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PHYTOCHEMICAL SCREENING, HEAVY METALS EVALUATION AND ACUTE TOXICITY STUDIES OF *Azadirachta indica and Mangifera indica* METHANOL LEAVES EXTRACT

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ABSTRACT
The present study was aimed to determine the phytochemical constituents, median lethal dose (LD_{50}) and levels of heavy metals in Azadirachta indica and Mangifera indica leaves obtained from Kano State, Nigeria. The preliminary phytochemical screening of Azadirachta indica and Mangifera indica leaves revealed the presence of Alkaloids, Flavonoids, Carbohydrate, Tannins, Saponins, Anthraquinones and Steroids while Cardiac Glycosides were absent in the methanol extracts. The results revealed that the concentrations of heavy metals in the leaves of Azadirachta indica were found to be 0.06± 0.01mg/kg, 0.05± 0.01 mg/kg and 0.07± 0.00mg/kg for Zn, As and Mn respectively and non-detectable concentrations of Cd, Cu, Ni, Co and Pb, and 0.02± 0.01mg/kg, 0.05±0.02mg/kg, 0.02±0.00mg/kg and 0.15±0.00mg/kg for Zn, As, Co and Mn respectively and non-detectable concentrations Cd, Cu, Ni and Pb in Mangifera indica. The LD_{50} of the Azadirachta indica and Mangifera indica methanol leaves extract were was between 500-5000 mg/kg body weight using Lorke's method, which showed that the extracts were slightly toxic. The levels of heavy metals detected were within the World Health Organization (WHO) permissible limits for metals in medicinal plant and herbal products. The findings of this study indicate the toxicity of the extracts as slightly toxic at LD_{50} which might suggest their use with caution and the need for further studies to assess their short and long-term cumulative risk with a view to improve their safety.
Keywords: Azadirachta indica, Mangifera indica, phytochemicals, median lethal dose, heavy metals.

INTRODUCTION

In the last decade, there has been a revival of herbal medicine, with resurgence in popularity even in Western countries. Medicinal plants are plants that possess therapeutic properties or exert pharmacological effect on humans. It has been established that plants which naturally synthesize and accumulate some secondary metabolites e.g. alkaloids, glycosides, tannins, volatile oils, certain minerals and vitamins possess some medicinal properties (Badyal *et al.,* 2020; Yusuf *et al*., 2021). Medicinal plants, often referred to as traditional medicines need to be evaluated so as to improve their efficacies, safety, availability and wider applications at low cost (Abubakar *et al*., 2017).

Azadirachta indica and *Mangifera indica* are trees of economical and medicinal importance popularly known as Neem and Mango respectively. They produce chemicals by primary or secondary metabolism called phytochemicals that have biological activity in the plant. Traditionally, all parts of neem tree including leaves, seeds, roots, bark and the flowers of the plant are used to cure different ailments, such as stomach ulcers, jaundice and to overcome a variety of infectious and parasitic diseases, ranging from leprosy, chicken pox, and malaria ((ref). Neem plants contain several thousands of chemical constituents such as Saponins, Flavanoids, Tannins, Alkaloids, Glycosides, Terpenes having insecticidal property (ref). Aluminum (Al), Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Mercury (Hg), Manganese (Mn), Nickel (Ni), Lead (Pb), and Zinc (Zn) are heavy metals found in leaves of the neem tree. And some of them are environmental pollutants and toxic to human health (Onder, 2006).

Different part of mango tree has been reported to exhibit anticancer, anti-inflammatory, Lipid profile, antibacterial, antioxidant, anti-parasitic, anti-tumor, anti-allergic, antihyperglycemic, antimicrobial, anti-malarial and anti-tumour activities (Ashifat, 2012). Phytochemicals present in *Mangifera indica* can be broadly categorized as polyphenols, terpenoids, carbohydrates, sterols, carotenoids, vitamins, fatty acids, and amino acids. Among them, total phenolic compounds (TPC), including phenolic acids, benzophenones, tannins, terpenoids, and flavonoids, are most abundant in *Mangifera indica* (Swaroop, 2018).

