Research Article

Phytochemical and Antimicrobial Screening of Some Weeds of Asteraceae Family and Widely Known Medicinal Herb *Paederia foetida* L.

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ABSTRACT

The four common weeds are selected for the screening of antimicrobial activity, of which 3 species belongs to Asteraceae family and 1 to Rubiaceae. Extract from plant samples, *Ageratum conyzoides* L.,*Mikania micrantha* H.B.& K., *Perthenium hysterophorus* and *Paederia foetida* L. were screened against 9 Gram negative bacteria, 3 Gram positive bacteria and 1 fungal isolate. Agar well diffusion method was used and an antibiotic disc of Ceftazidime was used as control and the leaf extracts of the samples prepared in 60% Alcohol and distilled water are poured into the well. The antibacterial potential of different leaf extracts was analyzed and determined by comparing with the antibacterial potential of the standard drug. The results reveals that the highest antibacterial property is shown by *Mikania micrantha* H.B. & K., followed by *Ageratum conyzoides* L., *Paederia foetida* L. and *Parthenium hysterophorus*. Moreover the fresh sample of the plant extracts shows less activity than the dry samples. It has been also observed that the alcohol extracts has more activity than the water extracts. The highest inhibition zone was recorded for *Parthenium hysterophorus* which is 20 mm. against *B. cereus* and the lowest was recorded for *Mikania micrantha* H.B. & K. which is 1mm. against *S. flexneri.* This antimicrobial screening of various leaf extracts reveals that they possess certain range of antimicrobial property. This differential result may be due to the presence of different biochemical compounds in the extracts which could be confirmed by further analysis.

Keywords: Antimicrobial activity, Asteraceae, Inhibition zone, Rubiaceae, Agar Well diffusion.

INTRODUCTION

Plants are very rich in various kinds of chemical constituents and widely used as traditional medicine. The use of different plants as medicine is an age old practice. According to World Health Organization[1], medicine plants would be the best source to obtain a variety of drugs and is the most effective way of curing different diseases without any side effect. In India most of the plants have medicinal properties. These medicinal plants are natural resources and are potential safe drugs[2]. Most of the microbial pathogens are now able to develop resistance against different commercially available antimicrobial agents.

Because of this more attention is given in discovering more and more effective but less toxic antimicrobial agents. As a result different plants have been tested for antimicrobial properties to develop less toxic and effective antimicrobial agents without any side effects. The use of herbs as complementary and alternative medicine has dramatically increased in the last 20-25 years[3].

Weeds are commonly defined as plants that grow out of place and is competitive persistent and pernicious[4]. Invasive weeds possess a variety of characteristics that enable them to disperse rapidly into new areas and out compete crops and native or desirable non-native vegetation for light water, nutrient and space[5] and are used as traditional medicine in most of the developing countries. They are found to be resistant to most of the microbial diseases when compared to cultivated crops[6]. These antimicrobial properties of weeds encouraged many workers to find out the cause behind such potentiality of the weeds. Antimicrobial activity of different weeds has been extensively studied in different parts of the world[7-13].

The use of plant extract with known antibacterial activity can be of great importance in disease prevention. The main aim of these study is to test the antibacterial and antifungal properties of four commonly available weeds namely- *Ageratum conyzoides* L., *Mikania micrantha* H.B.& K., *Parthenium hysterophorus and Paederia foetida* L. against 12 bacterial strains (both gram positive and gram negative) and a single fungal strain *Candida albicans*.

***Ageratum conyzoides* L.**

It is native to tropical America and considered as invasive weed. It is an annual herb about 50-100cm, sometimes less than 10cm. Leaves are often with axillary abortive buds. As a medicinal plant it has limited use due to its toxicity. It is also used as insecticide and nematicide as it contains certain chemicals.

***Mikania micrantha* H.B. & K.**

It is a widespread weed in the tropics. These are vines and perennial plants, stem usually twining to scrambling and branched. Leaves are cauline opposite and petiolate.

Florets 4, corollas usually white sometimes pink to rose or purplish. The extracts from Mikania slow the germination of variety of plant species.

