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Anti microbial activity of *Ocimum basilicum* and *Achyranthes aspera*

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ABSTRACT

The aim of the study was to investigate antibacterial and antifungal activity of plant extract taken from two different plants, *Achyranthes aspera* Linn., and *Ocimum basilicum* against *Streptomyces fulvissimus, Klebsiella pneumonia, Shigella flexneri, Escherichiae coli, Bacillus subtilis, Streptococcus pyogenes, Pseudomonus aeruginosa, Proteus mirabilis, Aspergillus niger, Candida albicans, Aspergillus flavus, Penicillium chrysogenum* using well diffusion method. The *in vitro* study revealed that ethanolic extract was more effective than aqueous extract. Plant extracts of *A.aspera* Linn. was reported to be more effective on fungal and bacterial species than *O.basilicum*.

Keywords: *Achyranthes aspera , Ocimum basilicum*, Aqueous extract, Ethanolic extract, Anti bacterial, Anti fungal.

INTRODUCTION

According to WHO report, 70-80 % of the world's population rely on non-conventional medicine mainly from herbal sources for their primary health care.[1] Ayurvedic, Yunani practitioner and Kabirajes use different parts of the plant to treat leprosy, asthma, fistula, piles, arthritis, wound, insect and snake bite, renal and cardiac dropsy, kidney stone, diabetes, dermatological disorders, gonorrhoea, malaria, pneumonia, fever, cough, pyorrhea, dysentery, rabies, hysteria , toothache etc. Ayurveda is one of the oldest medication system of disease prevention in the World and is called in its complete form under the name maharshi ayurveda. The World Health Organization has approved its efficacy[11].

The increase in microorganisms resistance to antibiotics, the use of antimicrobial drugs forced scientists to search for new antimicrobial substances from various sources including medicinal plants. The trend of using natural products has increased and the active plant extracts are frequently used for new drug discoveries and for the presence of antimicrobial substances.[2]

Phytochemical investigations revealed that the presence of sterols, alkaloids, saponins, cardiac glycosides, ecdysterone etc. *A. aspera* Linn. (family Amaranthaceae) and it is an annual, stiff erect herb, and found commonly as a weed throughout India.[3,4] The plant is used in indigenous system of medicine as emenagogue, antiarthritic, antifertility, laxative, ecbolic, anti-helminthic, aphrodisiac, antiviral, anti-plasmodic, antihypertensive, anti-coagulant, diuretic and anti-tumor. It is also useful to treat cough, renal dropsy, fistula, scrofula, skin rash, nasal, infection,

chron ic malaria, impotence, fever, asthma, piles and snake bites[5]. The root is astringent, diuretic and antispasmodic. It is used in the treatment of dropsy, rheumatism, stomach problems, cholera, skin diseases and rabies.[5,6] Many *in vitro* antimicrobial studies of different extracts of roots, leaves and stems of A. aspera has been carried out but none of them used as whole plant.[15,16]

Ocimum basilicum, commonly known as basil is a member of the family Lamiaceae.[7] It is used as food additive because of its flavoring properties. It is used in cosmetics, liqours and perfumes. It has also been used as a folk remedy to treat various ailments such as feverish illness, poor digestion, nausea, abdominal pain, gastroenteritis, migraine, insomnia, depression, gonorrhea, dysentery and chronic diarrhea[8]. Considering the aforesaid properties of two different plants a comparative analysis of their plant extracts was done for antibacterial and antifungal properties against *S.fulvissimus, K. pneumoniae, S. flexneri, E. coli, B.subtilis, S. pyogenes, P. aeruginosa, P. mirabilis and A. niger, C. albicans, A. flavus, P. chrysogenum*

METHODS

Collection

Fresh plants of *O.basilicum* and *A.aspera* were collected from the local area of Erode district, Tamilnadu. The plant materials were authenticated by Dr.P.Jayaraman Ph.D, Director, Plant Anatomy Research, Chennai, Tamilnadu, India and a voucher specimen no PARC/2014/2216 respectively. The whole plants were taken and washed under running tap water and rinsed with distilled water and air dried.

Extraction procedure

Fresh whole plants of *A. aspera* and *O. basilicum* were collected, dried under shade of room temperature for 15 days. In soxhlet extraction, dry powdered *A. aspera* and *O .basilicum* plants are placed in a thimble. The thimble is placed in an extraction chamber which is suspended above a flask containing the solvents (aqueous & ethanol) separately. At the end of the extraction process, the flask containing the solvent and extract is filtered and evaporated. [10]

Preparation of test organism.

S. fulvissimus, K.pneumonia, S. flexneri, E.coli, B. subtilis, S.pyogenes, P.aeruginosa, P. mirabilis and *A. niger, C. albicans, A.flavus, P. chrysogenum* were obtained from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh. The cultures of bacteria and fungi were sub-cultured on Nutrient Agar (NA) and Sabourds Dextrose Agar (SDA) slants respectively and stored at 4 ºC until required for study.

