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EVALUATION OF ANTIMICROBIAL POTENTIAL OF CHOSEN PLANTS

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ABSTRACT

Antimicrobial potential of aqueous, benzene and Ethanolic extracts of the green leaves of common herbs like Acalypha indica, Sesbania grandiflora, Alternanthera sessilis, Amaranthus viridis, Mentha piperita and Coriandrum sativum was evaluated by well diffusion method against Pseudomonas aeruginosa, Aeromonas hydrophila, Salmonella typhi, Klebsiella pneumonia, Vibrio cholera and Bacillus subtilis. Ethanolic extracts of all the plants exhibited higher antimicrobial potential than benzene and aqueous extracts. Ethanolic extract of A.indica produced maximum zone of inhibition against K.pneumonia, whereas ethanolic extracts of S.grandiflora, A.sessilis, A.viridis, M.piperita and C.sativum were more potent in resisting B.subtilis, S.typhi, K.pneumonia, S.typhi, V.cholera respectively. Thus dietary intake of green leafy vegetables not only provides nutrients but also act as health boosters enhancing the body's immune system to combat a wide range of pathogens.

KEYWORDS: Antimicrobial Activity, Acalypha indica, Sesbania grandiflora, Alternanthera sessilis, Amaranthus viridis, Mentha piperita, Coriandrum sativum.

INTRODUCTION

According to an estimate of world health organization (WHO) about 80% of the world population still uses herbs and other traditional medicines for their health care needs.[1] More than two thirds of the world's plant species - at least 35,000 of which are estimated to have medicinal value come from the developing countries. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants.[2]

Due to the indiscriminate use of antimicrobial drugs the microorganisms have developed resistance to many antibiotics. This has created immense clinical problem in the treatment of infectious diseases.[3] In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppression and allergic reactions.[4] Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. So the use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are combing the earth for phytochemicals that could be developed for treatment of various diseases. One approach is to screen local medicinal plants for possible antimicrobial properties. Medicinal herbs represent a rich source from which novel antibacterial and antifungal chemotherapeutic agents may be obtained.

"Food as Medicine" is one of the basic concepts of traditional Siddha Indian Medicine. The house hold recipes containing greens are part of Tamil culture. Even today one can find preparations containing greens (Keerai in Tamil) in the regular diet of South Indians. This tradition is passed through generations because of the immense medicinal properties which the greens posses. [5] Plants like *Acalypha indica, Sesbania grandiflora, Alternanthera sessilis, Amaranthus, Mentha piperita* and *Coriandrum sativum* are common green leafy vegetables with medicinal properties consumed by mankind as food and medicine.

Acalypha indica (Family-*Euphorbiaceae*) is an annual herb, commonly consumed as vegetable and traditionally used to treat bronchitis, asthma, pneumonia, rheumatism, vomiting, skin disease and cold.[6] *Sesbania grandiflora* (Family-*Fabaceae*) is a fast growing tree, leaves are regular and rounded and the flowers are white and red in colour. The flowers and young leaves are edible and are often used as a vegetable to supplement meals. In folk remedy, it is used for bruises, catarrh, dysentery, fevers, headaches, smallpox, sores, sore throat and stomatitis. It is a potential antidote for tobacco and smoking-related diseases. [7] *Alternanthera sessilis* (Family-*Amaranthaceae*) is a perennial herb, the leaves and young shoots are used as vegetable, the plant is used to treat gastrointestinal problems, headaches, bronchitis, asthma, vertigo, wounds and diabetes.[8,9]

Amaranthus viridis (Family-*Amaranthaceae*) is an annual herb, the leaves and stems are consumed as vegetables. It is used to treat anorexia, leprosy, blood diseases, burning sensation, bronchitis, piles and leucorrhoea.[10] *Mentha piperita* (Family-*Lamiaceae*) is a

herbaceous rhizomatous perennial plant, besides its use in food it is also used in herbal tea preparations, confectionaries and as medicine. It is used to treat nausea, bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints.[11] *Coriandrum sativum* (Family-*Apiaceae*) is a soft plant growing to 50cm tall. All parts of the plant are edible, but the fresh leaves and dried seeds are the parts most traditionally used in cooking. It is used in the house hold medicine preparation to cure bed cold, seasonal fever, nausea and stomach disorders.[12]

The present study was carried out to evaluate the antimicrobial potential of the commonly used green leafy vegetables like Acalypha indica, Sesbania grandiflora, Alternanthera sessilis, Amaranthus viridis, Mentha piperita and Coriandrum sativum.

MATERIALS AND METHODS

Collection of the Plant

The plant leaves were collected from the natural habitat, washed repeatedly with tap water in order to remove the adhered dusts, shade dried for 4 to 5 days and ground well to powder. The powders were packed in air tight container and stored in a refrigerator.

Preparation of plant extracts

5grams each of the plant powder was soaked in ethanol, Benzene and distilled water separately. The soaked material was shaken frequently and kept undisturbed for 48 hours. At the end of the soaking period the contents were passed through Whatman filter paper No.1. The filtrates obtained were concentrated by keeping in a water bath at 60^{o}C. The concentrated extracts were then stored in air tight vials in the refrigerator.

