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PHYTOMEDICINAL PROPERTY, PHYSICOCHEMICAL SCREENING AND BIOSAFETY EVALUATION OF EXTRACT OF *ICACINA TRICHANTHA* OLIVER SEED IN WISTAR ALBINO RATS

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ABSTRACT

Ethnomedicine provides a basic understanding of plant medicinal properties. The identification and standardization of active compounds in any medicinal plant is critical for the production of new drugs. The aim of this study was to evaluate the biochemical constituents and biosafety of the extract from *Icacina trichantha. Oliv. (Icacinaceae)* seed. Phytoconstituents of the plant material was assayed using standard biochemical methods and GCMS analytical procedures. Acute toxicity test was performed according to standard method, while sub-acute toxicity was determined by assessing haematological parameters in grouped rats, and were administered single daily doses of 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg of the extract for 14 consecutive days. Findings from the study revealed notable phytochemicals in sufficient amount; while in the GCMS, phytocompounds like erucic acid, hexadecanoic acid, stearic acid and phytol were identified. Physicochemical analysis on the studied plant material had appreciable amount of moisture (14.00%), crude lipid (2.92%), crude fibre (1.75%), proteins (5.25%), ash (12.26%) and carbohydrates (31.82%). The plant also had pronounced concentrations of notable vitamins. The result from the acute toxicity studies was assessed to be above 200 mg/kg, while the 14 days sub-acute test revealed that there was improving effect in all haematological parameters investigated without adverse interference. This study revealed that orally administered I. trichantha methanol seed extract was tolerated at a single graded dose, LD_{50} above 200 mg/kg. The data obtained tend to support the biosafety of the plant extract as reportedly used in indigenous herbal home remedies.

Keywords: Haematology, acute, sub-acute, toxicity, phytocompounds.

INTRODUCTION

Plant material possessing medicinal characteristics are well known to offer useful holistic measures, as well as chemical characters, which also can be used to define their pharmacological and therapeutic usefulness. Plant phytochemicals are secondary metabolites that are responsible for the medicinal properties of plants. In different extracts and parts of *Icacina trichantha*, the presence of phytochemicals has been reported (Otun et al. 2015). These phytochemicals/biomolecules are thought to be responsible for the plant's antihyperglycemic, anticonvulsant, sedative, analgesic, and antimicrobial properties (Alawode et al. 2018). They are biomolecules extracted from plants. Depending on their role in plant metabolism, these biomolecules are classified as primary or secondary constituents. Primary constituents include common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls, and so on. The remaining plant biomolecules, such as alkaloids (derived from amino acids), terpenes (a group of lipids), and phenolics, are classified as secondary constituents (derived from carbohydrates) (Krishnaiah et al. 2007).

Plant and plant-based therapies have a high potential for treating and controlling diseases and their complications, according to research conducted over the last several decades (Singh et al. 2008). Based on folklore medicine, many diseases have been treated orally with a variety of medicinal plants or extracts; without the knowledge of their bioactive principles and

safety. Hence, the foregoing study, searching for safer and more effective agents of plant origin possessing notable phytoconstituents of therapeutics implication in health sustainability. Looking back over the last 2000 years of medical history, mankind has primarily relied on plants as the best source of medicine (Nostro et al. 2000). Over 248,000 higher plant species have been identified, with 12,000 of these plants having medicinal properties (Mukharjee, 2002). According to the World Health Organization, approximately 80% of the Earth's inhabitants rely on traditional medicine for their primary health care needs, which primarily involves the use of plant extracts or their active components; and that phytochemical research based on ethnopharmacological information is widely regarded as an effective method for discovering new curative agents from natural plant products (Mukharjee, 2002).

Icacina trichantha Oliver belongs to the family *Icacinaceae*. The plant is a perennial shrub up to 2 m with scandent growth above, and commonly found in crop fields, forest regrowths and waste areas in the forest and savannah. The leaves are broadly elliptic, abruptly acute at the apex and rounded at the base. Leaf length is about 8.0 - 10.0 cm. while the width is up to 17.0 cm. Flowers are densely crowded and subsessile with calyx nearly as long as the petals. The fruits are tomentose on the surface, ellipsoid to globose in shape and they are about 2.5 cm long (Che et al. 2016). It is called as “Gbegbe” in Yoruba in the south west Nigeria and “Ibugo” in Igbo in the eastern Nigeria. The plant is extensively used in the rural areas; the leaves and tuber have folkloric uses in the treatment of malaria, constipation and food poisoning in Nigeria (Che et al. 2016).

