. Simple, opposite, thick, egg shaped with deeply toothed edges, and covered with dense hair are the leaves, borne on stalks. Like flower-heads, a daisy is born on long stalks. They consist of a central disc of several yellow tubular florets, including cream-colored florets, surrounded by 4-7 petals. The fruit is an achene topped with white bristles on a tuff.The antibacterial activity of Methanolic and ethyl acetate extracts of *Tridax procumbens* were examined against *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Bacillus cereus* and *Staphylococcus aureus.* Antibacterial activity was investigated by disc and agar well diffusion method. The ethyl acetate extracts of the *Tridax procumbens* showed effective inhibition against *staphylococcus aureus* than compared to another organism. *Tridax* has been used for wound healing and as an anticoagulant, antifungal and

repellent for insects in India. Juice derived from the leaves is added directly to the wounds. Its leaf extracts have been used in common medicines for infectious skin diseases. It is used for liver diseases, hepato-protection, gastritis and heartburn in ayurvedic medicine. Various pharmacological effects have been reported for *Tridax* extracts, such as mosquito repellent activity, leishmanicidal, hepatoprotective effect on the antioxidant system of the liver, immunomodulatory effect, activity of wound healing and antiprotozoal effects. Plants have an infinite potential to synthesise aromatic compounds, most of which are derivatives of phenols or oxygen substituted by them. Some are metabolites that are secondary. These compounds act as pathways for plant defence against predation by bacteria, insects and herbivores. Some terpenoids are plant dye, some are plant flavouring agents and others are having medicinal properties [45].

Materials And Methods

Preparation of plant extracts:

About 100 grams of fresh, green, healthy leaves of weed samples were collected from in and around waste lands of Bangalore and identified by the Department of Botany, St. Joseph's Post Graduate and Research Centre. The leaves were washed with running tap water and double distilled water to remove the surface debris and other contaminated organic contents. The leaves were shade dried for about 7-10 days depending on the plant samples. And then they were finely powdered using a mixer and stored in a clean dry air tight bottle. Collection of plant sample and drying has been depicted in figure-1.

About 10 grams of finely powdered leaf samples were homogenized with 100ml of solvent such as Acetone, Methanol, Petroleum ether and water using pestle and mortar. This extract was taken in beaker and covered with aluminium foil and heated in water bath for 20 minutes for complete exudation of all phytochemicals[46].This plant extract was centrifuged at 5200 rpm for about 10-15 minutes, the supernatant was filtered using Whatman number-1 filter paper to further purify the extracts and remove the other minute debris. This clear extract was stored in clean, dry, sterile, labelled glass vials [47].

Green synthesis and characterization of silver nanoparticles (AgNPs):

0.1M of 100mL Silver nitrate ($AgNO_3$) solution was prepared freshly in an Erlenmeyer flask and stored in clean, dry, brown, glass bottles.10mL of plant extract wastaken in a beaker and 10mL of $AgNO_3$ solution was added. This setup was incubated in dark chamber for 24 hours at room temperature to reduce photo-activation of silver nitrate. After 24 hours color change was noticed.Formation of silver nanoparticles was confirmed by the color change in the solution after 24-hour incubation in the dark [48].Silver nanoparticles are extraordinarily efficient at absorbing and scattering light and have a color that depends on the size and shape of the particles. In solution the larger the particles, more brownish red the solution is. Particles larger than 400nm have a reddish solution. The U.V Visible Spectroscopy was also used to confirm the formation of AgNPs which is based on their optical properties. Absorbance was measured between 200nm to 800nm with resolution of 1nm by taking the sample in quartz cuvette. Typical silver nanoparticles have peak in their absorbance values in the visible range of 446-448 nm [49,50].

Culturing of bacterial samples:

Pure ATCC Bacterial strains of Escherichiacoli, Klebsiellapneumonia, Staphylococcusaureuswere obtained from Apollo Hospital, Bannerghatta Road, Bengaluru. The cultures were obtained on Blood Agar Media. Each of the bacterial strains was sub cultured on Brain Heart Infusion media (BHI). A permanent stock was prepared on BHI agar for future sub culturing purposes and a working stock was prepared in the form of BHI broth for immediate purposes. The subcultures were prepared in the microbiology lab of St. Joseph's College, Post Graduate and Research Centre.

