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Research Article

STUDIES ON BIO EFFICACY OF WEEDS IN TANJORE DISTRICT, TAMILNADU, INDIA

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ABSTRACT

The present study was aimed to investigate the bio efficacy i.e., antibacterial, antifungal and free radical scavenging activity of ten common weeds which were collected in Tanjore district of Tamil Nadu. The selected weeds were *Acalypha indica, Amaranthus spinosus, Argemone mexicana, Cardiospermum halicacabum, Cassia angustifolia, Corchorus olitorius, Euphorbia pulcherrima, Hyptis suaveolens, Leucas aspera* and *Solanum trilobatum.* The weeds were initially screened for the presence of various phytoconstituents using aqueous extracts. Methanolic extracts of the samples were prepared by cold percolation technique. Antibacterial activity was carried by disc diffusion method against *Bacillus subtilis, Klebsiella pneumoniae, Salmonella typhi* and *Vibrio cholerae* in which *Acalypha indica* and *Leucas aspera* showed significant activity against 3 out of 4 strains used. Antifungal activity was carried out by well diffusion method against *Aspergillus niger, Aspergillus flavus* and *Candida albicans* in which none of the methanolic extracts of the weeds showed any activity. The free radical scavenging activity (FRSA) was tested against DPPH (2, 2-diphenyl-1-picryl hydrazyl) and the highest FRSA was found for *Cardiospermum halicacabum* with an IC_{50} value of 0.532mg/mL.

Keywords: Weeds, Phytochemistry, Antioxidant activity, Free radicals, DPPH, Antibacterial activity, Antifungal activity, Bio efficacy.

INTRODUCTION

A weed is commonly defined as a plant that grows out of place and is competitive, persistent and pernicious[1]. Weeds are also found to be resistant to most of the microbial disease when compared to the crops that shows disease symptoms[2]. World Health Organization (WHO) has reported that more than 80 % of the world's population relies on the traditional medicine for their primary healthcare. This is because the plant kingdom represents an enormous reservoir of biologically active compounds called phytochemicals. There is currently immense interest in natural antioxidants and their role in human health and nutrition. Similarly, Due to increased evolution of multiple antimicrobial resistant strains of organisms, there is need for continuous search for new antimicrobial agents[3]. Plants contains secondary metabolites which has anti-infective properties that be used as substitutes for antibiotics resistant to pathogenic bacteria and fungi.

Since ancient times, plants have been utilized as an important source of medicines as they are a reservoir of chemical agents[4] they have always been a common source of medicaments, either in the form of traditional preparations or as pure active principles, it is reasonable to make use of locally available plants like weeds that could help replace the pharmaceutical preparations. Hence, the present study was conducted to know the bio-efficacy, i.e. antibacterial, antifungal and anti-oxidant properties of common weeds present in Tanjore District of Tamil Nadu state, India. Further, major phytoconstituents of the plants were also analyzed.

MATERIALS AND METHODS

Source

Ten different weeds namely *Acalypha indica, Amaranthus spinosus, Argemone mexicana, Cardiospermum halicacabum, Cassia angustifolia, Corchorus olitorius, Euphorbia pulcherrima, Hyptis suaveolens, Leucas aspera* and *Solanum trilobatum* were collected from different parts of Tanjore district of Tamil Nadu State in India. Healthy leaves of the weeds were used for the study. Bacterial cultures of *Bacillus subtilis* (MTCC121), *Klebsiella pneumonia* (MTCC109), *Salmonella typhi* (MTCC733) and *Vibrio cholera* (MTCC3906) were procured from IMTECH, Chandigarh, India. Fungal cultures of *Aspergillus niger* (MLRDAN08) and *Aspergillus flavus* (MLRDAF03) belonging to culture collection of R&D, Marina Labs and *Candida albicans* (MTCC 183) procured from IMTECH, Chandigarh were used for the study.

Sample preparation

The healthy leaves of the collected weeds were cleaned thoroughly in running tap water. They were shade-dried for 4 days. The dried leaves were made powder using electric blender and stored for further use.

Preparation of plant – methanol extracts

The methanol-plant extract was prepared using cold-percolation method. To 10g of each dried pulverized sample 150ml of methanol was added and stirred in temperature-controlled shaker at 30 ± 2°C. After 48 hours the extract was filtered and concentrated using rotary evaporator. This extract was used for screening antibacterial, antifungal and anti-oxidant properties.

Phytochemical analysis

The dried pulverized plant material (1-5g) was extracted with double distilled water. The aqueous extracts were filtered using Whatman No.1 filter paper and the qualitative phytochemical analysis for the presence of tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides and steroids was carried out immediately without storage according to standard procedures[5].