From the scientific point of study, there is however, dearth of information in literatures on the seed of this plant. The present study therefore seeks to assess the phytoconstituents properties and biosafety activities of *Icacina trichanthaseed with the view of being* important data source for species prolific utilization in drug formulation.

MATERIALS AND METHODS

Chemicals and Reagents

Chemicals such as 1,1-diphenyl-1-picrylhydrazile (DPPH), hydroxytoluene butylated (BHT), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4'-disulphonic acid, hydrogen peroxide, ferrous chloride, nicotinamide adenine dinucleotide (NADH), trichloroacetic acid (TCA), phosphate buffer, sulfanilic acid, glacial acetic acid, and naphthylethylenediamine dichloride were used. Sigma provided the foline-ciocalteum reagent, sodium carbonate, vanillin, aluminum chloride, ascorbic acid, and potassium acetate (Sigma-Aldrich GmbH, Sternheim, Germany). All of the other chemicals and reagents used, including the solvents, were of analytical grade.

Collection of Plant Material Sample

The plant material (seed of *Icacina trichantha*) were collected in November 2020 within the campus of Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria. The samples were identified by the herbarium Curator, Botany Programme, Department of Biological Sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria where a specimen with voucher number (no: OAUSTECH/HB-BOT/075) was deposited.

Preparation of Plant Extract

The plant extract was prepared by basic extraction and fractionation procedures for experimental purposes as previouslydescribed and published by Abubakar and Haque (2020). The seeds were air dried at room temperature to a constant weight. The dried seeds were then pulverized to powder using an electric grinding machine (Panasonic MX-337N, China). The powdered material was stored in air-tight containers. Two hundred and fifty grams (250 g) of the powdered seed sample was extracted using 1000mL of distilled water for 48hrs with continuous stirring. The mixture was filtered using Whatman paper No1. The filtrate was evaporated to dryness using a water bath to obtain 30% yield of the extract.

Phytochemical Analysis

The phytochemical analysis was conducted using the standard analytical procedures for determination of biomoleculesas previously described and published by Sofowora (1993).

Analysis by Gas Chromatography and Mass Spectrometry

The phytocompounds derivatives of the extract were identified using GC-MS apparatus as cited by Bakari et al. (2015). A HP-5 MS capillary column was used for the GC system. Helium was used as carrier gas at a flow rate of 1 mL/min. The injection volume was 2µL. Ionization energy EI of 70 eV was used for mass spectroscopy detector.
The GC-MS apparatus was carried out by Chem-Station software package (Agilent Technologies). Both the injector and detector temperature were 250°C. The oven temperature was held at 100°C for 1 min, increased to 260°C at a heating rate of 4°C/min, and held for 10 min. The identification of compounds was achieved as the same method reported by Bakari et al. (2015).

Physicochemical Analyses

The parameters determined for proximate analyses include ash, moisture, crude protein, fat, fiber and carbohydrate. All of these were carried out using the methods described by (AOAC, 2010).

Vitamin Composition Determination

The amount of vitamin A, E, C, B12 in the sample was determined using the method described by (Achikanu *et al.* 2013). Vitamin B1, B2, B3, Vitamin K, and folate were determined using the method described by Okwu and Ndu, (2006). The method described by Oulai *et al.* (2014) was used in the determination of β-carotene.

Experimental Animals Care and Use Protocols

Wistar albino rats (25 – 30 g) were employed for this study. The animals were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, University of Benin, Nigeria. The animals were housed in standard laboratory conditions of 12 h and fed with commercial rodent feed (Guinea feeds Nigeria Ltd) and had free access to food and water *ad libitum.* All animal experiments complied with the ARRIVE guidelines (Percie du Sert et al. 2020), and were performed in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. Cage-side clinical observations of the rats were made throughout the study period (NIH, 2011). The study protocols were approved by the Animal Care and Use Committee of our institution (ethical approval number: FS/UG/021/BOT/005).

