



## Antimicrobial Activity and Phytochemical Screening of Some Common Weeds of Asteraceae Family

Munmi Borkatakya<sup>1\*</sup>, Bibhuti Bhusan Kakoty<sup>2</sup>, Lakhi Ram Saikia<sup>3</sup>

<sup>1,3</sup>Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India.

<sup>2</sup>Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India.

\*Corresponding author's E-mail: [mbk139@gmail.com](mailto:mbk139@gmail.com)

Accepted on: 21-08-2013; Finalized on: 31-10-2013.

### ABSTRACT

Plants have been utilized as a source of nutrition and healthcare products since ancient times. A weed is a collective name given to plants that grow and compete with the cultivated crops and are found to be resistant to most of the microbial diseases when compared to the cultivated crops. *Ageratum conyzoides*, *Eupatorium odoratum* and *Mikania micrantha* are members of Asteraceae, which is an exceedingly large and widespread family of some common well known weeds. Phytochemicals are secondary metabolites and are known to exert profound influence on various activities of plants. Methanolic extract of the plants revealed the presence of major phytochemicals in all of them. The antimicrobial potential of the plant extracts was evaluated against standard test strains and compared to the standard drug by the Activity Index (A.I.). It revealed that *M. micrantha* showed highest inhibition of *B. cereus* among the three plants. *E. odoratum* exhibited the highest antifungal activity followed by *A. conyzoides*. The antimicrobial potential of the three plants was compared in terms of the Proportion Index (P.I.). Although *E. odoratum* and *M. micrantha* exhibited to be equally potent in inhibiting the test strains, *E. odoratum* showed activity against both bacteria and fungi while *M. micrantha* showed only antibacterial activity. *A. conyzoides* exhibited a lower inhibitory potential but exhibited both antibacterial and antifungal activities.

**Keywords:** Activity Index, Antimicrobial Activity, Asteraceae, Phytochemical Screening, Weeds.

### INTRODUCTION

Plants, since ancient times have been utilized as a source of nutrition and healthcare products. Plants are a reservoir of diverse kinds of bioactive chemical agents and have often been utilized either in the form of traditional preparations or as pure active principles. It is reasonable to make use of locally available plants, domesticated or wild, that could substitute the synthetic preparations. The healing powers of traditional herbal medications have been validated by various workers. The use of herbs as complementary and alternative medicine has increased dramatically in the last 20–25 years.<sup>1</sup> Moreover, emergence of multiple drug resistant strains of microorganisms due to indiscriminate use of antibiotics has generated a renewed interest in herbal medicines.<sup>2</sup> Weeds, commonly defined as a plants that grows out of place and are competitive, persistent and pernicious<sup>3</sup>, have been a part of civilization and many ancient documents mention about humans battling weeds in the crop fields. Traditional healers recognized their medicinal potential and utilized them for the treatment of human ailments. Weeds are also found to be resistant to most of the microbial diseases when compared to the cultivated crops.<sup>4</sup> The resistance of weeds towards the microbial diseases provoked many workers to explore the reasons for such potency. Antimicrobial potential of different types of weeds is being studied extensively all over the world.<sup>5-11</sup> The present study was conducted to know the major phytoconstituents of the plants and further analysis was

conducted to explore the antibacterial and antifungal potentials of common Asteraceae weeds.

Asteraceae or Compositae, commonly referred to as the aster, daisy, or sunflower family, is an exceedingly large and widespread family of Angiospermae. The members have a wide range of adaptation to suit the different ecological niche and can thrive well even in inhospitable areas. The plants of this family are mostly herbs, some are climbers but shrubs and trees are very rare. This family has a remarkable ecological and economic importance.<sup>12</sup> Some members of this family are in famous as important weeds. Three well known examples are- *Ageratum conyzoides* Linn., *Eupatorium odoratum* Linn., *Mikania micrantha* H. B & K.

***Ageratum conyzoides* Linn.** (Common name- Goat weed; Vernacular name- Gondhwabon) It is an annual herb, 30-40 cm. high, stems erect, hairy-green or purple. Leaves opposite, broadly ovate, creanate, coarsely hairy on both sides, 3- nerved at the base. The inflorescence is a terminal corymb of many small heads; flowers violet or white. The juice from the fresh plant and the extract of the dried plant are used for the cure of allergic rhinitis and sinusitis, in aqueous solution for nasal instillation. The juice is also useful in post-partum uterine fragrant. A hair-wash consisting of a decoction of the fresh plant makes the hair fragrant, soft and dandruff-free. The plant extract is also known to prevent tetanus.<sup>13</sup>

***Eupatorium odoratum* Linn.** (Common name- Siam weed; Vernacular name- German habi). A scandent semi-woody annual plant with perennial root stock and pungent smell.



