



PHYSICO-CHEMICAL AND TOXICOLOGICAL STUDIES ON *XYLOPIA AETHROPICA* LEAVES

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ABSTRACT

The leaves of *Xylopiya aethiopica* widely used in ethno-medicine were studied. The hexane extract (HE), methanol extract (ME), methanol-water extract (MWE) and distilled water extract (DWE) of *X. aethiopica* were subjected to phytochemical and toxicity screening. Results showed that flavonoids and tannins were present only in the HE and ME. However; the ME, MWE and DWE were containing alkaloids, saponins, terpenoids, carbohydrates and resins while neither proteins nor oils were not detected in any of the solvent extracts. Furthermore, although all the extracts had results, which were higher than the control in varying amounts, that of HE and ME showed quite potent toxic activity against the tested organs at 400 mg/Kg body weight and 0.81 mL of extracts. Altogether, the study suggests that the solvent extracts of *X. aethiopica* may serve as source for compounds with therapeutic potentials and dietary energy. The study therefore, prompts further investigations, including anti-microbial screening to harness the possible potential use of *X. aethiopica* from Owerri-North-Nigeria either as drug, food or pharmafood.

Key words: *X. aethropica*, Physico-chemical, Toxicology, Health implications.

INTRODUCTION

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs¹⁻³. According to a report by World Health Organisation⁴, traditional medicine using plant extracts will continue to provide health coverage for over 80% of the world's population, particularly the developing countries. In Nigeria, traditional medicine accounts for more than 80% of rural populace health needs, with the practitioners formulating and dispensing the recipes and doses⁵. Traditional medical practitioners mostly administer these herbal medicines in most disease conditions over a long period of time without a proper dosage monitoring and consideration of toxic effects that might result from such a prolonged use. The warning

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regarding the potential toxicity of these therapies means that the practitioners should be abreast of the reported incidence of renal and hepatic toxicity associated with the ingestion of medicinal herbs⁶.

X. aethiopica is a well-known plant of great repute in West Africa, which produces a variety of complex chemical compounds most of which have been attested to be medicinal. It is used in traditional medicine against various ailments in Africa and other parts of the world. In Western part of Nigeria, it is used to prepare herbal remedy called 'agbo' for the treatment of stomach ache, bronchitis, biliousness and dysentery⁶. A decoction of the fruits of *X. aethiopica* is used to bathe children as an anticonvulsant⁷. External uses of the plant include a poultice for headache and neuralgia, and with lemon grass as douching solution. Even the odiferous roots of the plant are employed in tinctures, administered orally to expel worms and other parasitic animals from the intestines, or in teeth rinsing and mouth washing extracts against toothache⁸. However, most of the previous studies on *X. aethiopica* were not on its leaves and its toxicity. The present study therefore, aims at determining the various phytochemical components of distilled water extract (DWE), methanol-water extract (MWE), methanol extract (ME) and n-hexane extract (HE) of *X. aethiopica* and to evaluate their safety, that is their toxicity.

EXPERIMENTAL

Materials and methods

The solvents used for the experiment were Sigma-Aldrich[®] grade. Other chemicals and reagents used were of certified grade and quality and were used without further purification.

Collection and preparation of plant material

Fresh leaves of *X. aethiopica* were collected from Emii in Owerri-North Local Government Area in Imo state, Nigeria. The specimen was then identified at Bioresources Conservation and Development Programme Nsukka. Thereafter, the leaves were rinsed with water, sun-dried for 10 days and ground into powder using an electric grinder.

Extraction of *X. aethiopica* leaves

The leaves of *X. aethiopica* was extracted using distilled water, methanol-water mixture, methanol and n-hexane solvents.

To four separate 10 g of the powdered leaves, 500 mL of distilled water, methanol, a mixture of methanol and water in 50:50 ratio and n-hexane were added to each of the 10 g of

the powdered leaves and allowed to stand at room temperature for three days. The distilled water extract was filtered using Whatman filter paper and concentrated using water-bath. The extracts of the rest solvents were obtained using Soxhlet extractor for 2 hr and concentrated using rotary evaporator. Each of the extracts of the four solvents was preserved at 5°C in an air tight bottle until used for phytochemical and toxicity studies.