Figure.1: Collection and drying of samples.

Preparation of BHI media for subculturing:

37g of media was dissolved in one litre of distilled water in a conical flask.The solution was boiled for one minute with frequent agitation to completely dissolve the media. (17g of agar agar was added to one litre of media to prepare BHI agar if required) pH was maintained around 7.3 ± 0.1 at 25ºC.The media was Autoclaved at 121°C for 15 minutes. Later it was cooled to room temperature, labelled and stored at 4ºC for further use.

Sub culturing of bacteria:

In order to subculture the required bacteria in a broth, a scoop of bacteria was collected from the original pure culture plate and inoculated into sterilized broth to prepare the working subculture.The bacteria were streaked using quadrant streaking method on the prepared BHI agar plates to prepare the permanent stock from the original stock.

Assessment of anti-microbial assay using well diffusion method:

38g of Mueller-Hinton Agar (MHA) media was dissolved in one litre of distilled water.With frequent agitation the media was boiled for one minute to completely dissolve and it was autoclaved at 121°C for 15 minutes and later cooled to room temperature (The final pH was maintained at 7.3 ± 0.1 at 25ºC).Cooled Mueller Hinton Agar media was poured into clean, sterile petri dishes on a level, horizontal surface to give uniform depth. This was performed inside the Laminar Airflow Chamber. The plates were allowed to cool at room temperature.After solidification of the media, 200 µL of bacterial sample were inoculated into the petri plates using sterile, autoclaved ear budsor L shaped Glass rod and spread uniformly. Four wells were punched on each plate. 500 µL of Acetone extract, Methanol extract, Petroleum ether Extract, Water extract of plant sample was loaded in each of the wells. In another plate consisting of four wells, 500 µL of Acetone extract, Methanol extract, Petroleum ether Extract and Water extract of plant samples containing Silver Nanoparticles (AgNP's) were loaded in each of the wells. Control (solvent used for extraction like Acetone, Methanol, Petroleum ether)and commercially available anti-biotic disc like Chloramphenicol and Norfloxacin was loaded in another plate with four wells. The plates were incubated at 37°C in the incubator for 24 hours. Results were observed, diameter of the Clearing zones were noted down, photographed and the data was tabulated.

Results

Antimicrobial activity of Lantana:

Table-1 shows the clearing zone activity of various extracts of Lantana while figure-2 depicts their graphical representation. In case of all 3 bacteria the plant extracts give no or minimal clearing zone indicating that the plant extracts have no antimicrobial activity.However, in case of *E.coli*, Acetone and Methanol extracts show minimal clearing zone of diameter 2.0 mm and distilled water and Petroleum ether extracts show no clearing zones. In case of *K.pneumoniae*, Acetone, Distilled water and Methanol extracts showed minimal antimicrobial activity with diameter of 2.0 mm, 2.0 mm, and 4.0 mm respectively. In case of *S.aureus* none of the plant extracts showed any antimicrobial activity. Hence it is indicated that Methanolic leaf extracts are slightly effective against *E.coli* and *S.aureus* with a high MIC value [51]. The Acetone extract usually contains components accounting like Linoleic acid (54.9%), palmitic acid (5.4%) and oleic acid (5.4%). Water is an excellent solvent for extraction of polar compounds; the dissolved components usually are responsible for the antimicrobial activity.The AgNPs obtained from the extracts on the other hand showed comparatively more antimicrobial activity than the plant extracts. In case of *E.coli*, Methanol and Petroleum ether AgNPs show the maximum antimicrobial activity with a clearing zone of diameter 20 mm each. The Acetone AgNPs showed a little less antimicrobial activity compared to the other two extracts with a clearing zone of 16 mm whereas the AgNPs from the distilled water extracts did not show any antimicrobial activity. The dissolved components in the plant extracts act as reducing agents that reduce silver nitrate into silver ions [52].

Table.1: Clearing zone of *Lantana* plant extract against the bacterial samples.