Heavy metals are hazardous non-biologically degradable metals or metalloids that cause an environmental problem or pollution, examples of such elements include lead, chromium, arsenic, mercury, copper, nickel, cadmium, cobalt etc. (Cheng, 2003; Lasat, 2002). Because of the widespread presence of heavy metals in the environment, their residues also reach and are assimilated into medicinal plants (Hawkes, 1997). The WHO recommended that herbal products and medicinal plants should be thoroughly screened for the presence of heavy metals and their toxicity indexes. Therefore, this study was aimed to determine the phytochemical constituents, LD_{50} and levels of heavy metals such as cadmium (Cd), copper (Cu), nickel (Ni), arsenic (As), cobalt (Co), lead (Pb), manganese (Mn) and chromium (Cr) in the methanol leaves extract of *Azadirachta indica* and *Mangifera indica*.

MATERIALS AND METHODS

Animals

Adult Swiss albino mice of both sexes, weighing 25-30 g were obtained from the animal house of the Department of Pharmacology and Therapeutics, Bayero University Kano (BUK), Nigeria and maintained under normal laboratory conditions of humidity, temperature and light for 7 days before the experiment and allowed free access to food and water. This study were approved by the Ethical Committee for animal experimentation in Ethnobotany and Multidisciplinary Research Division, Bioresources Development Centre, Kano.

Study Site, Collection and Identification of Plant Materials

The study was conducted at Center for Dry Land Agriculture (CDA) laboratory and Pharmacognosy laboratory BUK. The plants were collected from BUK New site, Kano state, Nigeria, and was identified in the field and then taken to the Herbarium of Ethnobotany and Multidisplinary Research Division of Bioresources Development Centre, Kano for authentication, a reference voucher number: BDCKN/EB/1618 and BDCKN/EB/1625 was deposited at the Herbarium.

Extraction

The powdered plants material 20g each was macerated with 250 ml of methanol for 48 hours and the mixture was shaken occasionally. The filtrate obtained was evaporated to dryness at 40°C using a rotary evaporator.

Preliminary Phytochemical Screening of Methanol Extract of *Mangifera indica* and *Azadirachta indica* Leaves

The preliminary phytochemical screening was conducted using the standard laboratory procedures as described by Sofowora (2008) and Trease and Evans (2002).

Heavy Metal Evaluation of Plant Extracts

Approximately 0.20 g of powdered *Azadirachta indica* and *Mangifera indica* were weighted and vplaced into a medium pressure digestion rotor vessel, followed by 8 ml of HNO_3 and 2 ml of H_20_2, and then the mixture was digested with an advanced microwave digestion system (ref). The solution was cooled at room temperature and diluted to 10 ml with distilled water. The sample was then analyzed for the presence of Zn, Cd, Cu, Ni, As, Co, Pb and Mn using Micro Plasma Atomic Emission Spectrophotometer (Agilent 42100-MP-AES) available at CDA, BUK.

Determination of Median Lethal Dose (LD_{50})

Lorke`s Method

This was conducted in two phases using the method described by Lorke in 1983. In the first phase, mice were divided into three groups of three each. The first group received the extract (intraperitoneal) at a dose of 10 mg/kg body weight, followed by 100 and 1000 mg/kg to the second and third group respectively. The mice were then observed for 24 h for signs and symptoms of toxicity and death. In the second phase, mice were divided into three groups of one mouse each, the extract was then administered to group 1, 2 and 3 at the doses of 1600, 2900 and 5000 mg/kg body weight respectively. The mice were also observed for 24 h. After 24hours the number of death is recorded and the LD_{50} is calculated as the geometric mean of the highest non-lethal dose and least toxic dose.

$LD_{50} = \sqrt{a \times b}$ where a=highest non-lethal dose, b=least toxic dose.

