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Psychopharmacolgical Acitivities of Ethanol Leaf Extract of *Saccharum officinarum*

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ABSTRACT

Saccharum officinarum L. (Poaceae) is used in Ibibio ethnomedicine for the treatment of various diseases such as CNS disorders. The ethanol leaf extract of *Saccharum officinarum* (170, 340 and 510 mg/kg) was investigated for antidepressant and anticonvulsant activities in Swiss albino mice (20-25 g). Open field, force swimming and tail suspension tests were used to assess antidepressant activity, while pentylenetetrazol and aminophylline-induced convulsion models were used to assess anticonvulsant activity. The extract significantly ($p<0.05$-0.01) decreased the frequency of line crossing, rearing and walling activities of mice in open field test and also decreased significantly ($p<0.05$ - $p<0.001$) the duration of immobility of mice in forced swimming and tail suspension tests. The extract significantly ($p<0.005$ - $p<0.001$) offered protection against PTZ- and aminophylline-induced convulsions in mice. The findings of this study suggest that the leaf extract of *S. officinarum* has antidepressant and anticonvulsant activities and these supports its use in ethnomedicine for the treatment of central nervous system disorders.

Keywords: *Saccharum officinarum*, Anticonvulsant, Depressant, Central Nervous System.

Introduction

Depression and epilepsy affect a great number of people across the world irrespective of age. According to the World Health Organization Report about 450 million people worldwide suffer from one form of CNS disorder or the other.[1] Only a few of these individuals have access to basic healthcare and treatments. Most conventional drugs used in the management of these conditions are often associated with serious side effects despite their effectiveness. Thus, making a vast majority of the people to rely on natural products from plants which have been proven to be safe and affordable alternative to orthodox medicine. Medicinal Plant researches have increased over the years with efforts geared at demonstrating the pharmacological potentials of these plants.

*Saccharum officinarum*L.(Family-Poaceae) commonly known as sugarcane is widely cultivated throughout the tropical and sub-tropical regions. In folkloric medicine, it is used in the treatment of diarrhoea, dysentery, eyes, fever, arthritis, bedsores, boils, cancer, colds, cough, opacity, skin sores, sore throat, hiccups, inflammation, laryngitis, spleen, tumors, and wounds.[2] Biological activities reported on the leaf include antibacterial and anthelmintic,[3] anti-hyperglycaemic, anti-hyperlipidaemic,[4]antioxidant,[4,5]diuretic and antiurolithiatic[6]. Phytochemical screening of leaf extract of *Saccharum officinarum* reported the presence of glycosides, phytosterols, saponins, tannins, flavonoids.[3] Information on the biological activities of the leaf of *S. officinarum* is scanty. We report the antidepressant and anticonvulsant activities of the leaf extract to provide scientific proof for its use in traditional medicine for the management of central nervous system disorders.

Materials and Methods

Plant materials

Fresh leaves of *Saccharum officinarum* were collected in June 2018 from compounds in Uyo village in Uyo LGA, Akwa Ibom State, Nigeria. The leaves were identified and authenticated as *Zea mays* by Prof Margaret Bassey, a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at the Faculty of Pharmacy Herbarium, University of Uyo, Uyo with voucher number UUH. 42c.

Extraction

The plant parts (leaves) were washed and air- dried on laboratory table for 2 weeks. The dried leaves were pulverized using a pestle and mortar. The powdered leaf (1 kg) was macerated in 95% ethanol (5 L) for 72 hours. The liquid ethanol extract obtained by filtration was evaporated to dryness in a rotary evaporator 40˚C. The extract was stored in a refrigerator at 4˚C until used for experiment reported in this study.

Animals

The animals (Swiss albino mice) (20-25 g) of either sex were used for these experiments. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water *ad libitum.* All experiments were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of laboratory Animals (NIH Publications No. 80-23) revised in 2002.

Ethical Approval

Permission and approval for animal studies with reference number (CHS/AE/018/36) were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo, Uyo.

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Determination of median lethal dose (LD_{50})
The median lethal dose (LD_{50}) of the extract was estimated using Swiss albino mice by intraperitoneal (i.p) route using the method of Lorke[7] with a little modification. This involved intraperitoneal administration of different doses of the extract (100,500,1000,1500 and 2000 mg/kg) to groups of three mice each (n=3). The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, reduced breathing and death. The number of deaths in each group within 24 hours was recorded. The LD_{50} was calculated as geometrical means of the maximum dose producing 0% mortality (a) and the minimum dose producing 100% mortality (b).

