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Full Length Research Paper

Behavioural assessment of *Mikania micrantha* Kunth roots in Wistar albino rats

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The present study was designed to evaluate the adaptogenic activity of methanolic and aqueous extract of roots of *Mikania micrantha* kunth in Wistar albino rats using different experimental models such as anoxia stress tolerance, swimming endurance and immobilisation stress. The plant was subjected to preliminary phytochemical screening. The parameters like anoxia stress tolerance and swimming endurance time were recorded. The estimation of biochemical marker levels and determination of organs weight were carried out in immobilisation stress model. These activities are tested at oral doses of extract at 250 and 500 mg/kg and diazepam 2 mg/kg was used for comparison. Preliminary phytochemical screening revealed the presence of flavonoids, steroids and tannins. Pretreatment with methanolic extract showed increase in anoxia stress tolerance time and swimming endurance time. There was a dose dependant significant reduction in biochemical parameters like serum glucose, cholesterol and blood urea nitrogen levels exhibited methanolic extract treated animals. The stress induced increase in weight of liver, adrenal gland and decrease in weight of spleen were significantly reversed by the methanolic extract at higher dose. The results from the study indicated that methanolic extract of *M. micrantha* roots possessed significant antistress activity.

Key words: *Mikania micrantha* Kunth., Wistar albino rats, anoxia stress, methanolic extract.

INTRODUCTION

Stress can be defined as the sum of all the reactions of the body, which disturb the normal physiological condition and results in a state of threatened homeostasis. Normally stress induced changes are compensatory, self limiting and adaptogenic in nature. However in higher animals when stress events of any nature (physical, chemical, biological and emotional) over certain 'threshold' limits occur, the changes become rather irreversible. It leads to altered homeostasis and exhaustion, manifesting itself in the pathologic form of stress induced disease and maladjustment. There is no treatment in modern drug therapy for stress related diseases. The available techniques for increasing endurance performance include physical training for endurance work, yogic and meditation practices, supplementation of nutraceuticals and intervention by adaptogens. Present study will provide a scientific base for experimental research on Indian herb for stress related diseases. The term 'Adaptogen' denotes an agent that improves adaptation capacity of the organism during

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stress and “Antistress” agent is a pharmacological word for the same, meaning an agent which nullifies or prevents the effects of stress and improves adaptation (Sharma and Arora, 2006).

Mikania micrantha Kunth (Asteraceae) is found in the tropics of America and Asia, and is widely known as guaco, the plant is a branched, extensively scrambling and twining slender-stemmed vine. *Mikania* comprises about 300 identified species, but only 20 of them have been studied. It is used to treat fever, rheumatism, influenza and respiratory diseases. Terpenes such as mikanolide are the major constituents isolated from plants of this genus (Facey et al., 2010). In the present study, an attempt has been made to investigate the adaptogenic activity using aqueous and methanolic extract of roots of the plant *M. micrantha* in view of reported adaptogenic activity of other species of *Mikania* namely *Mikania cordata* (Bishayee and Chatterjee, 1994).

MATERIALS AND METHODS

Collection and identification of the plant materials

M. micrantha roots were collected from Kottayam, Kerala, India during the month of March, 2011 and were authenticated by Mr. Joby Paul, Botanist, School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala (Voucher No. 1461).

Preparation of extracts

Extraction of roots of *M. micrantha* was carried out using methanol by hot continuous extraction method using soxhlet apparatus. 500 g of dried roots were taken, size reduced, extracted with 2 L of methanol in the round bottom flask and extraction was continued for few hours. The extract obtained was collected and concentrated by gentle heating. The concentrated extract was then weighed and stored. Thus total methanolic extract is obtained. Aqueous extraction was carried out with the remaining marc by reflex method. The marc was packed in a round bottom flask and refluxed for 2 h using a reflex condenser. The extract was then concentrated to dry residue by heating. Percentage yield of methanolic and aqueous extracts were found to be 7.7 and 3.8% w/w.

Preliminary phytochemical analysis

The preliminary phytochemical studies were performed for testing different chemical constituents present in methanolic and aqueous extracts using standard methods (Kokate, 1994; Khandelwal, 2004).

Selection of animals

Healthy adult Wistar albino rats, weighing about 150 to 220 g obtained from the registered Animal house of University College of Pharmacy, MG University, Kottayam were used for the study. The study protocol was approved by the Institutional Animal Ethical Committee, University College of Pharmacy, Cheruvandoor Campus (001/MPH/UCP/CVR/12). All the animals were housed individually in polypropylene cages, maintained under standard husbandry conditions (12 h light and dark cycles, room temperature and 45 to 55% relative humidity). They had been given standard pellet diet and water *ad libitum* throughout the course of the study.

Acute toxicity studies (OECD Guidelines 423)

The acute toxicity studied was carried out in female albino rats by “acute toxic class method” (OECD guidelines 423). The animals were fasted overnight and extracts of the herb *M. micrantha* suspended in 0.5% Na. Carboxy methyl cellulose (CMC) was administered starting at 2000 mg/kg p.o, food was withheld for next 3 to 4 h. The animals were observed continuously for body weight, any changes in skin and fur, eyes , behavior pattern and also signs of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma every 30 min for the next 3 h and finally death after 24 h.

Evaluation of anti-stress activity

Anoxia stress tolerance test

Albino Wistar rats of either sex weighing 150 to 220 g were selected and divided into 5 groups of six each as Group I: Control (received only vehicle CMC 0.5% w/v p.o.), Group II: Methanolic extract of *M. micrantha* root (MEMMR 250 mg/kg p.o.); Group III: MEMMR (500 mg/kg p.o.); Group IV: Aqueous extract of *M. micrantha* root (AEMMR 500 mg/kg p.o.); and Group V: diazepam (2 mg/kg p.o.). Animals were treated as shown for 3 weeks. At the end of 1st, 2nd and 3rd week that is, on 7th, 14th and 21st day, 1 h after the treatment. Stress was induced by placing each animal individually in the hermetic vessel of 1 L capacity to record anoxia tolerance time. The time duration of entry of the animal into the hermetic vessel and the appearance of the first convulsion was taken as time of anoxia tolerance (Pawar and Shivakumar, 2011).

Swimming endurance test

Albino Wistar rats of either sex weighing 150 to 220 g were selected and divided into five groups of six each as mentioned. The rats were subjected to swimming stress by keeping them in propylene tank of dimension (37 × 37 × 30 cm), filled with water to a height of 25 cm. Extracts were given to rats, once daily for period of 7 days. On 8th day the rats were allowed to swim till complete exhaustion and the endpoint was taken when the animal started drowning. The mean swimming time for each group was calculated (Kothiyal and Ratan, 2011).

Immobilisation stress

Adult male albino rats of 150 to 220 g were selected and divided into 6 groups of 6 animals each as Group I: Negative control (unstressed, untreated); Group II: Positive control (Stressed, received vehicle); Group III: MEMMR (250 mg/kg p.o.); Group IV: MEMMR (500 mg/kg p.o.); Group V: AEMMR (500 mg/kg p.o.); Group VI: diazepam (2 mg/kg p.o.). The treatment was made as stated for 10 days, 1 h before the exposure of stress. Stress was induced by immobilizing rats with head down, supine position by fixing the forelimbs and hind limbs to a wooden board inclined at an angle of 60°, daily 2 h for a period of 10 days. The animals were sacrificed at the end of specified period and blood was collected by retro-orbital for estimation of biochemical parameters such as, serum glucose, cholesterol and blood urea nitrogen (BUN). The weight of organs, such as liver, spleen and adrenal glands after washing with alcohol was recorded per 100 g body weight of animal (Pawar and Shivakumar, 2011).

Table 2. Effect of *M.micrantha* on swimming endurance time.

Treatment	Swimming endurance time in minutes
Positive control CMC (0.5%W/V) p.o	24.83±2.21
MEMMR (250 mg/kg) p.o	35±1.98*
MEMMR (500 mg/kg) p.o	38.5±2.47**
AEMMR (500 mg/kg) p.o	31.5±3.27
Diazepam (2 mg/kg) p.o	39.66±3.11**

Statistical analyses

The statistical analyses were performed using 'Graph Pad Prism 6' Software by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. All data were expressed as mean ± standard error of mean (SEM), $P < 0.05$ was considered as statistically significant.

RESULTS

Preliminary phytochemical analysis

The preliminary phytochemical studies were performed for testing different phytochemical constituents present in methanolic and aqueous extracts of *M. micrantha*. The observations showed the presence of alkaloids, flavonoids, steroids, tannins and phenolics, which were found to be more in methanolic extract.

Acute toxicity studies

The methanolic and aqueous extracts of the plant *M. micrantha* was found to be safe up to 2,000 mg/kg body weight by oral route. After 24 h, animals were found well tolerated; there was no mortality and no signs of toxicity. The extracts were found to be safe, so the two dose levels that is, 250 and 500 mg/kg body weight were selected for the present study.

