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ANALGESIC EFFICACY OF ETHANOL LEAF EXTRACT OF *JUSTICIAINSULARIS* (T. ANDERS)

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ABSTRACT

The ethanolic leaf extract of *Justiciainsularis* (T. Anders) was evaluated for its analgesic properties using acetic acid-induced writhing and thermal (hot plate methods. Acytylsalicycli acid (ASA) 100mg/kg body weight/per day was used as standard drug. Phytochemical screening of the plant extract gave positive test for saponins, alkaloids, tannins, Flavoniriodsanthraquinones and cardiac glycosides. The extract was dose-dependent and significantly at (P = 0.05). The median Lethal dose (LD_{50}) was determined by intraperitoneal route which was calculated to be 2,449.49mg/kg using Lorke method. The experimental design used was a complete randomized type for the studied parameters. The result further justified the use of *Justiciainsularis* in herbal medicine for treatment of pains.

KEYWORDS: *Justiciainsularis,* Analgesic, Acetyl Salicylic Acid (ASA), Thermal Method.

INTRODUCTION

Scientific evaluation of relevant medicinal plants parts that are used in traditional medicine offer some hope for developing new agents that may be suitable for treatment of malarial infection and/or associated pathological complications such as pains that stem from the destruction of red blood cells during malaria attack. To this effect, the ethanol leaf extract of *Justiciainsularis* plant, was screened for this exercise.

Justiciainsularis is a common weed found growing on marshy ground. It is widely distributed in the tropics (Hutchinson and Dalziel, 1958). *Justiciainsularis* is a member of the family Acanthaceae L. (Odoemena*et al*, 2002). It is a perennial herbs commonly known as “Mmeme” in Effik/Ibibio language. The plant is extensively used in many traditional medicines. The people of Akwa-Ibom State of Nigeria, hot water extract of the leaves is used as an enema as well as a remedy for internal heat (Etuk, *et al*., 1997). The leaf extract on sequencing is often used as an eye drop for eye problems and as well as for the reduction of menstrual bleeding and cramp pains in young girls (Williams, 1967). It is also recommended for those having chest pain or cardiac problem (Etuk*et al*., 1997). The research on the plant was carried out to confirm its use by the traditional herbal practice.

MATERIALS AND METHODS

PLANT MATERIALS

Fresh leaves of *Justiciainsularis* (T. Anders) were collected in the month of July 2010 from Ediene.

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village in Abak Local Government Area of AkwaIbom State Nigeria and authenticated by Dr Margaret Bassey a taxonomist in the Department of Botany and Ecological studies University of Uyo, Uyo, Nigeria. Voucher specimen was deposited at the Departmental Herbarium with a voucher N0: Oyomah, UUH 1613 (Abak). The Frsh leaves (3.5kg) of the plant were dried on a laboratory table for ten days and reduced to coarse powder form using laboratory morter and pestle.

Two hundred and fifty grams (250g) were macerated with 1000ml of 70% ethanol for about 72 horus. The liquid extract obtained was concentrated in vacuo at 46^0C. The yield was solid residue of (9.8g) referred to as the extract. The extract was stored in a refrigerator at 4^0C until used.

PHYTOCHEMICAL SCREENING

Standard qualitative phytochemcial tests were carried out with the leaf extract to elucidate the presence or absence of bioactive compounds in the leaf. The compound include alkaloids, flavonoids, saponins, glycosides, phlabotannins, tannins and anthraquinones using standard procedures as described by Trease and Evans (1999).

ANIMALS USED

Animals used for the experiment were obtained from the animal unit of the Department of Pharmacology and Toxicology, University of UyoWistar albino rats (128-170g) of either sex and albino Swiss mice (20-25g) were obtained from the animal house unit. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the college of Health Sciences Animal Ethics committee University of Uyo.

DETERMINATION OF MEDIAN LETHAL DOSE (LD_{50})

The median lethal dose (LD_{50}) of the extract was estimated using albino mice by intraperitoneal (I.P) route using the method of Lorke (1983). It involved the administration of different doses of the extract to groups of five mice each, various signs were observed in the animals for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decrease respiration and death. The number of death in each group within 24hours was noted. The LD_{50} was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b) $LD_{50} = \sqrt{ab}$

EVALUATION OF ANALGESIC ACTIVITIES

Two methods were used for this experiment (acetic acid-induced and hot plate). Analgesia was assessed by the method of Koster, Anderson and De-Beer (1959). The number of abdominal constriction induced by acetic acid (0.6% V/V) was counted. The hot plate method described by Vaz*et al*., (1997) was used to evaluate analgesic activity of the plant extract

PROCEDURES FORFLAVONOIDAL PARTITIONING AND FRACTIONATION

Flavonoidal Partitioning and Fractionation five grams of the plant crude extract sample was extracted repeatedly with 10ml of 80% aqueons ethanol at room temperature. The whole solution was filtered through whattman (N0.1) Filter paper (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight of 2.6g according to the method of Bohm and Kocipal-Abyazan (1994).