$LD_{50} = \sqrt{ab}$.

Evaluation of antidepressant activity
Open field test
Swiss albino mice (20 -25 g) were randomly divided into five groups of 6 mice each and treated as follows for 5 days before open field test; group 1 (control) was treated with normal saline, (2 mL/kg *p.o.*), group 2 (Standard) was given imipramine (5.0 mg/kg, *p.o.*) and ethanol leaf extract of *S. officinarum* (170, 340, and 510 mg/kg, *p.o.*) were respectively administered to groups 3,4 and 5. The open-field arena was made of acrylic (transparent walls and black floor, 30 × 30 × 15 cm), divided into nine squares of equal areas. The open field was used to evaluate the exploratory activity of each animal.[8] The observed parameters were the number of squares crossed (with the four paws) and number of walling and rearing activities, recorded for 5 min testing period.

Forced swimming test
Swiss albino mice (20 -25 g) of either sex were randomly divided into five groups of 6 mice each and treated as follows for 5 days before the behavioural test; group 1 (control) was treated with normal saline (2 mL/kg *p.o.*), group 2 (standard) was given imipramine (5.0 mg/kg, *p.o.*) and ethanol leaf extract of *S. officinarum* (170, 340, and 510 mg/kg, *p.o.*) were respectively administered to groups 3,4 and 5. For assessing antidepressant activities, we employed the method described by Porsolt *et al.*[9,10] The development of immobility when mice were placed inside an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. Briefly, mice were individually placed in a circular tank (46 cm tall × 20 cm in diameter) filled with tap water (25 ± 1°C) to a depth of 20 cm and left there for 5 min. During this period, the behaviour of each animal was recorded by an observer. Mice were considered immobile when remained floating without struggling and making only slight movements necessary to maintain the head above the water.

Tail suspension test (TST)
Swiss albino mice (20 -25 g) of either sex were randomly divided into five groups of 6 mice each and treated as follows for 5 days before tail suspension test; group 1 (control) was treated with normal saline, (2 mL/kg *p.o.*), group 2 (Standard) was given imipramine (5.0 mg/kg, *p.o.*) and ethanol leaf extract of *S. officinarum*(170, 340, and 510 mg/kg, *p.o.*) were respectively administered to groups 3,4 and 5. The total duration of immobility induced by tail suspension was measured according to the methods described by Steru *et al.*[11] Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Mice were considered immobile only when they hung passively and were motionless.

Anticonvulsant activity
Pentylenetetrazol-induced convulsion
Anticonvulsant effect of the extract was assessed using a modified method of Vellucci and Webster [12] on overnight fasted mice. The mice were divided into five groups of six animals each and treated with 170, 340 and 510 mg/kg of the leaf extract respectively, phenobarbitone, 40 mg/kg one hour before induction of convulsion. Seizure was induced in each set of mice with pentylenetetrazol (PTZ) (70 mg/kg i.p). Control group received normal saline (10 mL/kg). The onset of clonic/tonic convulsion and the mortality rate was recorded and compared with the respective control groups. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity.[13]

Aminophylline-induced Convulsion
The extract was evaluated for activity against aminophylline–induced convulsion using the method of Juliet *et al.*[14] The mice were divided into 5 groups of six animals each and treated with 170, 340 and 510 mg/kg of the extract respectively. Phenobarbitone, 40 mg/kg, and normal saline (10 mL/kg) were given to the standard and control groups respectively. These treatments were done one hour before induction of convulsion. Seizure was induced using aminophylline (280 mg/kg,i.p). The animals were observed for 120 mins after the administration of aminophylline and the following parameters were noted: time to onset of myoclonic jerks in mins, time to onset of tonic convulsions in mins, time to death during the experimental time of 120 mins, and the number of mice dead/alive at 24 hours.

Statisticalanalysis
Data obtained from this work were analyzed statistically using ANOVA(One-way) followed by a post test (Tukey-kramer multiple comparison test). Differences between means were considered significant at 5% level of significance ($p \leq 0.05$).

Results and Discussion

Determination of Median lethal dose (LD_{50})
The median lethal dose (LD_{50}) was calculated to be 1732.05 mg/kg. The physical signs of toxicity included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death.

