ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SOME SELECTED MEDICINAL PLANTS OF HARYANA

SUMMARY
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Traditional medicinal practice has been known for centuries in many parts of the world for the treatment of various human ailments. The use of antibiotics has revolutionized the treatment of various bacterial infections. However, their indiscriminate use has led to an alarming increase in antibiotic resistance among microorganisms, thus necessitating the need for development of novel antimicrobials. Recent years have witnessed a renewed interest in exploring natural resources for developing such compounds. Medicinal plants are relied upon by 80% of the world’s population, and in India the use of plants as therapeutic agents remains an important component of the traditional medicinal system. A number of plants have been documented for their biological and antimicrobial properties.

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms. The worldwide emergence of *Escherichia coli*, *Klebsiella pneumoniae*, *Haemophilus* and many other β-lactamase producers has become a major therapeutic problem. Multi-drug resistant strains of *E. coli* and *K. pneumoniae* are widely distributed in hospitals and are increasingly being isolated from community acquired infections. *Candida albicans*, also a nosocomial pathogen, has been reported to account for 50-70% cases of invasive candidiasis. alarmingly, the incidence of nosocomial candidemia has risen sharply in the last decade. All this has resulted in severe consequences including increased cost of medicines and mortality of patients. Thus, in light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy.
For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against MDR microbe strains.

For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists. Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties.

In an effort to expand the spectrum of antimicrobial agents from natural resources, eighteen medicinal plants belonging to seventeen families, have been selected based on their traditional uses in Haryana to assess their antimicrobial potential along with minimum inhibitory concentrations, minimum fungal concentrations estimation and preliminary phytochemical estimation of medicinal plants.

**SELECTION OF MEDICINAL PLANTS**

Plants were selected on the basis of information provided in the ethnobotanical survey and local medicine men. On the basis on folklore medicinal properties eighteen medicinal plants were selected in the present study. Their names were A. aspera, A. tunifolius, C. fistula, C. indica, C. reflexa, C. sativa, E. hirta, E. prostrata, H. integrifolia, H. zeylanicum, K. pinnata, L. cehalotes, P. niruri, S. xanthocarpum, S. nigrum, P. minima, T. erecta, T. procumbens. Each specimen/ material was collected; labeled, identified and medicinal uses were recorded. The folklore medicinal value of these plants were following: the cure of urinary troubles, cough, epilepsy, scabies, eczema, Cholera, fever, diabetes, pneumonia, toothache, cuts, wounds, skin diseases, treatment of insect bite, malaria, intestinal worms, leprosy, ringworm, piles, fungal infections, relieve irritable bowels, jaundice, bronchitis asthma, and appetizer. The method used by the traditional healers for the preparation of medicine may vary with type, severity and
location of the diseases. The dose and method on drug intake also varies with the type of
disease of the patients. These medicinal plants may be used in various forms like in the
form of powder, pellets, decoction, infusion, paste, churns and can be applied topically on
the skin or through oral route

**IN VITRO ANTIMICROBIAL ACTIVITY**

The screening of different plant extracts for their antibacterial activity carried out using Mueller-Hinton and Nutrient agar media did not reveal any significant difference, thus further studies were carried out using nutrient agar medium only. Out of eighteen medicinal plants following mentioned plants exhibited promising antimicrobial activity, *A. aspera, A. tunifolius, C. fistula, C. indica, C. reflexa, C. sativa, E. hirta, T. erect* and, *L. cehalotes.*

