



ISSN 2320-3862

JMPS 2014; 2(5): 51-54

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Received: 27-07-2014

Accepted: 22-08-2014

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Antimicrobial activity of medicinal plant extracts on gram negative bacteria

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Abstract

Various types of antibacterial substances are present in higher plants. Plant derived medicines have made significant contributions towards human health and plants and are a great source of novel drug compounds. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Sensitivity to chemical substances vary from one strain of microorganisms to other. The plant extracts showing antibacterial activity can be further subjected to isolation of therapeutic antimicrobials and can carry out further pharmacological evaluation.

In the present investigation, the active leaf extract of six medicinally important plants obtained by aqueous and solvent extraction was tested against three bacterial organisms *E. coli*, *Pseudomonas* and *Klebsiella*. The aqueous lemon leaf extracts against *E. coli* showed a good inhibitory response. In *Klebsiella* the best antimicrobial activity was observed with Eucalyptus leaf ethanol extracts. *Pseudomonas* showed resistance to all the solvents except to Tulsi leaf ethanol extracts.

Keywords: Bacteria, antimicrobial activity, plant extracts, solvents.

1. Introduction

Nature has been a source of medicinal agents since ancient times. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in a management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [1]. Antibiotic resistance has become a global concern [2]. Recent work revealed the potential of several herbs as sources of drugs [3]. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of antibiotic prototypes [4]. Numerous studies have identified compounds within herbal plants that are effective antibiotics [5].

The characteristics of the plants that inhibit microorganisms and are important for human health have been researched in laboratories since 1926. Traditional healing systems around the world that utilize herbal remedies are an important source of discovery of new antibiotics [6]; some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria [7]. The results of this indicate the need for further research in to traditional health systems [8]. It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity [9]. Traditional medical treatments in daily life are now being used with empiric methods. The parts of the plant used are its leaves, seeds and oil. Fresh leaves are added to salads, vegetables and various cooked dishes in various countries. The seeds are used to flavour confectionery, dried figs, cakes, bread and curries. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents.

2. Materials and Methods

2.1 Materials Required

Sterile loops, petri plates, forceps, spreaders, incubator, zonal scale for measuring the zone of inhibition, cork-borer (0.6 cm), bottles for storage.

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2.2 Medias

Nutrient Broth: Peptone 0.5 gm, Beef extract 0.3 gm, sodium chloride 0.5 gm, Distilled water 100 ml, P^H 7

Nutrient Agar: Peptone 0.5 gm, Beef extract 0.3 gm, sodium chloride 0.5 gm, Agar 2.0 gm, Distilled water 100 ml , P^H 7.

2.3 Plant material: Fresh leaves of *Ocimum sanctum* (Tulsi), *Citrus limon* (Lemon), *Nerium oleander* (Nerium), *Azadirachta indica* (Neem), *Hibiscus rosasinensis* (Hibiscus), *Eucalyptus globulus* (Eucalyptus) are collected from the regions of Hyderabad, Andhra Pradesh, India. The plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

2.4 Preparation of Extracts:

2.4.1 For aqueous extraction: 2.5 g of air-dried powder was added to 25 ml distilled water and boiled on slow heat for 2 hr. It was filtered through muslin cloth and centrifuged at 5000 rpm for 10 min. The supernatant was collected and concentrated to make the final volume to one-fourth of the original volume. It was then autoclaved at 121 °C temperature

at 15 lbs pressure and stored at 4 °C.

2.4.2 For solvent extraction: 2.5 g of air-dried powder was taken in 100 ml of organic solvent(ethanol and chloroform) in a conical flask, plugged with cotton wool and then kept on an rotary shaker at 150 rpm . After 24 hours the supernatant was collected and the solvent was evaporated to make the final volume to one-fourth of the original volume and stored in airtight bottles.

