ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
Research Article
June 2016 Vol.:6, Issue:3

Antibacterial and Antiepileptic Activity of Ethanol Extract of Whole Plant of *Leucas martinicensis* (Jacq.)

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
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Submission: 7 June 2016
Accepted: 12 June 2016
Published: 25 June 2016

Keywords: *Leucas martinicensis*, Phytochemical, Antibacterial, Anticonvulsant

ABSTRACT

Leucas martinicensis is an annual herb which is used as mosquito repellant due to minty odor. It has been used traditionally to manage diverse medical ailments including infectious diseases, inflammatory conditions, rashes, diarrhoea, epilepsy and convulsions. The aim of this study is to ascertain the phytochemical constituents, antibacterial and anticonvulsant activity of the ethanolic extract of whole plant ethanol whole plant extract. The whole plant was air dried, powdered and extracted using 95% ethanol. The extract was then subjected to preliminary phytochemical screening and antibacterial susceptibility testing using cup plate method on nutrient agar. The anticonvulsant activity of the extract was investigated by studying the effects on seizures induced by pentylenetetrazole and strychnine in albino rats. The extract revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and cardiac glycosides. The antibacterial screening of the extract against clinical isolates of *Staphylococcus aureus, Salmonella typhi, Escherichia coli* and *Klebsiella species* showed a dose dependent inhibitory activity. The activity of the control gentamycin (gentamicin) was found to be significantly lower than the activity of the extract at the highest tested concentration against *Salmonella typhi* ($p<0.05$). The extract at doses between 125 and 1000 mg/kg dose dependently increased the onset of clonic convulsion induced by pentylenetetrazol and strychnine in albino rats. There was a statistical significant difference between the effect of the extract and the negative control ($p<0.05$) against the pentylenetetrazole and strychnine induced seizures in rats. The data obtained suggest that *Leucas martinicensis* possesses equipotent anticonvulsant activities against pentylenetetrazole and strychnine induced seizures in rats. The anticonvulsant and antimicrobial activity recorded for the plant extract validates their traditional uses to treat various acclaimed diseases.

INTRODUCTION

Plants are indispensable sources of medicine since time immemorial. Studies on natural products are aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses, and discovery of potential chemotherapeutic agents.[1] This plant based traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care.[2] Plants based on natural constituents can be derived from any part of the plant like leaves, barks, stems, flowers, roots, seeds etc.[3] Antimicrobials of plant origin have enormous therapeutic potential.[1-2] They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that often associated with synthetic antimicrobials. [1-2] Given the alarming incidence of antibiotic resistance in bacteria of medicinal importance, there is constant need for new and effective therapeutic agents. Therefore, there is need to develop alternative antimicrobials drugs for the treatment of infectious diseases from medicinal plants.[4] *Leucas martinicensis* (Jacq.) R. Br. belongs to family Lamiaceae and commonly called Whitewort which grows widely in the Northern part of Nigeria. It has been used traditionally to treat inflammatory conditions, rashes, diarrhoea, epilepsy and convulsions.[5]The present study was to investigate the phytochemical components, antibacterial and antiepileptic activity of ethanol leaf extract of the whole plant of *Leucas martinicensis* so as to justify scientifically the basis for traditional use of this plant in the management of diverse medical ailments including epilepsy and infectious diseases.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted in the Pharmacology and Toxicology Laboratory of the Faculty of Pharmacy, University of Maiduguri and Microbiology Laboratory of University of Maiduguri Teaching Hospital, Maiduguri, Borno state, Nigeria.

Plant collection, identification and preparation

Fresh whole plant of *Leucas martinicensis* was collected in the month of October 2015 in the staff quarters of the Gombe State University, Gombe State. It was identified by a plant taxonomist in the Department of Biological Science, University of Maiduguri, Borno State. The

plant was dried under the shade for 7 days. Dried plants were grounded in powder using mechanical milling machine, and stored in the airtight container for further use this was then stored in an airtight container till further use. One thousand gram (1000 g) of the powdered plant of *Leucas martinicensis* was weighed and defatted using petroleum ether for 24 hours; the marc was dried and soaked in 1500 ml of 95% ethanol for 24 hours at room temperature with occasional mechanical shaking. The filtrate obtained were concentrated using rotary evaporator to concentrate the extract and then air dried. The percentage yield of the dried extract was then calculated using the formula below: Percentage yield =X/Y x 100%; where X = weight of the dried concentrated extract, Y = weight of the dried powdered plant.