Statistical Analysis

All statistical analyses of data were carried out using SPSS statistical package programs version 17. One-way analysis of variance (ANOVA) was calculated, descriptive statistics such as mean ± standard deviation (SD) were used in the

analysis of the data. *P<0.05* was established as the limit of a statistical significance.

RESULTS

Preliminary Phytochemical Screening

Table 2 shows the results of phytochemical screening of *Azadirachta indica* and *Mangifera indica* methanol leaves extract. The results revealed the presence of Alkaloids, Flavonoids, Carbohydrate, Tannins, Saponins, Anthraquinones, Steroids and the absence of Cardiac Glycosides.

Heavy Metals Analysis in the Leaves of *Azadirachta indica* and *Mangifera indica*

The results of heavy metals analysis in the leaves extract of *Azadirachta indica* and *Mangifera indica* were recorded and shows the concentrations of analyzed metals (mg/kg) as; Zn (0.06±0.01), As (0.05± 0.01) and Mn (0.07± 0.00) in *Azadirachta indica* and Zn (0.02±0.01), As (0.05±0.02), Co (0.02±0.00) and Mn (0.15±0.00) in *Mangifera indica*. The concentrations of Cd, Cu, Ni, and Pb were undetected in the leaves of the two plants. The concentrations Co in the leaves of *Mangifera indica* was measured as 0.02±0.00 mg/kg and undetected in the leaves of *Azadirachta indica*.

Median Lethal Dose LD_{50} of the Leaves of *Azadirachta indica* and *Mangifera indica*

The acute toxicity study of methanol extract of *Azadirachta indica* was presented in table 4. The result revealed that methanol extract of *Azadirachta indica* recorded no mortality at first phase but mortality was observed at second phase at 5000 mg/kg body weight. The LD_{50} was estimated to be above 3808 mg/kg body weight thus the extract could be regarded as slightly toxic (500-5,000mg/kg) under the classification table of LD_{50} (Hodge, 2005). Table 5 shows the result of acute toxicity study of methanol extract of *Mangifera Indica*. The result obtained from the methanol extract of *Mangifera indica* revealed no mortality at first phase but mortality was observed at second phase at 5000 mg/kg body weight thus, the LD_{50} was estimated to be above 3808 mg/kg body weight thus the extract could be regarded as slightly toxic (500-5,000mg/kg) under the classification table of LD_{50} (Hodge, 2005).

Table 1: Result of phytochemical Screening of Methanol Leaves Extract of *Azadirachta indica* and *Mangifera indica*

		Inference	
S/N	Phytochemical constituents	*A. indica*	*M. indica*
1	Saponins	+	+
2	Carbohydrates	+	+
3	Flavonoids	+	+
4	Tannins	+	+
5	Alkaloids	+	+
6	Cardiac Glycosides	-	-
7	Anthraquinones	+	+
8	Steroids	+	+

Keys: (+) present and (-) absent

Table 2: Result of Heavy Metal Concentration (mg/kg) in the Leaves of *Azadirachta indica* and *Mangifera indica*.

S/N	Metal	*Azadirachta indica*	*Mangifera indica*	WHO permissible limits for medicinal plants (mg/kg)
1	Zn	0.06± 0.01	0.02± 0.01	50 (WHO, 2002, 2006)
2	Cd	ND	ND	0.3 (WHO, 2006)
3	Cu	ND	ND	10 (WHO, 2002)
4	Ni	ND	ND	1.5 (WHO, 2002)
5	As	0.05± 0.01	0.05±0.02	10 (WHO, 2007)
6	Co	ND	0.02±0.00	-
7	Pb	ND	ND	10 (WHO, 2006)
8	Mn	0.07± 0.00	0.15±0.00	200 (WHO, 1998)

All values were mean ± standard deviation of triplicate determinations (*P< 0.05*)

Key: ND = Not detected.