2.5 Microorganisms: *In vitro* antimicrobial activities were examined for aqueous and solvent extracts (ethanol and chloroform) from six medicinal plants. Microorganisms on which it was investigated were Gram negative bacteria *E coli*, *Pseudomonas*, and *Klebsiella*.

2.6 Isolation of microorganisms: Conventional methods were successful in isolating *E.coli*, *Pseudomonas*, *Klebsiella* from sewage samples and identified them based on cultural and biochemical tests as shown in table 1.

Table 1: Morphological and biochemical tests for identification of the isolates

Biochemical tests	E.coli	Klebsiella	Pseudomonas
Colony Morphology:			
Nutrient agar	Cream pinpoint colonies	Mucoid colonies	Cream coloured opaque colonies
Selective medium	EMB agar, greenish metallic sheen	Mac Conkey agar, Pink colour mucoid colonies	Cetrimide agar, bluish green pigmentation
Grams nature	Gram negative	Gram negative	Gram negative
Cellular morphology	Rods	Rods	Rods
Motility	Motile	Motile	Motile
Indole	+ve	-ve	-ve
Methyl red	+ve	-ve	-ve
Voges Proskauer	-ve	+ve	-ve
Citrate	-ve	+ve	+ve
Oxidase	-ve	-ve	+ve
Coagulase	-ve	-ve	-ve
Urease	-ve	-ve	-ve
Nitrate reduction	+ve	+ve	-ve
Catalase	+ve	+ve	+ve