Phytochemical screening

Basic phytochemical test on the ethanol plant extract of *Leucas martinicensis* was carried out based on standard method described by Trease and Evans[6] and Sofowora[7] to test for the presence of alkaloid, tannin, saponin, carbohydrate, glycoside, flavonoid, steroids and cardiac glycosides.

Sources of the microorganisms

Clinical isolates of the tests organisms (*Escherichia coli, Klebsiella spp, Salmonella typhi and Staphylococcus aureus)* were obtained from the Department of Microbiology, University of Maiduguri Teaching Hospital (UMTH).

Preparation of media for antibacterial screening

Sterilization of the equipment and disinfection

All work surfaces were moped with moist rag and was disinfected with cotton wool soaked in dettol (disinfectant) to prevent contamination during the process. A hot air oven was used to sterilize the conical flasks, forceps, wire loop, pipettes, and beakers at 160˚C for 45 minutes. All materials used in the course of this project are not sensitive to moist heat sterilization were adequately sterilized using autoclave. Materials such as glasswares, conical flasks were properly washed with detergent and water to remove dirt and contaminants and were allowed to dry prior to usage. These materials were then sterilized in a portable laboratory autoclave at 120^{o}C for 15 minutes.

Preparation of the nutrient agar plate

Prepared by dissolving 28 g of the agar in 1000ml one litre of distilled water and then covered with aluminum foil. The media was boiled to dissolution and then sterilized at 121˚C for 15 minutes. The media was allowed to cool to 45˚C and 30 ml of the sterilized medium was poured into the sterile Petri dishes and allowed to cool and solidify. The plates were dried at 37˚C for 30 minutes and were used for well diffusion method. The microbes were spread evenly over the surface of the medium with the aid of glass spreader at which the cup, were bored.

Antibacterial screening of *Leucas martinicensis* using cup plate method

Prepared agar plate was evenly spread with test organism after which 5 cups or holes were bored on the surface of the prepared plate using standard cup borer of 8mm in diameter. Ten grams (10 g) each of the extract was dissolved in 10 ml sterile distilled water to make an initial concentration of 1000 mg/ml. These were then serially diluted using 1:2 to produce concentrations of 500 mg/ml, 250 mg/ml and 125 mg/ml. The four different concentrations of the extract were poured into the cups respectively. Gentamicin (4ng/ml) being the control was poured into the fifth hole on the prepared nutrient agar plate. The bacteria plates were incubated at 37˚C for 24 hours and observation for the zone of inhibition of bacterial growth was made. The zones were measured with a transparent ruler and result recorded in millimeters. The screening was done using 5 replications of culture plate. Sterile distilled water was used as negative control.

Pentylenetetrazole Induced Seizure in Rat

Thirty (30) rats (110-150 g) were grouped into 6 groups of 5 rats each. The first group received water for injection B.P. 10 ml/kg, the second group received diazepam (by Roche) 10 mg/kg, the third, fourth, fifth and sixth groups received the extract 1000, 500, 250 and 125 mg/kg respectively. Sixty minutes later all the rats were administered PTZ 100 mg/kg and observed for onset of jerk, onset of convulsion and death. Absence of a convulsion of at least 5 seconds duration indicates a compounds ability to abolish the effect of pentylenetetrazole induced seizure threshold as earlier reported by Swinyard *et al*.[8]

Strychnine Induced Seizure in Rat

Thirty (30) rats (110-150 g) were grouped into 6 groups of 5 rats each. The first group received water for injection B.P. 10 ml/kg, the second group received diazepam (by Roche) 10 mg/kg, the third, fourth, fifth and sixth groups received the aqueous extract 1000, 500, 250 and 125 mg/kg respectively. Sixty minutes later all the rats were administered strychnine 5 mg/kg and observed for onset of jerk, onset of convulsion and death. Absence of a convulsion of at least 5 seconds duration indicates a compounds ability to abolish the effect of strychnine induced seizure threshold.[8]

Statistical Analysis

The results were analyzed with Graph Pad Instat software using pooled student T-test. A $p<0.05$ was considered significant, $p<0.01$ was considered highly significant and $p<0.001$ was considered extremely significant.