Acute Oral Toxicity Studies

This test was carried out according to the standard method described by Jothy et al. (2011). Sequel to the method, twenty-five albino Wistar rats of either sex selected randomly into five experimental groups of five rats each; one control, and four treatment groups were used in the study. The animals were fasted over-night with free access to water. The crude methanol extract (CME) of *Icacina trichantha* seed *at the doses of* 25, 50, 100 and 200 mg/kg were administered to the rats by gavage to determine the acute toxic dose. The rats were observed closely for obvious signs of toxicity and mortality for 24 h and for a total of 14 days.

Assessment of Sub-Acute Toxicity

The assessment of sub-acute toxicity were estimated by standard analytical procedures as previously described and published by Treasel et al. (2014). Twenty-five albino Wistar rats of either sex were allotted randomly into five experimental groups (n=5) and kept in separate cages. They included Group I (control), which received distilled water orally, while Group II-V were administered single oral doses of the extract at 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg respectively for consecutive 14 days. The animals were closely monitored for signs of morbidity and mortality. At termination of the treatment period, the rats were fasted into the following day, their body weights were noted before sacrificing under cervical dislocation. Blood samples were immediately collected via the abdominal aorta and aspirated into EDTA bottles for haematological analysis.

Statistical Analysis

The statistical analysis was estimated by basic statistical tools in research and data analysis as previously described and published by Ali and Bhaskar (2016). The statistical package for social sciences (SPSS) computer software version 16 was used for data analyses. The results were expressed as mean of replicate

determinations ± standard error of mean (S.E.M), results were analysed by using one way analysis of variance (ANOVA). Differences in values between means were considered statistically significant at $p<0.05$.

RESULTS

The presence of phytochemicals in *Icacina trichantha* seed studied is shown in Table 1. From the result, it was shown that flavonoids has the highest quantity (37.40±4.98), followed by phenol (35.42±3.01), alkaloids (20.31^{b}±0.21), steroids (20.31^{b}±0.21), and terpenoids (21.43^{b}±0.41), meanwhile, saponins (10.53^{c}±0.14), tannins (11.65^{c}±0.24), and glycosides (7.41^{d}±0.13) were found to have lower quantities compared to other secondary metabolites.

Table 1: Phytochemical composition of *Icacina trichantha seed*

S/N	Secondary Metabolites	Methanol Fraction
1	Alkaloids	22.23^{b}±0.25
2	Phenols	25.42^{a}±3.01
3	Saponins	10.53^{c}±0.14
4	Tannins	11.65^{c}±0.24
5	Flavonoid	27.40^{a}±2.17
6	Cardiac glycosides	7.41^{d}±0.13
7	Terpenoids	21.43^{b}±0.41
8	Steroids	20.31^{b}±0.21

**P<0.05 – Significant*; *Different letters in superscript down the column showed significant differences in the chemical response.*

The GC-MS analysis results of the biochemical compound composition of the methanol extract of *I. trichantha* is presented in Table 2. The GC-MS spectrum showed 13 peaks indicating 13 different bioactive compounds with Erucic acid, ($C_{18}H_{34}O_2$), RT 20.561, 29.01% as the major compound. Other notable compounds that are present in the methanol extract include stearic acid (24.65%, RT 17.637), 5-Hydroxymethylfurfural (17.67%, RT 7.485), 9, 12 octadecanoic acid methyl ester (6.31%, RT 21.530), Phytol (5.21%, RT 20.052) all representing 53.83% of the total compounds.

Table 2: *Icacina trichantha* seed methanol extract profile obtained from the GC-MS