Adaptogenic (anti-stress) activity

Anoxia stress tolerance time

The results obtained from the anoxia stress tolerance test was expressed as mean ± SEM. Anoxia stress tolerance time was significantly ($P < 0.05$) enhanced on 7th, 14th and 21st day in MEMMR (500 mg/kg) and diazepam (2 mg/kg) treated groups. There was increased anoxia tolerance time seen after 2nd and 3rd week of MEMMR (250 mg/kg) treated group but not statistically significant with result obtained on 7th day. However the effect of AEMMR (500 mg/kg) on anoxia stress tolerance time in rats was not statistically significant at the end of 1st, 2nd and 3rd week of treatment (Table 1 and Figure 1).

Swimming endurance test

The swimming endurance time was significantly ($P < 0.05$) enhanced on 8th day in MEMMR (250 mg/kg), MEMMR (500 mg/kg) and diazepam (2 mg/kg) treated groups when compared to the stressed group (Table 2 and Figure 2).

Immobilisation stress

Effect on biochemical parameters: The immobilisation stress caused marked increase in biochemical parameters such as glucose, cholesterol and blood urea nitrogen in stressed group when compared to the control group. This stress induced elevated level of biochemical parameters such as, serum glucose, cholesterol and blood urea nitrogen were significantly reversed in MEMMR 500 mg/kg treated groups whereas AEMMR failed to reverse the elevated levels of biochemical parameters significantly (Table 3).

Effect on organ weight: Weight of liver and adrenal gland was increased, while weight of spleen was reduced in stressed group when compared to unstressed group. Pretreatment with MEMMR extract at high dose significantly ($P < 0.01$) reduced the weight of the liver, adrenal gland and increased the weight of the spleen. However the MEMMR extract at low dose (250 mg/kg) and AEMMR (500 mg/kg) failed to protect the immobilisation stress induced changes in organ weight such as liver, adrenal gland and spleen (Table 4).

DISCUSSION

In the present investigation, methanolic and aqueous extracts of *M. micrantha* has been evaluated for the antistress (adaptogenic) activity against different types of stress: anoxia, swimming endurance and immobilisation models. Diazepam, a benzodiazepine anxiolytics was used for the comparison. Diazepam is reported to possess a non-specific anti-stress activity involving the mesocortical dopamine system and the norepinephrine and 5HT levels of whole brain and hypothalamus. It is proposed that this effect is produced through an enhancement of GABAergic neurotransmission (Kenjale

Table 3. Effect of *M. micrantha* on immobilization stress induced changes in biochemical parameters.

Treatment group	Biochemical estimation (mg/dl)		
	Glucose	Cholesterol	Blood urea nitrogen
Negative Control	78.66±2.41	75.66±2.12	24.16±1.25
Positive control CMC(0.5%W/V) p.o	110.33±4.16	95.16±3.07	36±1.36
MEMMR(250mg/kg) p.o	102.5±3.58	86.33±3.45	32±1.75
MEMMR(500mg/kg) p.o	96.66±3.71*	83.33±3.82*	28.83±1.62*
AEMMR(500mg/kg) p.o	104.5±4.45	90.5±2.14	35.33±2.26
Diazepam (2 mg/kg) p.o	97±2.17*	84±2.68*	28.66±1.76*

Values are expressed as Mean ± SEM (n=6), analysed by one-way ANOVA followed by Dunnett's post hoc test, * P<0.05, **P<0.001, ***P<0.0001.

Table 4. Effect of *M. micrantha* on immobilization stress induced changes in organ weight.

Treatment group	Organ weight (gm/100 gm B.W)		
	Liver	Adrenal gland	Spleen
Negative control	3.71±0.08	0.015±.001	0.410±0.018
Positive control	4.95±0.14	0.032±.002	0.287±0.025
MEMMR (250 mg/kg)	4.50±0.24	0.025±.002*	0.348±0.032
MEMMR (500 mg/kg)	4.41±0.024*	0.02±.0016**	0.384±0.016*
AEMMR (500 mg/kg)	4.90±0.21	0.03±.0025	0.301±0.017
Diazepam (2 mg/kg)	4.37±0.22*	0.026±.008*	0.312±0.028

Values are expressed as Mean ± SEM (n=6), analysed by one-way ANOVA followed by Dunnett's post hoc test, * P<0.05, **P<0.001, ***P<0.0001.

et al., 2007).

In anoxia stress tolerance model, depletion of oxygen in hermetic vessel leads to convulsions in animals, and pretreatment with methanolic extract of *M. micrantha* had increased the duration of stress tolerance indicating their adaptogenic/anti-stress activity. This effect may be due to that fact that; during stress the methanolic extract of *M. micrantha* was capable of increasing succinate dehydrogenase (SDH) in the brain (Pawar and Shivakumar, 2011). This enzyme is responsible for utilization and conservation of energy in the cellular system of the organism, which helps adaptive processes during stress. Adaptogens producing beneficial effects in stress are believed to act by increasing non-specific resistance.

In case of swimming endurance test, MEMMR exhibited significant antistress activity as indicated by increase in swimming endurance time. There are reports that plasma levels of adrenaline and noradrenaline are enhanced during stress induced by swimming endurance test. In addition, monoamine oxidase (MAO) levels in the brain are reportedly decreased during stress (Debnath et al., 2011). The swim endurance test results indicate clearly that the methanolic extract of *M. micrantha* has the properties whereby it increases the physical endurance as well as the overall performance in rats and possessed significant anti-stress activity. It may be possibly normalizing the plasma level of catecholamine and MAO.

The immobilisation stress caused marked increase in biochemical parameters such as glucose, cholesterol and blood urea nitrogen in stressed group when compared to the control group. In the present study, a significant hyperglycemia was observed with immobilization stress model. Under stressful conditions, cortisol in human and corticosterone in rats will be secreted by adrenal cortex. Hyper secretion of cortisol helps the maintenance of internal homeostasis through the process of gluconeogenesis and lipogenesis (Debnath et al., 2011). Methanolic extract of *M. micrantha* significantly reduced hyperglycemia probably by reducing the hyperactivity of adrenal cortex and also by maintenance of homeostatic mechanism in immobilization stress animals.

The mechanism by which stress rises serum cholesterol is likely to be related to the enhanced activity of hypothalamo-hypophyseal axis (HPA) resulting in liberation of catecholamines and corticosteroids. This could lead to increase in blood cholesterol level since epinephrine is known to mobilise lipids from adipose tissues. The increase in release of catecholamines leads to elevated levels of glucose and BUN (Tsigos and Chrousos, 2002). This stress induced elevated level of

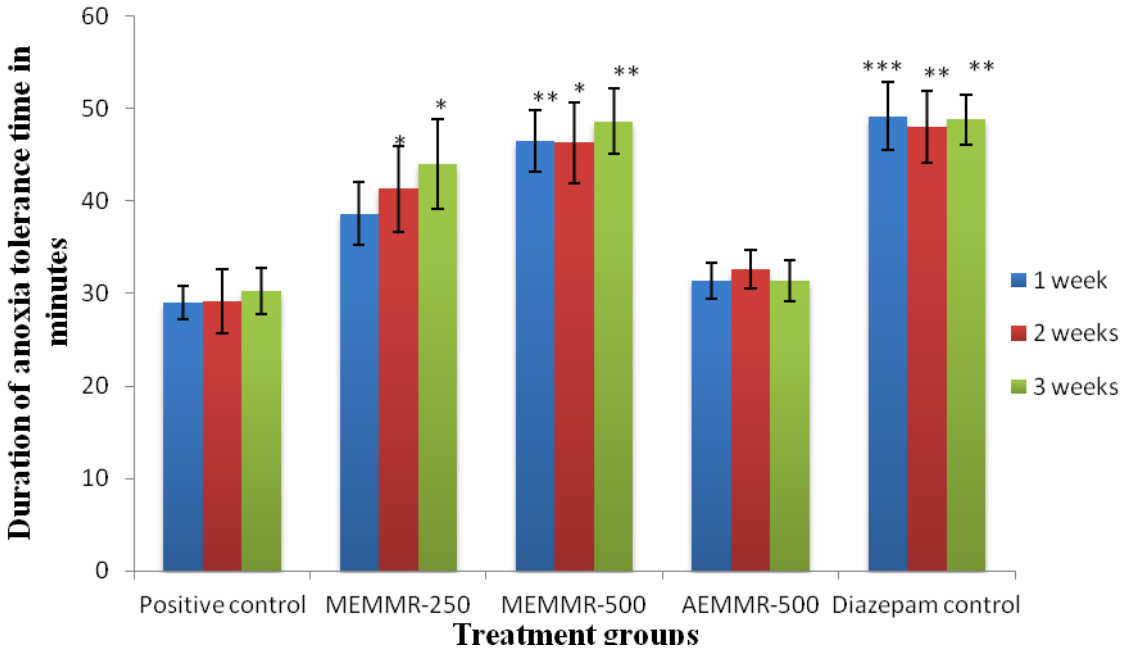

Figure 1. Effect of *M.micrantha* on anoxia stress tolerance time in rats.
Values are expressed as Mean ± SEM (n=6), analysed by one-way ANOVA followed by Dunnett's post hoc test, *Represents statistical significance vs. control ($p<0.05$).

