

## Antimicrobial activities and phytochemical screening of leaf extract of *Mikania micrantha* H.B.K

Anjana Devkota <sup>1</sup>✉ and Anita Sahu<sup>1</sup>

<sup>1</sup>Central Department of Botany, Tribhuvan University, Kirtipur Kathmandu, Nepal  
devkotaa@gmail.com

### ABSTRACT

Antimicrobial activities and phytochemical screening of leaves of *Mikania micrantha* was tested in laboratory against phytopathogenic fungi and human pathogenic bacteria. The leaves samples were extracted in distilled water and methanol. The crude extracts of leaves were assessed *in-vitro* for antimicrobial activity using different concentrations (50, 100, 150, 200, and 250 mg/ml) against five fungal strains (*viz.* *Sclerotium rolfsii*, *Phytophthora capsici*, *Alternaria brassicae*, *Fusarium oxysporum* and *Botrytis cinerea*) and six bacterial strains (*Klebsiella pneumoniae*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus subtilis*, *Enterococcus faecalis* and *Staphylococcus aureus*). Antifungal activity was tested by Poison Food Technique and Linear Mycelium growth Reduction (LMGR) percentage was determined. In crude extract of *M. micrantha*, the highest LMGR percentage was found in *F. oxysporum* in both distilled water and methanol extract. Antibacterial activity was carried out by Disc Diffusion method. In the crude leaf extract of *M. micrantha* Gram negative bacteria was found more resistant than Gram positive bacteria. Methanol extract was found more effective in determining the Zone of Inhibition for all the strains of bacteria in all the concentrations. The plant extracts were found more effective in showing antibacterial activity than antifungal activity. The phytochemical screening revealed that the selected species contained tannin, saponins, alkaloid, flavonoid, cardiac glycosides and terpenoids. This result supports the potential of this plant species used as a new chemotherapeutic drug.

**Keywords:** antimicrobial inhibition, mycelium, phytochemical, screening

### INTRODUCTION

*Mikania micrantha* Kunth ex H.B.K, commonly called mile a minute, is one of the devastating invasive species belonging to family Asteraceae. It is found to be growing best where fertility, organic matter, soil moisture and humidity are high enough. It suppresses the growth of other plants by shading light and smothering them. It is ranked amongst the top three worst invasive weeds of the world (Tiwari *et al.*, 2005). It releases the substances that inhibit the growth of other plants. Extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma *et al.*, 1999). *Mikania* species have multiple pharmacological actions. It is used in respiratory tract, anti-inflammatory, anti-allergic, analgesic, antioxidant and in central system

treatments (Rufatto *et al.*, 2012). ). However, there are no reports about the antimicrobial activities of *M. micrantha* extracts against human pathogenic bacteria and phytopathogenic microorganisms. Hence the present study aimed to identify the potential of *M. micrantha* against human pathogenic bacteria like *Klebsiella pneumoniae*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus subtilis*, *Enterococcus faecalis* and *Staphylococcus aureus* and phytopathogenic microorganisms such as *Alternaria brassicae*, *Botrytis cinerea*, *Fusarium oxysporum*, *Phytophthora capsici* and *Sclerotium rolfsii*. Further, it was also aimed to determine important phytochemicals present in the leaf of invasive plant *M. micrantha*, in order to highlight the medicinal values of the plant. Present study may contribute to the continuing search of new natural occurring products with antimicrobial activity and help not only for finding new drug but also for management of invasive plant *Mikania micrantha*.

## MATERIALS AND METHODS

### Collection and processing of the plant sample

The fresh and healthy leaves of *M. micrantha* were collected from Sauraha, Chitwan, Nepal. The leaves were chopped into small pieces and were shed dried at room temperature. The dried leaves were grinded into fine powder by using electric grinder and were preserved into zipper bag for the further experiment.

### Preparation of the extract

Twenty five grams of the dried ground leaf sample was soaked in 250 ml of distilled water and methanol (99.99%) separately for 72 hours. The samples were filtered using three layers of muslin cloth. The solvents distilled water and methanol were evaporated to semisolid form using water bath and rotary evaporator respectively as method followed by Mahida & Mohan (2007). The obtained semisolid extract was weighted and was kept into air tight container for further analysis.