Peaks	Compounds	Molecular Formula	Retention Time (mins)	Yield (%)
1	Acetoglyceride	$C_5H_{10}O_4$	5.396	2.54
2	1,1,2-triacetoxyethane	$C_{18}H_{12}O$	9.86	0.35
3	Erucic acid	$C_{18}H_{34}O_2$	20.561	29.01
4	3,5-diterbutyl phenol	$C_{14}H_{22}O$	11.047	1.39
5	Stearic acid	$C_{18}H_{36}O_2$	17.637	24.65
6	5-Hydroxymethylfurfural	$C_6H_6O_3$	7.485	17.67
7	2,5-Dihydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one	$C_{10}H_{12}O_3$	16.37	3.37
8	9,12-octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	21.530	6.31
9	Hexadecanoic acid	$C_{16}H_{32}O2$	24.433	1.30
10	4-((1E)-3 Hydroxy-1-propenyl)-2-methyoxyphenol	$C_{10}H_{12}O_3$	16.37	2.03
11	2,2-6,9-pentadecadien-1-ol	$C_{18}H_{28}O$	26.087	1.64
12	Palmitic acid and ethyl ester	$C_{18}H_{36}O_2$	18.078	4.53
13	Phytol	$C_{20}H_{40}O$	20.052	5.21

Table 3 is a result of physiochemical parameters of the studied herbal plant. The physicochemical analysis carried out on *Icacina trichantha* seed sample as presented in Table 3 revealed the moisture content to be 34.00%, crude lipid – 0.92%, carbohydrate – 31.82%, among other components. The moisture, crude lipid, crude fibre, protein, ash, carbohydrates and fat values were found to be within the range of 0.75 -34.00%. The results could serve as gateway to quality, authenticity and purity of the studied plant in herbal preparations. The moisture content of 34.00% could be due to the fruiting nature of the seed plant. The value was quite within the acceptable limit as such the seed plant of *I. trichantha* would not be susceptible to microbial attack if stored for a period of time and degradation of chemical reactions that may alter the nature of its active constituent.

Table 3: Physicochemical composition of *Icacina trichantha seed*

S/N	Proximate Component	Value (%)
1	Moisture	14.00
2	Crude lipid	2.92
3	Crude fibre	1.75
4	Protein	5.25
5	Ash	12.26
6	Carbohydrate	31.82
7	Energy (kcal)	56.00

The vitamins component of the studied herbal plant is depicted in Table 4. The result shows higher concentrations of carotenoid (6.55±0.09 mg/100g) and Vitamin A (6.06±0.04 mg/100g) while the B-Vitamins (0.46±0.03 mg/100g, 0.68±0.02 mg/100g and 0.36±0.03 mg/100g), Vitamin C (0.26±0.02 mg/100g), Vitamin D (0.13±0.05 mg/100g), Vitamin E (0.35±0.04 mg/100g) and Vitamin K (0.14±0.03 mg/100g) were in moderate amounts. Although vitamins C, vitamin E, vitamin D and vitamin K were in trace amount in the seed, they have very essential roles to play in the human health.

Table 4: Vitamin content of *Icacina trichantha seed*

S/N	Components	Concentration (%)
1	Vitamin A (mg/100g)	6.06±0.04
2	Vitamin E (mg/100g)	0.35±0.04
3	Vitamin B1 (mg/100g)	0.46±0.03
4	Vitamin C (mg/100g)	0.26±0.02
5	Vitamin B2 (mg/100g)	0.68±0,02
6	Vitamin B3 (mg/100g)	0.36±0.03
7	Vitamin D (mg/100g)	0.13±0.05
8	Vitamin K (mg/100g)	0.14±0.03
9	Carotenoid (mg/100g)	6.55±0.09

Table 5 is a result of the acute toxicity study (LD_{50} determination) of the crude methanol extract of *I. trichantha* seed. From the results, it is revealed that the extract is safe. This is because no lethality or toxic reactions were recorded in the study rats after 24 h and after 14 days of observation post-administration of extract and even at a maximum test dose of 200 mg/kg. In addition, the animals did not show any significant changes in behaviour, skin defects, breathing, impairment in food intake and water consumption, postural abnormalities or hair loss.

Table 5: Toxicity and mortality during the acute toxicity study of *Icacina trichantha* methanol seed extract in experimental rats.

