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Phytochemical Screening and Acute Oral Toxicity Study of Myrica Salicifolia (*Bayberry*) Root Extracts

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Abstract
Aim: *The present study was aimed at evaluating the possible acute oral toxicity and the major phytochemical constituents of Myrica salicifolia root extract.*
Study Design*: An experimental study design was used.*
Place and Duration of Study*: The phytochemical studies were done at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Department of Botany Laboratory, while acute oral toxicity studies were done at the SAFARI, JKUAT animal house. The study was done during the month of August to December 2020.*
Methodology*: Myrica salicifolia roots were harvested with the help of a plant taxonomist. The roots were chopped into small pieces and dried under shade for three weeks. They were then ground into powder. Organic extracts were prepared by sequential extraction (petroleum ether, dichloromethane (DCM), ethyl acetate and methanol), by use of cold maceration. Aqueous extracts were obtained by hot maceration. Phytochemical screening of extracts was done by standard phytochemical procedures. A total of 12 female albino rats were used in acute oral toxicity studies as per OECD 423 guidelines.*
Results: *Methanol extract had the highest composition of phytochemicals, i.e. alkaloids, flavonoids, saponins, sterols, and tannins. Aqueous and DCM extracts showed presence of alkaloids, saponins and cardiac glycosides, while petroleum ether and ethyl acetate extracts showed presence of alkaloids and cardiac glycosides. Steroids and tannins were absent in all extracts. In the acute oral toxicity study, there were no adverse effects at 2000 mg/kg extract administration.*
Conclusion*: It was found that Myrica salicifolia root extracts did not cause any toxic effects or mortality at the administered dose. No abnormality was noticed in all selected parameters in treatment groups as compared with their respective control groups. Thus, the possible oral lethal dose for Myrica salicifolia is more than 2000 mg/Kg body weight. These findings may require further verification using in vivo studies.*

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I. Introduction

Scientific evaluation of medicinal plants is important to the discovery of novel drugs and also helps to assess toxicity risks associated with the use of herbal preparations and other conventional drugs of plant origin. There are about over 5000 species of plants on earth but only 1 – 10% has been studied for their potential medicinal values (Verpoorte, 2000). *Myrica salicifolia* A Rich (Myricaceae) is a shrub of 1 m in height and is found in several central and east Africa countries such as Burundi, Ethiopia, Kenya, Malawi, Rwanda, Tanzania, Uganda, and Zaire. It belongs to the family Myricaceae found mostly in temperate to subtropical regions of the world (Dale and Greenway, 1961).Studies have shown that the root extract of M.salicifolia is a non-hypnotic (CNS) depressant with muscle relaxant, analgesic, hypothermic and antipyretic properties (Miaron, 2003). M. salicifolia is known by the number of vernacular names in Kenya viz. Olkitoloswa (Maasai), Mukikia, Muthogoya (Kikuyu) Kibogen (Marakwet) and Kabuneto (Kipsigis). In the Marakwet community in Kenya, its powder is mixed with honey and taken to treat hyperthyroidism (Korir et al., 2015). The study indicates that local people along with local herbalist use *M salicifolia* A Rich (Myricaceae) root and bark extract with tea for aliment of different disease such as chest congestion, pneumonia, diarrhea, nervous disorders, diabetes, hypertension, and respiratory diseases.[15]To verify the traditional uses of *M salicifolia*, various in vitro and in vivo studies have been conducted. The studies showed that this plant has most of the claimed activities, including antibacterial activities against Gram negative bacterial strains namely *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Salmonella kisarawe*, *Salmonella typhi*, and *Escherichia coli*.[16] The plant also has antipyretic[14] and antiplasmodic activities,[17] is a cough suppressant, and possesses wound healing and aphrodiasic

II. Materials and Methods

Collection of Plants and Preparation of Extracts

Roots of *M salicifolia* A Rich (Myricacae) were collected from Timboroa forest in Elgeyo Marakwet, Kenya, about 15 km from Eldoret town in October 2020. The plants were identified by a local herbal expert and authenticated by a taxonomist and have a voucher number of YK001 at the Department of Biology. The roots were chopped and air dried under shade in ambient temperature for three weeks. The dried roots were ground into coarse powder using an electric grinder made at the Mechanical Engineering Department in JKUAT. Organic extraction was done using petroleum ether, dichloromethane (DCM), ethyl acetate and methanol by cold maceration, whereas aqueous extraction was done using hot maceration. Organic extraction was done by adding 500 mL of petroleum ether, methanol, DCM and ethyl acetate to each quantity of 50 g of the myrica salicifolia root powder for 72 hours by cold maceration then the extract was concentrated by use of a rotary evaporator (BUCHI Vac® V-500) at 45°C and stored at 4°C [10]. In aqueous extraction, 50 g of the powder was added to 500 mL of distilled water in a 1 L flask then boiled for 15 minutes. The boiled mixture was then filtered using Whitman No. 1 filter paper and the extract was freeze-dried using a freeze dryer (BUCHI Lyovapor™ L-300). The lyophilized sample was kept at 4°C [11].