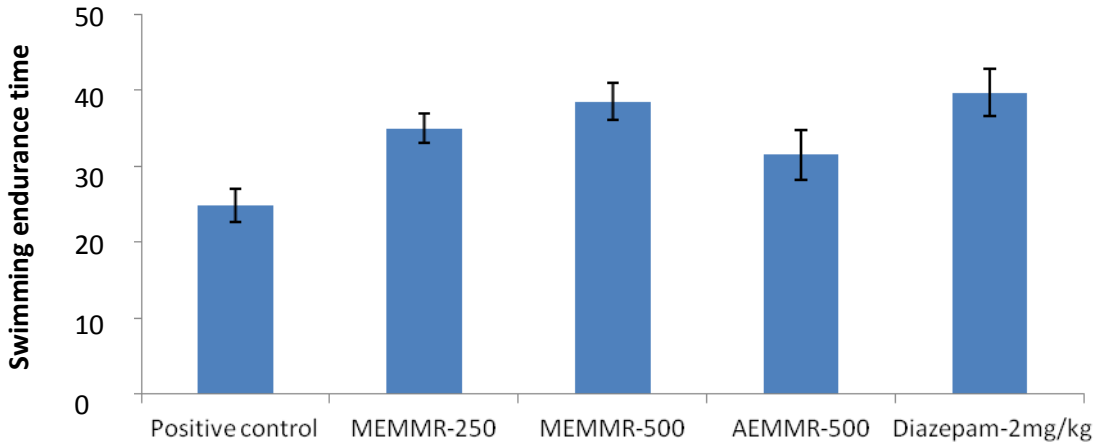

Figure 2. Effect *M.micrantha* on swimming endurance time in rats.
Values are expressed as Mean ± SEM (n=6), analysed by one-way ANOVA followed by Dunnett's post hoc test, *Represents statistical significance vs. control ($p<0.05$).

biochemical parameters were significantly reversed in MEMMR 500 mg/kg treated groups.

Adrenal glands and liver weights were significantly increased in immobilization stress models. Stress induces adreno-medullary response in man to release adrenaline which in turn stimulates β_2 receptors on the pituitary gland. It leads to greater release of adrenocorticotropic hormone (ACTH) that can stimulate the adrenal medulla as well as cortex resulting in further release of adrenaline and increase in weight of adrenal gland to a greater extent. Cortisol increases mRNA levels in liver cells, this lead to increase in weight of liver. Spleen constricts to release more blood cells (RBC) during stress. So its weight decreases during stress (Pawar and Shivakumar, 2011). This stress induced changes of organs weight were significantly reversed by the methanolic extract at higher dose 500 mg/kg.

Literature survey indicates that flavonoids, triterpenes

and tannins were reported to possess variety of pharmacological activities including antistress activity (Chethan et al., 2012). In this present investigation, preliminary phytochemical screening on MEMMR gave positive tests for flavonoids, steroids and tannins, this might be the reason for significant adaptogenic property. The result from the study showed an increase in duration of anoxia tolerance and swimming endurance time in rats treated with methanolic extract. The reversal of immobilization stress induced changes in biochemical parameters and organs weight were also exhibited in alcoholic extract treated groups. So the results suggest the adaptogenic activity of the plant *M. micrantha*, hence it can be categorized as plant adaptogen. The results are encouraging to pursue further studies on the bioactivity guided fractionation of these extracts to isolate and characterize probable bioactive molecule responsible for ant-stress activity.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Antimicrobial potential and bioactive constituents from aerial parts of *Vitis setosa* Wall.

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Plants still represent untapped sources of novel compounds with potential therapeutic effects. Hence, the present investigation was carried out to study the *in vitro* evaluation of antimicrobial potential and to assess the bioactive constituents of the aerial parts of *Vitis setosa* Wall. The powdered aerial material was extracted with different solvents and examined for antimicrobial activity against *Escherichia coli, Salmonella typhi, Bacillus subtilis, Streptococcus pyogenes, Candida albicans, Fusarium solani* and *Trichophyton rubrum* using well diffusion method. An ethanol extract of *V. setosa* showed significant antimicrobial activity against all the tested pathogens. The fresh aerial parts contained eight primary metabolites (chlorophylls, carotenoids, total soluble sugars, total soluble proteins, total free amino acids, total phenol, hydroxyl phenols and lipids) and alkaloids, tannins, saponins, triterpenoids and phenolic compounds. Gas chromatography-mass spectrometry (GC-MS) results also showed 26 bioactive compounds including n-hexadecanoic acid, 9,12,15-octadecatrienoic acid (z,z,z)- and τ-tocopherol. In conclusion, the aerial parts of *V. setosa* are a promising source of antimicrobial bioactive compounds.

Key words: Bioactive constituents, *Vitis setosa*, GC-MS analysis, ethanolic extract, antimicrobial activity, folk medicine.

INTRODUCTION

In the plant kingdom, there are thousands of plants, known and unknown, that yield medicines or drugs useful to man. These plants are the gold mines to treat the diseases of men and animals, and serve as cure in the natural way (Jain, 1979). Medicinal herbs constitute the cornerstone of traditional practice world-wide and they have been used for centuries as remedies for human diseases because they contain chemical components of

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therapeutic value (Christina and Muthumani, 2013) and the plants are known to possess various secondary metabolites which show inhibitory effect against the various human diseases (Bhardwaj and Laura, 2009; Gobalakrishnan et al., 2013). These herbs are relatively cheap, easily available and their use depends on ancestral experience (Jamilu et al., 2008). Plants still represent a large untapped source of novel compounds that might serve as leads for the development of novel drugs (Cowan, 1999) and they are an important source of new biochemical substances with potential therapeutic effects (Nadaf et al., 2012), inhibiting the growth of microbes by interfering with their specific physiological characters or metabolic functions (Arekemase et al., 2011).

The first step towards this goal is the biological and phytochemical screening of plant extracts from traditional preparations used in the popular medicines (Alonso-Paz et al., 1995). Drug resistance of human pathogenic bacteria has phytochemical substances which have been commonly and widely reported in literature (Sarac and Ugur, 2007). Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have focused their attention on the extracts and biologically active compounds isolated from medicinal plant species for herbal medicines (Essawi and Srour, 2000). Now-a-days, there is a growing interest in the antimicrobial screening of extracts and essential oils from plants to discover new antimicrobial agents. Keeping this in view, the present study was undertaken to investigate the natural bioactive constituent analysis and antimicrobial potential of the aerial parts extract of *Vitis setosa* (Family: Vitaceae; Pulinaralai in Tamil), a medicinal vine herb that grows only in selective areas of the Indian subcontinent.

MATERIALS AND METHODS

Plant

Aerial parts of fresh *V. setosa* plant were collected during January to April, 2011 from various regions of the Pudukkottai district in Tamilnadu, India. Plant was identified using the facility of Rapinat Herbarium, St. Joseph's College, Tiruchirappalli and the identified voucher specimen was deposited in the Research and PG Department of Botany, H.H The Rajah's college, Pudukkottai, Tamilnadu, India. Plants were thoroughly washed with tap water and aerial parts were separated and kept in between the filter papers in a dark room at room temperature to get rid of moisture until further analysis.

Preparation of extract

Dried aerial part materials were powdered with Waring blender, at room temperature and 2 g of the sample powder was soaked in 20 ml of different solvents (ethanol, ethyl acetate, chloroform, hexane, benzene and water) overnight. Later, the samples were filtered under vacuum using Whatman No.1 filter paper and stored in airtight screw-capped bottles at 5°C for further analysis.

Preparation of inoculum

Seven clinical pathogenic organisms were obtained from the Microbial Clinical Laboratory, KMC Hospital, Tiruchirappalli. Out of the seven, four strains were bacteria (*Escherichia coli, Salmonella typhi, Bacillus subtilis* and *Streptococcus pyogenes*) and three strains were fungi (*Candida albicans, Fusarium solani* and *Trichophyton rubrum*). Stock culture was maintained at 5°C on slopes of nutrient agar for bacteria and potato dextrose agar (PDA) for fungi. Under the sterile conditions, active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to the test tubes of respective media for respective tested organisms and incubated without agitation for 24 h at 37 ± 2°C for antibacterial activity and at 25 ± 2°C for 48 h for antifungal activity. Muller-Hinton broth (for bacteria) and PD broth (for fungi) were prepared for streaking and fresh slant cultures were prepared and stored in refrigerator at 5°C for future requirements.

In-vitro antimicrobial tests

Spectrum of antibacterial activity was studied by using the techniques described by Bauer et al. (1966). Gentamycin sensitivity disc (30 mg; Hi-Media) was used as a positive control and respective solvents were taken as negative controls. At the end of incubation, inhibition zones formed around the discs were measured with transparent ruler in millimeter. These studies were performed in triplicate.