Groups (Control)	Dose (mg/kg body weight)	Number of rats with indication of toxicity/normal behaviour (IT/UB)	Number of mortality/ survival (D/A)
1	10 mL/kg	0/5	0/5
2	25 mg/kg	0/5	0/5
3	50 mg/kg	0/5	0/5
4	100 mg/kg	0/5	0/5
5	200 mg/kg	0/5	0/5

Key: *(b.w) = body weight, (IT) = indications of toxicity, (UB) = usual behaviour, (D) = deceased, (A)= alive.*

The effect of sub-acute toxicity study for 14 days consecutive oral administration of methanol extract of *Icacina trichantha* seed in Wistar experimental rats is shown in Table 6. There was progressive increase in the leukocyte parameters of the rats in all dose levels of the extract used when compared with the control. Rats that received 25 mg/kg body weight of the extract had elevated white blood cell (WBC) count values of 12.73±1.18 than the control (8.37±1.27). At a dose of 50 mg/kg, 100 mg/kg and 200 mg/kg body weight of the extract, WBC count values were 11.93±2.38, 10.47±1.68 and 11.87±1.85 respectively. Extract treated rats also showed increase in lymphocytes count at all dose levels of the extract used compared with the control. The erythrocyte parameters namely, red blood cell, haematocrit and haemoglobin of the extract treated rats increased at all dose levels. At the doses of 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg body weight of the extract, red blood cell count values were 8.7±1.04, 8.8±0.44, 8.5±1.38 and 7.20±1.34 compared with the control (5.3±0.81) group that received only distilled water. Also, there is increase in haematocrit and haemoglobin concentrations in treated group of rats when compared to control group of rats at all dose levels of the extract used. However, the results of the thrombocytes indices (platelets and plateletcrit) as shown in Table 6 were found decreased significantly ($p<0.05$) in extract treated group of rats at all dose levels of the administered extract when compared to control group that received only distilled water.

Table 6: Mean haematological indices of various doses of *Icacina trichantha* methanol seed extract as it relates the effect of consecutive 14 days oral administration in wistar rats.

Parameters	Control 10mL/kg	25 mg/kg body wt	50 mg/kg body wt	100 mg/kg body wt	200 mg/kg body wt
WBC (10^3/μl)	8.37±1.27	12.71±1.18	11.93±2.38	10.47±1.68	11.87±1.85
LYM (%)	3.16±1.17	5.9±0.1	5.5±1.47	5.8±1.06	8.33±2.21
MON (%)	1.61±1.08	1.00±0.27	1.50±0.46	0.46±0.16	0.46±0.16
NEU (%)	10.72±3.49	11.68±2.03	11.76±1.93	9.65±2.10	9.65 ±2.10
RBC (10^3/6μl)	5.3^b±0.81	8.7^a±1.04	8.8^a ±0.44	8.5^a±1.38	7.20^a ±1.34
HCT (%)	33.50±4.21	41.90±1.70	39.90±2.01	37.80±1.51	45.43±1.68
HGB (g/dl)	13.0±1.58	15.3±0.6	14.4±0.87	13.7±0.82	14.68±0.53
PLT (10^3/μl)	812.10±191.71	727.0±121.23	635.0±75.55	575.70±24.06	517.5±67.8
PCT (%)	0.8±0.12	0.5±0.09	0.4±0.05	0.40±0.2	0.31±0.14

White blood cell (WBC), Lymphocytes (LYM), Monocytes (MON), Neutrophils (NEU), Red blood cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), Platelets (PLT), Plateletcrit (PCT). ***a, b Means in the same row with different superscripts differ significantly (p < 0.05).***