### Phytochemical screening

**Qualitative test:** Preliminary qualitative phytochemical screening was carried for the presence of cardio-glycosides, alkaloids, saponins, tannins, flavonoids and terpenoids according to established method of several scientists (Harborne, 1973; Obdoni & Ochuko, 2001; Sofowora, 1993; Trease & Evans, 1989)

**Qualitative analysis:** Preliminary qualitative phytochemical screening was carried out on aqueous and methanol extract applying the standard protocols described by Sofowara (1993), Trease & Evans (1989), and Harborne (1973) and for the result sharp change in color was noted.

**Terpenoids (Salkowski test):** Five ml of each extract was mixed in 2 ml of chloroform, and 3 ml of concentrated  $H_2SO_4$  was added carefully to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

**Saponin:** One gram of the powered samples were boiled in 10 ml of water and methanol separately in a water bath and filtered. Five ml of the filtrate was mixed with 2.5 ml of distilled water and shaken vigorously for a stable persistent froth.

**Steroids:** Five ml of extract was taken and to it few drops of acetic anhydride and 5 ml of chloroform was added. Finally few drops of  $H_2SO_4$  was added slowly along the wall of test tube. Green bluish colour indicated the presence of steroids.

**Flavonoids:** Five ml of dilute ammonia solution were added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated  $H_2SO_4$ . A yellow coloration observed in extract indicated the presence of flavonoids. The yellow coloration disappeared after some time.

**Tannins:** About 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

**Alkaloid:** Plant sample of 200 mg was taken in 10 ml methanol and was filtered. In 2 ml filtrate 1% HCL was added and was steamed and 1 ml of filtrate was treated with 6 drops of Mayer's reagents (Mayer's Test). Formation of yellow coloured precipitate indicated the presence of alkaloids. Filtrate was treated with Wagner reagent (Wagner's Test). Formation of brown/reddish coloured precipitate indicated the presence of alkaloids. Filtrate was treated with Dragondroff's reagents (Dragondroff's Test). Formation of red coloured precipitate indicated the presence of alkaloids.

**Cardiac glycosides (Keller- Killin Test):** 2.5 g of powdered sample was soaked in 10 ml of water. 5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. It was then treated with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

**Quantitative test:** Quantitative test was carried out for alkaloids, saponins and flavonoids following the methods of several scientists (Boham *et al.*, 1994; Harborne, 1973; Panthi & Chaudhary, 2006). Quantitative estimation was carried out in crude form.

**Alkaloid test (Harborne method 1973):** The sample (2.5 g) was weighed into a 250 ml beaker and 100 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter (25ml) of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed (initial and final weight of the filter paper was calculated).

**Saponin determination (Obadoni & Ochuko, 2001) :** The samples were grounded and 10 g of each were put into a conical flask and 50 cm<sup>3</sup> of 20 % aqueous ethanol were added. They were heated over a hot water bath for 2 h with continuous stirring at about 90°C. The mixture was filtered and the residue re-extracted with another 100 ml 20% ethanol. The combined extracts were reduced to 20 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 10 ml diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ethyl layer was discarded. The purification process

repeated. 30 ml of n- butanol was added. The combined n-butanol extracts were washed twice with 5 ml of 5% of aqueous sodium chloride. The remaining solution was heated in a water-bath. After evaporation the samples were dried in the oven to a constant wt; the saponin content was calculated as percentage.

**Flavonoid determination (Bohm & Kocipai-Abyazan, 1994):** 10g of each plant's leaf sample was extracted with 100 ml of 80% aqueous methanol at room temperature. The solution was filtered through Whatmans filter paper. The filtrate was later transferred into crucible and evaporated in dryness over a water bath and weighted to a constant weight.

### **Collection and maintenance of test organisms**

**Bacterial strains:** The bacterial strains used for the test were collected from Department of Microbiology, Teaching Hospital, Maharajgunj, Kathmandu. Six strains, viz. three Gram negative *i.e.* *Klebsiella pneumoniae* (ATCC 15380), *Proteus mirabilis* (ATCC 49132), and *Escherichia coli* (ATCC 25922) and three Gram positive *i.e.* *Bacillus subtilis* (ATCC6633), *Enterococcus faecalis* (ATCC 29212), and *Staphylococcus aureus* (ATCC 25923) were used for the test.