Phytochemical Screening

The solution of Petroleum ether, chloroform, ethylacetate, methanol and ethanolic extract was prepared using distilled water and subjected to preliminary phytochemical screening. Test for common phytochemicals were carried out by standard methods described in practical pharmacognosy by Kokate, Khandelwal and Trease and Evans [1]

Phytochemical screening was done by observing precipitate formation and colour change [12].

Saponins test

1 ml of the extract was put in a test-tube then 50mls of tap water was added. The mixture was shaken vigorously for 15 minutes. Formation of honeycombs foam that persists for 15 minutes after shaking was subjected to a confirmatory test, which involved dissolving 1 ml of the extract in anhydride tetrachloride to which 5 ml of concentrated sulphuric acid was added on the mixture. A blue, green or red color accompanied with a pink ring was indicative of saponins

Alkaloids

1ml of extract was tested with Mayer's reagent prepared by dissolving 35gm of mercury chloride in distilled water and a solution of 5 gm. potassium iodide in 10mls of water .the mixture then was diluted to 100mls. The appearance of opalescence or yellow color indicted presence of alkaloids.

Flavonoids

5 mL of hydrochloric acid solution and magnesium turnings was added to myrica salicifolia root extract. Appearance of a pink or magenta red was indicative of the presence of flavonoids [12].

Sterols and steroids test

Salkowaski method was used. To 1 mL of extract in a test tube, 0.5 mL acetic anhydride and 0.5 ml chloroform were added. Concentrated sulfuric acid was slowly added along the sides of the test tube. A red coloration was an indication of the presence of sterols and a green color was indicative of presence of steroids [3].

Tannins test

1 ml of extract was dissolved in water in which 1 %gelatin and 10% sodium chloride and salt solution (10%Nacl) was added. Allow tannins were indicated by presence of a blackish blue color while catechol tannins were indicted by a greenish back coloration.

Grouping and Dosing of Animals

Male Wister albino rats weighing between 160–200 g were used. The animals were purchased from the SAFARI animal house, JKUAT, and were housed in standard cages at the SAFARI JKUAT and provided with commercial rat pellets from Belmill feeds limited (Kenya) and water ad libitum. The temperature of the experimental room was maintained at 22°C±3 and relative humidity of 30–70%. The animals were exposed to 12 hours of light and 12 hours of darkness daily. Wood shavings were used for beddings and were changed on every other day. Acute toxicity testing was conducted using limit test dose of 2 g/kg according to the Organization for Economic Cooperation and Development (OECD 425, 2008) guidelines. Twelve male wister albino rats weighing 160–200 g divided into three groups of three were used. All animals were weighed before fasting, at day zero, day one, day two then day seven and day fourteen. . The first animal was given with a limit dose of 2000 mg/kg and no death was observed within 24 hours of dosing. The remaining rats were dosed and observed for toxicities like diarrhea, weight loss, and absence of tremor, lethargy, and paralysis periodically for the first four hours during the

24-hour period and later were followed for 14 days for any lethality.[27]
After the study, all the animals were humanely sacrificed by ndanyi et al.; EJMP, 31(7): 17-23, 2020; Article no.EJMP.56393 20 inducing hypoxia using CO2, thereafter the carcasses were incinerated

Statistical Analysis

Results of the study were expressed as a mean plus or minus standard error of the mean (M ± SEM). Data analyzed was undertaken using Windows SPSS Version 24. One-way analysis of variance (ANOVA) followed by Tukey's (post hoc) test to evaluate statistical significance for grouped data. The results were analyzed at 95 confidence interval and $P < .05$.

III. Results

Yield of Crude Extract of *Myrica salicifolia*

A total of 150 g of dried root crude extract was harvested at the end of the extraction process. In the preparation of crude 80% methanolic extract from the dried roots of *M salicifolia*, a yield of 21% was obtained.

Acute Toxicity Test of Crude Extract

The acute toxicity study indicated that the crude extract caused no mortality in limit dose of 2000 mg/kg within the first 24 hours as well as for the following 14 follow-up days. Physical and behavioral observations of the experimental rats also revealed no visible signs of overt toxicity. This indicates that LD_{50} of the extract is greater than 2000 mg/kg.

