

## Antimicrobial and Antioxidant Efficacy of Some Medicinal Plants Against Food Borne Pathogens

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**Abstract:** The medicinal plants are used in traditional treatments to cure variety of diseases for thousands of year. The aim of this study was to identify such plants with antimicrobial and antioxidant efficacy for controlling some food borne pathogens. The locally available plants viz., *Ficus religiosa*, *Leucas aspera*, *Holarrhena antidysenterica* and *Psidium guajava* were selected for the study. Various methods were tried to standardize the extraction of antimicrobial agents. Methanol extraction of *P. guajava* showed high antimicrobial activity against the food borne pathogens like *S. typhi*, *Pseudomonas spp* and *B. Subtilis*. Whereas in case of antifungal *H. antidysenterica* showed more activity with maximum of 23mm inhibition zone against *Aspergillus spp*. The high antioxidant activity of 36ig/100mg was observed in *Psidium guajava*. The GC-MS study also revealed the various phytic components for *H. antidysenterica*, which assured that it has high antimicrobial, anticancer activity against the food borne pathogens. It was concluded that plant extract can be used as a preservatives against the food borne pathogens.

**Key words:** Antioxidant • Antimicrobial • Antifungal activity • Medicinal plant

### INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Herbal medicine is the oldest form of healthcare known to mankind. India has rich medicinal plants flora of more than 7500 species. Of these, 4635 species are used commercially on a fairly large scale. Over 50% of all modern clinical drugs are of natural product origin and natural products play important roles in drug development in the pharmaceutical industry. Phytochemical compounds are found in plants that are not required for normal functioning of the body, but have a beneficial effect on health or play an active role in amelioration of diseases. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources.

In order to promote the use of medicinal plants as potential sources of antimicrobial compounds, it is pertinent to thoroughly investigate their composition and activity and thus validate their use [1]. Some phytochemicals produced by plants have antimicrobial activity allowing these plants to be studied and used for the development of new antimicrobial drugs [2]. The effectiveness of phytochemicals in the treatment of various diseases may lie in their antioxidant effects [3]. Secondary plant metabolites are largely unexplored in 'conventional' animal production systems. In the past, plant metabolites were generally considered as sources of antinutritional factors. Recent bans and restrictions on the use of animal antibiotic growth promoters stimulated interest in bioactive secondary metabolites of plant source as alternative performance enhancers [4].

In contrast to their regulated status in India, China and other countries, herbal medicines are regarded as dietary supplements for humans in the US and are widely used. It was also reported that approximately one quarter of adults used herbs to treat a medical illness within the past year in the US. According to World Health Organization, medicinal plants are the best sources to

obtain a variety of newer herbal drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants [5]. The present research has been conducted to study the medicinal properties like antimicrobial and antioxidant properties of some locally available medicinal plants against food borne pathogens.

## MATERIALS AND METHODS

The following medicinal plants were selected for the study from the local area based on their basic information available. The plants are, *Ficus religiosa* (Bodhi tree), *Leucas aspera* (Thumba), *Holarrhena antidysenterica* (Conissi bark) and *Psidium guajava* (Guava). Fresh samples of plants were collected, washed and air dried. The dried leaves were powdered and stored in air tight bottles separately for further studies.

### Preparation of Plant Extract

**Aqueous Extraction:** Samples of 10g were immersed in 100ml of distilled water, mixed and allowed to soak for 24hrs. Then the mixer was filtered through Whatmann No.4 filter paper to get pure extract.

**Methanol Extraction:** Air dried powder of 10g was placed in a conical flask containing 100 ml of organic solvent (Methanol) plugged with cotton and then kept on a rotary shaker at 190-220 rpm for 24 h. Later, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of its original volume.

**Ethanol Extraction:** Ten gram of sample was soaked in 100ml of 95% ethanol and kept in room temperature for 24hours. Then the extract solution was filtered through a Whatmann No.4 filter paper. Finally, the solvent was removed from the sample using a rotary vacuum evaporator until it reaches one-fourth of its volume.

All the above extracts were stored at 4°C in air tight bottles for further studies.

**Determination of Antimicrobial Activity:** Nutrient agar plates were prepared. The nutrient agar plates were heavily inoculated with the young bacterial culture (16-20hrs) by means of sterile cotton swab to ensure efficient growth of the organism. Six wells were made in each plate using cork borer. 0.5ml of various extracts were added to the wells of the plate which is pre swabbed with the culture to observe the antagonistic effect of various

microorganisms. The plates were incubated for 24 to 48 hours. Similar procedure was carried out for the rest of the isolates. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average value was tabulated.