Phytochemical screening

The phytochemical analysis of the DWE, MWE, ME and HE of *X. aethiopica* was by standard methods as described by Evans⁹ and Harbone¹⁰. Specifically, the four extracts were screened for alkaloids, flavonoids, glycosides, saponins, steroids, tannins, terpenoids, carbohydrates, reducing sugar, resins and oils.

Toxicity screening

Fifty healthy adult male wistar albino rats weighing between 192-202 g were obtained from the Department of Animal Health and Sciences, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The fifty rats were equally grouped into five: A, B, C, D and control. After the rats had been housed in standard cages for seven days to acclimatize to laboratory conditions, daily administration of rodent feed and water continued with subsequent administration of the extracts (DWE, MWE, ME and HE), which had already been dissolved in distilled water. The extract solutions were concentrated with distilled water to about 400 mg/Kg body weight of the rats and group A, B, C and D received 0.81 mL of the extract solutions. After administration of the extracts for seven days, the rats were sacrificed and relevant organs collected for albumin, bilirubin and creatinine test using standard methods.

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical results (Table 1) indicated the presence of alkaloids, saponins, terpenoids, carbohydrates, reducing sugar and resins for the DWE and flavonoids, glycosides, steroids, and reducing sugars for the HE as shown below.

Generally, the different solvent extracts of the *X. aethiopica* leaves indicated low to high presence of alkaloids, saponins, steroids, terpenoids, carbohydrates and resins. The results indicated that flavonoids and tannins were present only in the HE and ME. However, ME, MWE and DWE were containing alkaloids, saponins, terpenoids, carbohydrates and resins while neither proteins nor oils were detected in any of the solvent extracts.

Table 1: Phytochemical analysis results of the leaves of *Xylopi aethiopica* of DWE and HE

Secondary metabolites	HE	ME	MWE	DWE
Alkaloids	–	++	++	++
Flavonoids	++	++	–	–
Glycosides	+	–	++	++
Saponins	–	+++	+++	++
Steroids	+++	+++	+	–
Terpenoids	–	+++	++	++
Carbohydrates	–	+++	+++	++
Reducing sugars	+	–	++	++
Resins	–	++	+++	++
Tannins	+	++	–	–
Proteins	–	–	–	–
Oils	–	–	–	–

– : Not present, + : Present but low in abundance, ++ : Present and moderate in abundance, +++ : Present and high in abundance

Phytochemical/macronutrients screening and toxicity of ME and MWE of *X. aethiopica* leaves were studied in this work. Generally, secondary metabolites in plants have been reported to confer plants with therapeutic activities¹¹. In particular, alkaloids were reported as the most efficient, therapeutically significant plant substance¹². Hence, the varying amount of these secondary metabolites in tested extracts of *X. aethiopica* might explain the basis for its reported efficiency and use in ethnomedicinal therapy. However, the high abundance of saponins, which has been reported as an antioxidant phytochemical¹³ and to possess antifungi activity¹⁴ in all the extracts except in HE might be because of the closeness in the polarity of the three solvents and that goes to highlight the possibility that the therapeutic benefit(s) of *X. aethiopica* leaves might depend on its solvent medium and mode of extraction.

Tannins and flavonoids have biological activities that are of benefit in the prevention and management of many ailments¹⁵. Earlier, Erah et al.¹⁶ have associated antimicrobial activities with the presence of tannins and flavonoids. Tannins inhibit the growth of microorganisms by precipitating out the microbial protein and thus depriving them of nutritional

proteins needed for their growth and development¹³. Flavonoids as well inhibit platelets aggregation¹⁷ and could exert a membrane stabilizing action that may protect the liver cell from injury. These were linked to the possible efficient detoxification and antioxidant activities¹⁸ resulting, probably, from an enhanced induction of phase I and II enzymes by flavonoid¹⁹. Therefore, the presence of these two secondary metabolites in the ME and HE suggests that it is the metabolites are more extracted by the non-polar solvents. This likely explains the apparent wide spectrum antimicrobial activity of *X. aethiopica* leaves extracts as observed by Pieme et al.²⁰ and its hepatoprotective potential and therefore may be of benefit in any formulation intended for the management or prevention of liver disease.