The leaves are opposite, triangular to elliptical with serrated edges. Leaves are 4–10 cms long by 1–5 cms wide. Leaf petioles are 1–4 cms long. The white to pale pink tubular flowers are in panicles of 10 to 35 flowers that form at the ends of branches. Leaves and flower tops are used medicinally as emetic, cathartic and in healing cut wounds.<sup>14</sup>

***Mikania micrantha* H. B & K:** (Common name- Climbing hemp weed; Vernacular name- Assam lota) It is a perennial scrambling or climbing vine. Leaves opposite, petiolate, the blade up to 19 cm long, cordate to triangular with a broad cordate base. Flowers minute, white or cream coloured, borne in small densely packed heads which superficially resemble a single large flower. Ethnomedicinally, it is used to stop bleeding, to treat gastritis, insect bites and various skin irritations.<sup>13</sup>

## MATERIALS AND METHODS

### Preparation of plant extracts

The plant samples were collected locally and processed. The cleaned and shade dried plant material was ground into fine powder using electric blender. Plant extracts in methanol were prepared by cold maceration method. Fifty grams of dried powder was extracted by soaking in 500 ml methanol for 48 hours with intermittent shaking. The extracts were filtered into pre-weighed beakers. The filtrates were dried in an IKA RV 10 Digital rotatory vacuum evaporator until a constant dry weight of each extract was obtained. The residues were stored aseptically at 5°C for further use.

### Qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis of the plant extract for alkaloids, saponins, flavonoids, phenols and tannins, steroids and glycosides was performed by standard methods as outlined below.<sup>15,16</sup>

**Alkaloids:** (Extract was dissolved in 1% HCl and filtered) 1ml of filtrate was taken and add few drops of Dragendorff's reagents/Mayer's reagents/ Hager's reagents/ Wagner's reagent, Orange brown precipitate/ Cream colored precipitate/ Yellow precipitate/ Red brown precipitate respectively indicated the presence of alkaloids.

**Saponin:** a) Foam Test: small quantity of the residue was diluted with distilled water to 20 ml and shaken vigorously; formation of one cm layer of foam which was stable for 10 minutes indicated the presence of saponin. b) To the alcoholic extract, Sodium bicarbonate was added and shaken well; honey comb like frothing confirmed the presence of saponin.

**Flavonoids:** a) In the Plant residue 10% NaOH was added yellow coloration indicated the presence of flavonoids. (b) To the Extract few ml of con. H<sub>2</sub>SO<sub>4</sub> was added formation of yellow or orange color indicated the presence of flavonoids.

**Phenolics and tannins:** a) Small quantity of the extract dissolved in distilled water and added 10% Lead acetate solution, white precipitate indicated the presence of tannins; b) Small quantity of the extract dissolved in distilled water and added few ml of 1% gelatin and 10% sodium chloride, white precipitate indicated the presence of tannins.

**Steroids:** Salcowski test: Small quantity of the extract was diluted with distilled water and filtered. 2 ml of the filtrate is mixed with 2 ml of chloroform, further adding 2 ml con. H<sub>2</sub>SO<sub>4</sub>. Appearance of red colour in chloroform layer and greenish yellow fluorescence layer of H<sub>2</sub>SO<sub>4</sub> indicates the presence of steroids.

**Glycosides:** Kellar-Killani test: 2 ml of extract was taken and add 1 ml glacial acetic acid, one drop 5% FeCl<sub>3</sub> and Conc. H<sub>2</sub>SO<sub>4</sub>, reddish brown color appears at junction of the two liquid layers and upper appears bluish green, indicates the presence of glycosides.

### Antimicrobial screening

The methanolic extracts of the plants was screened against 8 bacterial strains, four Gram positive and four Gram negative and one fungal strain. The test organisms were *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96, *Proteus mirabilis* MTCC 1429, *Bacillus cereus* MTCC 430, *Escherichia coli* MTCC 739, *Salmonella enteric serv. typhi* MTCC 3917, *Pseudomonas aeruginosa* MTCC 1688, *Staphylococcus epidermidis* MTCC 435 and *Candida albicans* MTCC 3017. The test strains were obtained from the IMTECH, Chandigarh, India. The strains were maintained in the respective media as per the recommendations provided by the source.

### Preparation of inoculum

Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of culture from the stock to test tubes of Nutrient Broth for bacteria and Malt Yeast Broth for fungi and incubating for 24 hours at 37°C and 25°C respectively. The cultures were diluted with fresh Nutrient Broth and Malt Yeast Broth to achieve optical densities corresponding to 0.5 McFarland standard.