		Clearing zone in *E.coli* (mm)	Clearing zone in *K. pneumonia* (mm)	Clearing zone in *S. aureus* (mm)
Acetone Extract	**Plant extract**	**0.2**	**0.2**	**0.0**
	AgNPs Solu.	**1.6**	**1.0**	**1.0**
Distilled water extract	**Plant extract**	**0.0**	**0.2**	**0.0**
	AgNPs Solu.	**0.0**	**0.2**	**0.4**
Methanol extract	**Plant extract**	**0.2**	**0.4**	**0.0**
	AgNPs Solu.	**2.0**	**0.6**	**0.4**
Petroleum ether extract	**Plant extract**	**0.0**	**0.0**	**0.0**
	AgNPs Solu.	**2.0**	**0.4**	**0.6**

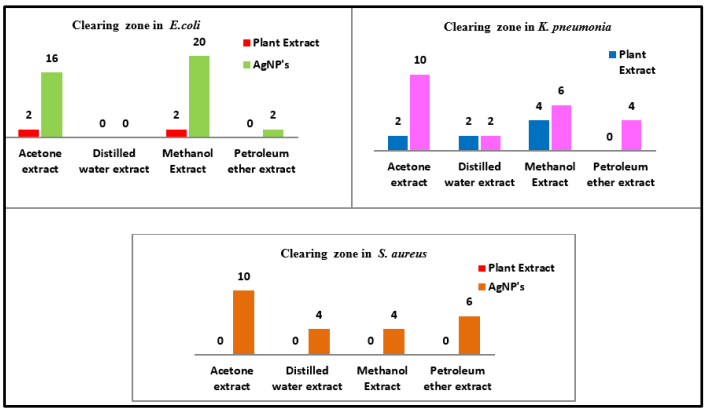

Figure.2: Graphical representation of Antimicrobial activity of *Lantana* sp.

Antimicrobial activity of *Leucas aspera*:

Table-2 shows the clearing zone activity of various extracts of *Leucas aspera* while figure-3 depicts their graphical representation. In case of *E.coli*, the Acetone plant extract gave the largest clearing zone with a diameter of 10 mm followed by Methanol extract which gave a clearing zone of diameter 2.0 mm. In case of *K.pneumonia*, Acetone and Methanol extracts show minimal clearing zones with a diameter of 2.0 mm each. Distilled water and Petroleum ether extracts do not show any clearing zones. In case of *S.aureus*, none of the plant extracts do not show any clearing zones indicating lack of antimicrobial activity. On the contrary,

according to Ai Lan Chew et al, the Methanolic leaf extracts of Leucas exhibited no antimicrobial activity against *E.coli* however the Methanolic leaf extracts showed minimal antimicrobial activity against *S.aureus*[53]**.** Against *E. coli*, the AgNP obtained from distilled water extract was the most effective, showing a clearing zone of diameter 24 mm followed by AgNP obtained from Methanol and Petroleum ether extract showing clearing zone of diameter 20 mm each. This is followed by the AgNP obtained from the Acetone extract, showing a clearing zone of diameter 16 mm. Against *K. pneumonia* AgNP obtained from Petroleum ether extract is the most effective showing a clearing zone of diameter 20 mm, followed by AgNP obtained from distilled water and Methanol with a clearing zone of diameter 18 mm and 12 mm. The AgNP obtained from Acetone are the least effective with a small clearing zone of 2.0 mm only. Against *S. aureus*, AgNP obtained from distilled water are the most effective giving a clearing zone of diameter 24 mm followed by AgNP obtained from Acetone, Methanol and Petroleum ether extracts with the diameter 20 mm, 12 mm, 8.0 mm respectively.