Preparation of inoculum

Stock cultures for *Pseudomonas aeruginosa, Aeromonas hydrophila, Salmonella typhi, Klebsiella pneumonia, Vibrio cholera* and *Bacillus subtilis* were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Muller-Hinton broth (MHB) and incubated without agitation for 24 hrs at 37°C.

Antimicrobial susceptibility test

Well diffusion method was used to screen the antimicrobial activity.[13] Three wells of 6 mm diameter were punched off into medium with sterile cork borer and filled with 50µl of plant extracts by using micro pipette in each well in aseptic condition. Plates were then kept in a

refrigerator to allow pre-diffusion of extract for 30minutes. Further the plates were incubated in an incubator at 37^0C for 24hours.The antimicrobial activity was evaluated by measuring the zone of inhibition in millimeters.

RESULTS AND DISCUSSION

Table-1. Antimicrobial Potential of Plant Extracts against different Bacteria. (Zone of Inhibition in mm)

Plant	Extract	*Klebsiella pneumonia*	*Vibrio cholerae*	*Bacillus subtilis*	*Salmonella typhi*	*Pseudomonas aeruginosa*	*Aeromonas hydrophila*
Acalypha indica	A	-	-	-	-	-	-
	B	-	9	28	12	9	8
	E	17	4	10	12	11	12
Sesbania grandiflora	A	-	8	-	-	-	8
	B	10	7	16	12	8	9
	E	8	14	16	11	10	12
Alternanthera sessilis	A	-	-	-	-	8	8
	B	9	15	15	22	7	8
	E	7	14	8	21	9	9
Amaranthus viridis	A	-	7	-	10	-	8
	B	18	14	-	15	9	11
	E	17	12	14	17	12	13
Mentha piperita	A	-	7	-	12	9	7
	B	12	9	28	13	8	10
	E	7	8	14	25	10	15
Coriandrum sativum	A	13	12	-	8	7	8
	B	21	14	7	18	10	8
	E	11	23	9	8	14	13

A=Aqueous, B=Benzene, E=Ethanol

The antimicrobial potential of Aqueous, Benzene and Ethanol Extract of *Acalypha indica, Sesbania grandiflora, Alternanthera sessilis, Amaranthus viridis, Mentha piperita* and *Coriandrum sativum* was tested against the bacteria *like Klebsiella pneumoniae, Vibrio cholera, Bacillus subtilis, Salmonella typhi, Pseudomonas aeruginosa and Aeromonas hydrophila*. The zone of inhibition observed against each bacterium was measured and tabulated (Table-1).

In *Acalypha indica* only the Benzene and Ethanol extracts were potent in resisting the growth of pathogens. In Benzene extract more zone of inhibition (28mm) was observed against *Bacillus subtilis*, whereas ethanolic extract is effective against *Klesiella pneumoniae, Bacillus subtilis, Salmonella typhi, Pseudomonas aeruginosa* and *Aeromonas hydrophila*. In

general extracts of *Acalypha indica* was effective in resisting the growth of *Bacillus subtilis and Salmonella typhi*.

The benzene and ethanolic extract of *Sesbania grandiflora* exhibited more potential in resisting the growth of all tested bacteria. Both ethanol and benzene extracts formed more zone of inhibition against *Bacillus subtilis* (16mm), Benzene extract was more potent against *Klebsiella pneumoniae, Basillus subtilis and salmonella typhi*, whereas ethanolic extract effectively resisted the growth of *Vibrio cholera, Bacillus subtilis, Aeromonas hydrophila, Salmonella typhi* and *Peudomonas aeruginosa*. In general the extracts of *Sesbania grandiflora* were effective against *Bacillus subtilis* and *Salmonella typhi*.

Aqueous extracts of *Alternanthera sessilis* did not show any noticeable antimicrobial potential. However Benzene and Ethanol extract exhibited more antimicrobial potential. Benzene extracts formed maximum zone of inhibition (22mm) against *Salmonella typhi*, whereas Ethanolic extract also formed maximum zone of inhibition (21mm) against *Salmonella typhi*. In short, *Alternanthera sessilis* extracts resists the growth of *Salmonella typhi, Bacillus subtilis, Vibrio cholerae* effectively.

In *Amaranthus viridis* also the Aqueous extract was less potent and Ethanolic extract produced more antimicrobial potential. The Ethanolic and Benzene extract exhibited effective resistance against the growth of almost all tested bacteria. Maximum zone of inhibition was formed in Benzene extract against *Klebsiella pneumonia* (18mm), and in Ethanol extract it was 17mm against *Klebsiella pneumoniae* and *Salmonella typhi*. In general *Amaranthus viridis* was more effective in resisting the growth of *Klebsiella pneumoniae, Vibrio cholera, Salmonella typhi, Pseudomonas aeruginosa* and *Aeromonas hydrophila,* especially *Klebsiella pneumonia* and *Vibrio cholera.*