### Antimicrobial susceptibility test

The agar well diffusion method<sup>17,18</sup> was used to screen the antimicrobial activity of the extracts. In vitro antibacterial activity was screened by using Nutrient Agar obtained from Himedia (Mumbai) and in vitro antifungal activity assay was performed by using Malt Yeast Agar obtained from Himedia (Mumbai). The plates were prepared by pouring 25 ml of molten media into sterile petri-plates (diameter 100 mm). The plates were allowed to solidify at room temperature and 100 µL inoculum suspension was spread uniformly with the help of a sterile glass spreader and allowed it to dry. The extracts were dissolved in DMSO to obtain a final concentration of 200 mg/ml. Five 6 mm diameter wells were bored into the medium with

the help of a sterile glass well borer and 100  $\mu$ L of each of the four extracts was loaded into each well. These were allowed to diffuse for 45 minutes at room temperature after which the plates were transferred for incubation at 35  $^{\circ}$ C for bacteria and 25  $^{\circ}$ C for fungi. After the 24 hours of incubation, inhibition zones formed around the well were measured with transparent ruler in millimeter. The experiment was performed in triplicate and results were expressed as mean along with standard deviation. The activities of the extracts were compared with the standard drugs- Ciprofloxacin (10  $\mu$ g/ml) for bacteria and Clotrimazole (30  $\mu$ g/ml) for fungi. DMSO was used as negative control.

#### Determination of activity index and proportion index<sup>19</sup>

The activity index of the crude plant extract was calculated as;

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

The proportion index was calculated as;

$$\text{Proportion Index (P.I.)} = \frac{\text{Number of positive results obtained for extract}}{\text{Total number of tests carried out for each extract}}$$

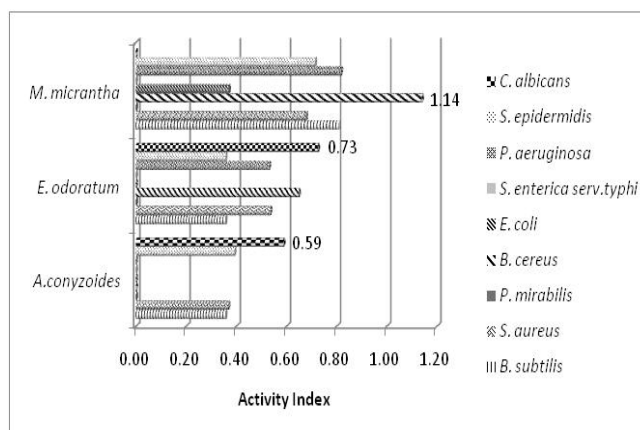
### RESULTS AND DISCUSSION

In the present study, the phytochemical analysis of methanolic extract of *A. conyzoides*, *E. odoratum* and *M. micrantha* revealed the presence of alkaloids, saponins, flavonoids, phenolics and tannins, steroids and glycosides (Table 1). Alkaloids, phenolics and tannins, steroids and glycosides were present in all the three plants whereas saponins were present only in *E. odoratum*. Also, flavonoids were present in *A. conyzoides* and in *E. odoratum* and absent in *M. micrantha*. The methanol extract of the three plants inhibited both the bacterial and fungal test strains with different efficacies at a concentration of 200 mg/mL (Table 2). The negative control did not inhibit the test strains. *A. conyzoides* showed highest inhibition of *S. epidermidis* followed by *B. subtilis* and *S. aureus*. *E. odoratum* showed a higher potency to inhibit gram positive strains as compared to gram negative strains. While *M. micrantha* inhibited both gram positive and gram negative strains with similar potency. The antimicrobial potential of the extracts was compared to the standard drug by the Activity Index (A.I.) (Figure 1). The methanol extract of *M. micrantha* showed highest activity index (A.I. = 1.14) against *B. cereus*, thus suggesting that its inhibitory activity is equipotent to the standard drug for the strain under consideration. *E. odoratum* exhibited the highest antifungal activity among the three plants (A.I. = 0.73) followed by *A. conyzoides* (A.I. = 0.59). *M. micrantha* failed to inhibit *C. albicans* at the test concentration. The antimicrobial potential of the three plants was compared in terms of the Proportion Index (P.I.) (Figure 2). Although *E. odoratum* and *M. micrantha* exhibited to be equally potent in inhibiting the test strains (P. I. = 0.67), *E. odoratum* showed broad spectrum activity against both bacteria and fungi while *M. micrantha* showed only antibacterial activity. *A. conyzoides* exhibited a lower