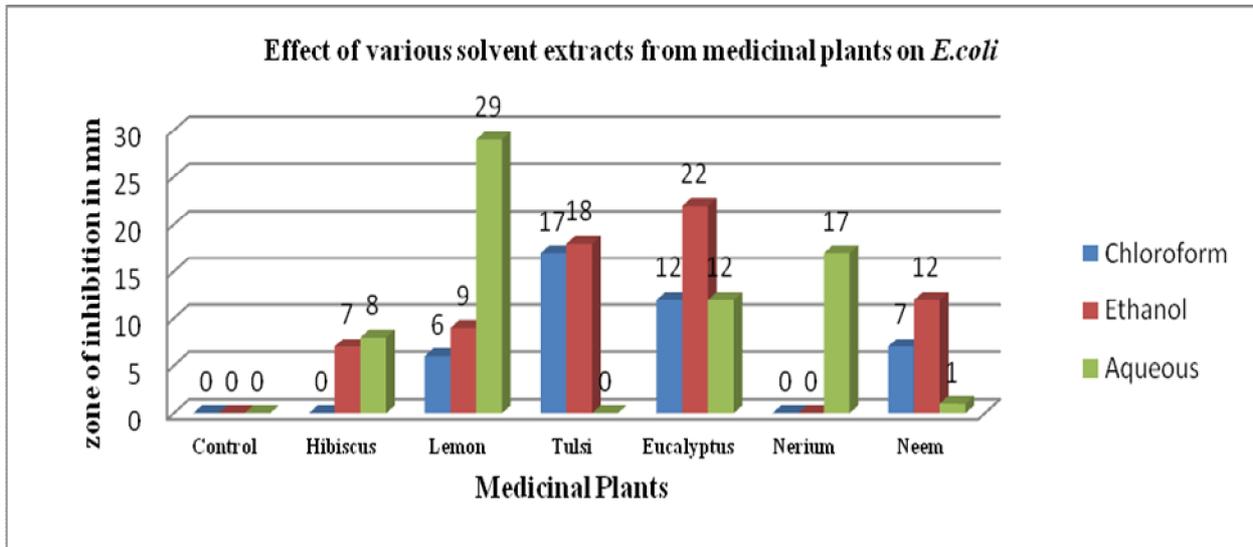
+ve positive -ve negative

2.7 Antimicrobial assay

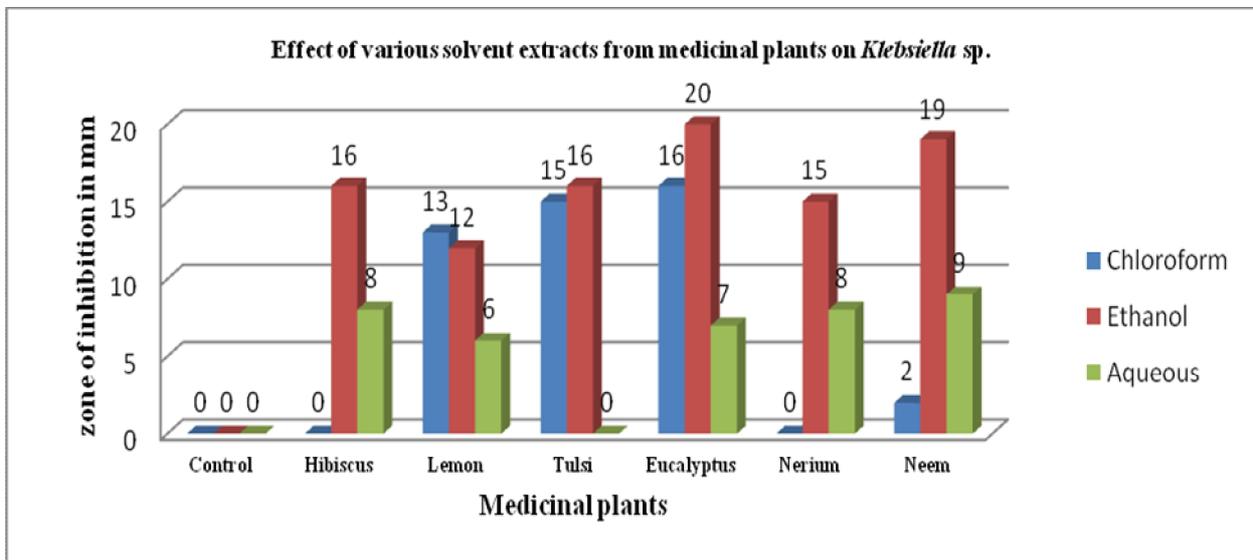
The antimicrobial assay was performed by an agar well diffusion method for both aqueous and solvent extracts. For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer (0.6 cm) 50ul of the test compound was introduced into the well. The plates were incubated overnight at 37 °C. Microbial growth was determined by measuring the diameter of the zone of inhibition. For each bacterial strain controls were maintained where pure solutions (aqueous and solvents) were used instead of the extracts. The result was obtained by measuring the zone diameter. The experiments were done in the duplicates and the mean values are presented.