Table.2: Clearing zone of *Leucas aspera* plant extract against the bacterial samples

		Clearing zone in *E. coli* (In mm)	**Clearing zone in *K.pneumonia*** (In mm)	**Clearing zone in *S. aureus*** (In mm)
Acetone Extract	Plant extract	1.0	0.0	0.0
	AgNPs Solu.	1.6	0.6	2.0
Distilled water extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	2.4	1.8	2.4
Methanol extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	2.0	1.2	1.2
Petroleum ether extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	1.0	0.6	0.8

Antimicrobial activity of oxalis:

Table-3 shows the clearing zone activity of various extracts of *Oxalis sps.* while figure-4 depicts their graphical representation.None of the plant extracts show any antimicrobial activity against any of the 3 bacteria except the Acetone extract which is effective against *S.aureus* and shows a clearing zone of diameter 10 mm. However Methanolic leaf extract of Acetone is found to be highly effective against human pathogens like *S.aureus* and *E.coli*[54]**.** Against *E.coli* the AgNP obtained from distilled water is the most effective showing a clearing zone of 40 mm, followed by Petroleum ether with a clearing zone of diameter 26 mm. AgNP obtained from Acetone and Methanol extract show a clearing zone of diameter 20 mm each. Against *K. pneumonia* AgNP obtained from Methanol and petroleum extracts are the most effective with a clearing zone of diameter 20 mm each followed by AgNP obtained from distilled water and Acetone with a clearing zone of diameter 10 mm and 6 mm respectively. However against *S.aureus*, AgNP obtained from Acetone extract is extremely effective showing a clearing zone of diameter 50 mm followed by the AgNP obtained from petroleum and Methanol extracts showing clearing zones of Diameter 10 mm and 5.0 mm respectively.

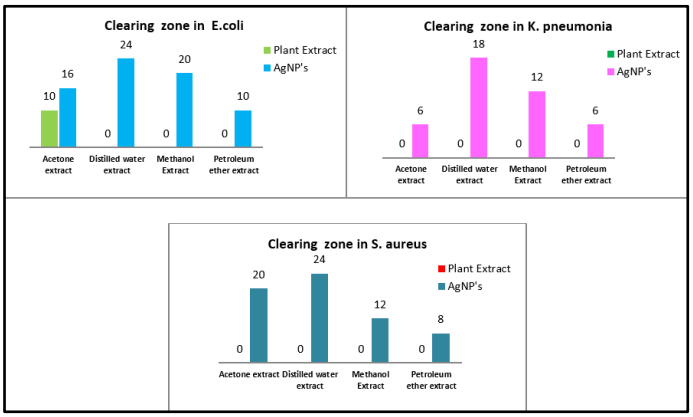

Figure.3: Graphical representation of antimicrobial activity of *Leucas aspera*

Table.3: Clearing zone of *Oxalis* plant extract against the bacterial samples

		Clearing zone in *E. coli* (In mm)	**Clearing zone in *K.pneumonia*** (In mm)	**Clearing zone in *S. aureus*** (In mm)
Acetone Extract	Plant extract	0.0	0.0	1.6
	AgNPs Solu.	2.0	0.6	5.0
Distilled water extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	4.0	1.0	0.0
Methanol extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	2.0	2.0	0.5
Petroleum ether extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	2.6	2.0	1.0

Antimicrobial activity of Parthenium:

Table-4 shows the clearing zone activity of various extracts of *Parthenium sps.* while figure-5 depicts their graphical representation.Against *E.coli* the Acetone extract is the most effective with a clearing zone of diameter 20 mm.

No other extract exhibited any antimicrobial activity against *E. coli*. Against *K. pneumonia* the Acetone extract is the most effective with clearing zone of diameter of 6.0 mm. No other extract exhibited any antimicrobial activity against *K. pneumonia*. None of the plant extracts show any antimicrobial activity against *S.aureus*.Only AgNP obtained from Acetone extract showed a clearing zone of diameter 34 mm against *E. coli*. Similarly only AgNP obtained from Acetone extract showed clearing zone of diameter 16 mm and 24 mm against *K. pneumonia* and *S.aureus* respectively.