**Fungal strains:** The fungal strains used for study were collected from Nepal Agriculture and Research Council, Khumaltar, Kathmandu. The five strains used for the test were *Sclerotium rolfsii*, *Phytophthora capsici*, *Alternaria brassicae*, *Fusarium oxysporum*, and *Botrytis cinerea*.

### **Preparation of culture inocula**

**For bacterial strains:** Nutrient broth was prepared with the help of manufacture (Hi-media) recommendation. It was sterilized by autoclaving at 15lbs pressure and 121°C for 2 hours in the conical flask. The cooled 10 ml Nutrient broth was poured in the sterilized Petri plates. The bacterial strain to be tested was aseptically taken with the help of needle from primary cultured slants and was transferred to the Petri plate containing nutrient broth and this was inoculated overnight in the incubator at 37°C.

**For fungus strains:** Potato Dextrose Agar (PDA) media was used for the fungal culture. 10 ml of PDA was poured in the sterilized Petri plate and 4 mm size of the pure culture from the slant was taken and was carefully placed in an inverted position. This was inoculated in the incubator at 27°C for 7 days.

### **Antibacterial Test**

**For bacteria:** Inhibition of bacterial growth was tested by using Disc Diffusion Method (Samie & Nefefe, 2012) with some modifications. 24 hours old bacterial culture was taken in sterilized Petri plates with the help of cotton swab and was suspended in 10 ml distilled water.

**Preparation of test discs:** 5 mm test discs were made by using Sartorius stadium (292) filter paper. The discs were prepared by saturating the sterilized filter paper in different concentrations of distilled water and methanol plant extracts (50, 100, 150, 200, 250 mg/ml) and control. For negative control (blank), distilled water and methanol paper were used, similarly for positive control antibiotics viz. ampicillin, and monocef were used by saturation the discs into 100 mg/ml of antibiotics used.

**Preparation of Nutrient Agar and transfer of bacteria:** Nutrient Agar (NA) was prepared by following HI media method. 10 ml media was poured into well labeled and pre-sterilized Petri plates and allowed to solidify for 10-15 minutes. The inoculum of bacteria was spread into Petri plates containing NA using sterile cotton swab. The discs dipped into different concentrations including control were aseptically placed per Petri plates with the help of forceps. After incubation for 24 hours at 37°C, diameters of inhibition zone around each disc were measured to the nearest millimeter (mm) with a ruler (including 5mm diameter of the test discs). The activity of extract was compared with standard antibiotics (ampicillin, monocef). Seven replicates were used for each test.

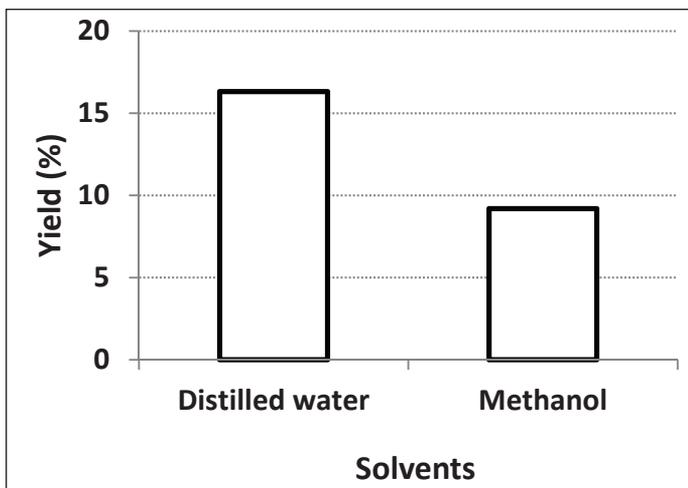
**For fungus:** The antifungal activity of plant extracts was determined by using Poisoned Food Technique applying the method of Samie and Nefefe (2012), with some modifications. The concentrations (50, 100, 150, 200, and 250 mg/ml) was prepared by using distilled water and methanol crude leaf extract.