3. Results and Discussion

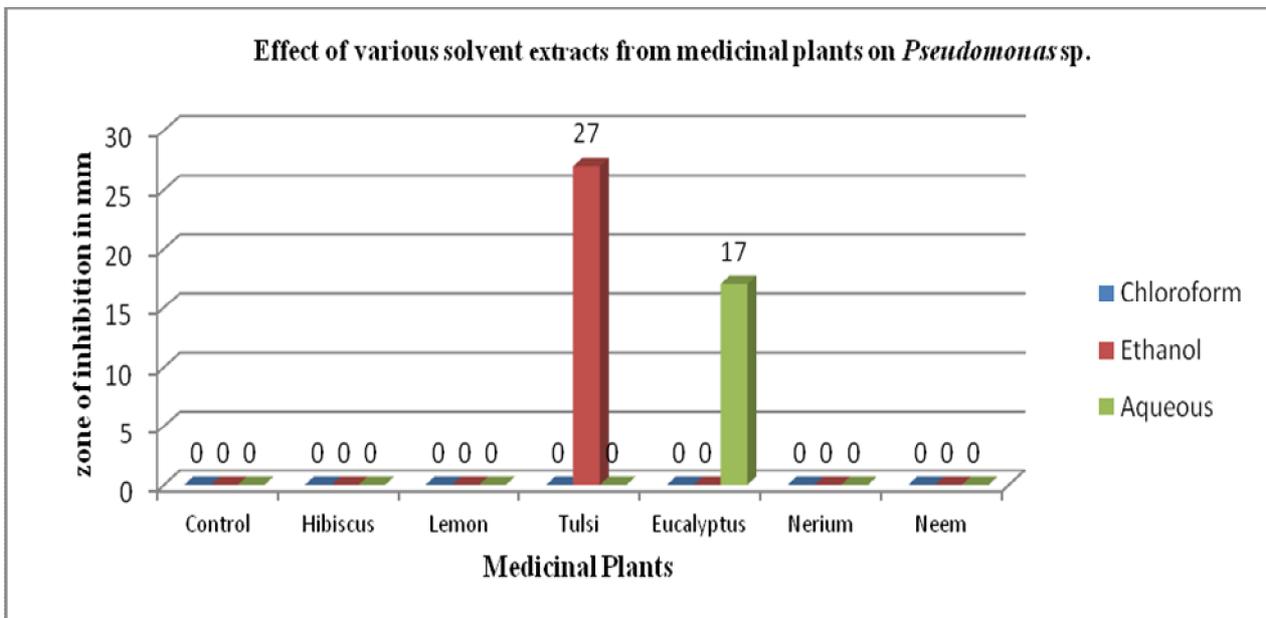
The presence of antibacterial substances in higher plants is well established. Plants have provided a source of inspiration for drug compounds as plants derived medicines have made significant contributions towards human health. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as solvent but it was found in this study that the plant extracts obtained by ethanol and chloroform extraction showed more consistent antimicrobial activity compared to those extracted by water. The results of antimicrobial activity of all the 6 plants against the three bacterial strains are given below.



Graph 1: Effect of solvent and aqueous extracts from medicinal plants on *E. coli* sp.



Graph 2: Effect of solvent and aqueous extracts from medicinal plants on *Klebsiella* sp.



Graph 3: Effect of solvent and aqueous extracts from medicinal plants on *Pseudomonas* sp.

The antimicrobial activity of the aqueous lemon leaf extracts against *E. coli* when performed showed a good inhibitory response. A relatively good inhibitory activity was also obtained from Eucalyptus ethanol extracts as shown in Graph 1. In *Klebsiella* the best antimicrobial activity was observed with Eucalyptus and Neem leaf ethanol extracts. The ethanol extracts of all the leaves showed good zone of hydrolysis compared to aqueous and chloroform extracts as in Graph 2. With respect to *Pseudomonas*, the organism showed resistance to all the solvents except to Tulsi leaf ethanol extracts and Eucalyptus leaf aqueous extract as in Graph 3.

4. Conclusion

In the present era, plant and herb resources are abundant. A significant number of studies have been used to obtain purified plant chemical, very few screening programmes have been initiated on crude plant materials has also been widely observed and accepted that the medicinal value of plants lies in the bioactive substances present in the plants. In the present investigation, the active leaf extract of six medicinally important plants obtained by aqueous and solvent extraction was tested against three bacterial organisms *E. coli*, *Pseudomonas* and *Klebsiella*. The aqueous lemon leaf extracts against *E. coli* showed a good zone of inhibition, while in *Klebsiella* the best antimicrobial activity was observed with Eucalyptus leaf ethanol extracts and in *Pseudomonas*, the organism showed resistance to all the solvents except to Tulsi leaf ethanol extracts. From the above studies, it is concluded that the traditional plants are new sources of antimicrobials with stable, biologically active components that can establish a scientific base for the use of modern medicine.

5. Acknowledgement

We thank the management of St. Pious X Degree and P.G College for Women, Nacharam, for permitting us to carry out this work and our dear Principal Rev. Dr. Sr. Nirmala for allowing us to use the facilities in the college laboratory. Our sincere thanks to Dr. Vindhya Vasini Roy, HOD of Microbiology for her constant support.

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