Biochemical screening

Biochemical tests: Chlorophylls *a*, chlorophylls *b*, total chlorophylls, total sugars and total proteins (Sadasivam and Manickam, 2005), carotenoids (Goodwin and Britton, 1988), total free amino acids (Troll and Cannan, 1953), total phenols and hydroxyl phenols (Swain and Hillis, 1959) and lipids (Jayaraman, 1981) were quantitatively revealed of the fresh aerial part of *V. setosa*. Secondary metabolites were tested by the standard methods of Harborne (1973) and Odebiyi and Sofowora (1978).

Preparation of extraction in GC-MS Analysis

Aerial parts of *V. setosa* were shade dried. 20 g of the powdered materials was soaked in 95% ethanol for 12 h. The extracts were then filtered through Whatman filter paper No. 41 along with 2 g sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulfate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant material used and these extracts (2 µl of injection sample) were employed for GC/MS analysis (Merlin et al., 2009).

GC-MS analysis

GC-MS analysis was performed in the Indian Institute of Crop Processing Technology (IICPT), Thanjavur, India. Prepared alcoholic samples were analysed in a Perkin Elmer GC Clarus 500 MS

Table 1. Antimicrobial activity of *V. setosa* extracts in different solvents.

Tested organisms	Inhibition zone of diameter (mm)[a].						
Bacteria	**A**	**B**	**C**	**ET**	**EA**	**H**	**Gentamycin (+) disc**
E. coli	-	-	-	15.0±0.3	-	-	23.5
S. typhi	-	-	-	11.3±0.5	-	-	28.3
B. subtitles	-	-	-	10.3±0.2	12±0.3	-	25.8
S. pyogenes	-	-	-	10.6±0.4	-	-	31.7
Fungi							
C. albicans	13.7±0.5	-	12.1±0.2	11.9±0.4	12.5±0.2	-	35.6
F. solani	-	-	-	12.3±0.5	13.5±0.3	-	28
T. rubrum	-	-	-	10.3±0.3	14.3±0.6	-	26.2

Aqueous (A), Benzene (B), Chloroform (C), Ethanol (ET), Ethyl acetate (EA), Hexane (H), No activity (-).[a] - Values are mean ± standard deviation of three determination.

system for different components present in the extract, under the following conditions: column – dimethyl polysiloxane DB-1 fused silica capillary column (30 m × 0.25 mm × 0.1 µm of film thickness); carrier gas - helium (1 ml/min); injector temperature 250°C; detector temperature 200°C; column temperature 35 to 180°C at 4°C/min; then 180 to 250°C at 10°C/min; MS electron impact 70 $_e$V. Identification of the constituents was achieved with the aid of the respective Kovarts Indices and comparison of the mass spectra with those in the library (NIST Ver.2.1).

RESULTS

Present study investigated the antimicrobial activity of aerial parts of *V. setosa* crude extract against four bacteria (*E. coli, B. subtilis, S. typhi, S. pyogenes*) and three fungi (*C. albicans, F. solani* and *T. rubrum*), and the results are presented in Table 1. Antimicrobial activity of ethanolic extract of *V. setosa* aerial parts showed significant antimicrobial activity against all the tested pathogens. Ethanolic extract had maximum (15.0 mm) inhibition against *E. coli*. Whereas, the ethyl acetate extract exhibited moderate significant antimicrobial activity and maximum (14.3 mm) inhibition against *T. rubrum*.

Results of the biochemical screening extract of fresh aerial parts of *V. setosa* were presented: Chlorophyll *a* (1.868 mg g^{-1}), chlorophyll *b* (1.350 mg g^{-1}), total chlorophyll (2.300 mg g^{-1}), carotenoids (0.448 mg g^{-1}), total soluble sugars (47.50 mg g^{-1}), total soluble proteins (51.30 mg g^{-1}), total free amino acids (5.78 mg g^{-1}), total phenol (12.27 mg g^{-1}), hydroxyl phenols (6.870 mg g^{-1}) and lipids (20 mg g^{-1}). Further, alkaloids, tannins, saponins, triterpenoids and phenolic compounds were also present in the *V. setosa*.

Gas chromatography and mass spectroscopy analyses were carried out on the alcoholic extract of aerial parts of *V. setosa* and various bioactive compounds were identified. Active principles with their retention time (RT), molecular formula (MF), molecular weight (MW), concentration (%) and nature of the compounds are presented in Table 2. In the present investigation, a variety of compounds has been detected: Pentanoic acid; 2-acetyl-4-methyl- ethyl ester, 1-Butanamine;2-methyl-N- (2-methylbutylidene), benzeneacetaldehyde, propan,1,2,3-triethoxy-, 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, β-D-glucopyranose;4-O-β–D-galactopyranosesyl, dodecanoic acid; methyl ester, 2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trymethyl-(R)-[Syn:Dihydroactinidiolide], dodecanoic acid, phosphonofluridic acid; (1-methylethyl)- cyclohexyl ester, tetradecanoic acid, 2-pentadecanone,6,10,14-trimethyl-[Syn:Hexahy drofarnesyl acetone], pentadecanoic acid, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-hexadecanoic acid, hexadecanoic acid ethyl ester, phytol, 9,12-octadecadienoic acid (z,z)-, 9,12,15-octadecatrienoic acid (z,z,z)-, Octadecanoic acid, 4,8,12,16-Tetramethylheptadecan-4-olide, 1,2-Benzenedicarboxylic acid; diisooctyl ester, Squalene, Cholesta-4,6-dien-3-ol,(3β), τ-Tocopherol and 9,10-Seco Cholesta-5,7,10(19)-trine-3,25,26- triol,(3β ,5Z,7E). GC-MS spectrogram showing the peak identities of the compounds is depicted in Figure 1.

DISCUSSION

In recent years, various scientists have accelerated research on the drugs and dietary supplements from the plants (Cowan, 1999) and used them as herbal medicines for the treatment of infectious diseases (Madureira et al., 2012), since no literature is currently available to substantiate antimicrobial prospective of the *V. setosa* aerial part. Therefore the present study was made on

Table 2. Compounds identified from ethanol extract of *V. setosa.*

RT	Compound	MF	MW	Peak area (%)	Compound Nature
3.57	Pentanoic acid,2-acetyl-4-methyl-,ethyl ester	$C_{10}H_{18}O_3$	186	1.38	Halogen- acids
4.13	1-Butanamine,2-methyl-N-(2-methylbutylidene)	$C_{10}H_{21}N$	155	1.55	Butyl alcohol
4.24	Benzeneacetaldehyde	C_8H_8O	120	5.05	Aromatic hydrocarbons
4.48	Propan,1,2,3-triethoxy-	$C_9H_{20}O_3$	176	1.24	Ether compound
5.79	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	5.05	Flavonoid fraction
7.85	β-D-Glucopyranose,4-O-β –D-galactopyranosesyl- [syn:-Lactose]	$C_{12}H_{22}O_{11}$	342	4.21	Sugar compound
10.34	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	0.34	Lauric acid ester
10.75	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro- 4,4,7a- trymethyl-(R)-[Syn:Dihydroactinidiolide]	$C_{11}H_{26}O_2$	180	1.32	Heterocyclic Compounds
10.97	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.21	Lauric acid
11.15	Phosphonofluridic acid,(1-methylethyl)-,cyclohexyl ester	$C_{18}H_{18}FO_2P$	208	1.52	Phosphorus Compounds
13.39	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	2.12	Myristic acid
14.4	2-Pentadecanone,6,10,14-trimethyl- [Syn: Hexahy drofarnesyl acetone]	$C_{18}H_{36}O$	268	5.35	Diterpenoids
14.75	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.59	Lauric acid
15.04	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.55	Terpene alcohol
16.16	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	19.68	Falmitic acid
16.46	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	248	2.19	Fatty acid
18.41	Phytol	$C_{20}H_{40}O$	296	4.98	Diterpene
18.73	9,12-Octadecadienoic acid(z,z)-	$C_{18}H_{32}O_2$	280	1.96	Linoleic (poly unsaturated fatty acid)
18.84	9,12,15-octadecatrienoic acid,(z,z,z)-	$C_{18}H_{30}O_2$	278	7.25	Linoleic acid
19.15	Octadecanoic acid	$C_{18}H_{36}O_2$	284	3.75	Myristic acid
21.95	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	2.36	Carbohydrates
24.68	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	2.92	Plasticizer compound
28.92	Squalene	$C_{30}H_{50}$	410	2.6	Triterpenoids
33.01	Cholesta-4,6-dien-3-ol,(3β)-	$C_{27}H_{44}O$	384	3.63	Steroids
33.78	τ-Tocopherol	$C_{28}H_{48}O_2$	416	5.53	Vitamin E groups
35.29	9,10-Secocholesta-5,7,10(19)-trine-3,25,26-triol,(3β ,5Z,7E)-	$C_{27}H_{44}O_3$	416	11.67	Steroids

on antimicrobial study of *V. setosa* aerial parts extract to provide scientific evidence for its use as a folk medicine. Results revealed that the plant has a potential antimicrobial activity against all the tested pathogens. Ethanolic extract showed effective antibacterial activity and it has better solubility compared to other solvents. It is worth mentioning here that the ethanol formulations are relatively safe for human consumption as com-pared with other organic solvents (Wendakoon et al., 2012).