RESULTS

Preliminary phytochemical screenings of ethanol plant extract of *Leucas martinicensis*

Table 1 shows the results of phytochemical analysis of the ethanol extract of *Leucas martinicensis.* Dragendroff's Reagent and Mayer's Reagent test for alkaloids were positive. Flavonoid was also detected in the leaf extract because Shinoda, $FeCl_3$ and lead acetate test were all positive. Combined anthraquinone was also tested positive for the presence of glycoside, whereas Saponin and cardiac glycosides were also tested positive in the extract. However, lead acetate test for tannins and free anthraquinones test for glycosides were found to be negative in the extract. Carbohydrates and steroids were not detected in the ethanol extract (Table 1).

Antibacterial activities of ethanol whole plant extract of *Leucas martinicensis*

The ethanol extract of *Leucas martinicensis* was found to inhibit the growth of *Staphylococcus aureus, Salmonella typhi, Escherichia coli (E. coli)* and *Klebsiella species* dose dependent (Table 2). The activities of gentamicin (4ng/ml) against *Staphylococcus aureus* was found to be extremely significant than the ethanol extract of *Leucas martinicensis* at 125-250 mg/ml ($p<0.001$). However, the activities of the extract at 500 and 1000 mg/ml was found to inhibit the

growth of *Salmonella typhi* more than the control (gentamicin) ($p<0.05$). Gentamicin (4 ng/ml) inhibited the growth of both *E. coli* and *Klebsiella species* significantly higher than the extract at 125-500 mg ($p<0.01$). There was no statistical significant difference in the activities of gentamycin and the highest tested dose of the extract against *E. coli* and *Klebsiella species* ($p>0.05$) (Table 2).

Anti-seizure activity of *Leucas martinicensis* extract on pentylenetetrazole induced seizure

The ethanol extract of *Leucas martinicensis* plant exhibited significant anticonvulsant activity, shown by a dose dependent increase in the onset time of clonic convulsion in albino rats. However, the extract at 250, 500 and 1000 mg/kg showed a statistical significant difference with the negative control ($p<0.05$). Diazepam used as positive control protected all the rats from clonic convulsion. The percentage protection of the extracts against the mortality of PTZ induced epileptic seizures was also dose dependent (Table 3).

Anti-seizure activity of *Leucas martinicensis* extract on strychnine induced seizure

The ethanol extract of *Leucas martinicensis* plant also exhibited significant anticonvulsant activity in strychnine induced seizures, shown by a dose dependent increase in the onset time of clonic convulsion in albino rats. The plant extract at 250 mg/kg and 1000 mg/kg showed a statistical significant difference with the negative control ($p<0.05$). Diazepam used as positive control protected all the rats from death but not from clonic convulsion. The plant extract did not protect the rats against the mortality of strychnine at all the tested doses (Table 4).

DISCUSSION

The phytochemical components of the *Leucas martinicensis* plant in which alkaloids, flavonoids, saponins, tannins, and carbohydrates were detected agrees with several literature reports in which similar constituents were detected.[5,9,10] These chemical compounds were speculated to account for the observed pharmacological effects of the plant extract.

The antibacterial activity of *Leucas martinicensis* plant observed in the present study has been attributed to the presence of phytochemical compounds like saponins, tannins and flavonoids contained in them which is in agreement with the reports of Muhammad *et al*[10] and Eze *et al*[5] in

which similar observations were made. There was a significant antibacterial activity against the clinical isolates tested especially at higher doses of the plant extract. In the present study, gentamicin was found to have a greater activity against the tested organisms than the ethanol extract of *Leucas martinicensis* plant except against *Staph. aureus* and *Salmonella typhi* at higher doses of the extract. The plant extract showed appreciable dose dependent broad spectrum antibacterial activities against *S. aureus, S. typhi*, *E. coli* and *K. species.* These pathogens are known to cause majority of the community and hospital acquired infections.[11]

The anticonvulsant activity demonstrated by *Leucas martinicensis* plant in this study agrees with several literature reports on the chemotherapeutic potential of plants as a good source of anticonvulsants.[12-14]The presence of phytochemical constituents in the plant such as tannins, saponins, flavonoids, glycosides and alkaloids may be responsible for the observed effects. *Leucas martinicensis* plant was able to protect the albino rats from PTZ and Strychnine induced convulsion may be attributable to the activity of GABA and glycine receptors.[15,16] The superiority of the plant extract on PTZ induced convulsion indicates that the extract might have higher activity for $GABA_A$ receptor via ion channel than the glycine receptors. Diazepam used as a positive control was able to protect the rats from convulsion and gives 100% protection from death due to pentylenetetrazole and strychnine. This confirms that diazepam is a $GABA_A$ agonist,[14,16] PTZ is a $GABA_A$ antagonist,[14] while the extract may be a $GABA_A$ agonist with less activity than Diazepam. The inability of the extract to protect the rats from death against strychnine-induced seizure suggests relative or lower activity on the glycine receptors. Therefore, the activity of the plant may be due to the presence of some bioactive phytochemical constituents detected such as alkaloid that has GABA potentiating effect.

