International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395 -0056
Volume: 03 Issue: 01 | Jan-2016 www.irjet.net p-ISSN: 2395-0072

A Comparative Study on Antibacterial Activity of Herbs with its Nanoparticle

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Abstract - *Since time immemorial, herbal plants were in use for medicinal and culinary properties. They have been traditionally used to cure cold, asthma, to treat digestive disorders, diarrhea and cholera. In this present study the methanol extracts of ten plants were prepared. In vitro antibacterial activity had checked for methanol extracts using well diffusion method against Escherichia coli and Staphylococcus aureus. Based on the zone of inhibition three herbs have been selected and nanoparticles had been prepared. The antibacterial activity of nanoparticle was investigated using well diffusion method. The antibacterial activity of methanol extract was compared with activity of nanoparticle. It is evident from the study that antibacterial activity of herbal extract was found better than its nanoparticles.*

Key Words: *Methanol, Well diffusion, Antibacterial, Nanoparticle, Herbal extract, Zone of Inhibition.*

1. Introduction

Plants have been used in traditional medicine for several thousand years. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as ayurveda, unani and siddha. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine Pei[25]

Herbal remedies are considered the oldest form of health care known to mankind on this earth. Traditionally this treasure of knowledge has been passed on orally from generation to generation without any written document Perumal swamy and Ignacimuthu[26] and is still retained by various indigenous groups around the world. Documenting the indigenous knowledge through ethnobotanical studies is important for the conservation and utilization of biological resources. Ethnobotanical survey has been found to be one of the reliable approaches to drug discovery, Fabricant and Farnsworth, 2001[16] Several active compounds have been discovered from plants on the basis of ethnobotanical information and used directly as patented drugs, Carney[8].

The extract of *Acacia nilotica* is used for tanning, dyeing of leather, for gastrointestinal disorders, syphilitic ulcers and tooth ache, Amos[2]. The pods have reported inhibited HIV-1 induced cytopathogenecity, Asres et al.,[3]. The leaves of *Aegle marmelos* is used in treatment of acute shigellosis, Haider[18], as diuretic in gonnorhea, Dhiman[15], typhoid Paricha,[24] and colitis Agarwal[1]. *Andrographis panniculata* for asthma, gonnorhea, piles, dysentery and dyspepsia, Bhall[5], influenza Dey[14], pharyngittonsillistis, Thamilikitkul[32], respiratory tract infections, Coon and Ernst[11], and jaundice Toma[34]. *Jatropha gossypifolia* is antibiotic, insecticidal Balee[4] and possess significant hepatoprotective and pesticidal activity Chaterjee[10]; Panda[23]. *Phyllanthus niruri* is used for vaginitis, as antiviral, antibacterial and ayurveda recommends its use for bronchitis, leprosy, anaemia, urinary discharge, asthma, etc, Paithankar[22].

Plectranthus amboinicus leaves have been traditionally used for chronic coughs, cold, bronchitis, asthma, nasal congestion as well as diarrhea Cano and Volpato[7]. In Unani and ayurveda systems of medicine, *Pongamia pinnata* is used as anti-inflammatory, Srinivasan[30], antinociceptive, anti–plasmodial ,antiglycemic, antilipidoxidative, antiulcer, antihyperammonic, CNS depressant Mahli[20]. *Sida cardifolia* is used in the folk medicine for several purposes: antirhuematic, antipyretic, laxative, diuretic, anti-inflammatory, analgesic, hypoglycaemic, aphrodisiac, antiasthmatic and to relieve nasal congestion Medeiros[21]. *Solanum trilobatum* is used as antibacterial, antifungal, antimitotic, antioxidant and antitumours , Shahjahan[29], Purushothaman[27]. *Withania somnifera* shows relaxant and antispasmodic effects against several plasmogens on intenstinal, uterine, blood vascular, bronchial and tracheal muscles Devi[13].

Nanotechnology has dynamically developed as an important field of modern research with potential effects in electronic and medicine Glomm[17], Chan[9], and Boisselier[6]. Nanotechnology can be defined as a research for the design, synthesis, and manipulation of structure of particles with dimension smaller than 100nm. A new branch of nanotechnology is nanobiotechnology. Nanobiotechnology combines biological principles with physical and chemical procedures to generate nano-sized particles with specific functions.

E. coli is gram negative, Facultative anaerobic and Non-sporulating organism. The cells are about 2µ long and 0.5µ in diameter with a cell volume of 0.6 to 0.7µm3 Kubitsche[19]. Some virulent strains of *E. coli* causes Gastroenteritis, Urinary tract infections, and neonatal meningitis, In rare cases, virulent strains are also responsible for Hemolytic-uremic syndrome (HUS), Peritonitis, Mastitis, Septicemia and Gram negative Pneumonia Todar[33].

