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Phytochemical Studies of the Extracts of *Mangifera indica* Linn

[1]Unemhilin, E.D., *[2]Onigbinde, M.O., [3]Okogun, J.I. and [1]Osuide, M.O.

[1]Department of Chemistry, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
[2]Department of Chemical Sciences, Samuel Adegboyega University, Ogwa, Edo State, Nigeria.
[3]Pax Herbal Clinic and Research Laboratories, Ewu, Edo State, Nigeria.
*monigbinde@sau.edu.ng

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ABSTRACT

In the recent times herbal medicine have gained high popularity both in developing and developed countries because of their natural origin and reduced side effects. More importantly, the claim that some plants have therapeutic action and are used for myriads of pathological conditions has generated more research interest globally. Mangifera indica known to possess varied medicinal properties in its back, leaves and fruits is a common example. In this study the phytochemical constituents in the ethylacetate and methanol leave extracts of M. indica were analyzed. The phytochemical screening results showed the presence of glycoside, saponin, flavonoids, tannins (condensed tannins), reducing sugar, alkaloids and terpenoids for methanol extract and glycoside, cardiac glycoside, saponins, flavonoids, tannins (hydrolysable tannins), terpenoids, reducing sugar, alkaloid and steroid for ethylacetate soluble fraction. Isolation was done with solvent extraction while purification was achieved with a chromatographic technique (HPLC). The structural elucidation of the pure compound isolated was attempted using mass spectroscopy and the presence of mangiferin in the methanol and ethylacetate soluble fraction was confirmed. However, the methanol fraction was discovered to have higher percentage of mangiferin considering the intensity of the peak in the HPLC-MS spectra.

1. INTRODUCTION

Medicinal plants play prominent roles in the maintenance of human health and several of them have been used as medicines since ancient times. According to the estimation of the World Health Organization, plant extracts are used as folk medicine in traditional therapies among 80% of the world population while more than 30% of the entire plant species have been used for medicinal purposes and are being promoted in

national health care programs (Joy *et al.*, 1998; Refaz *et al.*, 2017). Herbal drugs are widely prescribed, even when their biological ingredients are not known, due to their effectiveness, fewer side effects and low cost (Ajayi *et al.*, 2011; Nabam *et al,*2017).

Considering the local drug formulation and production from African plants, it is discovered that the need has since been expressed for industrial drug production from medicinal and aromatic plants in Africa in order to increase the economic and health potentials as well as the social benefits from our natural resources (Elujoba, 2005; Okigbo et al., 2009). To date, over 30% of the pharmaceutical products manufactured in Egypt are plant-derived. Rwanda and Zimbabwe also produced pharmaceuticals from plants' essential oils (Unemhilin, 2014).

In Nigeria, the "Village Chemist" outfit in the Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife has embarked on production of many standardized and efficacious herbal preparation in the management of different opportunistic infections in people living with HIV/AIDS (Unemhilin, 2014). Example of these medicinal plants in common usage include *Ammivisnaga, Glycyrrhi zaglabra, Aloe vera, Cajanus cajan, Datura metel, Phytolacca dodecandra, Tetrapleura tetraptera, Physostigma venenosum, Momordica charantia and Mangifera indica.*

Mangifera indica (Mango) belong to genus Mangifera which consists of about 30 species of tropical fruiting trees in the flowering plant family Anacardiaceae (Parvez, 2016). The chemical composition of this plant has been studied widely and reported to be rich in secondary metabolites such as triterpenes, phytosterols, flavonoids andpolyphenols (Suslebys *et al.*, 2014). As a result of the presence of these metabolites varied medicinal properties has been attributed to the different parts of the mango tree. Their therapeutic effects like anti-fungal, antibacterial, anthelmintic, anti-parasitic, anticancer, antiHIV, antispasmodic, antipyretic, antidiarrheal, immunomodulation, hypolipidemic, antiboneresorption, hepatoprotective, and gastro protective effect have been studied (Parvez, 2016).

The claim that some plants have therapeutic action and are used for the treatment of myriads of pathological conditions has aroused the curiosity and choice of this commonly used medicinal plant, *M. indica* in the Niger Delta area of Nigeria (Edo State). This study is therefore aimed at analysing the chemical composition of the extracts of *M. indica* and relating the composition to the treatment of ailments like diarrhea, dysentery, hiccup and the management of wounds. Equally, the leaf of *M. indica* is known to contain the physiological active mangiferin in foreign species of the plant. There is the need to establish its presence in the local species available in Nigeria and also isolate and characterize it for further pharmaceutical development.