PROCEDURE FOR GLYCOSIDAL PARTITIONING AND FRACTIONATION

A portion of the ethanol crude extract (3g) was put in a boiling tube and 10ml of 70% ethanol was gently added and then vigorously shaken for 2-4 minutes. The boiling tube contents were warmed over a water bath at 45^{o}C for 5 minutes and the contents allowed to cool. After cooling, 5% of lead acetate was gradually added until a precipitate was formed. As described by Gyang, Nyam and Sokomba (2004), the precipitate was then filtered out using Whattman (N0.1) filter paper and the filtrate evaporated to dryness over water bath at 45^{0}C. The solute left after evaporation was tested for the presence of glycoside using the method described by sofowora (1993).

EXPERIMENTAL DESIGN AND TREATMENT

The experimental design was complete randomized type with two independent treatments and five replicates. The infected animals were randomly allotted into seven groups each containing five animals viz:

1. Mice with acetic acid induced writhing and
2. Mice on hot plate for the study of analgesic potentials.

STATISTICAL ANALYSIS AND DATA EVALUATION

Data obtained from this research work were analysed statistically using students t-test and ANOVA (One and Two-way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means was considered significant at 1% and 5% level of significance i.e. P ≤ 0.01 and 0.05.

RESULTS

The preliminary qualitative phytochemical screening of the ethanol leaf extract of *Justiciainsularis* revealed the presence fo alkaloids, flavonoids, tannins, anthraquinone, glycosides and saponins. Cardiac glycoside was the most dominant secondary metabolites detected in the plant. The various colour intensities resulting from the qualitative phytochemcial tests were indicative of the concentration of the chemical constituents analysed in the leaf extracts.

Table 1.Result of Phytochemical Screening of Ethanol Leaf Extract of *Justiciainsularis*

Secondary Metabolites	Test Done	Inference
Alkaloids	Dragendorfs	++
	Meyer	++
Tannins	Ferric chloride	++
	Lead acetate	++
Flavonoids	Shinodas	++
	Sodium hydroxide	++
Saponnins	Frothing	+
	Fehling	+
Phlobatannins	Dilute Hcl	ND
	Formaldehyde	ND
Anthraquinones	Bortrager	++
Cardiac Glycosides	Keller-Killiani	+++
	Sakowski test	+++
	Liebermans test	+++

These are arbitrarily presented with the number of positive signs ascribed, the more the concentration of the constituents in the leaf.

Key:

+++ = Strongly Present

++ = Moderately Present

+ = Trace

ND = Not detected

ACUTE TOXICITY

The mice were treated intraperitoneally with a single dose of 1000-5000 mg/kg of *Justiciainsularis* crude extract. The result evaluated showed 100% mortality rate at dose range between 3000-5000mg/kg, while 0% mortality was obtained at 1000-2000 mg/kg respectively. Signs and symptoms including respiratory distress, sedation, decreased limb tone and coma were observed at the administration of higher concentration of the extract. The LD_{50} of the extract in mice was calculated to be 2,449.49 mg/kg.

ANALGESIC ACTIVITY

The result of the effect of *Justiciainsularis* extracts as an analgesic using two models viz: acetic acid-induced pain and hot plate model are shown in table 2 and 3 respectively. The extract doses gave. Significant ($P < 0.05$) pain reduction in the animals. The treatment with 734.85 mg/kg gave the lowest reduction time of 2.00± 0.32 minutes followed by the glycosidalfraction 2.60± 0.40 minutes are significant and reduced the number of writhes. The highest dose of the extract (734.85 mg/kg) was more effective than that of the flavonoidal and glycosidal fractions in both models used.

Table 2.Effects of *J. insularis* Extracts on Analgesic Activity on Experimental Mice(Mousewrithing assay)

Group	Treatment (doses)	Average Writhing (minutes)					
		5	**10**	**15**	**20**	**25**	**30**
1	Control 10ml/kg	21.20 ± 1.07	16.20 ± 0.86	16.20 ± 0.86	12.80 ± 0.86	8.86 ± 6,73	8.60 ± 0.98
2	Extract 244.95mg/kg	16.40 ± 1.03	13.00 ± 0.84	11.20 ± 1.07	9.40 ± 0.51	6.60 ± 0.68	5.20 ± 0.49
3	Extract 489.90mg/kg	12.00 ± 0.84	10.00 ± 0.32	8.40 ± 0.68	7.80 ± 0.86	6.00 ± 0.84	4.20 ± 0.58
4	Extract 734.85mg/kg	11.00 ± 1.00	8.80 ± 0.58	6.20 ± 0.58	6.00 ± 0.45	4.00 ± 0.32	2.00 ± 0.32
5	ASA 100mg/kg	3.60 ± 0.68	6.20 ±0.38	7.60 ± 0.51	2.60 ± 0.87	1.20 ± 0.58	0.40 ± 0.40
6	Flavoniod Fraction 489.90mg/kg	16.60 ± 1.17	11.80 ± 0.80	10.80 ± 0.77	9.60 ± 1.21	7.60 ±0.28	5.20 ± 0.58
7	Glycoside fraction 489.90mg/kg	1.80 ± 0.73	7.80 ± 0.86	8.60 ± 0.51	7.60 ± 0.51	3.00 ± 1.00	2.60 ± 0.40

Value are expressed as mean ± SE (n = 5). All columns are significant using students t-test, $p <$ 0.05 when compared to the control.