The high abundance of steroids in ME goes further to validate our suggestion that this particular solvent extract has more therapeutic potential and use over its other extracts counterpart like the MWE, which has low in abundance of steroids. This is based on the fact that steroids increase nitrogen level in the body, thereby producing proteins that help in the production of muscles. Steroids could enhance metabolism and thus inhibit the accumulation of fat, correct such disorders like anemia to increase the production of red blood cells in the body and contribute to the treatment of arthritis, asthma, brain injury and some types of cancer. However, steroids could enhance the outset and progression of cardiovascular and liver diseases as well as acne (by stimulating the sebum to produce oil). Therefore, *X. aethiopica* might be useful in management of cardiovascular and liver ailment. Hexane would have been concluded as completely inappropriate solvent for the *X. aethiopica* leaves extraction considering the scanty amount of phytochemicals it extracted were it not for the presence of these two very important metabolites-steroids and flavonoids, which distilled water and methanol-water system could not. The presence of carbohydrates, glycosides and reducing sugars in the plant leaves extracts at varying abundances seems to indicate the high energy content of the leaves of *X. aethiopica*. The MWE especially will favour those interested in exploiting the plant leaves as source for edible food or raw materials²¹. However, it might not be of benefit for regeneration, growth and overall biosynthesis owing to noted absence of protein and oil.

Toxicological study

Although all the solvent extracts (DWE, MWE, ME and HE) had results, which were higher than the control in varying amounts that of HE and ME showed quite potent toxic activity against the tested organs at 400 mg/Kg body weight and 0.81 mL of extract (Tables 2 and 3).

Table 2: Effect of the extracts on the animal organs

Organs	Control	A	B	C	D
Heart	1.20 ± 0.01	1.10 ± 0.12	1.28 ± 0.03	1.16 ± 0.01	1.20 ± 0.50
Liver	0.58 ± 0.02	0.61 ± 0.02	0.64 ± 0.06	0.52 ± 0.01	0.50 ± 0.04
Kidney	1.13 ± 0.05	1.27 ± 0.08	1.17 ± 0.04	1.17 ± 0.15	1.00 ± 0.02
Brain	3.16 ± 0.04	3.39 ± 0.14	3.24 ± 0.11	3.03 ± 0.07	2.48 ± 0.10
Lung	1.66 ± 0.02	1.59 ± 0.13	1.58 ± 0.02	1.72 ± 0.06	1.20 ± 0.02

Mean ± SEM (n = 3), A – DWE, B – MWE, C – ME and D – HE = 400 mg/Kg
Body weight = 0.81 mL of extracts

Table 3: Summary of the biochemical test on the animals (albino rats)

Parameters	Control	A	B	C	D
Bilirubin (mg/hl)	2.50	3.15	1.04	9.12	17.00
Albumin (%)	1.47	1.51	1.18	1.58	1.99
Creatinine (mg/dl)	0.23	0.20	0.08	0.29	0.343

The toxicity test results of the various extracts (Table 3) revealed that there was significant increase in the plasma creatinine levels of ME and HE relative to the control. The elevation in the plasma creatinine concentration indirectly suggests kidney damage specifically renal filtration mechanism²².

Hence, the increase in creatinine concentration in rats treated with ME and HE had a serious kidney damage compare to the rat, which received MWE and DWE treatment. Increase in bilirubin concentration in the serum or tissue is indicative of observation in the excretion of bile²³.

The increase in the level of bilirubin concentration observed in rats treated with ME and HE could be as a result of liver damage or a blocked bile duct as compared with rats treated with MWE and control. Although there was increase in bilirubin concentration in rats treated with DWE, it was very mild. Also, the high level of bilirubin in the blood stream can cause permanent damage to certain areas of the brain. According to the test results in Table 3, the rats treated with ME and HE had severe liver damage. This could account for the high level albumin tested by the animals in this test set.

CONCLUSION

Taken together, the study revealed the scientific basis for the possible use of *X. aethiopica* leaves in ethno-medication. The phytochemical/macronutrient components and the toxicological results of the present study suggest that the leaves of *X. aethiopica* could serve as a good source either for herbal medicine, energy giving foods or raw materials for pharmaceutical, food and biodiesel industries. Also the study revealed that the therapeutic potency and toxicity of *X. aethiopica* may be dependent on the extraction solvent used and therefore, n-hexane, though was able to extract some interesting phytochemicals, which distilled water could not, may not be a very good solvent for the extraction of *X. aethiopica* leaves because of the toxic effects observed in the administration of its extract. We recommend that distilled water alone should be used in the extraction of *Xylopia sp.* leaves.

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