Phytochemical screening test

In the present study the qualitative analysis of Myrica salicifolia root extracts were carried out for dried roots. The preliminary phytochemical screening results of petroleum ether, chloroform and chloroform/methanol (1:1) crude root ectract of Myrica salicifolia are presented in Table 1.

Table 1: Phytochemical screening results

solvent	Alkaloids	flavinoids	saponins	Sterols/ steroids	tannins	phenolics
MeOH	+	+	+	+	+	+
EtOAc	+	+	-	-	-	-
PE	+	-	-	-	-	-
DCM	+	-	+	-	-	-
aqueous	+	-	+	-	-	

*Key '+' Present, '-'absent; MeOH = Methanol; EtOAc = Ethyl acetate; PE = Petroleum ether; DCM = Dichloromethane

Table 2.means body weight +/- SEM for Myrica salicifolia treated groups and the control group

M.salicifolia (Mg/kg)	fasting day	day 0	day1	day 7	day14
Control group	177.80±2.747	171.90±1.751	178.67±0.998	184.78±1.358	188.95±2.193
300	183.74±1.529	168.21±3.292	182.51±1.150	188.63±3.549	194.97±3.703
1000	182.00±1.8179.11±1.67		177.09±1.58	176.21±2.11	178.00±1.20
2000	176.95±4.084	165.02±5.269	173.63±4.713	180.27±4.159	183.34±4.709
P-value	0.275	0.639	0.393	0.365	0.270

IV. Discussion

The safe use of the extracts tested and of active substances they contain, explain probably their common uses by traditional healers in the treatment of numerous human and animal diseases (DMP, 2004; CAPES, 2006,). Alkaloids, flavonoids sterols, phenolics and tannins were observed in the root extracts of *M. salicifolia*. The therapeutic properties of these large chemical groups have been reported by various authors (Delaveau, 1988; Cowan, 1999).

Its methanol extract was found to contain alkaloid, tannins, saponins flavonoid sterols and phenolics. Its ethanol extract has alkaloid, flavonoids,but saponins, sterols and phenolics were absent. The Petroleum ether extract contains alkaloids but, flavonoids and tannins, saponins and phenolics were absent. All extracts contain alkaloids. Tannins were reported to exhibit anidiabetic, anti-inflammory, antibacterial and antitumor activities [39, 40]. Additionally some tannins were able to inhibit HIV replication selectively beside their use as diuretics. It was reported saponins are used as mild detergents and in intracellular histochemistry staining to allow

antibody access intracellular proteins [39, 41]. Medicinally, saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anti-cancer, anti-inflammatory, central nervous system activities and weight loss [41]. It is also known to have antifungal properties [39, The plant saponins generally help humans to fight fungal infections, combat microbes and viruses and knock out some tumor cells, particularly lung and blood cancers [44]. Alkaloid help in controlling development system of living organisms plays some metabolic role and have protective property [42]. Alkaloids have been reported from Morella salicifolia and their medicinal values indicated these compounds serve as natural antibiotics, which help the body to fight infections and microbial invasion [45]. Plant with alkaloids have been determined to exhibit analgesic, antispasmoic and antibacterial properties and are used in medicines for reducing headache and fever [46, 47]. The presence of alkaloids in the stem bark of M. salicifolia might be one of the reasons for its traditional medicinal values. Secondary metabolites serve as chemical defense against micro organisms. While phenolic compound and flavonoids are known to possess biological activity such as antibacterial activity, antioxidant, anti-inflammatory, etc [42]. Therefore, the phytochemical screening results reveal that the presence of these phytochemical constituents supports the use of Myricaa salicifolia plant in folklore medications.

Treatment with methanol extracts of *M. salicifolia root extract* (300 mg/kg, 1000 mg/kg, and 2000 mg/kg) was tolerated by all the animals, the signs noticed within 24 hours included loss of appetite, reduced mobility, dullness and general weakness; these signs were not seen in the 300 mg/kg dose group but progressed and became increasingly pronounced as the dose increased towards 2000 mg/kg. However no death was recorded, indicating the LD_{50} of *M.salicifolia root extract* is approximately higher than 2000 mg/kg, and thus it is relatively safe and non-toxic to rats (Lorke, 1983) in acute usage. It was also observed that the plant extract caused a slight but insignificant decrease in the body weight of the test animals; this may be due to a decrease in appetite, which may be secondary to a feeling of fullness after administration of the extract. It may also be due to the effect of the plant on the body fat metabolism. This, however, remained to be rationalized.

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The products used for this research are commonly and predominantly used products in our area of research and country. absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the author

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical review was sought from Biosafety, animal use and ethics committee of JKUAT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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