The preliminary biochemical tests are significant and helpful in finding chemical constituents in the plant materials that might lead to the source of pharmacologically active compounds (Iqbal, 2012). Chanda and Kaneria (2011) reported that the bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration could vary in different plant

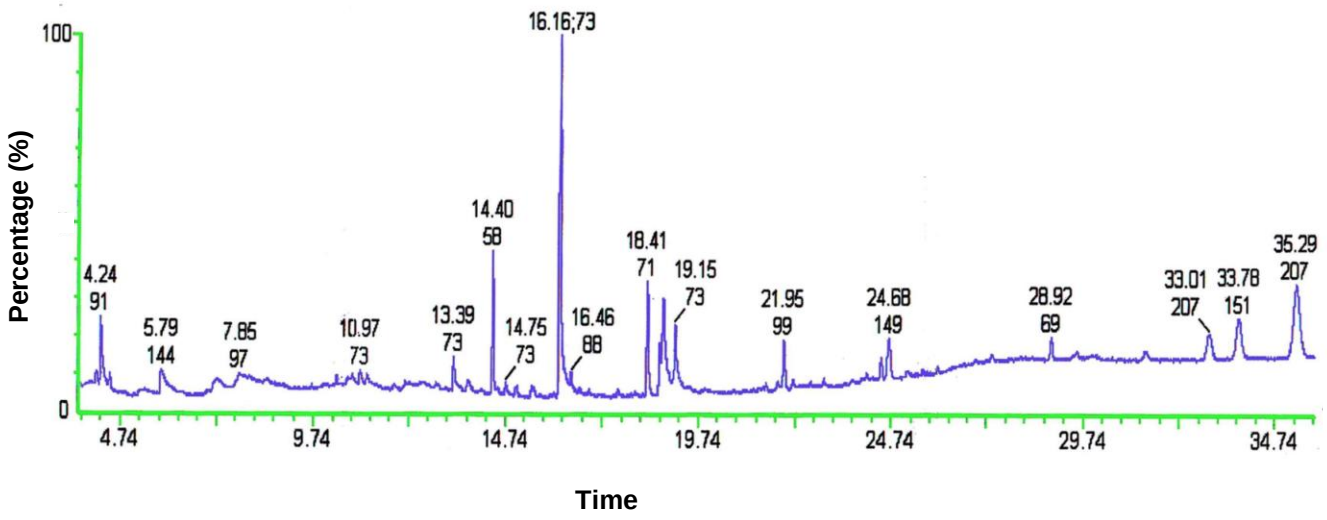

Figure 1. GC-MS spectrogramof ethonolic extract of the *V. setosa.*

parts. In this regard, aerial part is one of the highest accumulatory plant parts and its compounds are generally preferred for therapeutic purpose (Pires De Abreu et al., 2003).

Generally, alkaloids, saponins, tannins, flavonoids and phenolic compounds are important antimicrobials (Yadav and Agarwala, 2011). This lends support to the present study which revealed that those phytochemicals were identified in *V. setosa*. Similarly, previous studies have revealed that plant phytochemicals obtained from *V. setosa* leaf could act as antimicrobial drugs (Misra, 2009; Hemayet et al., 2012). Action mechanism of such compounds has not been unequivocally established, but they may interfere with peptidoglycon bacterial cell wall synthesis in the effected organisms (Rasooli and Mirmostafa, 2002) and in many other ways such as inhibiting protein synthesis, interfering with nucleic acid synthesis, breaking the peptide bonds, acting as chelating agents, inhibiting the metabolic pathway, lysis of cells and preventing the utilization of available nutrients by the microorganisms.

Knowledge of chemical constituents of plants is desirable because such information will be important for synthesis of chemical substances (Yadav and Agarwala, 2011). It could be qualified for application in pharmaceutical industry (Iqbal, 2012). Therefore, present study revealed that in the aerial part extract of *V. setosa* were identified 26 different compounds by GC-MS. Especially, n-Hexadecanoic acid, 9,10-Seco Cholesta-5,7,10(19)-trine-3,25,26-triol,(3β,5Z,7E, 9,12,15-octade-catrienoic acid (z,z,z) and τ-Tocopherol contributed more percentage than the other compounds. Compounds except pentanoic acid; 2-acetyl-4-methyl-ethyl ester, 1-butanamine-2-methyl-N-(2-methylbutylidene)-benzene-acetaldehyde, propan,1,2,3-triethoxy, 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trymethyl-(R)-phosphonofluridic acid;(1-methylethyl)- cyclohexyl ester, 2-Pentadecanone,6,10,14-trimethyl and Cholesta-4,6-dien-3-ol,(3β) were identified.

n-Hexadecanoic acid was extracted from the *V. setosa* aerial part (19.69 %), which is higher than that of the other extracted compounds. Harada et al. (2002) reported that this compound acts as an anticancer drug and Graikou et al. (2011) observed antioxidant and antimicrobial property for this compound. Similarly, Praveenkumar et al. (2010) reported that n-Hexadecanoic acid act as an antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic and 5- alpha reductase inhibitor.

9,12,15-Octadecatrienoic acid-(z,z,z) belonging to linoleic acid group was extracted from *V. setosa* (7.25%) and this compound acts as antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Praveenkumar et al., 2010) and also as an antimicrobial agent (Senthilkumar and Kamaraj, 2010). τ-Tocopherol was obtained from *V. setosa* (5.53%) and it belongs to vitamin E which has reactivity with radicals and shows some antioxidant effect (Yoshida et al., 2007). It also has important implications in the anti-inflammatory effects and can control neutrophil oxidative burst (Varga et al., 2008). Further, vitamin E is associated with a decreased risk of heart diseases and certain cancers (Dickinson, 2002;

Jaialal and Devaraj, 2003).

In conclusion, ethanolic extract of aerial parts of *V. setosa* possess significant antimicrobial activity and this potential may be due to the presence of bioactive compounds like alkaloids, tannins, saponins, triterpenoids and phenolic compounds. Hence, the present study was justified on its use in the traditional folk medicine. GC-MS analysis also identified a variety of natural bioactive compounds including n-hexadecanoic acid, 9,12,15-octadecatrienoic acid (z,z,z)- and τ-tocopherol. However, a further detailed study on *V. setosa* is necessary for the development of novel drugs in the arena of antioxidant, anti-inflammatory, anticancer, antiandrogenic, antiarthritic and anticoronary activity.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

In vitro killing rate of *Euphorbia heterophylla* and *Pterocarpus lucens* extracts minimum bactericidal concentration (MBC) on some clinical bacterial isolates

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In vitro **antibacterial activity of methanol and ethanol leaf extracts of** ***Euphorbia heterophylla*** **and** ***Pterocarpus lucens*** **were investigated against six bacterial clinical isolates using the tube dilution and agar diffusion methods.** ***Salmonella typhi*** **was the most susceptible to methanol leaf extracts of** ***E. heterophylla*** **with a zone of inhibition ranging from 16 to 24 mm for 12.5 to 100 mg/ml concentration. This was followed by** ***Streptococcus lactis, Escherichia coli, Staphylococcus aureus,*** **and** ***Shigella*** **species in that order with** ***Proteus vulgaris*** **not susceptible to the different test concentrations of both plant extracts.** ***E. heterophylla*** **had the least minimum inhibitory concentration (MIC) of 6.25 mg/ml against** ***E. coli*** **and** ***S. typhi*** **while** ***P. lucens*** **extract MIC of 25.00 mg/ml was the least against** ***S. typhi*****. Since there is an inverse relationship between MIC value and susceptibility of the clinical test isolates, the MIC values also shows that** ***E. heterophylla*** **methanol leaf extracts were more potent to the susceptible test organisms having lower MIC values than the corresponding ethanol leaf extract MIC value.** ***E. heterophylla*** **extract minimum bactericidal concentration (MBC) was 25.00 mg/ml for the sensitive isolates except for methanol extract with 12.50 mg/ml against** ***S. typhi*** **and ethanol extract with 12.50 mg/ml against** ***S. aureus. P. lucens*** **extract MBC was 100.00 mg/ml for the sensitive test isolates except for ethanol leaf extracts with 50.00 mg/ml against** ***S. typhi.*** **The killing rate of** ***E. heterophylla*** **methanol leaf extract MBC shows that** ***E. coli*** **was most rapidly killed at a rate of 4.53×10^6 CFU/min with** ***S. aureus*** **as the least killed at a rate of 0.62×10^6 CFU/min.** ***S. lactis*** **and** ***E. coli*** **were the most rapidly killed by** ***P. lucens*** **leaf extract MBC at a rate of 1.90×10^6 CFU/min. The killing rate of the extracts showed a positive support in the potential use of these plants in curing some infections as done by the traditional herbal healers in Anyigba, Kogi State, Nigeria.**

Key words: *In vitro*, antibacteria, extract, inhibitory.