Parthenium hysterophorus

It is a species of flowering plant in the aster family, Asteraceae, that is native to American tropics[14]. It invades all disturbed land. This are annual herbs about 30-120 cm. leaf blades ovate to elliptic, 2-pinnately lobed lobes lanceolate to linear. Heads obscurely radiate, borne in open, paniculi form arrays. Pistillate florets 5 and disc florets 12.

***Paederia foetida* L.**

It is native to tropical Asia and has great value as folk medicine. Strong sulphurous odour exuded when its leaves or stems are crushed or bruised.

The oil responsible for the smell is found primarily within the leaves which contain sulphur compound including largely disulphide. Leaf stalks are commonly up to 6cm long. The flowers are small, greyish pink or lilac in colour. The petals are joined to form a corolla with 5 spreading lobes.

MATERIALS AND METHODS

Collection of Materials and Preparation of Extracts

All the three plant samples were collected from the RMRC campus, Dibrugarh. The fresh leaves were first washed with tap water and then rinsed thoroughly with distilled water. Few leaves from each sample were dried in the hot air oven for 72 hours at 40°C. Two solvents aqueous and 60% ethanol were used for preparation of the extract and both fresh and dried leaves extract were used for the test. Fresh leaf extract was prepared by crushing the fresh leaves and dissolving 5g in 100ml water for aqueous extract and 100ml of 60% ethanol for ethanol extract. Oven dried leaves were crushed and 1g of each leaf sample was weighed. These leaves were dissolved in 10ml of water and 60% ethanol separately. The combination was allowed to settle at room temperature for 24 hours. Both the fresh and dried leaves extract were filtered using Whatman no.1 filter paper and poured in air tight bottle and stored in 4°C refrigerator for further use.

Qualitative Phytochemical Analysis of the Plant Extracts

Qualitative Phytochemical analysis of the four different plant extracts (both water and alcoholic) for alkaloids, tannins, flavonoids, terpenes, glycosides and saponins were performed following the given standard methods:

Alkaloids

Presence of alkaloid is detected by adding few drops of Meyer's reagent to the extracts. Occurrence of cream colour precipitation indicates the presence of alkaloids (Siddique and Ali, 1997).

Tannins

1ml of 5% ferric chloride is added to the extract and formation of bluish black or greenish black precipitate indicate the presence of Tannins.

Flavonoids

Few drops of 10% concentrated H_2SO_4 was added to the extract, followed by 1ml of ammonia formation of greenish yellow ppt. indicate the presence of flavonoids**.**

Terpenes

5ml chloroform and 2ml conc. H_2SO_4 was added to 2ml of extract. Reddish brown coloration indicate the presence of terpenes (Harbourne, 1971).

Glycosides

Few ml of Extract was taken and 2ml of glacial acetic acid was added. Few drops of 5% $FeCl_3$ and conc. H_2SO_4 were added to the extract.

Saponins

20ml water was added to 150mg extract and shaken vigorously. Layer of foam formation indicates the presence of saponins .

Preparation of Test Organism

The plant extracts were screened against 13 bacterial strains and one fungal strain. Isolates of gram negative bacteria *Salmonella typhimurium*ATCC51812*, Proteus vulgaris* ATCC8427*, Escherichia coli* ATCC 25922*, Shigella flexneri* ATCC9799*, Edwardsiella tarda* ATCC 15947, *Shigella sonnei* ATCC 9290, *Salmonella enteritidis* (D) ATCC 13076*, Salmonella paratyphi* A (A) ATCC 9150 and *Klebsiella pneumonia* ATCC 10031 and gram positive bacteria *Staphylococcus aureus* ATCC 29213*, Bacillus cereus* ATCC 11778 and *Listeria monocytogenes* (4b)ATCC 13932.and fungal strain *Candida albicans* ATCC 10231 were taken for the test. ATCC strains were purchased from Hi Media, India and maintained as glycerol stock at Regional Medical Research Centre(ICMR), N. E. Region and were subcultured in Nutrient Agar and Mac Conkey agar. 0.5 McFarland standard of each bacterium was prepared using normal saline.