Antibacterial activity

The methanol extracts of the 10 weeds were screened against 4 bacterial strains: *Bacillus subtilis* (MTCC121), *Klebsiella pneumonia* (MTCC109), *Salmonella typhi* (MTCC733) and *Vibrio cholera* (MTCC3906). The cultures for screening were maintained in saline and were suitably diluted prior to use. Disc diffusion method[6] was used to screen the antibacterial activity using Mueller Hinton Agar (MHA). Onto the sterile MHA plates 0.1mL of the saline suspension was swabbed uniformly. Different concentrations of the extract (4.5, 6, 7.5 mg/disc) that were loaded prior a day on 5 mm sterile discs were placed on the medium along with the control disc streptomycin. The plates were incubated at 37°C for 24 hours. After the incubation period, inhibition zones formed around the discs were measured in millimetre. These studies were performed in duplicates for all the weed samples against the 4 bacterial cultures.

Antifungal activity

Well diffusion method was used to screen the weeds against three fungal species, *Aspergillus niger* (MTCC 9652), *Aspergillus flavus* (MTCC 9639) and *Candida albicans* (MTCC 183). Sabourauds Dextrose Agar (SDA) was used for *Candida albicans* whereas Potato Dextrose Agar (PDA) was used for *Aspergillus niger* and *Aspergillus*

flavus. Dispersed fungal spores (0.1mL) were added on SDA & PDA plates and were spread using UV sterilized cotton. *C. albicans* was dispersed in saline whereas the *Aspergillus* sp. were dispersed in distilled water. Three half wells were drilled with a cork borer and 15, 20 and 25µL of the plant extracts were added to the wells. The control disc (Ketoconazole) was placed in each plate at the bottom. All the plates were prepared in duplicate. The plates were incubated at 37°C for 72 hours.

DPPH free radical scavenging assay

The free radical scavenging activity of 10 plant methanolic extracts was measured using DPPH (2,2 diphenyl-1-picryl hydrazyl), employing the method of Blois (1958). To 0.5mL of methanolic extract of each plant and the reference compound in various concentrations (15, 7.5, 3.75, 1.87, 0.93 mg/mL), 0.5mL methanol and 0.5mL of 0.1mM solution of DPPH in methanol was added. After 30 minutes of incubation in dark condition at room temperature, absorbance was measured at 517nm using spectrophotometer. The same solution of DPPH in methanol was used as control, whereas BHA was used as reference.

Percentage inhibition was calculated using the formula:

% Inhibition = [(Control absorbance - Test absorbance)/ Control absorbance] x 100

RESULTS

Antibacterial activity

Out of ten weeds selected from Tanjore district, *Acalypha indica* and *Leucas aspera* showed significant antibacterial activity against bacterial species used. Among the ten weeds 5 showed positive results against *Klebsiella pneumoniae.* The maximum zone of inhibition was shown by the methanol extract of *Acalypha indica* (18mm) followed by *Leucas aspera* (12mm). Against *Bacillus subtilis* 6 weeds showed positive results. Out of these *Acalypha indica* and *Leucas aspera* exhibited maximum zone of inhibition (12mm). Among the weeds studied, 5 weeds inhibited the bacteria, *Salmonella typhi*. All of these showed a minimum zone of inhibition (7mm). *Vibrio cholerae* was inhibited by only 3 plant species. *Cardiospermum halicacabum* did not show anti-bacterial activity against any of the bacterial species used. The results are depicted in Table 1.

Antifungal activity

Zone of inhibition was not formed for any of the 3 species, i.e. Aspergillus niger, Aspergillus flavus and Candida albicans by any of weed extracts.

Free radical scavenging activity (FRSA)

From the above study, it is evident that the weeds were performed FRSA using DPPH. Table 2 shows the % inhibition and IC_{50} values obtained by the weeds. It can be seen that, *Cardiospermum halicacabum* showed maximum percentage inhibition at the least concentration i.e., 88.23% inhibition at 0.937mg/mL and obviously showing the least IC_{50} value of 0.532mg/mL. Next to that, *Acalypha indica* shows the same percentage inhibition at 1.875mg/mL with an IC_{50} value of 0.732mg/mL. This value is same as that of the reference compound butylated hydroxyl anisole (BHA). With the exception of *Solanum trilobatum (*IC_{50} value=6.356mg/mL), possessing the least value of the selected samples, other extracts show moderate % inhibition and IC_{50} values. The values recorded for the assay is presented in Table 2.

Phytochemical analysis

The tests for the presence of phytochemicals were carried out on aqueous extracts prepared from each weed sample and the results are reported in the Table 1. It shows that out of the ten selected weeds, 70% weeds showed the presence of saponins, 50% flavonoids, 40% steroids, 30% Tannins and terpenoids, 20% phlobatannins and 10% cardiac glycosides. The aqueous extracts belonging to the weeds have given positive indication for Cardiac glycosides, Flavonoids, Saponins, Steroids, Tannins, Phlobatannins and Terpenoids.

DISCUSSION

The bio efficacy of the plant is attributed to its phytoconstituents and it is clearly evident in this present study. The bio efficacy of the common weeds studied from the Tanjore district of Tamil Nadu has showed significant difference in antibacterial, antifungal and anti-oxidant properties.