The antibacterial activity of *Achyranthus aspera* revealed that the ethanol extract exhibit inhibition against *S. aureus* (16 mm), *S. flexneri* (14 mm) and *V. cholera* (14 mm). The aqueous extract shows antibacterial activity against *S. aureus* (14 mm) and *S. flexneri* (16 mm). On the other hand no antifungal activity was found in all four extracts of *A. aspera.* The ethanol extracts of *A. tenuifolius* showed maximum inhibition against *V. cholera* (16 mm) followed by *P. mirabilis* (14 mm), *S. typhi* (14 mm), *S. flexneri* (14 mm), *S. aureus* (13 mm), *P. aeruginosa* (13 mm) and *S. marcescens* (11 mm). In case of *C. fistula* the ethanol extracts exhibited antibacterial activity against *S. typhi* (16 mm), *V. cholera* (16 mm). The antifungal activity was also exhibited by ethanol extract against *C. albicans* (22 mm). The aqueous extract shows antibacterial activity against *S. aureus* (16 mm), *S. typhi* (16 mm) and *V. cholera* (13 mm). In case of *C. indica* the ethanol extracts exhibited antibacterial activity against *P. aeruginosa* (12 mm), *V. cholera* (11 mm) and the methanol extract exhibited antibacterial activity against *S. aureus* (15 mm), *P. aeruginosa* (14 mm), *P. mirabilis* (13 mm) and *V. cholera* (12 mm). The antibacterial was exhibited by the ethanol extract against *S. aureus* (15 mm) and antifungal activity against *C. albicans* (16 mm). The methanol extract exhibited antibacterial activity only against *V. cholera* (13 mm) and the aqueous extract exhibited antibacterial activity against *S. aureus* (14 mm) with *V. cholera* (16 mm). The methanol extract of *E. hirta*
showed the antibacterial against *S. aureus* (22 mm), *E. coil* (20 mm), *P. aeruginosa* (13 mm), *P. mirabilis* (16 mm), *S. typhi* (21 mm), *S. flexneri* (17 mm), *V. cholera* (12 mm) and *S. marcescens* (16 mm). Similarly the aqueous extract antibacterial activity against *E. coil* (23 mm), *P. aeruginosa* (16 mm), *P. mirabilis* (19 mm), *S. typhi* (19 mm), *S. flexneri* (18 mm), *V. cholera* (17 mm) and *S. marcescens* (14 mm). The ethanol extract of *T. erect* exhibited strong antibacterial activity against *S. aureus* (19 mm), *P. mirabilis* (13 mm) and *P. aeruginosa* (13 mm). The methanol extract exhibited strong antibacterial activity against *S. aureus* (18 mm) followed by *P. mirabilis* (16 mm) and *P. aeruginosa* (14 mm,). *L. cephalotes* exhibited good antibacterial activity against *S. aureus*, ethanol (14 mm), methanol (12 mm) and aqueous (11 mm).

**MINIMUM INHIBITORY CONCENTRATION AND MINIMUM FUNGICIDAL CONCENTRATION**

In our study the MIC ranges from 2 mg/ml to 32 mg/ml and the MFC ranges from 4 mg/ml to 32 mg/ml. In case of *A. aspera* the methanol extract showed MIC 2 mg/ml against *S. aureus* and the ethanol extracts (2 mg/ml) against *S. flexneri*. The MIC was observed 8 mg/ml in ethanol, aqueous extract and 2mg/ml in methanol extracts against *S. aureus*. In case *V. cholera* 4 mg/ml MIC was observed in aqueous extracts and 8mg/ml in ethanol and methanol extracts. In *S. flexneri* 2 mg/ml MIC was shown by ethanol extract and 4 mg/ml by ethanol and aqueous extracts. The minimum MIC was exhibited by methanol extracts (8 mg/ml) against *S. aureus*. In case of *A. tunifolus* the minimum MIC was observed in case of methanol extract against *S. aureus*. The ethanol extract of *C. fistula* showed minimum MIC (2 mg/ml) against *V. cholera*. In case of *C. indica* the minimum MIC (4 mg/ml) was found in case of methanol extract against *P. aeruginosa* and the ethanol extract of *C. reflexa* showed minimum MIC (2mg/ml) against *S. aureus*. The methanol extract (8mg/ml) of *C. sativa* and the aqueous extracts (4mg/ml) of *E. hirta* exhibited minimum MIC against *S. typhi*. The ethanol, aqueous extract of *E. prostrate* exhibited minimum MIC (2mg/ml) against *S. flexneri*. In case of *H. integrifolia* the minimum MIC (2mg/ml) was observed against *S. flexneri*. In case of *L. cephalotus* the MIC was observed ranged between 4 mg/ml to 16 mg/ml and *P. niruri* MIC ranged from 2 mg/ml to 18 mg/ml. The MIC was found ranged from 4 mg/ml to 16
mg/ml in *T. erecta*, 16 mg/ml to 32 mg/ml of *T. procumbens*. The MIC ranged from 2 mg/ml to 32 mg/ml in case of *S. nigrum* and 2 mg/ml to 16 mg/ml in case of *S. xanthocarpum*.