DISCUSSION

Plants contain phytochemicals that can be used therapeutically or as a precursor for pharmaceutical synthesis (Makut et al. 2008). As a result, global demand for herbs and their products has skyrocketed in recent years. Non-nutritive plant chemicals with protective or disease-preventive properties are known as phytochemicals. They are secondary metabolites or plant products with pharmacological, medicinal, and nutritive properties in terms of organoleptic properties. The presence of tannins in *Icacina trichantha* seed makes it an excellent source for treating wounds caused by varicose ulcers and hemorrhoids (Njoku and Akumufula, 2007). Tannin-containing plants are used as astringents, diuretics, and to treat stomach and duodenal tumors. Plant flavonoids have medicinal properties which are use for disease prevention against cancer, inflammation, and atherosclerosis. (Onyeka and Nwambekwe, 2007). The presence of alkaloids in the seed of *Icacina trichantha* supports the findings by Oyeleke *et al.* (2008), that the antibacterial activity of this plant may be attributed to the presence of alkaloids. And that pure isolated alkaloids and their synthetic compounds have been used in medicine as an analgesic, antispasmodic and bactericidal agents (Oyeleke *et al.*2008). Saponins derived from fruits and vegetables are important dietary supplements with antimicrobial properties that protect plants from microbial pathogens (Sczkowski *et al.* 1988). They may be useful in modulating blood lipids, lowering cancer risks, improving blood glucose response, and possessing antioxidant activity (Igidi and Edene, 2014). Terpenoids found in *Icacina trichantha* seed support its use in the treatment and management of cancer, ulcers, and malaria. They also have medicinal properties like anticarcinogenic, antimalarial, anti-ulcer, antimicrobial, and diuretic activity (Dudareva *et al*. 2004). As a result of the presence of these terpenes, the seed of *Icacina trichantha* could be used in ethnomedicine to treat a variety of ailments. Plant extracts containing glycosides have the potential to be used as flavoring agents in pharmaceutical formulations (Dudareva *et al.* 2004). Therefore, the presence of glycoside in the seed of *Icacina trichantha* supports its pharmacological use as a flavouring agent and in the management of cancer. This report contradicts reports by Oyeleke *et al*. (2008) which reported that his studied plant material contain no glycosides.

The GCMS analysis of the studied herbal plant revealed appreciable number of phytocompounds. These compounds were known for their beneficial health effects such as unsaturated fatty acids which can prevent cardiovascular diseases (Baskaran et al. 2014). Phytocompounds are the major important compounds which are responsible for the medicinal properties of any herbal plants. Hence, medicinal plants could be a potential source for nutraceutical (Baskaran et al. 2014). The GCMS analysis of the methanol extract of *Icacina trichantha* seed resulted many compounds which have diverse uses. The comparison of the compounds' mass spectra with the data base gave more than 100% match as well as confirmatory compound structure match. Compounds having antioxidants, anti-inflammatory, antimicrobial, skin conditioning properties have been identified. The extract also contained a variety of fatty acids. The compounds hexadecanoic acid, 9, 12-octadecadienoic acid, etc. are having antioxidant, anti-inflammatory, acne reductive and cancer preventive properties (Baskaran et al. 2014). Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant. The biological importance and toxicity of erucic acid, the major compound identified in the studied plant (Table 2), which is a monounsaturated fatty acid have remained controversial according to literature. Studies carried out on laboratory animals in the early 1970s showed that erucic acid appears to have toxic effects on the heart at high enough doses, although an association between the consumption of rapeseed oil and increased myocardial lipidosis or heart disease has not been established for humans (FSANZ, 2003). Meanwhile, phytol (3, 7, 11, 15-tetramethylhexadec-2-en-1-ol) is a diterpene, a member of the group of branched-chain unsaturated alcohols. It is the product of chlorophyll metabolism in plants; hence, phytol is abundantly available in nature. Phytol is known to inhibit the growth of *Staphylococcus aureus* and to block the teratogenic effects of

retinol (Inoue *et al*. 2005). In the light of the various reports on the toxicity of erucic acid, the therapeutic effect of the plant should be weighed alongside its toxicity when administered in folkloric medicine. The presence of these compounds is responsible for the antioxidant and antimicrobial efficacy of this plant.

` The physicochemical analysis of plant drugs is important for detecting adulteration or improper handling of drugs (Kaskoos, 2014); as well as an important index to classify the nutritional value of a food material. Our investigation revealed that the dominant components are carbohydrate, moisture and ash; however, fibre content was relatively lower. A sample with high level of carbohydrates can regulate nerve tissue. Ash contents give an idea about the inorganic content. They are also expected to facilitate the metabolic processes, growth and development while moisture contents display more information about the storage/shelve life and the viability of microorganism's growth. Proteins, on the other hand, can serve as enzymatic catalyst, growth control and cell differentiation. Crude fibre content of this plant could aid in the absorption of trace elements in the gut and therefore increases intestinal bowel movement, lower cholesterol, triglycerides, and protect against cancer and digestive disorders (Abolaji *et al.* 2007). The moderate amount of ash content in the studied herbal plant provides a measure of total amount of mineral matter in a plant. Measuring ash content is important because mineral matter may be the cause of a pharmacological effect. Based on the concentration of the proximate components of the studied plant material, it can be inferred and justify as a good source of nutrient. As reported in the results of the studied plant material (Table 4), the presence of vitamins in appreciable amount of concentration in any natural compound of plant origin have very essential roles to play in the human health. Vitamin C and E are very important antioxidants which protect the cell membranes from oxidative stress/damage caused by free radicals (Guyton and Hall, 2006). *Icacina trichantha seed* contain ascorbic acid and flavonoids, both of which are effective antioxidants. Vitamin C possesses an antioxidant property and required for maintenance of normal connective tissues, wound healing and also facilitates the absorption of dietary iron from the intestine (Guyton and Hall, 2006). This justifies the antioxidant activity of the leaves as reported by Oyeleke *et al.* (2008). The seed of *Icacina trichantha* contains moderate amount of vitamin A, and therefore essential for clear vision.