CONCLUSION

The plant extract of *Leucas martinicensis* contains bioactive compounds that may have antibacterial and anticonvulsant properties which may justify the use of this plant in traditional medicine for the treatment of infections and epilepsy.

Acknowledgement

The authors are sincerely thankful to Mr Innocent of Department of Pharmaceutical Chemistry and Mr. Umar Ibrahim of Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri for their technical assistance and support.

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Table 1: Qualitative phytochemistry of ethanol plant extract of *Leucas martinicensis*

Phytoconstituents	*Results*
Alkaloids	
(a) Dragendroff's Reagent	+
(b) Mayer's Reagent	+
Carbohydrates	
(a) Molish's Test	-
(b) Fehling's Test	-
Flavonoids	
(a) Shinoda's Test	+
(b) $FeCl_3$ Test	+
(c) Pb acetate Test	+
Glycosides	
(a) Free anthraquinones	-
(b) Combined anthraquinones	+
Saponins	
(a) Frothing Test	+
(b) Fehling's Test	+
Steroids	-
Tannins	
(a) $FeCl_3$	+
(b) Pb acetate	-
Cardiac glycosides	+

– = not detected; + = present

Table 2: Antibacterial activity of ethanol plant extract of *Leucas martinicensis*

Treatment (mg/ml)	*Zones of Inhibition (mm)*			
	Staph. aureus	*S. typhi*	*E. coli*	*Klebsiella spp*
Control (4 ng/ml)	37.20±2.42	31.20±2.48	39.00±1.00	37.80±2.52
LM 125	13.40±0.93***	12.80±0.74***	-	-
LM 250	22.00±1.14***	27.60±0.93	20.20±2.40***	15.25±1.25***
LM 500	33.40±1.36	38.60±1.03*	22.80±2.04***	24.40±1.36**
LM 1000	40.60±1.08	43.60±1.36**	39.00±2.92	33.20±1.72

LM = Leucas martinicensis, Staph. aureus = Staphylococcus aureus, S. typhi = Salmonella typhi, E. coli = Escherichia coli, Klebsiella spp = Klebsiella specie, Control = gentamicin, n = 5, - = no activity

Table 3: Effect of ethanol extract of *Leucas martinicensis* on pentylenetetrazole induced seizures in albino rats

Treatment	*Dose (mg/kg)*	*Onset of Clonic Convulsion in Seconds (Mean±SEM)*	*Protection (%)*
Control	Vehicle (10 ml/kg)	163.50±19.25	00
Diazepam	10	A	100
LMEE	1000	950.00±95.24***	80
LMEE	500	550.00±84.12**	60
LMEE	250	340.00±61.20*	20
LMEE	125	195.00±32.02	00

LMEE = Ethanol Extract of *Leucas martinicensis*, Vehicle = Water for injection B.P., A = absence of convulsion,

***= p<0.001 (extremely significant), **= p<0.01 (highly significant), *= p<0.05 (significant), N = 5

Table 4: Effect of ethanol extract of *Leucas martinicensis* on strychnine induced seizures in albino rats.

Treatment	*Dose (mg/kg)*	*Onset of Clonic Convulsion in Seconds (Mean±SEM)*	*Quantal Protection*
Control	Vehicle (10 ml/kg)	115.00±21.28	0/5
Diazepam	10	1530.00±220.42	5/5
LMEE	1000	410.00±54.20**	0/5
LMEE	500	302.00±25.20*	0/5
LMEE	250	184.00±20.20	0/5
LMEE	125	99.00±8.30	0/5

LMEE = Ethanol Extract of *Leucas martinicensis*, Vehicle = Water for injection B.P., A = absence of convulsion,

**= p<0.01 (highly significant), *= p<0.05 (significant), N = 5