Staphylococcus aureus is a facultative anaerobic, Gram positive Coccus, which appear as grape like cluster when viewed through a microscope and has large round, golden- yellow colonies, often with hemolysis when grown in blood agar plate. *Staphylococcus aureus* may occur as a commensal on human skin sometimes it infect other tissue when normal barrier have been breached, this leads to furuncles (boils) and carbuncles (a collection of furuncles). In infant *Staphylococcus* infection can cause a severe disease *Staphylococcus* scalded skin syndrome Curran[12]. It also causes some disease like Atopic dermatitis, Toxic shock syndrome, and Mastitis in cow. Plants have many compounds that possess different properties which are used in drugs nowadays. Nanotechnology is a developing field and is implemented in many areas. So, the main aim of the study is to compare the antibacterial activity of herbal extract with its nanoparticle.

Materials and method:

Strains used:

- *Staphylococcus aureus* ATCC 6538
- *Escherichia coli* ATCC 8739

Nutrient Agar Medium:

Beef extract - 0.3gm
Yeast extract - 0.2gm
Sodium chloride - 0.3gm
Peptone - 0.5gm
Agar agar - 1.5gm
Distilled water - 100ml
pH - 7.0

Chemicals used:

- Ethanol
- Methanol
- Sodium alginate
- Calcium chloride

Collection of medicinal plants:

The medicinal plants selected for the present study was collected from in and around Coimbatore district, Tamilnadu, South – east coast of India.

Drying:

After harvesting, plant parts were analyzed for their moisture content of 60 – 80%. The collected plants were dried with in a temperature range of 100 – 140 o F as they cannot be stored without drying to avoid breakdown of important compounds and contamination by microorganisms. The collected leaves of the plants were shadow dried in room temperature. The moisture content of the plant was reduced to less than 14 % with proper drying.

Garbling process:

Garbling refers to the separation of that portion of the plant to be used from other parts of the plants, dirt and other extraneous matter. This step was performed by hand.

Extraction:

The active component from the plants was extracted in a stepwise manner as mentioned below.

Grinding process:

Grinding or mincing of an herb denotes mechanical breaking down of leaves, roots, seeds or other parts of a plant into very small units ranging from larger course fragments to fine powder. Grinding was employed in the production of initial phases of plant extracts. Grinding or mincing of the leaves was carried out in a mixer. The fine powder obtained after grinding was used for extraction and the fine powered was stored under good condition to reduce the risk of the contamination.

Methanolic extract:

Methanolic extracts of the plants were prepared by mixing 4 g of medicinal plant powder with 50ml of 80% methanol and 20%distilled water in an airtight conical flask and kept at room temperature overnight. After 12 hours of extraction, the solution was filtered using musclincloth and the filtrate was kept at room temperature for evaporation of methanol. The solution was filtered to get the concentrated extract.

Antibacterial activity by well diffusion method Rojas[28]:

The antibacterial activity of the different plant extracts was evaluated by Agar well diffusion method. Sterile nutrient plates were prepared. The plates were allowed to solidify for 5 minutes and wells of 6 mm were punctured using a well borer. 0.1% inoculum suspension of *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739) were swabbed uniformly over the surface of the agar. 20 µl of each herbal extract was loaded into the well and the plates were kept for incubation at 37ºC for 24 hours. The antibacterial activity was evaluated in terms of zone of inhibition, measured and recorded in millimeters.

Preparation of Nanoparticles Sumithra[31]:

The Nanoparticles were synthesized by using 10ml of herbal methanolic extracts for selected herbal plants. Initially 25ml of sodium alginate (base solution) (3.35mg / ml) was prepared, followed by 15ml of calcium chloride (3mg / ml) was prepared. The calcium chloride ($Cacl_2$) solution was added a drop wise into sodium alginate solution with constant stirring at 1500 rpm for 30minutes at room temperature. Then the herbal extract was added to the mixture very carefully drop wise to the above solution with constant stirring for 45-60 minutes. The reaction mixtures were kept undisturbed for overnight. After incubation the uppermost layer is discarded and the pellet was collected and checking its

activity against the *E.coli* and *S. aureus* by well diffusion method.

Result

Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. Continued further exploration of plant-derived antimicrobials is needed today.