Open field test
Administration of leaf extract of *S. officinarum* (170-510 mg/kg) for 5 days caused significant ($p<0.05 – 0.01$) dose-dependent decreases in the frequency of line crossing when compared to control. The standard drug, imipramine (5 mg/kg), caused a significant ($p<0.001$) higher increase in the locomotor activity of the mice as evident in the frequency of the line crossing (Table 1).

Table 1: Effect of ethanol leaf extract of *Saccharum officinarum* on locomotive behaviour of mice during open field test.

TREATMENT	DOSE (mg/kg)	LINE CROSSING	WALLING	REARING
Control normal saline	-	98.6 ± 5.23	32.33 ± 1.45	8.66 ± 0.88
Immipramine	5	130.75 ± 5.72[b]	65.7 ± 1.25[a]	12.00 ± 0.57[b]
Saccharum officinarum	170	89.66 ± 3.37[b]	24.33 ± 5.20[a]	6.33 ± 0.91[b]
	340	78.33 ± 2.90[b]	19.66 ± 0.88[a]	5.66 ± 1.20[b]
	510	68.66 ± 4.05[a]	19.66 ± 1.20[a]	3.33 ± 0.88[b]

Data are expressed as MEAN ± SEM, Significant at [a]$p < 0.05$, [b]$p < 0.001$, when compared to control. (n = 6).

Saccharum officinarum leaf extract (170 –510 mg/kg) caused prominent decreases in walling frequency of the mice which was significant ($p < 0.05$ - $p < 0.001$) when compared to control. These effects were dose-dependent. The standard drug, imipramine (5 mg/kg), produced a significant ($p < 0.001$) increase in the walling frequency of the animals (Table 1).
The leaf extract of the *S. officinarum* (170 – 510 mg/kg) caused significant ($p < 0.001$) dose-dependent decreases of the rearing frequency of mice administered with the extract for five days. Similarly, the standard drug, imipramine (5 mg/kg), exerted a significant ($p < 0.001$) increase in the rearing frequency when compared to control (Table 1).

Effect on force swimming test
Administration of the ethanol leaf extract of *S. officinarum* (170 – 510 mg/kg) to mice for five days significantly ($p < 0.01$) reduced immobility duration dose-dependently in mice during force swimming test when it was compared to control. The standard drug, imipramine (5 mg/kg) similarly produced a significant ($p<0.01$) reduction in the immobility time of the mice when compared to control.(Table 2).

Effect on tail suspension test
Administration of the ethanol leaf extract of *S. officinarum* (170 – 510 mg/kg) to mice for five days significantly ($p<0.01$-0.001) reduced immobility duration dose-dependently during tail suspension test when it was compared to control. The standard drug, imipramine (5 mg/kg), exerted a significant ($p < 0.001$) reduction of the immobility time of the mice when compared to control (Table 3).

PTZ –induced convulsion
Administration of leaf extract of *Saccharum officinarum* (170-510 mg/kg) provided a considerable degree of protection for the mice against seizure induced by pentylenetetrazol. The extract prolonged the time for onset of myoclonic convulsion in a dose-dependent fashion and this was only significant ($p < 0.01$ - $p < 0.001$) at high doses (340 and 510 mg/kg) and comparable to that of the standard drug, Phenobarbitone (Table 4). Similarly, the extract exerted a prolongation of time for onset of tonic convulsion in a dose-dependent manner which was significant ($p < 0.05$) at the highest dose (510 mg/kg) (Table 4). The time of death of the treated animal was not significantly ($p<0.05$) different statistically when compared with the control. The standard drug, phenobarbitone also offered 100% protection to the animals treated with it.

Aminophylline-induced convulsion
Administration of the leaf extract of *S. officinarum* (170-510 mg/kg) caused a significant ($p<0.001$) delay in the onset of seizure induced by aminophylline in a dose-dependent fashion. The delay was pronounced in both myoclonic and tonic convulsion (Table 5). The time of death of treated animal was not significantly ($p < 0.05$) different statistically when compared with the control. The standard drug, Phenobarbitone offered a more significant ($p < 0.001$) protection to the mice treated with it.

Table 2: Effect of ethanol leaf extract of *Saccharum officinarum* on behavior of mice during forced swimming test.