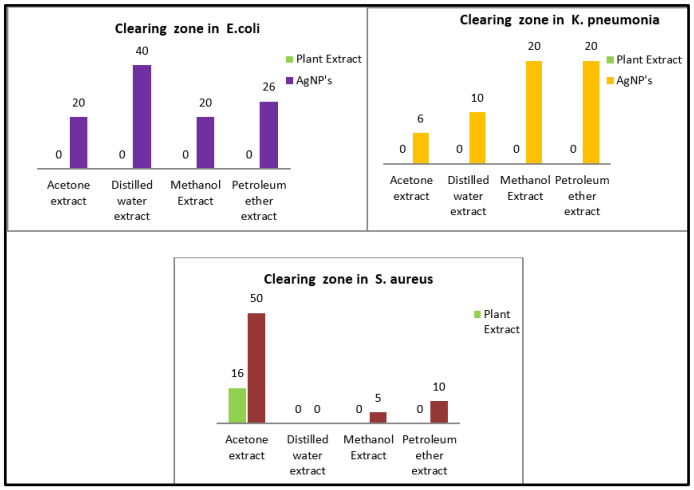

Figure.4: Graphical representation of Antimicrobial activity of *Oxalis* sps.

Table.4: Clearing zone of *Parthenium* plant extract against the bacterial samples

		Clearing zone in *E. coli* (In mm)	**Clearing zone in *K. pneumonia*** (In mm)	**Clearing zone in *S. aureus*** (In mm)
Acetone Extract	Plant extract	2.0	0.6	0.0
	AgNPs Solu.	3.4	1.6	2.4
Distilled water extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	0.0	0.0	0.0
Methanol extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	0.0	0.0	0.0
Petroleum ether extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	0.0	0.0	0.0

Antimicrobial activity of Tridax:

Table-5 shows the clearing zone activity of various extracts of *Tridax sps.* while figure-6 depicts their graphical representation.None of the plant extracts show any antimicrobial activity against any of the 3 bacteria. However contrasting results were obtained by V.Bharathi et al using Methanolic extracts of Tridax against *E.coli* showing a clearing zone of diameter 11 mm and of 15 mm against *S.aureus* . However, no clearing zone was seen against *K. pneumonia*[55]. Against E. coli AgNP obtained from Petroleum ether is the most effective showing a clearing zone of diameter 20 mm followed by AgNP obtained from Acetone and Methanol extracts showing clearing zone of diameter 6.0 mm and 4.0 mm respectively.AgNPs obtained from distilled water had no antimicrobial activity. Against K. pneumonia AgNP obtained from Acetone extract is the most effective with the clearing zone of diameter of 20 mm followed by AgNP obtained from Methanol and Petroleum ether extracts showing clearing zones of diameter 6.0 and 2.0 mm respectively. The distilled water extract AgNPs have no antimicrobial activity.

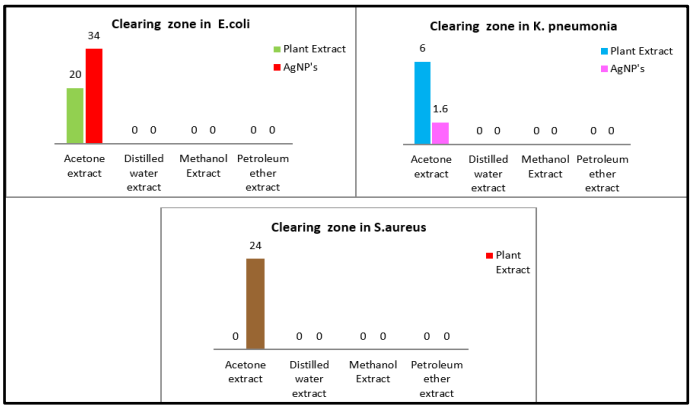

Figure.5: Graphical representation of Antimicrobial activity of *Parthenium* sp.

Table.5: Clearing zone of *Tridax* plant extract against the bacterial samples

		Clearing zone in ***E. coli*** (In mm)	Clearing zone in ***K. pneumonia*** (In mm)	Clearing zone in ***S. aureus*** (In mm)
Acetone Extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	0.6	2.0	0.4
Distilled water extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	0.0	0.0	0.0
Methanol extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	0.4	0.6	0.4
Petroleum ether extract	Plant extract	0.0	0.0	0.0
	AgNPs Solu.	0.6	0.2	0.2