Table 3: Acute Toxicity Study of Methanol Extract of *Azadirachta indica*

First Phase		Second Phase		Classification based on dose range
Dose (mg/kg)	Mortality	Dose (mg/kg)	Mortality	
10	0/3	1600	0/1	
100	0/3	2900	0/1	
1000	0/3	5000	1/1	Slightly toxic

Table 4: Acute Toxicity Study of Methanol Extract of *Mangifera indica*

First Phase		Second Phase		Classification based on dose range
Dose (mg/kg)	Mortality	Dose (mg/kg)	Mortality	
10	0/3	1600	0/1	
100	0/3	2900	0/1	
1000	0/3	5000	1/1	Slightly toxic

DISCUSSION

The presence of alkaloids in *Mangifera indica* and *Azadirachta indica* supported the use of these plants parts i.e. leaves in the treatment of ulcer, malaria and fever in Nigerian folk medicine(Srimal, 1997). However, this work is in agreement with the work of (Katsayal, 2008), who reported that the leaves of Neem tree contained positive Alkaloids, Flavonoids, Carbohydrate, Tannins, Saponins, Anthraquinones, Steroids and negative Cardiac Glycosides, but is in contrary with the work of (African networks on ethno medicines, 2004*)* who reported the presence of Alkaloids, Flavonoids, Carbohydrate, Tannins, Anthraquinones, Saponins, Cardiac Glycosides and absence of Steroids in the leaves of Mango. This could be due to the differences in soil, season during which the plant was collected and the solvent used, which are the main factors that influenced the presence or absence of phytochemical constituents (Eldridge, 1983).

The heavy metals evaluation of *Azadirachta indica* and *Mangifera indica* leaves has shown that Zn, As and Mn were present in both the plants while toxic elements like Cd, Cu, Ni, Co and Pb were not detected in the leaves of *Azadirachta indica* and Ni was also not detected in *Mangifera indica* leaves. However, the presence of Ni and Pb in the leaves of *Azadirachta indica* collected from Funtua town in Kaduna State, Nigeria was reported by (Lawal *et al*., 2011). This could be attributed to the differences in the concentration and chemical constituents (Guala, 2010).The levels of Arsenic detected in the leaves of *Azadirachta indica* and *Mangifera indica* did not exceed WHO permissible limits for metals in medicinal and herbal drugs (ref). Results of acute toxicity test of *Azadirachta indica and Mangifera indica* extract in mice were shown in Table 3 and 4.

The effects of *Azadirachta indica* and *Mangifera indica* methanol extract in mice after intraperitoneal administration was estimated to be above 3,808 mg/kg body weight thus the extract could be regarded as slightly toxic (500-5,000mg/kg) under the classification table of LD_{50} (Hodge, 2005). The findings in this work were in agreement with the work of (Bnouham *et al.,* 2006) who reported that the aqueous leaves extract of *Azadirachta indica* revealed an LD_{50} below 5,000 mg/kg body weight thus the extract could also be regarded as slightly toxic.

Also the findings of this work were in contrary to that of (Nsaka *et al*., 2012) who reported that the aqueous extract of the leaves of *Mangifera indica* revealed an LD_{50} less than 5,000 mg/kg body weight. The difference in LD_{50} value may be due to polarity of the solvents used, method of extraction and phytochemical constituents present.

CONCLUSION

The present study shows the presence of carbohydrates, tannins, saponins, flavonoids, anthraquinones, alkaloids, and steroids in *Azadirachta indica* and *Mangifera indica* leaves indicate some medicinal properties. The levels of Zn, Ar, and Mn detected in the leaves of *Azadirachta indica* did not exceed the permissible limits approved by the WHO while the levels of Zn, Ar, Cu and Mn detected in the leaves of *Mangifera indica* were below the maximum tolerable limits recommended by WHO. The LD50 of methanol extract of *Azadirachta indica* and *Mangifera indica* leaves showed that, 3808mg/kg body weight was slightly toxic, suggesting it use with caution and the need for further studies to assess their short and long-term cumulative risk with a view to improve their safety.

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