**Transfer of the fungal strains:** A volume of 1ml the concentration to be used was poured aseptically into 9 ml of PDA and was swirled to mix the content completely. 10 ml of this mixture (PDA and extract) was then poured into the pre sterilized and well labeled Petri plates applying the method of Samie & Nefefe (2012). It was allowed to solidify for 15-20 minutes. The negative control plates received only distilled water and methanol in PDA without extract, while for positive control, fungicide Bavistin and Mancozeb were used. 4 mm inoculums from actively growing margin of fungus culture isolate (7 days old culture) was placed face down in the center of the petriplate with the help of sterilized needle. The test fungi were inoculated for 7 days at 27°C. The fungal colony was measured on the seventh day of incubation. Minimum and maximum readings of the colony diameter were taken using millimetre ruler. For each treatment seven replicates were used and mean value was taken. The result was compared with the positive control. The percentage of linear mycelium growth reduction of pathogenic fungi compared with control was calculated using the following formula as Khalil & Dababneh (2012).

Linear growth reduction (%) =  $\frac{\text{growth in control} - \text{growth in treatment}}{\text{growth in control}} \times 100$

## RESULTS AND DISCUSSION

The crude leaf extract yield was found higher in the distilled water (16.32%) than methanol (9.2%) (fig. 1).



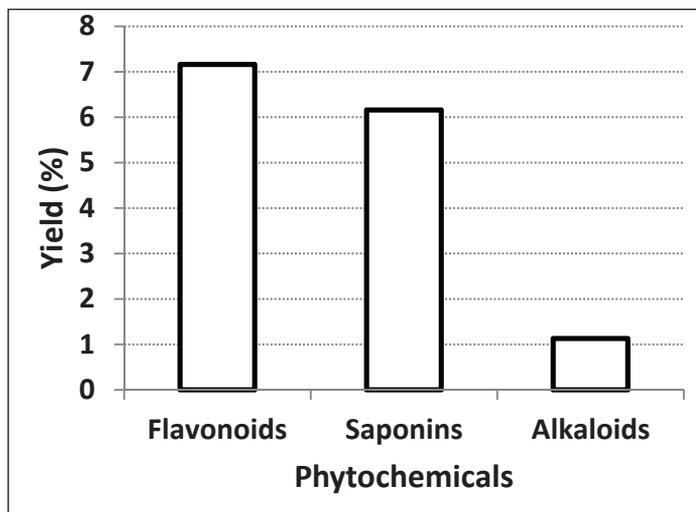
**FIG.1. Yield of chemical compounds in different solvents.**

Results of preliminary phytochemical screening of leaf extracts of *M. micrantha* showed the presence of important bioactive compounds. It also showed the presence of tannin, saponins, cardiac glycosides, terpenoids, steroids, flavonoids and alkaloids. Cardiac glycosides and steroids were found in greater amount in the methanol solvent (table 1).

**TABLE 1. Preliminary phytochemical screening of leaf extract of *Mikania micrantha*.**

Solvents	Phytochemical constituents						
	Tannin	Saponins	Cardiac glycosides	Terpenoids	Steroids	Flavonoid	Alkaloid
Methanol	+	++	+++	+	+++	++	+
Distilled water	+	++	-	-	-	+	+

+++ = strong reactions, ++ = moderate, + = weak, - = no reaction



**FIG. 2.** Yield of different phytochemicals.

Yield of flavonoids was found highest (7.16 %) followed by saponins (6.16 %) and alkaloids (1.13%) (fig. 2). Highest growth reduction was observed in *F. oxysporum* and *B. cinerea* in bavistin and mancozeb (table 3) than in distilled water and methanol (table 2).

**TABLE 2.** Percentage of linear mycelium growth reduction of different phytopathogenic fungi in distilled water (DW) and methanol (M) crude leaf extract of *M. micrantha*.

Fungi	Linear mycelium growth reduction (%)									
	50		100		150		200		250	
	DW	M	DW	M	DW	M	DW	M	DW	M
<i>Sclerotium rolfsii</i>	0	0	0	0	0	24.29	0	34.29	19.64	38.71
<i>Phytophthora capsici</i>	0	0	0	14.64	0	17.43	0	18.79	0	27.71
<i>Alternaria brassicae</i>	33.96	18.98	41.49	25.62	45.84	29.00	47.33	33.24	57.53	43.68
<i>Fusarium oxysporum</i>	62.04	36.16	66.70	40.78	69.40	50.00	79.76	55.30	81.90	64.75
<i>Botrytis cinerea</i>	29.44	16.23	32.89	19.48	41.91	30.52	46.68	41.23	54.91	46.75

0= no action / no growth reduction, values are mean of seven replica.