TREATMENT	DOSE (mg/kg)	Duration of immobility
Control normal saline	-	65.33 ± 5.33
Immipramine	5	46.0 ± 3.05[a]
Saccharum officinarum	170	49.33 ± 0.88[b]
	340	50.0 ± 1.15[b]
	510	56.33 ± 5.36[b]

Data are expressed as MEAN ± SEM, Significant at [a]$p < 0.05$; [b]$p<0.01$, when compared to control. (n = 6).

Table 3: Effect of ethanol leaf extract of *Saccharum officinarum* on behavior of mice during Tail suspension test.

TREATMENT	DOSE (mg/kg)	Duration of immobility
Control normal saline	-	140.5 ± 6.93
Immipramine	5	78.66 ± 7.88[c]
Saccharum officinarum	170	67.33 ± 7.33[c]
	340	70.33 ± 5.17[c]
	510	76.33 ± 6.33[c]

Data are expressed as MEAN ± SEM, Significant at [c]$p < 0.001$, when compared to control. (n = 6).

In this study, evaluation of the effect of ethanol leaf extract of *S. officinarum* on central nervous system was carried out in mice using different models; Open field test, tail suspension test and force swimming test for depressant effect, while PTZ- and aminophylline-induced convulsion were used to evaluate the anticonvulsant effect. The leaf extract (170 – 510 mg/kg) was found to cause significant dose-dependent decreases in the frequency of line crossing, walling and rearing activities of the pretreated mice. It also reduced significantly the immobility time of the mice in force swimming and tail suspension tests.
Monitoring of locomotor activity of animals has been used in assessing effect of drug on the CNS. An increased movement is a measure of the level of excitability of the CNS [15] and its decrease may be intimately related to sedation resulting from depression of the CNS.[16] Central nervous system stimulants are known to increase locomotor activity, while agents with depressant activity cause reduction in movements.[17] The leaf extract was found to decrease significantly line crossing, walling and rearing activities during open field test suggesting depressant effect on the CNS as evidenced by the significant reduction of psychomotor activities of the test animals. The leaf extract understudy may in part be exert its depressant activity by enhancing the GABAergic inhibition of the CNSthrough membrane hyperpolarization that lead to a reduction in the firing rate of critical neurons in the brain or the extract may simply activate the GABA receptorsdirectly.[17] Researches have shown that plants containing alkaloids, flavonoids and tannins are useful for the treatment of many CNS disorders as they reduce the locomotor activity of the CNS[18] which led to the postulation that these compounds may act as benzodiazepine- like molecules.[19]However, it is worthy to note that several established antidepressants decrease locomotor activity as was the case in this study.[20]
The leaf extract was found to reduce the immobility time of mice during force swimming and tail suspension tests suggesting CNS stimulatory effect and antidepressant effect of the leaf extract. Forced swimming and tail suspension tests are two of the most commonly used animal models of depression for antidepressant screening. In the forced swimming test, the development of immobility when mice are placed into an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior.[21] The tail suspension test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. Various antidepressants are able to reverse the immobility and promote the occurrence of escape related behavior. Both models of depression are widely used to screen new antidepressants.[9-11] These tests are quite sensitive to major antidepressant drugs including tricyclics, serotonin-specific reuptake inhibitors, MAO inhibitors, and atypical antidepressant.[9,11]
Forced swimming and tail suspension tests which represent the behavioural despair model, claimed to reproduce a condition similar to human depression.[9,11,22] The tests are based on the observation that animals, following initial escape-oriented movements, develop an immobile posture when placed in an inescapable chamber. The immobility is thought to reflect either a failure of persistence in escape-directed behaviour (i.e. behavioural despair) or the development of passive behaviour that disengages the animal from

Table 4: Effect of ethanol leaf extract of *Saccharum officinarum* on aminophylline-induced convulsion.

TREATMENT	DOSE (mg/kg)	Onset of myoclonic	Onset of Tonic	Time of death	No. of death
Control normal saline	-	6.59 ± 0.26	8.30 ± 0.14	31.11 ± 7.20	6/6
Phenobarbitone	40	29.91 ± 1.14[a]	0.00 ± 0.00[a]	0.00 ± 0.00	6/6
Saccharum	170	8.06 ± 0.25	10.06 ± 0.33[a]	27.51 ± 2.53	6/6
officinarum	340	13.36 ± 0.64[a]	17.85 ± 1.29[a]	31.70 ± 18.73	6/6
	510	14.31 ± 0.53[a]	23.14 ± 0.54[a]	31.86 ± 7.21	6/6

Data are expressed as MEAN ± SEM, Significant at [a]p< 0.001, when compared to control. (n=6).