INTRODUCTION

The primitive man lived at the mercy of nature in constant harassment of diseases from the earliest time. The search for agents to cure disease began long before people were aware of the existence of microbes (Larry and Judy, 1996). The use of medicinal plant in the treatment of disease is as old as the diseases themselves. Ijomah et al. (1997) noted that herbalism was the earliest form of medicine. It was the introduction of orthodox medicine that suppressed the growth and development of herbal medicine, the early indigenous health care system. Hence

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long before mankind observed the existence of microbes, the idea that plants contained healing potentials was accepted (Rios and Recio, 2005).

Records of early civilization in all parts of the world revealed that a considerable number of drugs that are used in modern medicine were in use even in the ancient times (Johnson, 2005). It was estimated that 25% of all prescribed medicines today are substances derived from plants (Belloin et al., 2005). In spite of the improved health system and longevity in the US and Europe, millions of people in this countries are turning back to herbal medicines in order to prevent or treat many illness (WHO, 2006) and to circumvent the resistance of many human pathogens to conventional drugs, some of which produced side effects like hypersensitivity and immuno-suppression (Beardsley, 1996). Medicinal plants are used in traditional societies all over the world for centuries to cure many infectious diseases. These early attempts used natural substances usually native plants or their extracts as remedies for the treatment of human diseases. This is largely based on experience handed down from one generation to another. The traditional herbal healing techniques are passed on as trade secrets in the families of certain communities, a practice protected by tradition. Indigenous people have demonstrated the therapeutic value and healing power of plants over the years (Edward and Ayansu, 1983) and over 60% of Nigeria rural population depends largely on traditional medicine for their health care needs (Ghani et al., 1986), while up to 80% of African population uses traditional medicine for their primary health care (WHO, 2006).

As part of the unabated search for plants with antimicrobial activity, this study was carried out on *Euphorbia heterophylla* and *Pterocarpus lucens* to find out the antibacterial potential of these plants against some test clinical bacterial isolates and determine the killing rate of the extracts minimum bactericidal concentration (MBC). The choice of these plants was predicated on their use by the traditional herbal healers in Anyigba, Kogi State, Nigeria in the treatment of infections such as typhoid fever, gastrointestinal disorder, urinary tract infections, infected wounds and topical ulcers.

E. heterophylla Linn belongs to the family Euphorbiaceae with more than 1000 species included in this family. Members of the family are found in most parts of the world, but their diversity is greatest in the tropics (Peter et al., 1992). The plants ranged from prostrate herbs to tall trees. Several species of Euphorbia exhibit xerophytic adaptation that made them to resemble some members of the cactus family. The superficial similarity also found in some species of milkweed family is as a result of convergent evolution in unrelated plant species (Peter et al., 1992). Members of the Euphorbiaceae are of considerable importance by providing us with food; drugs, rubber and other products (Walter et al., 1999). They are also used as purgatives (Peter et al., 1992*). E. heterophylla* is an annual medicinal herb with common name 'spurge weeds' (Falodun et al., 2004). The plant extracts is used in ethno medicine for the treatment of constipation, bronchitis and asthma by traditional practitioners (Falodun et al., 2006). The herbal healers in Anyigba, Kogi State, Nigeria use *E. heterophylla* Linn commonly called 'Salime' in 'Igala' in the treatment of typhoid fever by drinking the tea made from cooking the leaves.

MATERIALS AND METHODS

Plant source and identification

The plant stem with leaves and reproductive structures were collected from different locations in Anyigba, Dekina Local Government Area, Kogi State, Nigeria. The plants were identified by Professor F. A. Oladele and Mr. S. A. Adebayo of the Herbarium, Department of Biological Sciences, University of Ilorin, Kwara State, Nigeria.

Plant treatment

The plant leaves were washed with distilled water and dried at room temperature in the Microbiology Laboratory, Kogi State University, Anyigba, for several days until the leaves became crispy and of constant weight. The dried leaves were ground separately using sterile pestle and mortar.

Preparation of crude extracts

Plant extracts were prepared using the modified method of Alade and Irobi (1993). 500 g of the powered dried leaves were soaked separately in 500 ml of 90% methanol and 98% ethanol for 72 h in the dark. It was then agitated at 200 rpm for 1 h on a mechanical shaker. The resulting suspension was filtered using sterile Whatman filter paper No. 1. The filtrate obtained was evaporated to dryness using a rotary evaporation (Falodun et al., 2006) and weighed on chemical balance.

Source of test clinical bacterial isolates

The test organisms were collected from the culture collections of the Federal Medical Centre, Owo and the Public Health Laboratory, Akure, both in Ondo State, Nigeria.

Culture media

The medium used for the activation and standardizations of inoculums of the test clinical bacterial isolates was nutrient broth, while the Mueller Hinton Agar was used for the antimicrobial sensitivity test. They were prepared according to the manufacturers' instruction.

Standardization of inoculums

The test clinical bacterial isolates were grown in a nutrient broth and incubated at 37°C for 24 h. Both cultures were standardized using the McFarland nephrometric method. McFarland standard No. 4 was chosen and prepared by adding 9.6 ml of 1.0% Na_2SO_4 solution to 0.4 ml of 1.0% BaCl which gives a corresponding

Table 1. Methanol and ethanol extracts filtrates.

Plant	Methanol extract filtrate (g)	Ethanol extract filtrate (g)
Euphorbia heterophylla	10.23	8.02
Pterocarpus lucens	10.01	7.3

approximate bacterial density of 1.2×10^7 CFU/ml of solution (Lenette et al., 1985; Bryant, 1981). The bacterial broth culture used was diluted to give the same turbidity with the solution prepared.

Preparation of extract concentrations

In preparing 100 mg/ml concentration, 1 g of the filtrate was reconstituted in 2 ml of the appropriate solvent (methanol or ethanol). 50 mg/ml concentration was prepared by adding equal volume of solvent and 100 mg/ml concentration, that is, double fold dilution. Double fold dilution was carried out on the 50 mg/ml concentration to give 25 mg/ml, while it was repeated for 25 mg/ml to get 12.5 mg/ml concentration.

Antibacterial activity of extracts

Zone of inhibition

The modified agar well diffusion method of Perez et al. (1990) was used. 0.2 ml of the standardized bacterial broth culture of each test clinical bacterial isolate was mixed with 20 ml of molten Mueller Hinton Agar at 40°C. The seeded agar was poured aseptically into sterile Petri dishes and allowed to solidify. The solidified agar was punched with a 6 mm diameter sterile cork borer to create wells on the agar.

The wells were filled with 0.1 ml of each prepared extract concentration (one concentration per well). Sterile distilled water was used to fill one of the wells which served as the solvent control, while gentamycin sulphate (1 µg/ml) was used as the positive control. Tests were carried out in duplicates and plates incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the inhibition zone diameter (IZD) in millimeter (mm)

Determination of minimum inhibitory concentration (MIC)

Sterile nutrient broth was used to prepare the different extract concentrations. 4 ml of each extract concentration was introduced into sterile test tube. 1 ml of the standardized bacterial broth culture of test clinical bacterial isolate was added to each set of the extract concentrations.

The control test tube was inoculated with sterile distilled water. All the tubes were cotton plugged and incubated at 37°C for 24 h. The MIC was taken as the lowest inoculated extract concentration that did not permit any visible growth when compared with the turbidity of the test tube containing sterile nutrient broth and inoculated with water (control) (Rojas et al., 2006).

Determination of minimum bactericidal concentration (MBC)

The content of all the MIC tubes with no visible growth were plated out on sterile Mueller Hinton Agar and incubated at 37°C for 24 h. The MBC was taken to be the lowest inoculated extract concentration that did not produce bacterial colonies when platted out on sterile Mueller Hinton agar (Rojas et al., 2006).

Determination of the MBC killing rate

The modified method of Olowosulu et al. (2003) was used. 1 ml of the plant extract MCB was mixed with 3 ml of sterile nutrient broth and incubated at 37°C with 1 ml of standardized culture of the test isolate broth. 1 ml of standardized culture of the test isolate broth was added to 4 ml of normal saline to serve as control. The tubes were incubated at 37°C. 0.1 ml of the incubated tubes was removed at regular interval between 0 and 180 min. Each withdrawn sample was dispensed and serially diluted 10 fold to dilution of 10^{-5} with normal saline. 0.1 aliquot of the 10^{-5} dilution was plated out in duplicate on sterile Mueller Hinton Agar and incubated at 37°C for 24 h. The number of colony forming units (CFU) on each plate was counted. The total number of viable bacteria in the original sample per ml was computed from the number counted from the 0.1 ml aliquot of 10^{-5} dilution at time 0 min. The number of deaths of the bacteria was extrapolated from the number of bacterial survivors at each time withdrawn when compared with the number of bacteria counted at time 0 min.