### Gas Chromatography-Mass Spectrum Analysis:

GC-MS (Gas chromatography Mass Spectrum) technique was used in this study to identify the compounds present in the plants extract. The powdered leaf materials (20 g) was soaked in 50ml of absolute alcohol for 12hrs and then filtered through Whatmann filter paper No.4 along with 2g sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering the samples, it was concentrated by nitrogen gas which reduces the volume to 1 ml. Then the plant extract was injected in the Gas chromatography-mass spectrometer.

Gas Chromatography technique was carried out in GC Clarus 500 Perkin Elmer with column Elite-1 (100% Dimethyl poly siloxane). Mass detector used was Turbo mass gold-Perkin Elmer and nitrogen was used as a carrier gas. Temperature of column was maintained at 200-280°C. The spectrum of the unknown component is compared with the spectrum of the known components stored in the NIST library and ascertained the name, molecular weight and structure of the components of the test materials.

**Estimation of Antioxidant:** The antioxidant property was determined using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), which is the oxidizing radical to be reduced by the antioxidant present in the given sample. The antioxidant is measured spectrophotometrically at 517nm [6].

## RESULTS AND DISCUSSION

The antimicrobial potential of plants was compared according to their zone of inhibition against the several pathogenic organisms. The plant extract from various extraction processes showed their activity against both bacteria and fungi. Aqueous extract of *F. religiosa* showed high antimicrobial activity against all four selected pathogenic organism. High activity was found on *B. subtilis* with about 24mm inhibition zone. Infections organism *P. aeruginosa* and *B. subtilis*, especially those with multi-drug resistance, are among the most difficult to treat with conventional antibiotics [7]. In the present study, the growth of *P. aeruginosa* was remarkably inhibited by the plant extract of *F. religiosa* (Fig. 1).

