



Original Research Article

Antibacterial activity of methanolic extracts of selected weeds against two phosphorous solubilizing bacteria

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A B S T R A C T

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The methanolic extract of the leaves of four common weeds *viz.*, *Ageratum conyzoides*, *Eupatorium odoratum*, *Mikania micrantha* and *Centella asiatica* were investigated for their *in vitro* antibacterial activities. The agar well diffusion method was used to determine the antibacterial activity on *Bacillus subtilis* and *Bacillus pumilus* at different concentrations. The results indicated that the extracts inhibited the growth of the two phosphorous solubilizing bacteria. *Eupatorium odoratum* was found to have the highest antibacterial activity.

Introduction

Weed is commonly defined as plants that grow out of place and is competitive, persistent, and pernicious (James *et al.* 1991). 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, nutrients, and space (Westbrooks 1998, 2001). Plants are sources of very potent and powerful drugs with antibacterial properties (Chopra *et al.* 1992; Iyengar 1985). An antibacterial is a compound or substance that kills or slows down the growth of bacteria. Most of green plants represent a reservoir of effective chemo-therapeutants and can

provide valuable sources of natural drugs, natural pesticides and biofertilizers. They have a long evolution of resistance against microbial agents which has lead to alternative directions in drug development. Therefore, extracts of plants and phytochemicals are getting more importance as potential sources for viral inhibitors during the recent decade. Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes (Bhattarai & Shreshta 2009). The common dominant weeds of Mizoram that are used for the

study of the antibacterial activity are: *Ageratum conyzoides*, *Eupatorium odoratum*, *Mikania macrantha* and *Centella asiatica*.

Phosphorus (P) is one of the major essential macronutrients for biological growth and development. Plants excrete organic acids such as citric, oxalic and tartaric acid to increase phosphorus solubility and availability in rhizosphere (Marschner *et al.* 1990). There are considerable populations of phosphate-solubilizing bacteria in soil and in plant rhizospheres (Sperberg 1958; Katznelson *et al.* 1962; Raghu & Macrae 1966; Alexander 1977). A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere in comparison with non-rhizosphere soil (Katznelson *et al.* 1962; Raghu 1966). P-solubilizing bacteria have been reported to increase the P uptake and yield of many crops, and to change insoluble P in soils into soluble forms by producing organic acids.

Materials and Methods

Plant collection and extraction

1kg of each fresh leaves of *Eupatorium odoratum*, *Ageratum conyzoides*, *Mikania micrantha* and *Centella asiatica* were collected from MZU campus, Tanhril. The collected plant material were washed with distilled water and then washed with 70% ethanol and dried under sunlight. After completely dried the plant material were ground into powder with a mixer grinder under sterilized condition. 100g of each plant powder were taken and washed separately with 1L of petroleum ether in a clean glass container (a round bottom flask) and closed with aluminium foil. The solution was subsequently shaken and then

filtered with Whatman filter paper No.1. The residue was again washed with 500ml of methanol, closed tight with aluminium foil, again shaken every 30mins for one whole day and filtered. The filtrate was centrifuged at 2000rpm for 10mins and the clear supernatant was collected. The extract solvent was kept in a wide open beaker and evaporated in an oven below 45°C and kept overnight. The final extract should be dried completely and kept immediately in a refrigerator or 0°C until use. This is the crude extract which should be processed for the experiment.

Preparation of crude extract for antibacterial assay

The crude extract of different plants were weighed into 100mg, 75mg, 50mg and 25mg and kept separately in eppendorf tubes. Each of the extracts was dissolved in 0.5ml of dimethylsulphoxide (DMSO). To these, 1ml distilled water was added to obtain 100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml concentrations.

Test microorganisms

Bacillus subtilis (ATCC 11774) and *Bacillus pumilus* (ATCC 14884) which are both phosphorus solubilizing bacteria were used for test organisms. They were re-cultured by nutrient broth medium and then incubated at a B.O.D incubator at around 30°C for 24hrs. 1 ml of the suspended culture was then transferred to nutrient agar medium.

Evaluation of antibacterial activity

The agar well diffusion method as described by Esimone *et al.* (1998) was adopted for the study. 15 ml of molten nutrient agar was seeded with 1.0 ml of standardized broth cultures of the bacteria

by introducing the broth cultures into sterile Petri dishes, incorporating the molten agar rotating slowly to ensure uniform distribution of the microorganisms and then allowed to solidify on a flat surface. Three holes were made in the plates (5.0 mm diameter) using a sterile cork borer and equal volumes of the extracts were transferred into the holes using a Pasteur's pipette.

The plates were allowed to stand for one hour for pre-diffusion of the extract to occur and were incubated at 27°C for 24 hrs at a B.O.D incubator. At the end of incubation the plates were collected and zones of inhibition that developed were measured.