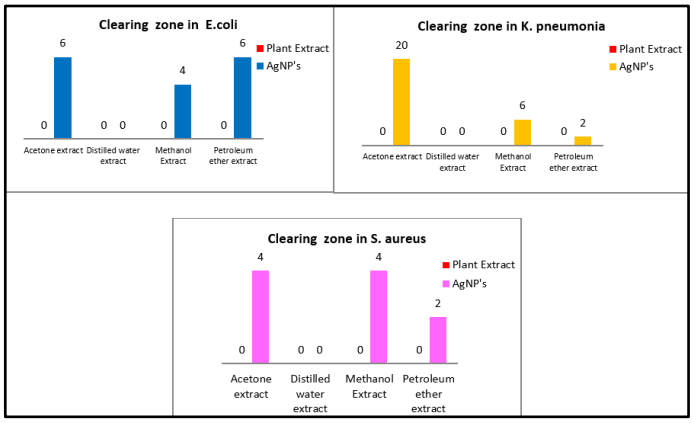

Figure.6: Graphical representation of Antimicrobial activity of *Tridax* sp.

Conclusion

In all cases of plant samples taken, the plant extracts show minimal or no antimicrobial activity. However, the AgNPs obtained from the extracts are far more effective when compared to the mere plant extracts. From this project it can be concluded that the AgNPs obtained from the leaf extracts of the weeds readily available in and round Bangalore cannot be exploited industrially due to availability of far better competitors in the market. However, it gives us an idea of the components of the leaf extracts that may have helped in the formation of the AgNPs. Leaves contain polyphenols (Flavonoids). Phenolic compounds have hydroxyl and ketonic groups which bind to metals to reduce the metal salt and provide stability against agglomeration. Plant extract gives protein and enzymes to the $AgNO_3$ solution in which Ag^+ ions combine with the enzyme to form enzyme substrate complex. The enzyme released from the plant extract act on the silver ions and there is a release of silver nanoparticle from the enzyme. That silver nanoparticle combines with the protein released from the plant extract and there is a formation of protein capped silver nanoparticles. This experiment might indicate that selected plant samples have lower number of polyphenols.

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Conflict of Interest

No known conflict of interest.

References

1. Sergeev GB, Shabatina TI. Cryochemistry of nanometals. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2008; 313:18-22.
2. Williams DF. The Williams dictionary of biomaterials. Liverpool University Press; 1999.
3. Gubin SP, Gulayev YV, Khomutov GB, Kislov VV, Kolesov VV, Soldatov ES, Sulaimankulov KS, Trifonov AS. Molecular clusters as building blocks for nanoelectronics: the first demonstration of a cluster single-electron tunnelling transistor at room temperature. Nanotechnology. 2002;13(2):185.
4. Prabhu S, Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International nano letters. 2012 ;2(1):32.
5. Stark WJ, Stoessel PR, Wohlleben W, Hafner AJ. Industrial applications of nanoparticles. Chemical Society Reviews. 2015;44(16):5793-805.
6. Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS. Nanoparticulate material delivery to plants. Plant science. 2010;179(3):154-63.
7. Nel A, Xia T, Mädler L, Li N. Toxic potential of materials at the nanolevel. science. 2006;311(5761):622-7.
8. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. Journal of biomedical materials research. 2000;52(4):662-8.
9. Bosetti M, Massè A, Tobin E, Cannas M. Silver coated materials for external fixation devices: in vitro biocompatibility and genotoxicity. Biomaterials. 2002 Feb 1;23(3):887-92.
10. Zhang F, Wu X, Chen Y, Lin H. Application of silver nanoparticles to cotton fabric as an antibacterial textile finish. Fibers and Polymers. 2009;10(4):496-501.
11. Abou El-Nour KM, Eftaiha AA, Al-Warthan A, Ammar RA. Synthesis and applications of silver nanoparticles. Arabian journal of chemistry. 2010;3(3):135-40.
12 Raimondi F, Scherer GG, Kötz R, Wokaun A. Nanoparticles in energy technology: examples from electrochemistry and catalysis. Angewandte Chemie International Edition. 2005;44(15):2190-209.
13. Furno F, Morley KS, Wong B, Sharp BL, Arnold PL, Howdle SM, Bayston R, Brown PD, Winship PD, Reid HJ. Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection?. Journal of Antimicrobial Chemotherapy. 2004;54(6):1019-24.
14. Richard JW. Acticoat versus Silverlon: the truth. J Burns. 2002; 1:11-9.
15. Ingle A, Gade A, Pierrat S, Sonnichsen C, Rai M. Mycosynthesis of silver nanoparticles using the fungus Fusarium acuminatum and its activity against some human pathogenic bacteria. Current Nanoscience. 2008 May 1;4(2):141-4.
16. De Sousa CP. Escherichia coli as a specialized bacterial pathogen. Rev biol cienc Terra. 2006;2(2):341-52.