**TABLE 3. Reduction in mycelium growth (mm) in negative and positive controls.**

Fungi	Negative control (blank)		Positive control (fungicide)	
	Distilled water	Methanol	Bavistin	Mancozeb
<i>Sclerotium rolfsii</i>	100	100	46.5	21.42
<i>Phytophthora capsici</i>	100	100	100	32.57
<i>Alternaria brassicae</i>	72.14	50.60	44.85	21.35
<i>Fusarium oxysporum</i>	76.57	31	13	15.35
<i>Botrytis cinerea</i>	26.92	22	16	13.42

Values are mean of seven replica.

The percentage reduction in mycelium was higher in methanol than in distilled water extract. The significant difference ( $p > 0.05$ ) was found in effect for the two solvents. In distilled water and methanol the highest reduction was found for *F. oxysporum* and least i.e. No reduction was found for *S. rolfsii* in distilled water and *P. capsici* in methanol (table 2).

There was significant difference ( $p > 0.05$ ) between the two solvents at different concentrations. The highest percentage of linear mycelium growth reduction in distilled water was found in *F. oxysporum* (81.89%) followed by *A. brassicae* (57.53 %) and *B. cinerea* (54.91%). In case of *S. rolfsii* percentage reduction (19.64 %) was observed only at 250 mg/ml, while for *P. capsici* no reduction was observed in distilled water. For methanol extract *F. oxysporum* had the highest percent growth reduction in all concentrations. *B. cinerea* had second highest reduction from 150-250 mg/ml but its reduction (16.22 and 19.48%) at 50 mg/ml and 100 mg/ml was lower than *A. brassicae*, similarly *A. brassicae* had 18.98, and 25.61% reduction at 50 mg/ml and 100 mg/ml while other values are lower (29%, 33.24%, 43.68%) than *B. cinerea* which was found 30.52, 41.23, and 46.75% at 150, 200 and 250 mg/ml, respectively. The growth reduction of 24.26, 34.29, and 38.71 % for *S. rolfsii* was observed at 150, 200, and 250 mg/ml, respectively while no growth reduction was found at 50 and 100 mg/ml concentrations. *P. capsici* showed least percent in the growth reduction (14.64, 17.43, 18.79, and 27.71%) at different concentrations used while no growth reduction was observed in 50 mg/ml concentration.

There was significant difference ( $p > 0.05$ ) between the effect of two solvents used. The crude leaf extract of *M. micrantha* at all concentrations was found more effective than bavistin in reducing the mycelium growth of *A. brassicae* (table 3). For *F. oxysporum* the concentrations at 200 mg/ml for distilled water and 150 mg/ml, 200 and 250 mg/ml for methanol were more effective in reducing mycelium growth than bavistin and mancozeb. Similarly, for *B. cinerea* the reduction in the growth was found more at 150, 250, 200 and 250 mg/ml concentrations for both distilled water and methanol than bavistin, in the same way 200 mg/ml of methanol and 250 mg/ml of distilled water and methanol was found inhibiting the growth more than mancozeb (table 3). Methanol extract was found more effective than distilled water for all the concentrations.

TABLE 4. Zone of inhibition (mm) displaced by different concentrations for different strains of bacteria.

Bacteria	Concentrations (mg/ml)											
	50		100		150		200		250			
	Distilled water	Methanol	Distilled water	Methanol	Distilled water	Methanol	Distilled water	Methanol	Distilled water	Methanol	Distilled water	Methanol
<i>Klebsiella pneumoniae</i>	6.50± 0.41	9.07± 1.43	7.00± 0.65	11.57± 1.64	7.57± 0.79	13.36± 1.99	7.79± 0.70	14.14± 3.24	8.43± 0.79	16.57± 1.21		
<i>Proteus mirabilis</i>	6.14± 0.38	10.43± 1.62	6.79± 0.64	11.57± 1.67	7.29± 0.27	13.64± 1.60	7.86± 0.38	14.43± 3.13	8.36± 0.24	15.14± 1.63		
<i>Escherichia coli</i>	0.00± 0.00	11.43± 1.21	4.71± 0.39	12.64± 1.11	5.21± 0.39	13.71± 1.52	6.57± 0.45	15.86± 1.21	7.29± 0.49	17.36± 2.23		
<i>Bacillus subtilis</i>	6.57± 0.35	14.00± 1.08	7.50± 0.50	15.79± 1.15	7.71± 0.76	16.00± 1.76	8.14± 0.63	16.0± 1.21	8.86± 0.24	18.43± 3.27		
<i>Enterococcus faecalis</i>	7.07± 0.53	12.29± 2.63	7.00± 0.58	14.07± 0.35	7.79± 0.70	14.86± 1.75	7.93± 0.53	17.57± 1.90	8.50± 0.41	17.87± 3.18		
<i>Staphylococcus aureus</i>	6.21± 0.27	12.57± 2.34	7.00± 0.29	15.00± 0.91	7.71± 0.49	16.07± 1.40	8.00± 0.29	16.79± 1.65	8.64± 0.38	16.86± 1.46		