Results and Discussion

Results of the antibacterial screening of different concentrations of the extracts on the test isolates are shown in Table 1 (a) and (b). The results show that the increase in concentration of the extracts increased the zones of growth inhibition of the bacteria (Fig. 1 and 2). The assessment of the antibacterial activity was based on the measurement of diameter zone of inhibition (mm) that formed around the hole made by the borer filled with the extract. Maximum inhibition zone was recorded at 100 mg/ml and the minimum inhibition zone at 25 mg/ml in both the bacteria for all the extracts (Table 1a and 1b; Fig 1 and 2).

Among the four common weeds tested, *Eupatorium odoratum* gave the largest area of inhibition zone against the two bacteria tested *i.e.*, *B. pumilus* and *B. subtilis* (Photo 2 and 6 respectively). *Mikania micrantha* gave the least

inhibition zone against *B. pumilus* (Photo 3) and *Centella asiatica* also gave the least inhibition zone against *B. subtilis* (Photo 8). This shows that *Eupatorium odoratum* had the highest antibacterial activity against the two Phosphorous solubilizing bacteria.

In this study, the results obtained indicated that the methanolic extract of the plants inhibited the growth of the two Phosphorous solubilizing bacteria. This therefore, showed that the extracts contained substances that can inhibit the growth of the selected bacteria. Other workers have also shown that extracts of plants inhibit the growth of various microorganisms at different concentrations (Akujobi *et al.* 2004; Esimone *et al.* 1998; Nweze *et al.* 2004; Ntiejumokwu & Alemika 1991).

From the review of *A. conyzoides* by Okunade (2011) a wide range of chemical compounds including alkaloids, flavonoids, chromenes, benzofurans and terpenoids have been isolated. The phytochemical screening of *E.odoratum* showed the presence of saponin, flavonoids, proteins, oil, tannin and alkaloids (Okonkwo 2009). Some flavonoids and dicaffeoylquinic acid butyl esters have been recently described as bioactive for *M. micrantha* (Wei *et al.* 2004). Active chemical compounds of the plant *Centella asiatica* are asiatic acid, asiaticoside, madecassic acid, pectic acid, ascorbic acid, two saponins: brahmoside and brahminoside, three triterpene acids: brahmnic acid, isobrahmnic acid and betulic acid and stigmasterol (Chopra *et al.* 1956) So, it may be due to the presence of these chemical compounds and substances that the plant extracts can inhibit the growth of the bacteria.

Table.1 (a) Zone of inhibition in *B. subtilis*

Concentration of extracts (mg/ml)	Zones of inhibition (mm) of <i>B. subtilis</i>			
	<i>Ageratum</i>	<i>Eupatorium</i>	<i>Mikania</i>	<i>Centella</i>
100	7.33	10.66	6.66	6.66
75	6.0	9.33	6.66	4.0
50	5.33	8.0	4.0	4.0
25	2.66	6.0	2.66	2.0
Total	21.32	33.99	19.98	16.66

Table.1(b) Zone of inhibition in *B. pumilus*

Concentration of extracts (mg/ml)	Zones of inhibition (mm) of <i>B.pumilus</i>			
	<i>Ageratum</i>	<i>Eupatorium</i>	<i>Mikania</i>	<i>Centella</i>
100	8.0	16.66	5.33	10.66
75	6.0	13.33	4.0	5.33
50	4.66	10.66	2.0	4.0
25	2.66	4.66	2.0	2.0
Total	21.32	45.31	13.33	21.99

Fig. (a, b, c, d) Effect of extracts of *Ageratum*, *Eupatorium*, *Centella*, and *Mikania* respectively against *Bacillus subtilis*