Antibacterial Assay

Agar well diffusion[15,16] technique was used to determine the antibacterial activity of different plant extracts. *In vitro* antibacterial and antifungal activity of plant extracts were screened on MH Agar. A sterile cork borer of 7 mm diameter (Hi Media) was used to cut four different wells on the surface of each agar plates. The wells were filled with the 3different leaf extracts of *Ageratum conyzoides, Mikania micrantha, Partheium hysterophorus and Paederia foetida*. One of the well was filled with solvent i.e. distilled water for aqueous extract and 60% ethanol for alcoholic extract which was used as negative control. An antibiotic disc of Ceftazidime (30 µg/ml) was used as positive control. The plates were then allowed to stand for proper diffusion of the extract and all the plates were incubated in 37°C for 24 hours and observed for zone of inhibition. A zone of clearance around each well signified

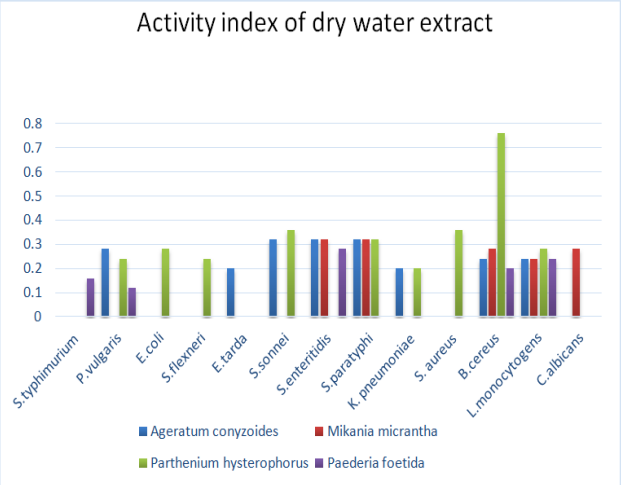
inhibition and the diameter of each zone was measured in millimeter using transparent scale.

Determination of Activity of Index[17]

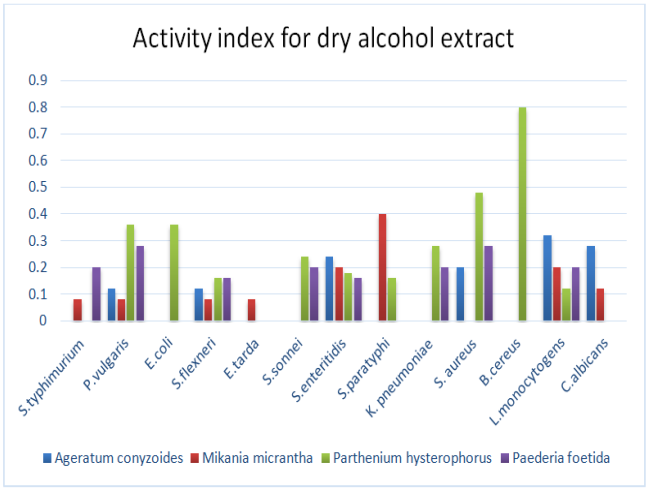
The Activity Index of the plant extract was determined as follows:

$$Activity\ Index\ (A.I) = \frac{Mean\ of\ zone\ of\ inhibition\ of\ the\ extract}{Mean\ of\ zone\ of\ inhibition\ of\ the\ standard\ antibiotic\ drug}$$

RESULTS AND DISCUSSION



Graph 1: Activity Index of Water Extract



Graph 2: Activity Index of Alcohol Extract

Table 1: Phytochemical Analysis of Plant Extracts

Phytochemicals	*Ageratum conyzoides*		*Mikaniamicrantha*		*Paederiafoetida*		*Parthenium hysterophorus*	
	Water Extract	Alcohol Extract	Water Extract	Alcohol Extract	Water Extract	Alcohol Extract	Water Extract	Alcohol Extract
Glycosides	+	+	+	+	-	-	+	+
Tannins	+	+	+	+	+	+	+	-
Flavonoides	-	+	+	+	+	-	-	+
Terpenes	+	+	-	-	_	-	-	-
Saponins	+	+	+	+	+	+	+	+
+ = Present					- = Absent			