Antibacterial activity of methanol extracts:

Extract has been prepared for selected herbs using methanol as a solvent. The antibacterial activity of methanol extracts was assayed by well diffusion method against *E.coli* and *S.aureus.* The methanol extract of *Sida cardifolia* and *Plectranthus amboinicus* showed good activity against *E.coli* as their zone of inhibition (ZOI) was 17mm. It is followed by *Acacia nilotica, Pongamia pinnata* and *Withania somnifera* as their ZOI was 14mm against *E.coli. Jatropha gossypifolia, Andrographis panniculata, Phyllanthus niruri* showed moderate activity. *Solanum trilobatum* showed lesser activity against the gram negative bacteria. *Phyllanthus niruri* and *Plectranthus amboinicus* showed good activity against *S.aureus* as their ZOI was 14mm. *Acacia nilotica* and *Aegle marmelos* showed moderate activity against *S.aureus* as their ZOI was 13mm and 14mm respectively. Other herbs like *Andrographis panniculata, Jatropha gossypifolia, Pongamia pinnata, Sida cardifolia, Solanum trilobatum* and *Withania somnifera* showed least activity against *S.aureus.* The methanol extract of herbs showed better activity against gram negative bacteria as compared to gram positive bacteria (Chart 1).

Antibacterial activity of herbal nanoparticle:

Based on the ZOI against *E.coli* and *S.aureus,* three herbs viz, *Acacia nilotica, Phyllanthus niruri* and *Plectranthus amboinicus* had selected. Herbal nanoparticle had been prepared for selected herbs using sodium alginate and calcium chloride solutions. The antibacterial activity of herbal nanoparticle was investigated using well diffusion method. Herbal nanoparticle of *Acacia nilotica* showed moderate activity against *S.aureus* as 10mm was its ZOI. It was followed by *Plectranthus amboinicus* with 4mm as its ZOI. For *E.coli*, *Acacia nilotica* and *Plectranthus amboinicus* showed moderate activity as 8mm was their ZOI. *Phyllanthus niruri* showed least activity as their ZOI was 6mm for both the organisms.

Herbal nanoparticle of *Acacia nilotica* showed moderate activity against *S.aureus* as 10mm was its ZOI. It was followed by *Plectranthus amboinicus* with 4mm as its ZOI. For *E.coli*, *Acacia nilotica* and *Plectranthus amboinicus* showed moderate activity as 8mm was their ZOI. *Phyllanthus niruri* showed least activity as their ZOI was 6mm for both the organisms.

The antibacterial activity of methanol extracts of selected herbs and nanoparticle against *E.coli* was compared (Chart 2). The methanol extracts of herbs showed better activity than herbal nanoparticle against *E.coli.*

Antibacterial activities of herbal extract with nanoparticle against *S.aureus* for selected three herbs were compared (Chart 2). The herbal extracts showed better activity than the nanoparticle.

On comparing the methanol extracts with herbal nanoparticle, the methanol extracts of herbs possess better antibacterial activity.

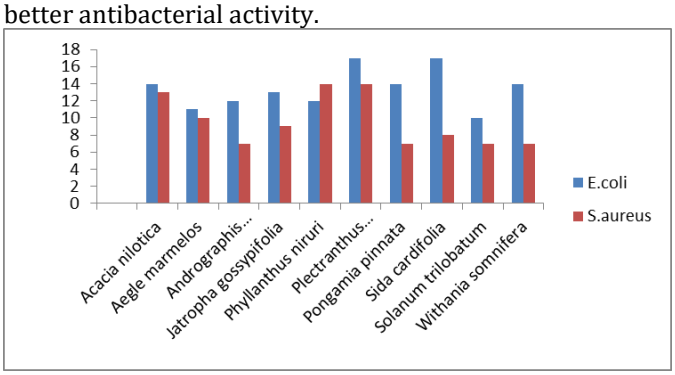


Chart 1: **Antibacterial activity of methanol extracts**

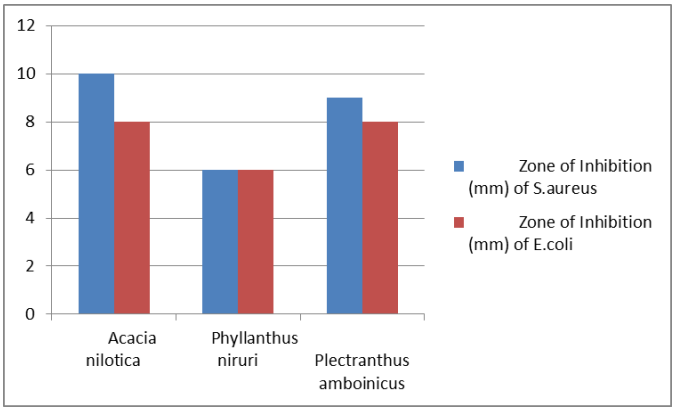
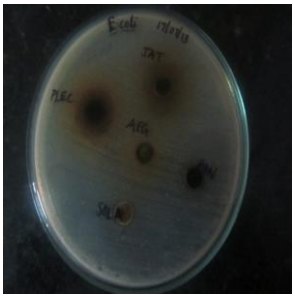
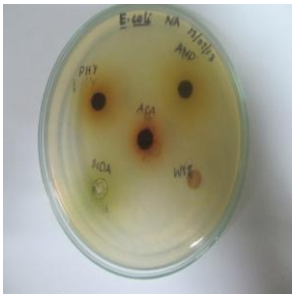