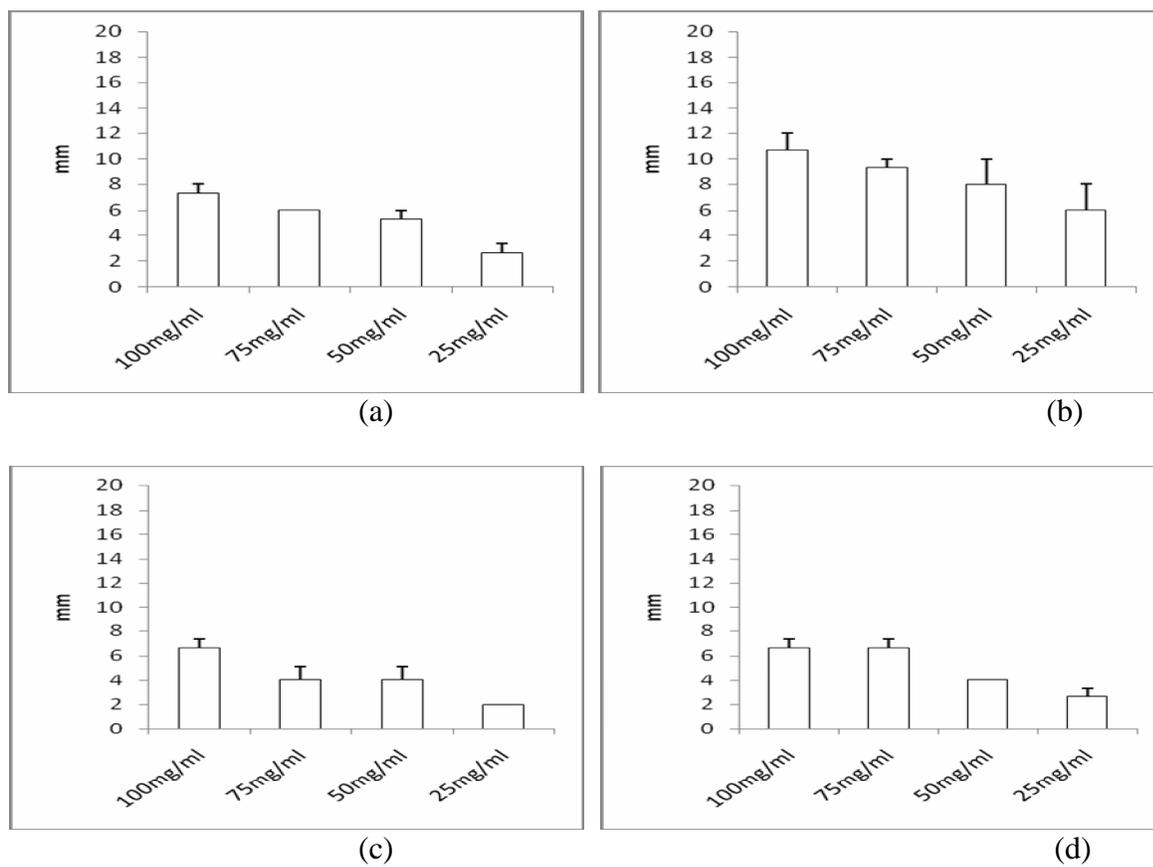
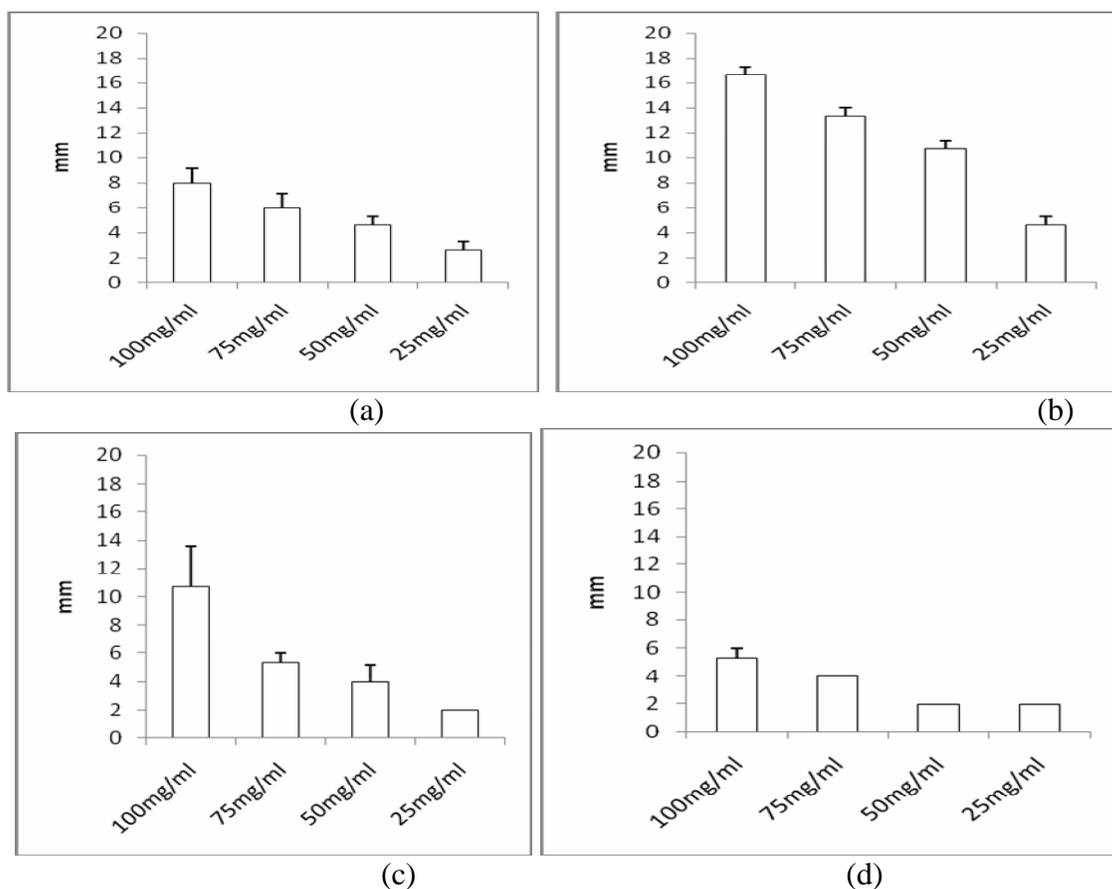


Fig. (a, b, c, d) Effect of extracts of *Ageratum*, *Eupatorium*, *Centella* and *Mikania* respectively against *Bacillus pumilus*



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