Out of ten weeds studied *Acalypha indica* and *Leucas aspera* showed good inhibition against 3 out 4 bacterial species used. *Salmonella typhi* was not inhibited by the control Streptomycin but 50% of weeds showed activity against the same pathogen. 50% of the weeds showed inhibition of *Klebsiella pneumoniae* species and 30% proved to control *Vibrio cholerae*. *Bacillus subtilis* was inhibited by 60% of plants. The plant extracts were more active against the Gram-positive microorganisms than against the Gram-negative microorganisms. This is in agreement with Vlietinck *et al*[7]. Flavonoids[8], terpenoids[9], tannins and phlobatanins[10] are the phytochemicals that have been demonstrated to have antimicrobial activity.

Traditional healers use primarily water as the solvent but according to Nair *et al*[11] the plant extracts in organic solvent (methanol) provided more consistent antimicrobial activity compared to those extracted in water. Many studies confirm that methanolic extracts of the plant showed more antibacterial activity among the other solvents when compared[12, 13]. Besides, methanolic extracts also possess the ability to dissolve or diffuse in different media[14].

It is seen that *Cardiospermum halicacabum* possess highest FRSA among the selected samples. It is closely followed by *A. indica, C. angustifolia* and *E. pulcherrima.* Previous reports[15-17] concluded that the FRSA in these samples could be attributed to the presence of polyphenols. Polyphenols such as tannins and flavonoids can absorb or neutralize free radicals, quench singlet and triplet oxygen or decompose peroxides[18]. They are known to contain potent antioxidant, anti-inflammatory and cancer-preventive activities and also retard the oxidative degradation of lipids.

Weeds are home to many types of phytochemicals, which are the reason for the protective property of weeds against pests[19]. The presence of Flavonoids and Saponins in the plant extracts indicates that they could be used for antifungal activity. Herbs rich in tannins are used for treating intestinal disorders such as diarrhoea and dysentery[20].

Table 1: Zone of Inhibition (In mm) recorded for weed sample against the Bacteria

S. No.	Plant Name	*Klebsiella pneumoniae* (MTCC109)			*Bacillus subtilis* (MTCC121)			*Salmonella typhi* (MTCC733)			*Vibrio cholerae* (MTCC3906)		
		Extract in µl/disc											
		15	20	25	15	20	25	15	20	25	15	20	25
1	*Acalypha indica*	10	11	18	8.5	10.5	12	-	-	-	8	8	8.5
2	*Amaranthus spinosus*	-	-	-	6.5	6.5	6.5	6.5	6.5	7	6.5	6.5	7
3	*Argemone mexicana*	-	-	-	7	7	7	7	7	7	-	-	-
4	*Cardiospermum halicacabum*	-	-	-	-	-	-	-	-	-	-	-	-
5	*Cassia angustifolia*	-	-	-	7.5	8	8.5	6.5	7	7	-	-	-
6	*Corchorus olitorius*	-	-	-	-	-	-	6.5	6.5	7	-	-	-
7	*Euphorbia pulcherrima*	8	9	10	-	-	-	-	-	-	-	-	-
8	*Hyptis suaveolens*	7	7	7.5	7	7	8	7	7	7	-	-	-
9	*Leucas aspera*	11	11.5	12	8.5	11.5	12	-	-	-	8.5	9	9.5
10	*Solanum trilobatum*	7	7.5	7.5	-	-	-	-	-	-	-	-	-

- indicates absence of zone

Table 2: Inhibition percentage of the plant extracts & BHA to DPPH and their respective IC_{50} values at different concentrations in mg/mL

Species	15mg/mL	7.5mg/mL	3.750mg/mL	1.875mg/mL	0.937mg/mL	IC_{50} values
Acalypha indica	-	-	32.35	88.23	64.70	0.732
Amaranthus spinosus	-	70.58	41.17	29.41	14.70	5.357
Argemone mexicana	35.29	67.60	41.17	20.58	17.64	5.597
Cardiospermum halicacabum	-	-	-	23.52	88.23	0.532
Cassia angustifolia	-	-	26.47	41.17	50	0.938
Corchorus olitorius	-	94.11	38.23	23.52	17.64	3.989
Euphorbia pulcherrima	-	-	-	26.47	50	0.938
Hyptis suaveolens	-	-	85.29	50	26.47	2.206
Leucas aspera	-	-	52.94	64.70	32.35	3.544
Solanum trilobatum	41.17	58.82	32.35	23.52	17.64	6.356
BHA (reference)	35.29	64.70	82.35	88.23	94.11	0.499

Table 3: Prevalence of Phytochemical constituents in weeds of Tanjore

Species	Phytochemicals						
	Cardiac Glycosides	Tannins	Phlobat annins	Flavanoids	Terpenoids	Saponins	Steroids
Amaranthus spinosus	+	-	-	+	-	+	-
Cassia angustifolia	-	+	+	-	+	+	+
Acalypha indica	-	-	-	+	-	+	-
Euphorbia pulcherrima	-	+	+	-	+	+	+
Hyptis sauveolens	-	-	-	+	-	-	-
Leucas aspera	-	+	-	-	-	+	+
Corchorus olittorius	-	-	-	-	-	+	-
Argemone mexicana	-	-	-	-	-	-	+
Cardiospermum halicacabum	-	-	-	+	+	-	-
Solanum trilobatum	-	-	-	+	-	+	-

+ indicates presence and - indicates absence

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