Values are mean ± standard deviation of seven replica.

Negative control (distilled water and methanol) did not show any action against bacterial strains. Among the positive control, ampicillin was more effective than monocef for different bacteria used. The plant extract at the concentration used were found less effective than the standard antibiotics in inhibiting the growth of the different strains of the test bacteria used in the study (table 5).

**TABLE 5. Zone of inhibition displaced by negative and positive controls.**

Type of strains	Bacteria	Negative control (Blank)		Positive control (Antibiotics)	
		Distilled water	Methanol	Ampicillin	Monocef
Gram negative	<i>Klebsiella pneumonia</i>	NA	NA	37.79± 4.89	28.1 ± 3.87
	<i>Proteus mirabilis</i>	NA	NA	27.14± 1.31	34.43± 4.41
	<i>Escherichia coli</i>	NA	NA	33.21± 4.06	29.64± 3.45
Gram positive	<i>Bacillus subtilis</i>	NA	NA	28.57± 7.44	29.71± 4.46
	<i>Enterococcus faecalis</i>	NA	NA	34.57± 0.45	29.07± 3.17
	<i>Staphylococcus aureus</i>	NA	NA	26.86± 7.73	28.29± 3.99

Values are mean ± standard deviation of seven replica.

In different concentrations of distilled water extract gram positive bacteria were found more susceptible than Gram negative bacteria. *B. subtilis* was found most susceptible with largest ZOI among Gram positive bacteria, while *E. coli* was found most resistant bacteria among Gram negative bacteria at different concentration in distilled water extract (table 4). In methanol leaf extract, *B. subtilis* showed the largest ZOI (18.43 mm) and smallest for *E. faecalis* among Gram positive bacteria while among Gram negative bacteria *E. coli* had the largest ZOI (17.36 mm diameter) and *K. pneumoniae* had the smallest ZOI (16.57 mm diameter). *B. subtilis* was the most susceptible while *K. pneumoniae* was the most resistant bacteria for methanol extract.

The higher yield of crude extract was in distilled water (16.32 %) than methanol (9.2%) (fig.1). Successive isolation of chemical compounds from the plant material is largely dependent on the type of solvent used in the extraction procedure (Aiyegroro & Okoh, 2010). Analysis of the preliminary phytochemical screening of leaf extract of *M. micrantha* revealed the presence of phytochemicals such as, tannins, flavonoids, saponins, cardiac glycosides, steroids, terpenoids, and alkaloids (table 1). This result is in accordance with the finding of Matawali *et al.* (2016). The quantitative analysis showed that the leaf contained highest percentage of

flavonoid (7.16%) followed by saponins (6.16%) and alkaloid (1.14%) (fig.2). This result is similar to the findings of Salah (1995). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Just *et al.*, 1998). Flavonoids are synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganism *in vitro* (Sodipo *et al.*, 2000). Saponins are known to produce inhibitory effect on inflammation and have property of precipitating and coagulating red blood cells (Okwu, 2001). Steroids have antibacterial properties (Antherden, 1969) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Stray, 1998). Some workers have reported the analgesic (Okwu & Okwu, 2004) antispasmodic and antibacterial (Nyarko & Addy, 1990) properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowora, 1993).

This study reveals that leaf extract of *M. micrantha* has different degrees of inhibition against different test microorganisms at different concentrations (tables 4, 5). The diameter of zone of inhibition produced depends on extrinsic parameters like pH of the medium, period and temperature of incubation, volume of the well, concentrations of the plant extracts and size of the inoculums can be fixed and standardized during the experiment, hence no error results due to extrinsic factors. However, intrinsic factors such as nature of the medicinal plants including its components, solubility and diffusing property are predetermined. Due to variable infusibility, the antibacterial with very high potency may not determine ZOI commensurate to its efficacy (Farnsworth, 1993).