2. MATERIALS AND METHODS

2.1. Collection and Identification of the Plant

Mangifera indica was collected from Ujemen community in Ekpoma town of Esan West Local Government Area of Edo State, Nigeria in December, 2012 and was identified by Professor B.O. Obadini of the Department of Botany, Ambrose Alli University, Ekpoma. The final authentication was done at the Federal Forestry Research Institute of Nigeria, Ibadan, Oyo State, Nigeria and voucher specimen deposited in Professor J.C. Okafor Herbarium, Pax Herbal, Ewu, Edo State, Nigeria.

2.2. Preparation of Plant Leaves

Foreign matters in the collected *M. indica* were removed and the plant washed twice with large quantity of deionized water, spread on a clean sack and placed under shade for air drying at room temperature. The dried *M. indica* was pulverized using modern laboratory electric milling machine.

2.3. Plant Extract Preparation and Purification

Maceration method was used for the extraction of the coarsely powdered crude plant. *M. indica* powder (100 g) was macerated for three days in hexane and methanol (all of analytical grade). The mixture was constantly agitated and filtered after 72 hours for each solvent. The crude extracts were concentrated by evaporation

and final extracts were obtained, weighed and stored in the refrigerator at 4 °C. The purification of the extracted was done using High Performance Liquid Chromatography (HPLC).

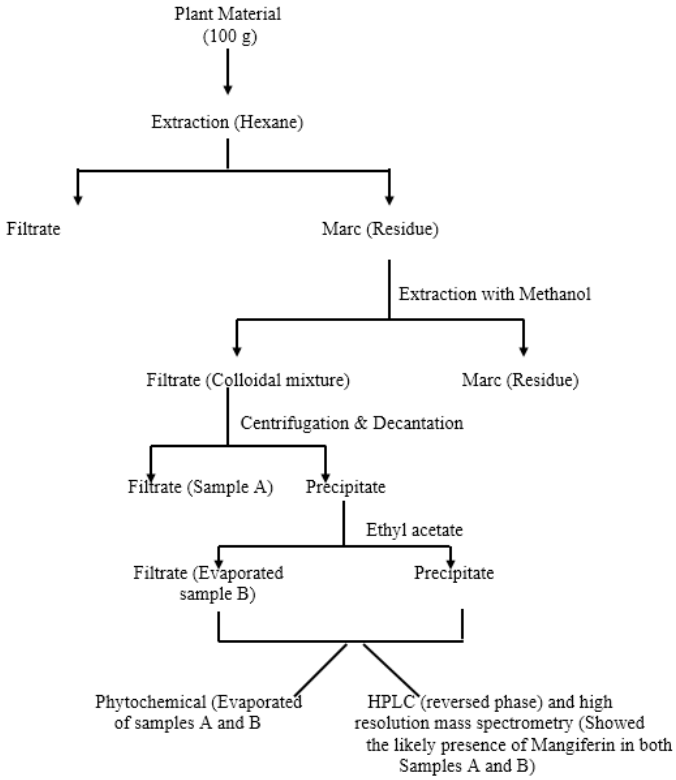

Figure 1: Flow chart for analysis of *Mangifera indica*

2.4. Phytochemical Screening of Crude Extract

Phytochemical screening was carried out using standard method described by Soforowa (1993), Trease and Evans (2002) and Khandelwal (2006).

Test for alkaloids: The sample extract (2 cm^3) was added to 2 cm^3 of 1% HCl and steamed for 15 minutes, then left to cool and finally centrifuged. The filtrate from the centrifugation (1 cm^3) was mixed with 6 drops of Wagner's reagent to check for turbidity in the solution mixture or reddish brown precipitate which confirmed the presence of alkaloids.

Test for tannins: The sample extract (2 cm^3) was mixed with 2 cm^3 $FeCl_3$ solution. The presence of blue-black colour or brownish green colour indicated positive result.

Test for saponins: The sample extract (0.5 cm^3) was mixed with 5 cm^3 of distilled water and the mixture shaken vigorously. Frothing persistence indicated the presence of saponins.

Test for flavonoid: The sample extract (1 cm^3) was mixed with 5 cm^3 of dilute ammonia solution followed by the addition of 1 cm^3 of concentrated H_2SO_4. Yellow colouration which disappeared upon standing indicated the presence of flavonoids.

Test for polysaccharides/starch: 6 drops of iodine solution was added to the sample extract (2 cm^3). The presence of blue-black colouration indicated the presence of polysaccharide/starch.

Test for reducing sugar/glycoside: The sample extract (2 cm^3) was mixed with 5 cm^3 of Fehling's solution followed by steaming. The presence of red colouration indicated the presence of reducing sugar after boiling.

Test for terpenoids: 6 drops of Brady's reagent was added to the sample extract (2 cm^3). The presence of yellowish orange indicated positive result.

Test for phlobatannin: The sample extract (2 cm^3) was mixed with 2 cm^3 of 1% HCl followed by steaming. The presence of red deposit at the base of test tube indicated a positive result.