RESULTS

Preparation of crude extracts

The leaf extract prepared showed that methanol gave a higher filtrate for *E. heterophylla* and *P. lucens* when compared with the ethanol extract filtrate for both plants as shown in Table 1.

Antibacterial activity of leaf extracts

Zone of inhibition

The methanol and ethanol leaf extracts of *E. heterophylla* were active against all the test clinical bacterial isolates except for *Proteus vulgaris* which was not sensitive to the extract concentrations as shown in Tables 2 and 3. *P. lucens* leaf extracts did not show antibacterial activity against *P. vulgaris*, *Staphylococcus aureus* and *Shigella* species as shown in Tables 2 and 3.

Minimum inhibitory concentration (MIC) of leaf extracts

The MIC of the methanol leaf extracts and ethanol leaf extracts were the same for both plants against all the test clinical bacterial isolates except for *Streptococcus lactis* which differs in both plants extracts as shown in Table 4. *E. heterophylla* extract concentration of 6.25 mg/ml was the least against *Escherichia coli* and *Salmonella typhi* as shown in Table 4.

Table 2. Antibacterial sensitivity of methanol leaf extract.

Test organism	*Euphorbia heterophylla* (mm) (Concentration in mg/ml)				*Pterocarpus lucens (mm)* (Concentration in mg/ml)				Gentamycim (1 µg/ml)
	100	50	25	12.5	100	50	25	12.5	
Staphylococcus aureus	18	15	13	9	6	6	6	6	12
Escherichia coli	20	16	12	8	10	7	6	6	12
Proteus vulgaris	6	6	6	6	6	6	6	6	25
Salmonella typhi	24	18	18	16	11	10	9	8	18
Streptococcus lactis	20	18	15	13	10	8	6	6	15
Shigella species	15	14	13	12	6	6	6	6	20

Table 3. Antibacterial sensitivity of ethanol leaf extract.

Test organism	*Euphorbia heterophylla* (mm) Concentration in mg/ml				*Pterocarpus lucens* (mm) Concentration in mg/ml				Gentamycin (1 µg/ml)
	100	50	25	12.5	100	50	25	12.5	
Staphylococcus aureu s	18	12	10	8	6	6	6	6	12
Escherichia coli	20	15	12	8	10	6	6	6	12
Proteus vulgaris	6	6	6	6	6	6	6	6	25
Salmonella typhi	20	17	15	11	13	12	11	10	18
Streptococcus lactis	18	17	15	14	10	8	8	6	15
Shigella species	15	14	13	12	6	6	6	6	20

Table 4. MIC of methanol and ethanol leaf extract of *E. heterophylla* and *P. lucens*.

Test organism	Methanol leaf extracts (mg/ml)		Ethanol leaf extracts (mg/ml)	
	E. heterophylla	*P. lucens*	*E. heterophylla*	*P. lucens*
Staphylococcus aureus	12.5	Nil	12.5	Nil
Escherichia coli	6.25	50	6.25	50
Proteus vulgaris	Nil	Nil	Nil	Nil
Salmonella typhi	6.25	25	6.25	25
Streptococcus lactis	12.5	50	6.25	25
Shigella species	12.5	Nil	12.5	Nil

Minimum bactericidal concentration (MBC) of leaf extracts

E. heterophylla methanol and ethanol leaf extract of 12.5 mg/ml was the least extract concentration bactericidal against *S. typhi* and *S. aureus*, respectively as shown in Table 5. *E. heterophylla* showed MBC of 25 mg/ml against all the other sensitive test clinical bacterial isolates, while *P. lucens* had MBC of 100 mg/ml against all the sensitive test clinical bacterial isolates except against *S. typhi* (Table 5).

Killing rate of plants extracts MBC

The killing rate of *E. heterophylla* methanol leaf extract MBC is as shown in Figure 1, while that of *P. lucens* is as shown in Figure 2. *E. coli* was the most rapidly killed by *E. heterophylla* methanol leaf extract MBC at the rate of 4.53×10^6 CFU/min (Figure 1). *E. coli* and *Streptococcus lactis* were the most rapidly killed by *P. lucens* methanol leaf extract MBC at the rate of 1.09×10^6 CFU/min (Figure 2).

DISCUSSION

Methanol was found to be a better extracting solvent for *E. heterophylla* and *P. lucens* than ethanol with respect to the extract filtrate yield, in which methanol yielded 10.23 and 10.01 g for *E. heterophylla* and *P. lucens*, respectively,

Table 5. MBC of methanol and ethanol leaf extract of *E. heterophylla* and *P. lucens*.

Test organism	Methanol leaf extract (mg/ml)		Ethanol leaf extract (mg/ml)	
	E. heterophylla	***P. lucens***	***E. heterophylla***	***P. lucens***
Staphylococcus aureus	25	Nil	12.5	Nil
Escherichia coli	25	100	25	100
Proteus vulgaris	Nil	Nil	Nil	Nil
Salmonella typhi	12.5	100	25	50
Streptococcus lactis	12.5	100	25	100
Shigella species	25	Nil	25	Nil

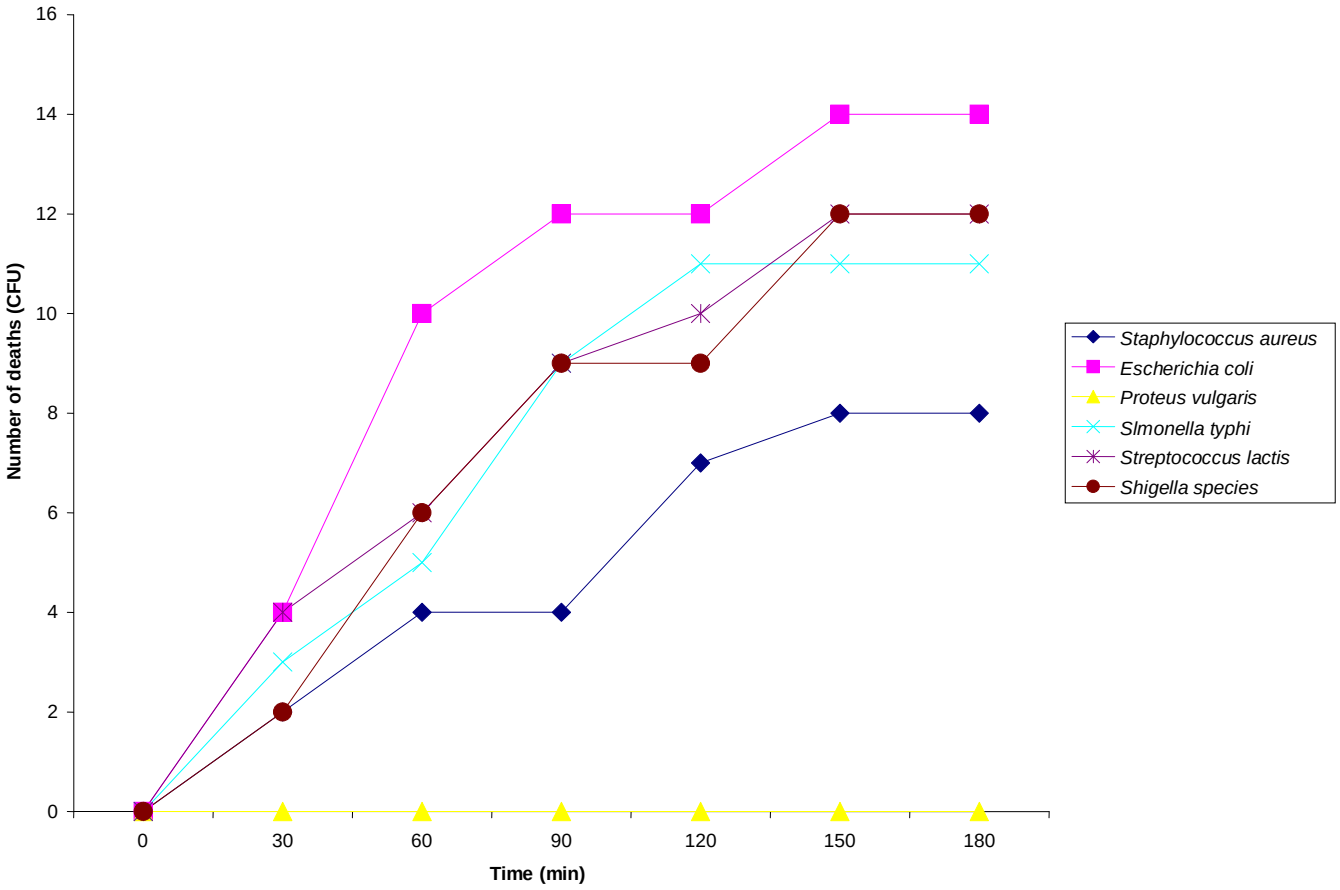

Figure 1. Killing Rate of *Euphorbia heterophylla* Methanol Leaf Extract MBC.

higher than ethanol extract filtrate yield of 8.02 and 7.34 g for the plants, respectively as shown in Table 1.