17. Burke RL, Whitehouse CA, Taylor JK, Selby EB. Epidemiology of invasive Klebsiella pneumoniae with hypermucoviscosity phenotype in a research colony of nonhuman primates. Comparative medicine. 2009 Dec 15;59(6):589-97.
18. Harris LG, Foster SJ, Richards RG. An introduction to Staphylococcus aureus, and techniques for identifying and quantifying S. aureus adhesins in relation to adhesion to biomaterials: review. Eur Cell Mater. 2002;4(3):100-20.
19.Wallmark G, Finland M. Phage types and antibiotic susceptibility of pathogenic staphylococci: Results at Boston City Hospital 1959-1960 and comparison with strains of previous years. jama. 1961 ;175(10):886-97.
20.Coonrod JD, Eickhoff TC. Combined action of 6-mercaptopurine and antibiotics on gram-negative bacteria in vitro. Proceedings of the Society for Experimental Biology and Medicine. 1972 ;140(2):524-7.
21. Janick J. Horticultural science. Macmillan; 1986 Feb 15.
22. Willis RJ. The history of allelopathy. Springer Science & Business Media; 2007.
23. Ashraf M, Öztürk M, Ahmad MS, Aksoy A, editors. Crop production for agricultural improvement. Springer Science & Business Media; 2012 Jun 2.
24. Annecke DP, Moran VC. Insects and mites of cultivated plants in South Africa. Butterworth; 1982.
25. Sastri RL. Studies in Lauraceae. III. Embryology of Cassytha. Botanical Gazette. 1962;123(3):197-206.
26. Prajapati SK, Tripathi BD. Biomonitoring trace-element levels in PM10 released from vehicles using leaves of Saraca indica and Lantana camara. AMBIO: A Journal of the Human Environment. 2007;36(8):704-5.
27. Nascimento GG, Maestro V, Campos MS. Ocorrência de resíduos de antibióticos no leite comercializado em Piracicaba, SP. Revista de Nutrição. 2001;14(2):119-24.
28. Mostafa AA, Al-Rahmah AN, Abdel-Megeed A. Evaluation of some plant extracts for their antifungal and antiaflatoxigenic activities. Journal of Medicinal Plants Research. 2011 ;5(17):4231-8.
29. Rai V, Agarwal M, Agnihotri AK, Khatoon S, Rawat AK, Mehrotra S. Pharmacognostical Evaluation of Leucas aspera Link. Natural product sciences. 2005;11(2):109-14.
30. Srinivasan R, Ravali B, Suvarchala P, Honey A, Tejaswini A, Neeraja P. Leucas aspera-medicinal plant: a review. International Journal of Pharma and Bio Sciences. 2011;2(1):153-9.
31. Deb. D.B., The Flora of Tripura State, Scholarly Publications,1983
32. Devi OS, Komor P, Das D. A checklist of traditional edible bio-resources from Ima markets of Imphal Valley, Manipur, India. Journal of Threatened Taxa. 2010 :1291-6.
33. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC complementary and alternative medicine. 2006 ;6(1):35.
34. Thakur DK, Misra SK, Choudhuri PC. In vitro trials of plant extracts and chemicals for their antifungal activity. Indian J animal health. 1987; 26:31-5.
35. Selvaraj Pandian R, Revathy C, Manoharan AC. Toxicity evaluation of herbal smoke and synthetic mosquito mat on Culex quinquefasciatus Say. GEOBIOS-JODHPUR-. 1994; 21:166-.
36. Mangathayaru K, Lakshmikant J, Sundar NS, Swapna RG, Grace XF, Vasantha J. Antimicrobial activity of Leucas aspera flowers. Fitoterapia. 2005 ;76(7-8):752-4.
37. Botelho MA, Nogueira NA, Bastos GM, Fonseca SG, Lemos TL, Matos FJ, Montenegro D, Heukelbach J, Rao VS, Brito GA. Antimicrobial activity of the essential oil from Lippia sidoides, carvacrol and thymol against oral pathogens. Brazilian Journal of Medical and Biological Research. 2007 Mar;40(3):349-56.
38. Christenhusz MJ, Byng JW. The number of known plants species in the world and its annual increase. Phytotaxa. 2016;261(3):201-17.
40. Mohan SM, Pandey B. Antimicrobial activity of Oxalis corniculata Linn. International Journal of Science and Research. 2016;5(7).
41. McConnachie AJ, Strathie LW, Mersie W, Gebrehiwot L, Zewdie K, Abdurehim A, Abrha B, Araya T, Asaregew F, Assefa F, Gebre-Tsadik R. Current and potential geographical distribution of the invasive plant Parthenium hysterophorus (Asteraceae) in eastern and southern Africa. Weed research. 2011 ;51(1):71-84.
42. Oudhia P. Parthenium hysterophorus: a new weed in upland rice fields of the Chhattisgarh Plains (India). International Rice Research Notes. 2000;25(1).
43. Kaur M, Aggarwal NK, Kumar V, Dhiman R. Effects and management of Parthenium hysterophorus: A weed of global significance. International scholarly research notices. 2014;2014.
44. Kaur M, Aggarwal NK, Dhiman R. Antimicrobial activity of medicinal plant: Parthenium hysterophorus L. Res. J. Med. Plant. 2016;10(1):106-12.
45. Karunaratne UK, Karunaratne MM. Evaluation of methanol, ethanol and acetone extracts of four plant species as repellents against Callosobruchus maculatus (Fab.).
46.Bhalodia NR, Acharya RN, Shukla VJ. Evaluation of in vitro Antioxidant Activity of hydroalcoholic seed extratcs of Cassia fistula linn. Free Radicals and Antioxidants. 2011 ;1(1):68-76.
47. Ahmed S, Chaudhry SA, Ikram S. A review on biogenic synthesis of ZnO nanoparticles using plant extracts and microbes: a prospect towards green chemistry. Journal of Photochemistry and Photobiology B: Biology. 2017; 166:272-84.
48. Veerasamy R, Xin TZ, Gunasagaran S, Xiang TF, Yang EF, Jeyakumar N, Dhanaraj SA. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. Journal of saudi chemical society. 2011;15(2):113-20.