Test for steroid (Liebermann-Burchard's test): The sample extract (2 cm^3) was mixed with 0.5 cm^3 of acetic acid anhydride followed by cooling in an ice bath. This was followed by the addition of 1 cm^3 of chloroform and 1 cm^3 of concentrated H_2SO_4 carefully added with a pipette until the presence of a reddish-brown ring formed at the separation level between the two liquids which indicated positive result.

Test for cardiac-glycoside (Keller-Kiliani test): The sample extract (2 cm^3) was mixed with (2 cm^3) of glacial acetic acid followed by the addition of 1 cm^3 of $FeCl_3$ and finally 1 cm^3 of concentrated H_2SO_4. The presence of green-blue colour indicated a positive result.

3. RESULTS AND DISCUSSION

3.1. Yield of the Plant Extracts

The yield of the plant extract studied in this research is presented in Table 1. Maceration of 100 g of the powdered aerial part of *M. indica* Linn which was extracted with different solvents yielded 6.25 g corresponding to 6.25% yield (using hexane); 32.78 g corresponding to 32.78% yield (Sample A); 11.38 g corresponding to 11.38% yield (sample B); and 0.37 g corresponding to 0.37% yield (the precipitate). The highest yield was obtained using methanol for the extraction. Methanol has the highest polarity when compared to the other solvents used and this is a likely reason for the higher yield because extraction of biomolecules in plants has been found to be solvent polarity dependent (Altemimi, et al., 2007). This is similar to the report where the yield from *Mangifera indica* leaves was 31.1% (Venkatesh *et al.*, 2010).

Table 1: Yield of the extracts of *M. indica*

Extracts	Yield
Hexane extract	6.25%
Sample A (Methanol extract)	32.78%
Sample A (Ethylacetate extract)	11.38%
Yellow precipitate	0.37%

3.2. Phytochemical Screening

Phytochemical screening results of *M. indica* Linn is presented in Tables 2.

Table 2: Phytochemical screening of *M. indica* Linn. (Sample A and B)

Parameters	Sample A	Sample B
Glycoside	+++	+++
Cardiac glycoside	-	+
Saponin	+	++
Flavonoids	+++	++
Phenolic compounds (tannins)	+++	+++
Hydrolysable tannins (blue-black)	-	+++
Condensed tannins (brownish green)	+++	-
Phlobatanins	-	-
Terpenoids	++	+++
Polysaccharide/starch	-	-
Reducing sugar	+++	+++
Alkaloids	+++	+++
Steroids	-	++

+ = Presence of compound; - = Absence of compound

From Table 2 compounds such as glycosides, saponins, flavonoids, phenolic compounds, reducing sugar, terpenoids and alkaloids were seen to be present in both samples. But, phlobatanins and polysaharide/starch were not found in any of the sample. However, cardiac glycosides, hydrolysable tannins and steroids were found in sample B extracted with ethylacetate while condensed tannins were only present in sample A extracted with methanol. This could be attributed to the differential effect of the solvents used for extraction of the plant extracts and it is based on the differences in the polarity of both the solvents and the biomolecules or solutes of interest (Altemimi, 2017). As reported by Suslebys *et al.* (2014) the isolation of different constituents present in raw material is limited by the type of extraction process used and this include the solvents employed.

3.3. Structural Elucidation of the Isolated Compound

3.3.1. Mangiferin in the extract

The extract of mangiferin was analysed using reversed phased chromatography and accurate mass spectrometry. The extract was separated and mangiferin eluting at retention time of 1.96 minutes was identified by the mass spectrometer. Though the molecular formula of mangiferin is $C_{19}H_{18}O_{11}$, the compound identified as mangiferin in the mass spectrometer is $C_{19}H_{19}O_{11}$. This is because ionization of the compound was accomplished in positive mode; $M+H = MH^+$. Figures 2 to 3 gave the elemental composition and molecular mass of mangiferin in both samples A and B.