**PRELIMINARY PHYTOCHEMICAL ESTIMATION**

Phytochemicals isolated from *A. tenuifolius* were anthraquinones and anthocyanins. Phytochemicals isolated from *A. aspera* were alkaloids, amino acids, flavonoids, saponins, tannins and phenols. Phytochemicals isolated from *C. fistula* were alkaloids, tannins, flavonoids, amino acids, phenols, anthraquinones and anthocyanins respectively and from *C. indica* phytochemicals were alkaloids, tannins, anthraquinones and anthocyanins. Phytochemicals isolated from *C. reflexa* were alkaloids, saponins, tannins, flavonoids, amino acids, sterols and phenols. Phytochemicals isolated from *C. sativa* was only cardiac glycoside. The phytochemicals isolated from *E. hirta* were tannins and anthocyanins. Phytochemicals isolated from *E. prostrate* were alkaloids, tannins, phenols and anthocyanins. The *H. integrifolia* was having alkaloids, flavonoids, phenols and sterol. *H. zeylanicum* showed presence of tannins, flavonoids, amino acids, phenols and *L. cephalotes* was having tannins, amino acids, phenols and anthraquinones. The phytochemicals isolated from *P. niruri* were saponins, tannins, flavonoids, phenols and anthraquinones. *T. erecta* was found to be contained alkaloids, tannins, flavonoids, amino acids, sterols and anthraquinones, cardiac glycosides and anthocyanins. *S. nigrum* contained saponins and sterols only but *S. xanthocarpum* was having alkaloids, tannins, flavonoids, amino acids, phenols and anthraquinones. The phytochemicals found in *P. minima* were alkaloids, flavonoids, amino acids and anthraquinones.

So the antibacterial activity of studied plants namely; *A. aspera*, *A. tunifolius*, *C. fistula*, *C. indica*, *C. reflexa*, *C. sativa*, *E. hirta*, *T. erect* and *L. cephalotes* may be due to presence of various isolated phytochemicals which are known to be synthesized by plants in response to microbial infection.
The results of the present study are encouraging as three out of eighteen plants tested possessed antibacterial properties against most of the tested bacteria. Only three plants extract viz. *S. nigrum*, *C. reflexa* and *C. fistula* exhibited antifungal activity against *C. albicans* while two plants extract revealed antifungal activity against fungus *A. niger*. Petroleum ether extracts of mostly plants did not show the antimicrobial activity. Four bacterial species ( *S. aureus*, *P. aeruginosa*, *S. flexneri*, *V. cholerae*) are susceptible to ethanolic, methanolic and aqueous plants extracts. No single plant was found to be equally effective against all the bacteria tested. The % activity of ethanolic extract of *A. tunifolius* was highest (63.63%). Methanolic extract % activity was 72.72% for *E. hirta* while aqueous extract was 63.63 % of *E. hirta*. Six of the plants tested here (*A. aspera*, *A. tunifolius*, *C. fistula*, *C. sativa*, *E. hirta*, *P. niruri*) exhibited antibacterial activity against more than three bacteria. All these plants have been in use for many years as decoctions or infusions prepared in water to treat various ailments. This work thus provides a scientific basis for the use of these aqueous plant extracts in home-made remedies and their possible application against microorganisms such as *Staph. aureus* and *Ps. aeruginosa*, etc., that cause nosocomial infections. Further studies may lead to their use as safe alternatives.