Chart 2: **Antibacterial activity of herbal nanoparticle against *E.coli & S.aureus***





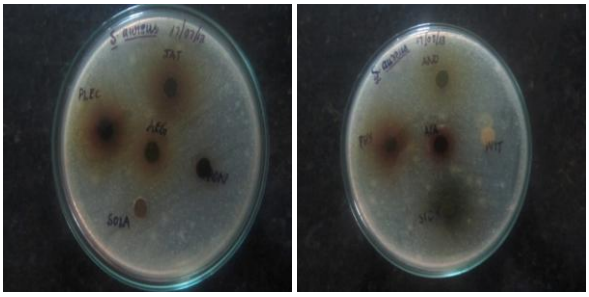


Fig -1: Antibacterial activity of methanol extract against *E.coli* and *S.aureus* by well diffusion method

Fig -2: Antibacterial activity of herbal nanoparticle against *E.coli* and *S.aureus* by well diffusion method

Table -1: Antibacterial activity of methanol extracts

Methanolic extract	Herbal part used	Zone of Inhibition (mm)	
		E.coli	*S.aureus*
Acacia nilotica	Bark	14	13
Aegle marmelos	Leaf	11	10
Andrographis panniculata	Leaf	12	7
Jatropha gossypifolia	Leaf	13	9
Phyllanthus niruri	Leaf	12	14
Plectranthus amboinicus	Leaf	17	14
Pongamia pinnata	Leaf	14	7
Sida cardifolia	Leaf	17	8
Solanum trilobatum	Leaf	10	7
Withania somnifera	Root	14	7

Table -2 Antibacterial activity of herbal nanoparticle

Herbal nanoparticle	Zone of inhibition(mm)	
	E.coli	*S.aureus*
Acacia nilotica	8	10
Phyllanthus niruri	6	6
Plectranthus amboinicus	8	9

Bacteria cause many infectious diseases in humans, animals and plants. *E.coli* and *S.aureus* causes many common infections like diarrhea, food poisioning and skin infections. Traditionally herbs have been used to treat many diseases. Nowadays nanotechnology has been applied in many fields. So, the main aim is to apply nanotechnology for bacterial infections, by preparing herbal nanoparticle without using any heavy metals. The antibacterial activity of herbal nanoparticle was compared with herbal extracts.

3. CONCLUSIONS

In this study herbal extract were prepared from selected herbs using methanol as a solvent. Their antibacterial activity was checked against *E.coli* and *S.aureus* by well diffusion method. *Plectranthus amboinicus* and *Sida cardifolia* showed good activity against *E.coli* than *Pongamia pinnata, Acacia nilotica* and *Withania somnifera. Phyllanthus niruri* and *Plectranthus amboinicus* showed good activity against *S.aureus* than *Acacia nilotica.* Based on ZOI of the three herbs viz., *Acacia nilotica, Phyllanthus niruri* and *Plectranthus amboinicus*, the herbal nanoparticle was prepared using sodium alginate and calcium chloride with herbal extract. The antibacterial activity of herbal nanoparticle was checked against *E.coli* and *S.aureus* by well diffusion method. Herbal nanoparticles showed lesser activity against both tested organisms. On comparing the herbal extract with its nanoparticle, herbal extract showed better activity than herbal nanoparticle. The antimicrobial activity of aqueous extract of *Phyllanthus niruri* was checked against *E.coli, Klebseilla pnuemoniae, Staphylococcus aureus, Salmonella typhirium, Candida albicans, Aspergillus sp.* and *Fusarium sp.* Silver nanoparticle had been prepared for extract and their antimicrobial activity was checked at different concentrations. At higher concentration the silver nanoparticle showed good activity against bacterial strains. Silver nanoparticle showed lesser activity against fungus mainly against *Fusarium sp.* On comparision with silver nanoparticle and plant extracts, silver nanoparticle showed potent antimicrobial activity. The antimicrobial activity of silver nanoparticle may be because of usage of silver nitrate for preparation of nanoparticle.

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