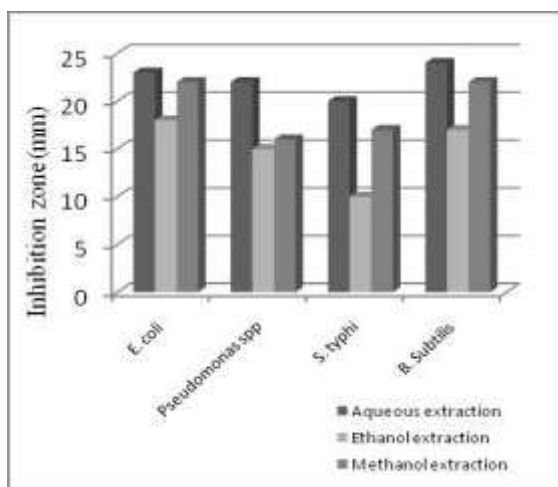


Fig. 1: Antimicrobial activity of *Ficus religiosa*

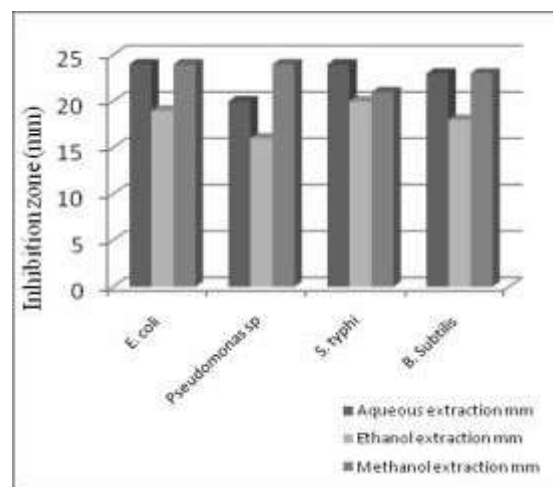


Fig. 4: Antimicrobial activity of *Psidium guajava*

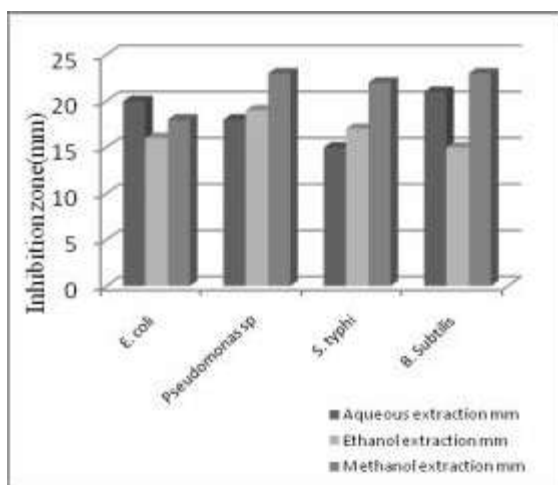


Fig. 2: Antimicrobial activity of *Leucas aspera*

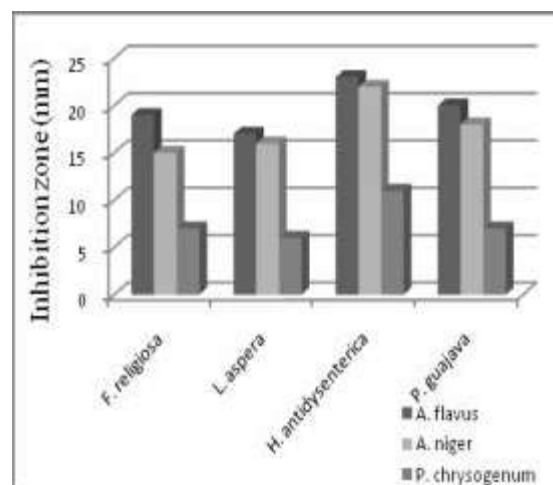


Fig. 5: Antimicrobial activity of all four selected plants against fungi

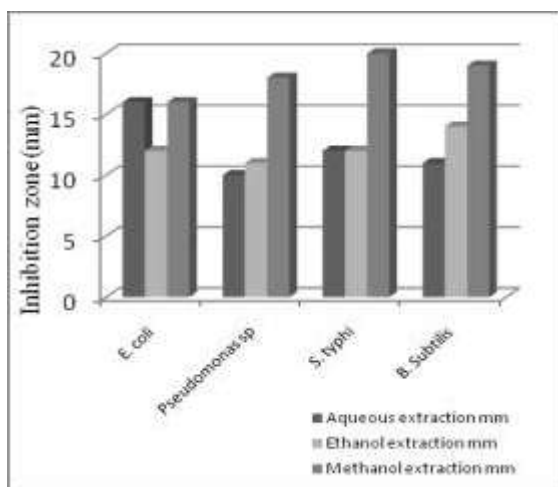


Fig. 3: Antimicrobial activity of *Holarrhena antidysenterica*

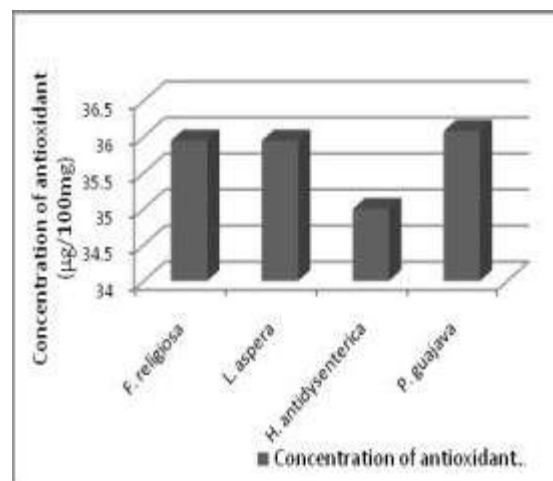


Fig. 6: Estimation of antioxidant activity for four plant extract

Methanol extraction of *L. aspera* showed activity in *Pseudomonas spp* and *B. Subtilis* with 22-23mm, that the aqueous and ethanol extraction showed low activities (Fig. 2). The methanol extraction of *H. antidyserterica* showed high activity on the pathogens above 16mm inhibition zone. *S. typhi* showed more sustainability to the extract. Both aqueous and ethanol extraction of *Holarrhena* showed low antimicrobial activity then the other plants (Fig. 3). Selected pathogens showed susceptible against solvent extraction of *P. guajava*. Among the four selected plants *Psidium spp* have good antimicrobial activity against the pathogen (Fig. 4). In case of fungi, only methanol extracts showed antimicrobial activity against all four pathogens. *H. antidyserterica* showed high activity against all three fungi with 23, 22 and 11mm inhibition zone. Whereas the *P. guajava* played next to *Holarrhena* with 20, 18 and 7mm inhibited zone (Fig. 5). The other two plant extracts showed similar results to each other. Due to the high activity of *Holarrhena spp.*, it was taken for the phytochemicals studies through GC-MS. Phyto-components identified in the ethanolic extract of the *Holarrhena antidyserterica* are 2-Methoxy-4-vinylphenol, Megastigmatrienone, 3-O-methyl-D-glucose, Tetradecanoic acid, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, Phytol, 9,12,15-Octadecatrienoic acid, Octadecanoic acid, Eicosanoic acid and Squalene. These 11 useful photochemical compounds were identified which have high antimicrobial, anticancer and antioxidant activity.

Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria [8, 9]. These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure [10]. Antioxidants protect cells against damage caused by molecules known as free radicals. The antioxidant effects of plant extracts are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes [11]. Numerous studies with plant phytochemicals showed phytochemicals with antioxidant activity may reduce risk of cancer and improve the health. All the plants extracts showed antioxidant activity with about 35-36µg/100mg and among those. *Psidium guajava* had more antioxidant property with 36µg/100mg (Fig. 6).

In conclusion, the contamination of food by microorganisms is a worldwide public health concern and is a leading cause of trade problems internationally.

To avoid these problems, medicinal plants can be used to prevent the food borne microorganisms as a tool of preservatives during food processing and to design a drug.

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