Zone of Inhibition

Photographs showing Antibacterial Activity of Extract against Bacteria

Table 2: Measurement of Zone of Inhibition

a) ***Ageratum conyzoides***

Bacteria (Gram negative)	Extract	Diameter of zone of inhibition (mm.)	
		FRESH	DRY
S.typhimurium	Water	No Zone	No Zone
	Alcohol	No Zone	No Zone
P.vulgaris	Water	6	7
	Alcohol	No Zone	3
E.coli	Water	No Zone	No Zone
	Alcohol	No Zone	No Zone
S.flexneri	Water	No Zone	No Zone
	Alcohol	2	3
E.tarda	Water	No Zone	5
	Alcohol	No Zone	No Zone
S.sonnei	Water	7	8
	Alcohol	No Zone	No Zone
S.enteritidis	Water	3	8
	Alcohol	No Zone	6
S.paratyphi	Water	No Zone	8
	Alcohol	No Zone	No Zone
K.pneumoniae	Water	No Zone	5
	Alcohol	No Zone	No Zone
Bacteria (Gram positive)	**EXTRACT**	**Diameter of zone of inhibition (mm.)**	
		FRESH	**DRY**
S.aureus	Water	No Zone	No Zone
	Alcohol	No Zone	5
B.cereus	Water	4	6
	Alcohol	No Zone	No Zone
L.monocytogens	Water	No Zone	6
	Alcohol	No Zone	8
FUNGUS *C. albicans*	Water	No Zone	No Zone
	Alcohol	No Zone	7

b) ***Mikania micrantha***

Bacteria (Gram negative)	EXTRACT	Diameter of zone of inhibition (mm.)	
		FRESH	DRY
S.typhimurium	Water	No Zone	No Zone
	Alcohol	No Zone	2
P.vulgaris	Water	No Zone	No Zone
	Alcohol	No Zone	2
E.coli	Water	No Zone	No Zone
	Alcohol	No Zone	No Zone
S.flexneri	Water	No Zone	No Zone
	Alcohol	1	2
E.tarda	Water	No Zone	No Zone
	Alcohol	No Zone	2
S.sonnei	Water	No Zone	No Zone
	Alcohol	No Zone	No Zone
S.enteritidis	Water	No Zone	8
	Alcohol	No Zone	5
S.paratyphi	Water	No Zone	8
	Alcohol	No Zone	10
K.pneumoniae	Water	No Zone	No Zone
	Alcohol	No Zone	No Zone
Bacteria (Gram positive)	**EXTRACT**	**Diameter of zone of inhibition (mm.)**	
		FRESH	**DRY**
S.aureus	Water	No Zone	No Zone
	Alcohol	No Zone	No Zone
B.cereus	Water	4	7
	Alcohol	No Zone	No Zone
L.monocytogens	Water	No Zone	6
	Alcohol	No Zone	5
FUNGUS C.albicans	Water	6	7
	Alcohol	No Zone	3

c) ***Parthenium hysterophorus***

Bacteria (Gram negative)	EXTRACT	Diameter of zone of inhibition (mm.)	
		FRESH	DRY
S.typhimurium	Water	No Zone	No Zone
	Alcohol	No Zone	No Zone
P.vulgaris	Water	No Zone	6
	Alcohol	No Zone	9
E.coli	Water	No Zone	7
	Alcohol	No Zone	9
S.flexneri	Water	No Zone	6
	Alcohol	No Zone	4
E.tarda	Water	No Zone	No Zone
	Alcohol	No Zone	No Zone
S.sonnei	Water	No Zone	9
	Alcohol	No Zone	6
S.enteritidis	Water	No Zone	No Zone
	Alcohol	No Zone	3
S.paratyphi	Water	No Zone	8
	Alcohol	No Zone	4
K.pneumoniae	Water	No Zone	5
	Alcohol	No Zone	7
Bacteria (Gram positive)	**EXTRACT**	**Diameter of zone of inhibition (mm.)**	
		FRESH	**DRY**
S.aureus	Water	No Zone	9
	Alcohol	5	12
B.cereus	Water	No Zone	19
	Alcohol	5	20
L.monocytogens	Water	No Zone	7
	Alcohol	No Zone	3
FUNGUS *C.albicans*	Water	No Zone	No Zone
	Alcohol	No Zone	No Zone