The sizes of the zone of inhibition are indicative of the level of the antimicrobial activities of the extracts. Therefore, it can be said that the methanol extracts of *E. heterophylla* and *P. lucens* had higher antibacterial activity against the susceptible test clinical bacterial isolates than the ethanol extracts as shown in Table 2. The diameters of the zones of inhibition were larger for *E. coli* methanol extract against *S. aureus*, *Salmonella typhi* and *S. lactis* than for *E. heterophylla* ethanol extracts (Table 2). Unlike in the case of *E. heterophylla*, ethanol extracts of *P. lucens* had a higher antibacterial activity than methanol extract against *S. typhi* as shown in Table 2.

S. typhi was the most susceptible to *E. heterophylla* of all the test clinical bacterial isolates followed by *S. lactis, E. coli, S. aureus* and *Shigella* species with inhibition zone diameter range of 16 to 24 mm, 13 to 20 mm, 8 to 20 mm, 9 to 18 mm and 12 to 15 mm, respectively while

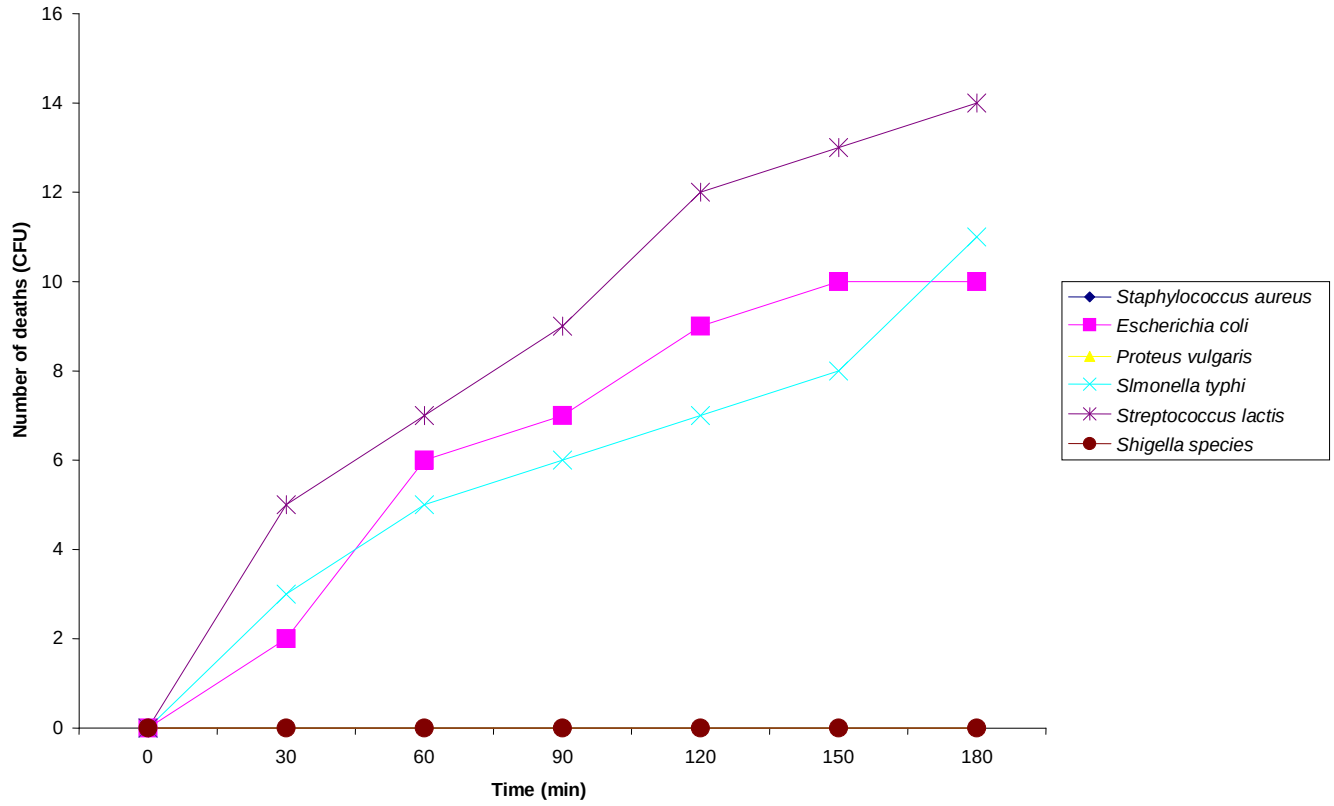

Figure 2. Killing rate of *Pterocarpus lucens* methanol leaf extract MBC.

Proteus vulgaris was not susceptible to both plant extracts as shown in Table 2. Resistance of Gram negative bacteria is well known (Irvin et al., 1981). All the test clinical bacterial isolates were sensitive to *E. heterophylla* except against *P. vulgaris*. Extract of plants from different parts of the world have been shown to possess antimicrobial activity (Ummulkaltum et al., 2002; Ogbulie et al., 2004; Beloin et al., 2005, Bello et al., 2005; Ariyo and Akande, 2005). In addition to *P. vulgaris* that was not sensitive to *P. lucens, S. aureus* and *Shigella* spp. were also not sensitive to *P. lucens* methanol and ethanol extracts, while *S. typhi* was also the most susceptible to *P. lucens* extracts followed by *S. lactis* and *E. coli* with inhibition zone diameter range of 8 to 11 mm, 8 to 10 mm and 7 to 10 mm, respectively. The different antibacterial activity of the plants may not be unconnected to the different phytochemicals present in the plants.

E. heterophylla had minimum inhibitory concentration (MIC) of 6.25 mg/ml against the most susceptible test clinical bacterial isolate and *E. coli*. The MIC values also indicated that *E. heterophylla* leaf extracts were more potent to the susceptible test clinical bacterial isolates having shown lower MIC values than the corresponding *P. lucens* leaf extract MIC as shown in Table 3. This is implied based on the fact that there is an inverse relationship between MIC and susceptibility of the test clinical bacterial isolates. Apart from the less antibacterial activity of *P. lucens* extracts, it did not show activity against 50% of the test isolates with 16.6% being very susceptible as shown in Table 3.

The minimum bactericidal concentration (MBC) had a similar pattern to the MIC as shown in Table 4. *E. heterophylla* methanol and ethanol extracts had MBC of 12.5 and 25 mg/ml, respectively against *S. typhi* while *P. lucens* methanol and ethanol extracts had MBC of 100 and 50 mg/ml, respectively (Table 4). The MBC result shows that the action of *E. heterophylla* ethanol extract on *S. aureus* was bactericidal having the same MIC and MBC of 12.5 mg/ml (Tables 3 and 4). Unlike the other extracts with different MIC and MBC values, an indication of a possible bacteriostatic and bactericidal mode of action by the extracts as shown in Tables 3 and 4.

The antibacterial activity of the *E. heterophylla* extracts was pronounced against *S. typhi* and *S. lactis. E. coli* and *S. aureus* were also very susceptible to the *E. heterophylla extracts* and *P. lucens* except for *S. aureus*. The implication of this result is that bacteria species that have been implicated in infections such as typhoid fever, as in the case of *S. typhi* (Julie, 2000); gastrointestinal disorder such as *S. typhi* and *E. coli* (Lederberg, 2000) and urinary tract infections as in the case of *E. coli* and *Shigella* spp (Ebie et al., 2001) were found to be

susceptible to these plant extracts. Therefore, it can be said that the effort of the herbal healers in Anyigba using water extracts of *E. heterophylla* and *P. lucens* in treating and curing infections like typhoid fever, gastrointestinal disorder and urinary tract infection is probably in the right direction since this work has shown the antibacterial potential of these plants extracts on the etiologic agents of these infections.

The *in vitro* killing rate of *E. heterophylla* methanol extract MBC shows that *E. coli* that has been implicated in gastrointestinal disorder (Lederberg, 2000) was the most rapidly eliminated at a rate of 4.53×10^6 CFU/min, an indication of the fact that the use water extracts of *E. heterophylla* by the Anyigba herbal healers in treating gastrointestinal disorder may not be out of place as supported by the result of this work as shown in Figure 1. In the same vein, the *in vitro* rapid killing rate of the *E. heterophylla* extract on *S. typhi*, an organism implicated as causative agent of typhoid fever, at a rate of 1.32×10^6 CFU/min also shows the antibacterial potential of the plant extracts against the causative agent of typhoid fever has been used by the herbal healer in Anyigba, Kogi State, Nigeria. The *P. lucens* methanol extract MBC killing rate of 190×10^6 CFU/min on *E. coli* and *S. typhi* also shows the antibacterial potential of the plant extracts against etiologic agents of gastrointestinal disorder and typhoid fever as shown in Figure 2. In conclusion, if these extracts did not undergo biotransformation in the body to chemicals of different potencies from their crude extracts, the *in vitro* killing rate of the extracts of *E. heterophylla* and *P. lucens* show the potential of these plants in eliminating some of the etiologic agents of the infections which the herbal healers use the plants to treat in Anyigba, Kogi State, Nigeria.

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