Comparing effects on Gram positive and Gram negative bacteria, the maximum ZOI (table 4) was displaced by Gram positive bacteria (*B. subtilis*, *E. faecalis* and *S. aureus*) than by gram negative bacteria (*K. pneumoniae*, *P. mirabilis* and *E. coli*) in both distilled water and methanol extracts. Similarly, various workers have already shown that gram positive bacteria are more susceptible towards plant extracts as compared to gram negative bacteria (Gatsing *et al.*, 2010; Aiyegroro & Okoh, 2010). These differences may be attributed to fact that the cell wall in Gram positive bacteria is of a single layer, whereas gram negative cell wall is multilayered structure (Yao *et al.*, 1995). Microorganisms show variable sensitivity to chemical substances related to different resistant levels between strains (Cetin & Gurler, 1989).

Similarly, negative control (distilled water and methanol) did not show any action against the test bacteria. For the antibiotics, *K. pneumonia* showed the largest ZOI (37.79 mm) in ampicillin, but had the lowest ZOI (28.1 mm) in monocef while *P. mirabilis* had lowest ZOI (27.14 mm) in ampicillin and largest (34.34 mm) in monocef. The plant extract showed lower antibacterial activity than the standard antibiotics used. This may be due to the reason that active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed (Taylor *et al.*, 2001). Lack of activity can only be proven by using large doses (Farnsworth, 1993). The plant extracts were found more effective in showing antibacterial activity than antifungal activity. The resistance of fungi to the tested extracts may be due to the presence of more complex cell wall with rigidity than the thin cell membrane of bacteria. Also, this may be due to their ability to produce extracellular enzymes

that helps them to degrade and metabolize substrate such that the extract becomes a source of food to the fungi instead of inhibiting their growth after they have been rendered nontoxic due to degradation (Gatsing *et al.*, 2010).

The extracts were found effective against the fungal pathogens and human pathogenic bacteria that may be due to the presence of different bioactive compounds in the plants and their action towards the pathogens. The methanolic extracts of plants provided more consistent antimicrobial activity compared to those extracted by distilled water. This might have resulted from lack of solubility of the active constituents in the aqueous solutions while methanol extract showed some degree of antibacterial activity. The distilled water and the methanol leaf extract of *M. micrantha* exhibited antibacterial and antifungal properties with the presence of various phyto-constituents such as alkaloids, tannins and polyphenols, saponins and triterpenoids. The methanolic extracts of plants provided more consistent antimicrobial activity compared to those extracted by distilled water. It was proven that *M. micrantha* offered roles as good antimicrobial agents. This preliminary study had paved a way in effort to discover new and noble therapeutic antimicrobial. This study help not only for finding new drug but also for proper management of invasive plant *Mikania micrantha*.

#### ACKNOWLEDGEMENTS

We are thankful to University Grants Commission, Nepal, for providing financial support for this research work. We are also grateful to National Agricultural Research Council, Khumaltar and Microbiology Department of Teaching Hospital providing tested microorganisms.

#### REFERENCES

- AIYEGRORO, O A; OKOH A I (2010) Preliminary phytochemical screening and *in vitro* antioxidant activities of aqueous extract of *Helichrysum longifolium*. *BMC Complementary and Alternative Medicine* 10:21–32.
- ANTHERDEN, L M (1969) *Textbook of pharmaceutical chemistry*. Oxford University Press, London, UK (8th edition).
- BOHAM, B A; KOCIPAI-ABYAZAN, R (1994) Flavonoids and condensed tannin from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium*. *Pacific scientific* 48: 458–463.
- CETIN, T E; GURLER, N (1989) Bacterilerin antibiyotiklere duyarlılık deneyinin yapitnasi. *Kukem Dergisi* 12: 2–5.
- FARNSWORTH, N R (1993) Biological approaches to the screening and evaluation of natural products. In RASOANAIVO, P; RATSIMAMANGA-URVERG, S (eds) *Biological evaluation of plants with reference to the Malagasy flora, Madagascar*. pp 35–43.
- GATSING, D; NKEUGOAUAPI, C F N; NKAH, B F N; KUIATE, J R; TCHOUANGUEP, F M (2010) *International Journal of Pharmacology* 6: 173–182.
- HARBORNE, J B (1973) *Phytochemicals methods*. Chapman and Hall Ltd., London, UK.
- JUST, J; RECIO, M J; GINER, R M; CUÉLLAR, M J; MÁÑEZ, S; BILLIA, A R; RIOS, J L (1998) Antiinflammatory activity of unusual lupine saponins from *Bupleurum frutescens*. *Planta Medica* 64: 404–407.
- KHALIL, A; DABABNEH, B F (2007) Inhibition of phytopathogenic fungi by extracts from medicinal plants