Table 5: Effect of ethanol leaf extract of *Saccharum officinarum* on Pentylenetetrazol-induced convulsion.

TREATMENT	DOSE (mg/kg)	Onset of myoclonic	Onset of Tonic	Time of death	No. of death
Control normal saline	-	0.63 ± 0.28	2.71 ± 0.29	10.77 ± 4.08	6/6
Phenobarbitone	40	5.02 ± 0.28[c]	18.28 ± 1.82[c]	13.17 ± 1.61	6/6
Saccharum	170	1.43 ± 0.02	5.21 ± 0.89	11.48 ± 4.53	6/6
officinarum	340	2.48 ± 0.31[b]	5.86 ± 0.58	13.89 ± 2.66	6/6
	510	3.06 ± 0.32[c]	8.32 ± 0.47[a]	14.64 ± 2.72	6/6

Data are expressed as MEAN ± SEM, Significant at [a]p < 0.05; [b]p < 0.01; [c]p < 0.001, when compared to control. (n = 6).

active forms of coping with stressful stimuli.[21] It is well known that clinically effective antidepressants (such as imipramine) typically increase the swimming efforts of the animal seeking a solution to the problem and, therefore, they decrease the duration of immobility in the forced swimming test.[9] This was observed in this study.

Similarly, the results of this study suggest that the leaf extract exhibited significant antidepressant activity without psychomotor stimulation. Phytochemical constituents such as flavonoids have been implicated in antidepressant action on the CNS,[23] while polyphenols especially flavonoids like quercetin and rutin have also been reported to exhibit antidepressant effect.[24] The leaf extract of *S. officinarum* have been reported to contain some phytochemical compounds such as glycosides, phytosterols, saponins, tannins, flavonoids.[3] These phytochemical constituents may be responsible for the observed activity of the leaf extract in this study.

The leaf extract was also found to offer considerable protection against PTZ- and aminophylline-induced convulsions by prolonging the time for onset of tonic and clonic convulsions. The exact mechanisms of seizures induced by aminophylline appear to be diverse, multiple and complex, and also unclear. Evidence suggests that seizures induced by aminophylline, could be the result of adenosine receptor antagonism or due to inhibition of cerebral nucleotidase activity,[25,26] which lowers the adenosine content in the brain and eventually lead to a process of disinhibition. However, report has it that di-phenylhydantoin a potent inhibitor of adenosine uptake was ineffective in preventing these seizures.[27] Apart from non-specific adenosine receptor antagonism,[28] aminophylline is thought to have an inhibitory influence on adenosine synthesis. At higher doses inhibition of phosphodiesterase activity including mobilization of intracellular calcium ions from labile stores are said to be implicated in aminophylline-induced seizures.[29] However, a report by Ray *et al.*,[30] has implicated oxidative stress due to the generation of free radicals and reactive oxygen species to be responsible for the seizures induced by aminophylline. This suggest that plant extract with antioxidant activity are able to prevent convulsion induced by aminophylline which is likely to be the mechanism of action of this extract as *S. officinarum* has been reported to possess antioxidant activity.[4,5]

According to De Sarro *et al.*,[31]pentylenetetrazol (PTZ) is suggested to exert its anticonvulsant effect by inhibiting the activity of gamma aminobutyric acid (GABA) at $GABA_A$ receptors. Gamma-aminobutyric acid is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion, respectively.[32] Phenobarbitone and diazepam, standard epileptic drugs, have been shown to exert their antiepileptic effects by enhancing GABA-mediated inhibition in the brain.[33] These drugs are reported to antagonise PTZ-induced convulsion[34] by enhancing GABA neurotransmission. Phenytoin was unable to prevent PTZ-induced seizure because it is thought to exert its antiepileptic effect by blocking sodium ions into brain cells thus inhibiting generation of repitative action potential.[33] Since the leaf extractof *S. officinarum* was able to delay PTZ-induced convulsion it maybe effecting this action by enhancing GABA-mediated inhibition in the brain, this also confirms its CNS depressant effect.

Conclusion

The results of this study suggest that the leaf extract of *S. officinarum* possess antidepressant and anticonvulsant activities which may be due to the phytochemical compounds present in the leaf.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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