The Aqueous, Benzene and Ethanolic extracts of *Mentha piperita* were potent in resisting the growth of tested pathogens. However Benzene and Ethanol extract resisted more effectively than Aqueous extract. The Benzene extract resisted the growth of *Bacillus subtilis, Salmonella typhi, Klebsiella pneumonia* and *Aeromonas hydrophila,* whereas Ethanol extract resisted the growth of *Salmonella typhi, Aeromonas hydrophila, Bacillus subtilis* and *Pseudomonas aeruginosa*. Maximum zone of inhibition formed in Aqueous extract was 12mm against *Salmonella typhi*, in Benzene extract it was 28mm for *Bacillus subtilis* and in Ethanol extract the zone of inhibition was 25mm against *Salmonella typhi*. In general all

extracts of *Mentha* can effectively curb the growth of *Salmonella typhi, Psedomonas aeruginosa, Aeromonas hydrophila* and to some extent *Vibrio cholerae*. In short, extracts of *Mentha* effectively resisted the growth of *Bacillus subtilis* and *Salmonella typhi.*

The only plant in which all the three extracts were potent against *Klebsiella pneumonia* and *Vibrio cholera*e was *Coriandrum sativum*. However ethanolic extract, followed by Benzene extract were more potent than Aqueous extract. Benzene extract formed more zone of inhibition against *Klebsiella pneumonia, Vibrio cholera* and *Salmonella typhi*, whereas ethanolic extract formed more zone of inhibition against *Vibrio cholera, Peudomonas aeruginosa, Aeromonas hydrophila and Klebsiella pneumoniae.*

DISCUSSION

Medicinal plants are used by large proportion of the Indian population. The reasons for this include (a) true improvement of disease conditions after herbal treatment (b) harmful side effects and the high cost of the other forms of treatment.

The word "Phyto" is the Greek word for plant. Phytochemicals are not only non nutritive plants chemicals that have protective or disease preventive properties but also protect human from a host of disease. Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins. Alkaloids and flavonoids have been used as antiviral, antibacterial, antimicrobial and anticancer agents. Phenolic and polyphenolics are the other groups of secondary metabolites. [14] The bioactive compounds of plants interfere in the metabolism of microbes. The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effect of the phytochemicals in them.[15]

Acalipha indica is rich in secondary metabolites like Alkaloids, saponins, sugars, Terpenoids, flavonoids, tannins, alkaloids, cardiac glycosides, steroids and phenols. The extract of *A.indica* has antimicrobial potential against *E.coli, S.aureus, B.subtilis* and *Pr.mirabilis.* [16,17] Presence of flavonoids, tannins, steroids, saponins, alkaloids, glycosides, anthraquinone, pholobatannins, terpenoids, proteins and carbohydrates were reported in *Sesbania grandiflora* and its extract was effective in resisting the growth of *P.aurigenosa, S.aureus, E.coli, C.albicans, A. niger.*[18,19] Presence of the secondary metabolites like alkaloids, steroids, terpenoids, glocosides, phenolic compounds, carbohydrates and saponins are reported in

Alternanthera sessilis[20] and its antimicrobial potential against *P. vulgaris, S. pyogenes, B.subtilis* and *S. typhii* was also evaluated.[21]

The extracts of *Amaranthus viridis* is positive for tannins, flavonoids, cardiac glycosides, alkaloids, saponins and proteins, and also effective in resisting the growth of *S.aureus, E.coli, F.solani* and *R.oligosporus.*[22] Qualitative phytochemical analysis has revealed the presence of alkaloids, tannins, flavonoids, steroids and phenols in *Mentha piperita.* Effective antimicrobial potential was also been recorded against *B. subtilis, S. pneumonia, E.coli, P.vulgaris, K.pneumonia.*[23] *Coriandrum sativum* is rich in flavonoids, saponins, carbohydrates, terpenoids, sterols and phenols and its extract has potentially resisted the growth of S.*typhi, S.aureus, B.cereus, Klebsiella* and *Candida.*[14]

The significant antimicrobial potential exhibited by the different plant extracts in the present study may be due to presence of rich amount of different secondary metabolites. Ethanol, however, is the most commonly used organic solvent by herbal medicine manufacturers because the finished products can be safely used internally by consumers of herbal extracts.[24] Ethanolic extracts of *A.indica* exhibited highest antimicrobial activity.[17] In our previous study on *Marsilea quadrifolia* we have observed that benzene and ethanolic extracts were potent in resisting the growth of different pathogens,[25] in the present study also ethanol extracts exhibited higher zone of inhibition.

Thus screening for antimicrobial potential of a plant not only will be helpful in the identification of new antimicrobial compound but also inculcates awareness about the conservation of plants.

CONCLUSION

From the present study it was inferred that the common green plants consumed primarily as food too provides significant potential to resist pathogenic microbes due to the presence of a wide range of secondary metabolites. In the present investigation it was observed that the ethanolic extracts of Acalypha indica, Sesbania grandiflora, Alternanthera sessilis, Amaranthus viridis, Mentha piperita and Coriandrum sativum has significant microbicidal potential against Klebsiella pneumoniae, Vibrio cholera, Bacillus subtilis, Salmonella typhi, Pseudomonas aeruginosa and Aeromonas hydrophila.

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