in Jordan. *Journal of Biological Science* 7(3): 579–581.

MATAWALI, A; CHIN, L P; ENG, H S; GANSAU, J A (2016) Antibacterial and phytochemical investigations of *Mikania micrantha* H.B.K. (Asteraceae) from Sabah, Malaysia. *Transactions on Science and Technology* 3(1–2): 244–250.

MAHIDA, Y; MOHAN, J S (2007) Screening of plants for their potential antibacterial activity *Staphylococcus* and *Salmonella* spp. *Natural Product Radiance* 6(4): 301–305.

NYARKO, A A; ADDY, M E (1990) Effects of aqueous extract of *Adenia cissampeloides* on blood pressure and serum analyte of hypertensive patients. *Phytotherapy Research* 4(1): 25–28.

OKWU, D E (2001) Evaluation of chemical composition of medicinal plants belonging to Euphorbiaceae. *Pakistan Veterinary Journal* 14: 160–162.

OKWU, D E; OKWU, M E (2004) Chemical composition of *Spondias mombin* Linn. plant parts. *Journal of Environment & Sustainable Agriculture* 6(2): 140–147.

OBDONI, B O; OCHUKO, P O (2001) Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science* 8(b): 203–208.

PANTHI, M P; CHAUDHARY, R P (2006) Antibacterial activity of some selected folklore medicinal plants from West Nepal. *Scientific World* 4( 4): 16–21.

RUFATTO, L C; GOWER, A; SCHWAMBACH, J; MOURA, S (2012) Genus *Mikania*: chemical composition and phytotherapeutical activity. *Brazilian Journal of Pharmacognosy* 22(6): 1384–1403.

SAMIE, A; NEFEFE, T (2012) Antifungal activities of essential oils from Southern African medicinal plants gainst five *Fusarium* species. *Journal of Medicinal Plants Research* 6(3):465–478.

SALAH, N, MILLER, N J ; PAGANGA, G; TIJBURG, L; BOLWELL, G P; RICE- EVANS, C (1995) Polyphenolic flavonoids as scavengers of aqueous phase radicals as chain-breaking antioxidant. *Archives of Biochemistry and Biophysics* 322:339–346

SODIPO, OA; AKINIYI, JA; OGUNBAMORU, JV (2000) Studies on certain characteristics of extracts of bark of *Pansinystalia macruceras* (K. Schemp) Pierre Exbeille. *Global Journal of Pure and Applied Science* 6: 83–87.

SOFOWORA, A (1993) *Medicinal plants and traditional medicine in Africa*. Spectrum Books Ltd., Ibadan, Nigeria.

STRAY, F (1998) *The natural guide to medicinal herbs and plants*. Tiger Books International, London, UK.

TAYLOR, J L S; RABE, T; MACGRAWL, L J; JAGER, A K; VAN STADEN, J (2001) Towards the scientific validation of traditional medicinal plants. *Plant Growth Regulator* 34: 23–27.

TIWARI, S; SIWAKOTI, M; ADHIKARI, B; SUBEDI, K (2005) *An inventory and assessment of invasive alien plant species of Nepal*. IUCN, The World Conservation Union, Nepal.

TREASE, G E; EVANS, W C (1989) *Pharmacognosy*. Bailliere Tindall, London, UK (11th edition).

VARMA, J; DUBEY, K (1999) Prospectives of botanical and microbial products as pesticides of tomorrow. *Current Science* 76: 172–179.

YAO, J; MOELLERING, R (1995) Antibacterial agents. In MURRAY, P; BARON, E; PFALLER, M; TENOVER, F; YOLKEN, R; ASM, (eds) *Manual of Clinical Microbiology*. ASM Press; Washington DC, USA; pp 1281–1290.