Anti-microbial activity [9,10]

The anti-microbial assay was performed by agar well diffusion method. For assessing the antibacterial activity of the prepared extracts, 0.6ml of standardized bacterial stock suspension was thoroughly mixed with 60 ml of sterile nutrient agar.[12,21] 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes.[13] The plates were allowed to dry for atleast 15 minutes. A sterile cork borer No.4 was used to make wells of 6 mm diameter in each plate for extracts. A total of 0.2ml of plant extract were poured into the wells with concentrations as 1000µg/ml, 500µg/ml, 250µg/ml, and incubated overnight at 37 ºC. Each test was repeated triplicate.[17,18,19] The obtained results are compared with the standard (Oxytetracycline). The same procedure was adopted for fungal species except SDA was used with the standard (Ketoconazole).

RESULTS AND DISCUSSION

The preliminary test screened for the phytochemicals such as alkaloids, flavonoids, triterpenoids, glycosides, steroids, tannins and saponins. The aqueous and ethanolic extract showed more active constituents particularly flavanoids as compared to other extract and was selected for the antimicrobial study. Various concentrations of aqueous and ethanolic extracts (1000µg/ml, 500µg/ml, 250µg/ml) were used for testing the antimicrobial activity. The results were shown in the table1.

The results indicated that, the extracts obtained from the plants showed inhibition of growth against tested microorganisms. Successful prediction of extracted compounds from plant materials largely dependent

on the type of solvent used in the extraction procedure. The traditional practitioners make use of water as a primer solvent, but on the first observation ethanol was a better solvent for extracting antimicrobial substance. Ethanolic extract of *A.aspera* reported to be more effective against fungal and bacterial species showing highest inhibition such as 18mm against *A.niger*, 16 mm against *C.albicans*, 21 mm against *B. subtilis*, 20 mm against *E.coli* and *S. fulvissimus*. The ethanolic extract of *O.basilicum* was more effective against bacteria such as 13mm against *B.subtilis*, 11 mm against *S. fulvissimus* than the fungal species such as 11 mm against *C.albicans* and 07 mm against A.flavus. The overall comparative studies showed that the ethanolic extract of *A.aspera* showed highest degree of antimicrobial activity than ethanolic extract of O. basilicum.

Comparative analysis of antibacterial and antifungal activities of these plant extracts against these microorganisms indicated that there is possibility of discovering alternative antibiotic substance in these plants for the development of newer antimicrobial agents.

Table 1: Preliminary Phytochemical Screening

Phytoconstituents	***A.aspera***				***O.basilicum***			
Solvents	Water	Ethanol	Ethyl acetate	$CHCl_3$	Water	Ethanol	Ethyl acetate	$CHCl_3$
Flavanoids	+	+	+	+	+	+	+	+
Tannins	+	+	-	-	+	+	-	-
Alkaloids	+	+	+	-	+	+	+	-
Glycosides	-	+	-	-	+	+	-	-
Saponins	+	+	-	-	+	+	-	-
Steroids	+	+	-	-	-	-	-	-
Volatile oil	-	-	-	-	+	+	+	+

Table: 2 *In vitro* Anti bacterial assay of Aqueous and Ethanolic plant extracts of *A.aspera* and *O.basilicum*

Test organism	**Zone of inhibition(mm) of *A.aspera***						**Zone of inhibition (mm) of *O.basilicum***						**Oxytetracycline (mg/ml)**
	Aqueous extract(µg/ml)			Ethanolic extract (µg/ml)			Aqueous extract (µg/ml)			Ethanolic extract (µg/ml)			
	1000	500	250	1000	500	250	1000	500	250	1000	500	250	1
E.coli	18	11	06	20	13	09	06	04	02	08	06	03	24
K.pneumoniae	15	08	07	17	11	08	04	03	00	06	04	02	22
P.mirabilis	17	11	09	19	14	12	05	03	02	07	05	03	20
S.flexneri	15	13	08	18	15	11	08	05	02	10	07	04	25
S.pyogenes	14	10	06	17	12	08	05	03	00	07	0	02	28
S.fulvissimus	17	15	10	20	17	12	09	06	04	11	09	06	25
B.subtilis	19	12	08	21	14	10	10	08	05	13	10	07	26
P.aeruginosa	10	00	00	12	02	00	07	04	00	09	06	02	25

Table: 3 *In vitro* Antifungal assay of Aqueous and Ethanolic Plant extracts of *A.aspera* and *O.basilicum*.

Test organism	Zone of inhibition(mm) of *A.aspera*						Zone of inhibition(mm) of *O.basilicum*						Ketoconazole (mg/ml)
	Aqueous extract(µg/ml)			Ethanolic extract (µg/ml)			Aqueous extract (µg/ml)			Ethanolic extract (µg/ml)			
	1000	500	250	1000	500	250	1000	500	250	1000	500	250	1
C.albicans	14	04	02	16	06	03	09	04	02	11	06	03	18
A.niger	16	08	04	18	10	06	08	04	00	10	05	02	20
A.flavus	13	08	03	15	12	08	05	03	02	07	05	03	18
P.chrysogenum	14	11	05	17	14	09	03	02	00	06	04	03	17

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