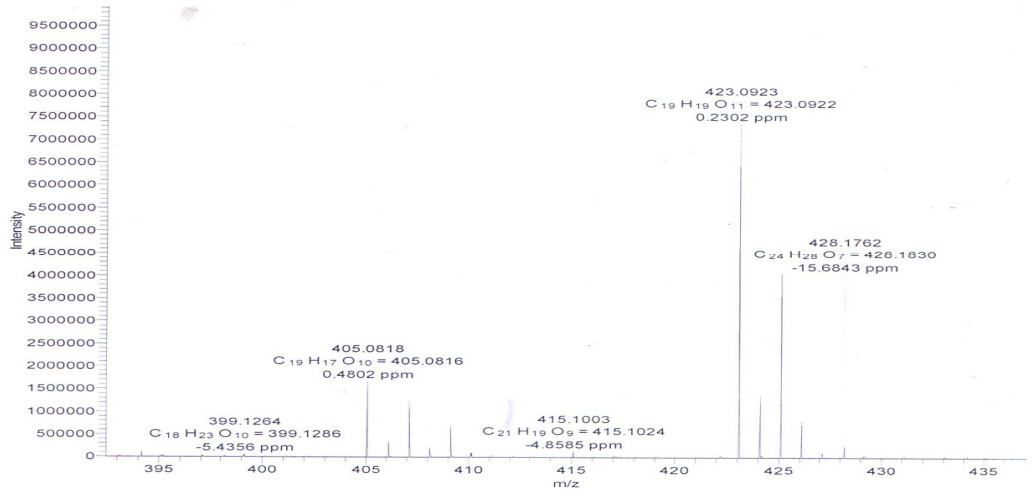

Figure 2: Mass spectrum of sample A showing the accurate mass and elemental composition of Mangiferin

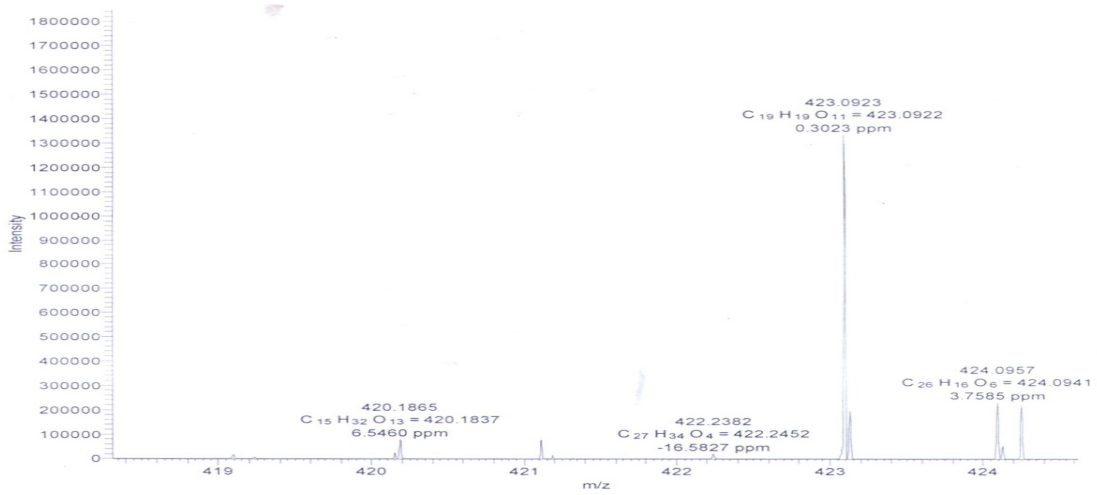

Figure 3: Mass spectrum of sample B showing the accurate mass and elemental composition of Mangiferin

The accuracy of the result was assessed by comparing the experimental value of the molecular mass with theoretical value. The difference expressed in Δm = 0.2302 ppm using Equation 1 is within the range of the value acceptable. Any value obtained within (±) 5 ppm is considered acceptable (Unemhilin, 2014).

$$\Delta m = (\text{theoretical mass} - \text{experimental mass})/\text{nominal mass} \times 1000000 \quad (1)$$

The peaks shown in Figures 2 and 3 shows the mass fragments which confirm the presence of mangiferin (2C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone) a xanthone-C-glycoside with molecular formula $C_{19}H_{19}O_{11}$ and molecular weight 423.09 in the sample analysed. The ions pattern of manifestation in the spectra of both samples contain the positive ion mode of mangiferin alongside with other fragment ions. This is similar to what is obtained in literatures and this has allowed the possibility of the structural determination of compounds using the specific molecular ions and fragment ions along with their chromatographic retention times (Stohs et al., 2018). The results of the mass spectra of this work have provided reliable information for confirming the presence of mangiferin in the extracts of *M. indica* studied.

4. CONCLUSION

This study is part of the on-going research effort to gain insights into the chemical compositions of some local plants found in Edo State, Nigeria. The plant *M. indica* was characterized and the presence or absence of some important chemical compounds such as glycosides, saponins, cardiac glycosides, flavonoids, tannins, phlobatanins, reducing sugar, alkaloids, terpenoids and steroids were established. Also, the presence of mangiferin, the active ingredient for the treatment of diabetes, heart and urinary problems, and also an antioxidant was identified, following extraction with solvents, purification using HPLC and analysis with high resistant accurate mass spectrometry. This study therefore, has supported the claim that some African plants like *M. indica* have therapeutic action and could be used for some of the pathological conditions.

5. ACKNOWLEDGMENT

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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