49.Banerjee P, Satapathy M, Mukhopahayay A, Das P. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresources and Bioprocessing. 2014 ;1(1):3.
50. Tripathy A, Raichur AM, Chandrasekaran N, Prathna TC, Mukherjee A. Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of Azadirachta indica (Neem) leaves. Journal of Nanoparticle Research. 2010 ;12(1):237-46.
51.Barreto FS, Sousa EO, Rodrigues FF, Costa JG, Campos AR. Antibacterial activity of Lantana camara Linn Lantana montevidensis Brig extracts from Cariri-Ceara, Brazil. Journal of Young Pharmacists. 2010 ;2(1):42-4.
52.Singh G, Maurya S, DeLampasona MP, Catalan CA. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food and chemical toxicology. 2007 ;45(9):1650-61.
53.Chew AL, Jessica JJ, Sasidharan S. Antioxidant and antibacterial activity of different parts of Leucas aspera. Asian Pacific Journal of Tropical Biomedicine. 2012 ;2(3):176-80.
54.Raghavendra MP, Satish S, Raveesha KA. Phytochemical analysis and antibacterial activity of Oxalis corniculata; a known medicinal plant. My science. 2006;1(1):72-8.
55.Bharathi V, Varalakshmi B, Gomathi S, Shanmugapriya A, Karpagam T. Antibacterial activity of Tridax procumbens Linn. Int. j. pharma sci. res. 2012;3(4):364-7.