d) ***Paederia foetida***

Bacteria (Gram negatiive)	EXTRACT	Diameter of zone of inhibition (mm.)	
		FRESH	DRY
S.typhimurium	Water	No Zone	4
	Alcohol	No Zone	5
P.vulgaris	Water	7	3
	Alcohol	No Zone	7
E.coli	Water	No Zone	No Zone
	Alcohol	No Zone	No Zone
S.flexneri	Water	No Zone	No Zone
	Alcohol	3	4
E.tarda	Water	No Zone	No Zone

	Alcohol	No Zone	No Zone
S.sonnei	Water	No Zone	No Zone
	Alcohol	No Zone	5
S.enteritidis	Water	No Zone	7
	Alcohol	4	4
S.paratyphi	Water	No Zone	No Zone
	Alcohol	No Zone	No Zone
K.pneumoniae	Water	No Zone	No Zone
	Alcohol	No Zone	5
Bacteria (Gram positive)	**EXTRACT**	**Diameter of zone of inhibition (mm.)**	
		FRESH	**DRY**
S.aureus	Water	No Zone	No Zone
	Alcohol	No Zone	7
B.cereus	Water	No Zone	5
	Alcohol	No Zone	No Zone
L.monocytogens	Water	No Zone	6
	Alcohol	No Zone	5
FUNGUS *C.albicans*	Water	No Zone	No Zone
	Alcohol	No Zone	No Zone

In this study the phytochemical screening of water extract of *Ageratum conyzoides, Mikania micrantha, Parthenium hysterophorus* and *Paederia foetida* revealed the presence of glycosides, tannins, flavonoids, terpenes (Table 1). Tannins and saponins were found to be present in all the four plants. Glycosides is found absent only in *Paederia foetida* while terpene was found present only in *Ageratum conyzoides*. Flavonoids was found to be present only *Mikania micrantha* and *Paederia foetida*. Phytochemical screening of ethanolic extract showed same results for the Glycosides, Terpenes and Saponins (Table 2). But different results were found in for tannins and flavonoids. Tannin unlike water extract was found absent in *Parthenium hysterophorus* and flavonoids unlike water extract was found to be absent only in *Paederia foetida.*

The highest antibacterial property is shown by *Mikania micrantha*, followed by *Ageratum conyzoides*, *Paederia foetida* and *Parthenium hysterophorus* (Table 2). Moreover the fresh sample of the plant extracts shows less activity than the dry samples. It has been also observed that the alcohol extracts has more activity than the water extracts (Graph 1 and 2). The highest inhibition zone was recorded for *Parthenium hysterophorus* which is 20 mm. against *B. cereus* and the lowest was recorded for *Mikania micrantha* which is 1mm against *S. flexneri.*

This differential result of the various plant extracts was may be due to the presence of different biochemical compounds in the extract. A compound may not be equally dissolved in water and alcohol. Therefore water and ethanol extracts shows different result. Moreover, the activity of the phytochemical compounds may depend upon the type of solvents used during extraction.

CONCLUSION

This antimicrobial screening of various leaf extracts against thirteen different Gram positive and Gram negative bacteria and a fungus reveals that they possess certain range of antimicrobial property. No doubt this screening seems very ordinary but in real sense it may give some information regarding antimicrobial properties present in commonly available weeds. Moreover the weeds are unwanted and easily available and thus may lead to production of natural antibiotics which are eco-friendly and less expensive. Therefore, the isolation, purification and identification of the compounds is must for further study.

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