The *Icacina trichantha* (*I. trichantha*) toxicity test is a biological assay considered as one of the most widely used tools for preliminary toxicity assessment of plant extract. The acute toxicity study showed that the crude methanol extract of *I. trichantha* seed has a wide safety margin since no death was recorded in the study animals used at a maximum dose of 200 mg/kg. So, the extract is considered practically safe and can be used for long term oral administration. This may be attributed to the incomplete absorption brought about by inherent factors limiting absorption in the gastro intestinal tract (Dennis, 1984). Toxicity results from animals are highly essential in judging the safety of crude drugs if they are found to have promising pharmacological activities. From the foregoing, the crude extract from *I. trichantha seed* could be assigned class 5 status (LD_{50}> 200 mg/kg) which is the lowest toxicity class. In general, simple interpretation of this result is that the plant material extract is practically non-toxic. The therapeutic value of most herbs is indisputable but their toxicity sometimes limits their clinical applications. Thus, the toxicity profile of the herb must always be taken into consideration especially as the dosing and dosing regimens of their preparations are not usually determined (Chan, 1997). The very high LD_{50} observed is not a conclusive finding about the safety of the extract of *I. trichantha* as higher doses could be tested for better understanding of its effects if used for a longer period of time and for proper recommendation on its future utilization.

Blood parameters act as pathological indicators of status to deleterious substances and underlining disorders (Olafedehan *et al.* 2010). From the foregoing, findings reported that extract treated group of rats has elevated leukocyte counts, and are in accordance with the results observed by *Abdulrahman et al*. (2010) who observed increased WBC production in hyperlipidemic rats which may lead to possible stimulation of the immune defense system. It was also observed that the methanol extract of the *materia medica*used in this study could stimulate and thus increase the level of

erythrocyte counts, and this agrees with the results observed by *Effraim et al*. (1997) who stated that the aqueous extract of *Corynanthe yohimbe* significantly increased the heamoglobin (Hb), erythrocyte count and packed cell volume, with the increasing doses of the extract, and improvement in these parameters is likely to improve upon the well-being of the patients thereby justifying its empirical use in conditions like loss of libido. Equally importantly, the present study described that there is a decrease in thrombocytes indices in extract treated groups of rats. The primary physiological function of platelets is to mediate the haemostatic response. Sequel to the sub-acute toxicity study undertaken in this study, it is evidently possible from the results (Table 6) that administration of *Icacina trichantha* methanol seed extract at all dose levels used in this study, justifying human dose in animal experimental model, may not contribute to the risk for cardiovascular diseases. Further studies are however required using high doses on the possibility of toxicity in conditions of more chronic exposure or use of the extract. It is also important to investigate the toxicity of the extract on other biological factors, such as biochemical and histopathological paramaters in animal models.

CONCLUSION

The present study shows the presence of biochemical compounds in *Icacina trichantha* seed which may therefore justify both its nutritional and ethnomedicinal benefits to human health. Crude methanol extract of *I. trichantha seed* had good safety profile as it is tolerable at a single dose levels used in this study. Furthermore, 14 days accumulated daily doses of the extract have no adverse effect on the haematological indices in animal model; evidenced by zero mortality and non-negative alterations in heamatological parameters studied in experimental animals. The results presented a broad spectrum of reliable andnon-toxic biomolecules in *I. trichantha seed* which could be useful in the therapeutic intervention on series of human ailments.

COMPETING INTERESTS

Authors declared none competing interest.

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