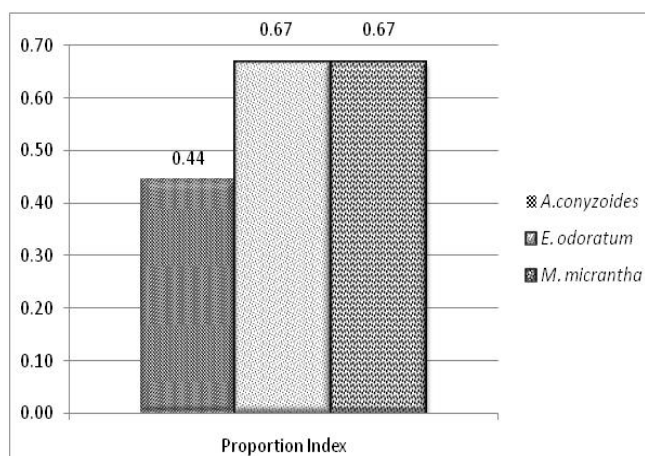
inhibitory potential (P.I. = 0.44) but exhibited both antibacterial and antifungal activities.

**Table 1:** Phytochemical analysis of methanol extract of plants

Phytochemicals	<i>A. conyzoides</i>	<i>E. odoratum</i>	<i>M. micrantha</i>
Alkaloids	+	+	+
Saponins	-	+	-
Flavonoids	+	+	-
Phenolics and tannins	+	+	+
Steroids	+	+	+
Glycosides	+	+	+
+ =	Present	- =	Absent



**Figure 1:** Activity Index of the methanol extracts of plant



**Figure 2:** Proportion Index of the methanol extracts of plant

Weeds, like any other plant, are a reservoir of many types of phytochemicals. As the literature suggests, the phytochemicals, which are the different kinds of secondary metabolites, are often the reason for the various protective properties of plants.<sup>20-23,5,10</sup> The presence of flavonoids and saponins has been shown to be responsible for antifungal activity of the plant.<sup>10</sup> In the present study, similar observations have been made where the *E. odoratum* extract shows the presence of both flavonoids and saponins and exhibits highest



antifungal activity as compared to *A. conyzoides* extract which contains only flavonoids. *M. micrantha* extract lacked both flavonoids and saponins and did not exhibit antifungal activity. Although *A. conyzoides* lacked saponins, it had phytochemical composition similar to *E. odoratum* but exhibited antibacterial activity much lower than *E. odoratum*. *M. micrantha* lacked flavonoids and saponins and showed antibacterial activity higher than *E. odoratum*. This suggests that the antibacterial activity may not be influenced by flavonoids or saponins. Further study in this regard is required to understand the influence of phytochemicals on antibacterial and antifungal properties of the plants and to identify the

bioactive principles responsible for their activities. Herbs rich in tannins have been used for treating intestinal disorders such as diarrhoea and dysentery.<sup>10, 24</sup>

The choice of solvent used for extraction procedure affects the type of compounds extracted from the plant material.<sup>9</sup> Traditional healers primarily use water as solvent for the extraction purpose but according to (Nair et al 2005)<sup>25</sup> the plant extracts in organic solvent (methanol) provided consistent antimicrobial activity when compared to water extract. Similar studies confirm this finding.<sup>5,9,10</sup> Moreover, methanol extracts possess the ability to dissolve and diffuse in wide variety of media.<sup>14</sup>

**Table 2:** Antibacterial and antifungal activity of methanol extract of plants expressed as diameter of zone of inhibition (in mm).

MTCC NO.	Microorganism	<i>A. conyzoides</i>	<i>E. odoratum</i>	<i>M. micrantha</i>	Standard Drug
441	<i>B. subtilis</i>	10±0	10±0	23±1	28±1
96	<i>S. aureus</i>	10±0	15±1	19±1	28±1
1429	<i>P. mirabilis</i>	NA	NA	NA	26±1
430	<i>B. cereus</i>	NA	12±1	22±1	19±1
739	<i>E. coli</i>	NA	NA	10±0	27±1
3917	<i>S. enteric serv. typhi</i>	NA	NA	NA	27±1
1688	<i>P. aeruginosa</i>	NA	12±1	18±2	22±1
435	<i>S. epidermidis</i>	11±1	10±0	20±1	28±1
3017	<i>C. albicans</i>	10±0	12±1	NA	17±0

## CONCLUSION

Hence, from the present study, it may be concluded that the phytochemicals play a definitive role in the biological activities of the plants and the kind of phytochemicals extracted depends on the kind of solvent used for the purpose of extraction. Further, this knowledge may be applied for targeting compounds with desired activities with minimal efforts.

**Acknowledgement:** Authors are grateful to UGC, New Delhi for providing financial assistance to the first author and to the Dibrugarh University for providing the necessary facilities for conducting the research.

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Source of Support: Nil, Conflict of Interest: None.