Table 3.Effect of *J. insularis* Extract on Analgesic Activity on Mice (Hot plate model)

Group	Treatment (doses)	Mean Reaction time on hot plate at 56 $\pm$ 1^{0}C (Seconds)
1	Distilled water 10ml/kg	4.33 $\pm$ 0.27
2	Extract 244.95mg/kg	8.60 $\pm$ 0.33
3	Extract 489.90mg/kg	11.66 $\pm$ 0.47
4	Extract 734.85mg/kg	16.80 $\pm$ 0.42
5	ASA 100mg/kg	20.30 $\pm$ 0.54
6	Flavonoids Fraction 489.90mg/kg	15.18 $\pm$ 0.28
7	Glycosides Fraction 489.90mg/kg	19.09 $\pm$ 0.50

Value are expressed as mean $\pm$ SE (n = 5). Value are significant using students' t-test, $P < 0.05$, when compared to control.

The ability of *Justiciainsularis* leaf extract to inhibit pain using hot plate model is shown in Table 3. A significant ($P < 0.05$) analgesic protection to the animals against the thermal induced pain was achieved with all the doses of the extract used, (244.95, 489.90 and 734.85mg/kg respectively). Also the standard drug (ASA) (100mg/kg), flavonoid and glycoside fraction (489.90mg/kg) respectively gave significant ($P < 0.05$) pain relief to the animals. The inhibitory effect of the extract on the thermally induced pain on the animals was dose dependent with the highest mean reaction time level from the 734.85mg/kg concentration in which the animals were able to stay for 16.80 seconds on the hot plate, while glycoside fraction treated animals were able to withstand the heat for 19.09 seconds and ASA, the standard drugs treated animals could withstand the heat for 20.30 seconds. Therefore, the extracts and the fractions compared favourable with the ASA.

DISCUSSION

Justiciainsularis ethanol leaf extract was evaluated for its analgesic efficacy in acetic acid-induced writhing and hot plate pain induced on mice. The acute toxicity test of the ethanol leaf extract was also carried out. The qualitative phytochemical analysis of *J. insularis* ethanol leaf extract revealed that the leaf contain various degrees of secondary metabolites. The presence of alkaloids, glycosides, flavonoids, tannins, saponnins, terpenes and anthraquinones in the plant (Table 1), confirms the major bioactive constituents found in medicinal plants (Trease and Evans 1989, Odoemena and Essien, 1995). This is a lead way in support of some traditional healer claims on the plant efficacy from the results presented. These chemical compounds are known to show medicinal activity as well as exhibiting physiological action in animal metabolisms (Sofowora, 1993, Zaidi, 1998). The presence of alkaloids in the plant is in line with the observation of Sofowora (1993) who reported that alkaloids from medical plants are used as synthetic materials for some useful drugs.

Result from this investigation suggest that the leaf extract, as well as the flavonoids and glycosides fraction of *J. insularis* showed higher analgesic activities. The two models used in this study, yielded the same result. The extract inhibited the acetic acid-induced writhing in mice and as well increased the reaction time of hot plate test. There was no sharp difference between the different doses of the extract administered and that of the flavonoid fraction. However, the extract at 734.85mg/kg level and that of the glycosidal fraction compared favourably with that of the standard drug (Acetyl Salicyclic Acid). This showed that the presence of glycoside in the plant is responsible for its ability to reduce pains. The presence of alkaloids in the plant extract suggests its use as a pain reliever, as pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agent for

analgesic purposes (Stray, 1998). The analgesic results indicates that the plant extract, flavonoids and glycoside fractions exhibit central analgesic properties, since it extorted a significant protective effect on thermic (heat) painful stimulus from respective doses. Such efficacy on this stimulus is characteristic of central analgesics like morphine, aspirin etc. (Lewis *et al.,* 1987; Hughes, Smith and Kosterllitz, 1975).

CONCLUSION

The preliminary result from this study revealed that the ethanol leaf extract of the plant has analgesic properties, one of the vital properties necessary for the management of clinical symptoms referred to as malaria fever (Aguwa, 1996). Also the higher efficacy of the glycosidal fraction of the plant extract than the flavonoidal fraction suggest that the main active ingredient for its analgesic activity of the leaf resides in the glycosidal fraction. Therefore, it would be interesting if the other active principle is isolated, identified and characterised.

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