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Studies on the Crude Extract of Moringa Oleifera Leaf for Preliminary Identification of Some Phytochemicals and Organic Functions

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Abstract: *Moringa oleifera is a tropical plant that grows rapidly with well established leaves and well documented nutritional, economic and medicinal benefits. The preliminary investigation of some phytochemicals as well as identification of organic functions has been achieved from the crude extract of the leaves by n-hexane using standard procedures. Phytochemical screening tested positive for saponins, phenols, glycosides, terpenoids and alkaloids as useful natural products that have been found potent for use as therapeutics. The infrared spectral data obtained revealed the following bonds: C-H, C=O, C-C, C=C, N-O and C=C-H that are diagnostic markers of aliphatic as well as aromatic compounds. However, because of the fingerprint region which has a pattern that is specific for every molecule, the presence of -OH function and N-O stretch, suggest that aromatic or aliphatic phenols or alcohols and nitrogen containing molecules are major components of the Moringa oleifera leaf that was studied.*

Keywords: *extraction, preliminary investigation, natural product, infrared spectral data, Moringa oleifera, n-hexane*

I. Introduction

Medicinal plants contain substances known to modern and ancient civilization for their healing properties. Until the development of chemistry, and particularly of the synthesis of organic compounds in the 19th century, medicinal plants and herbs were the sole source of active principles, capable of curing man's ailments. They continued to be important to people that do not have access to modern medicines, and moreover, modern pharmaceuticals rely heavily on the same active principles, be they natural or synthetic. The active principles differ from plant to plant due to their biodiversity, that is, to the plant genetic coding ability to produce them. (Liu et. al., 2009). Medicinal plants are also known to be involved in the performance of many biological functions and defence against attack from predators, such as fungi, insect, among others.

One of such plants that are of immense benefits to man is Moringa oleifera a small tropical tree that grows to about 25 feet (8 meter). It has tuberous roots and fern-like edible leaf. The bark has a whitish colour and is surrounded by thick cork; young shoots have purplish or greenish– white hairy bark. The tree has an open crown of dropping fragile branches. The flowers are bisexual and surrounded by fine unequal tiny veined yellowish-white petals. The flowers are approximately 1-1.5 cm long and 2 cm broad. Flowering begins within the first six months after planting. In seasonally cool region, flowering will occur once a year between April and June. The fruit is a hanging three-sided brown capsule of 20-45 cm size which holds dark brown globular seeds, with a diameter of approximately 1 cm. Moringa leaves are small, thick, tear-drop shaped which grow rapidly as the plant matures; they are strong and firm, and can easily be picked from the tree's branches (Wikipedia, 2013).

India's ancient traditional medicine survey report says the leaves of the Moringa oleifera prevent about 300 diseases. It has been posited that it is perhaps the most nutritious of all leaf crops (Jed et. al., 2005). Moringa oleifera leaves have 7% protein and have extremely high level of foliates. The leaves of this miracle tree, as its sometimes described, contains 7 times the vitamin C in oranges, 4 times the calcium in milk, 2 times the protein in yoghurt, 4 times the vitamin A in carrot and 3 times the potassium in banana (Jed et. al., 2005). Moringa goes by many names: In the Philippines, where the leaves of Moringa are cooked and fed to babies, it is called 'mothers best friend'; other names for it include the benzolive tree (Haiti), horseradish (Florida), nebeday (Senegal), miracle tree and drumstick tree (Indis). In Nigeria, the Hausa tribe calls it zogale; the Yorubas call it ewelgable, while the Igbos know it as okwe oyibo. It is also native to the sub-Himalaya tracts of India, Pakistan, Bangladesh, Central America, Afghanistan and Africa (Wikipedia, 2013).

Its nutritional, economic and medicinal benefits are well documented (Mughal et. al., 1999; Chumark et. al.,2008). As an antioxidant, it has been reported to contain over 46 antioxidants that help to block enzymatic activities that tend to promote growth of cancer in humans (Bajpai et. al., 2005). The leaves have also been found potent in inhibiting growth of pathogenic bacteria including staphylococcus aureus, Bacillus subtills, E.coli, etc. (Saadabi and Abu, 2011). The alkaloids in the leaves closely resemble ephedrine that can serve as

therapeutic agent against diabetes (Kirtikar and Basu, 2003); moreover, phytoconstituents such as sterols are bioactive with beneficial effects on asthma, pain and high blood cholesterols (Ghasi et. al., 2000)

Consequent upon the medicinal, nutritional and economic benefits of this plant, a number of workers have ventured into several studies to seek means by which these benefits can be realised **(**Rev et. al., 2001; Ayanbimpe et. al., 2009; Huda et. al., 2012; Souravh and Ramica, 2014). The present study is therefore conducted to complement such efforts by qualitatively determining some phytochemicals and their associated functional groups in the crude n-hexane extract of Moringa oleifera leaf.

II. Materials And Methods

Fresh leaves of Moringa oleifera were collected within the premises of Ignatius Ajuru University of Education, Iwofe, Rumuolumeni, Port Harcourt, and along Port Harcourt International Airport Road, Omagwa in Ikwerre Local Government Area of Rivers State. The fresh leaves were air–dried for two weeks, and pulverized in a mortar into fine powder. This was set aside in a herbarium for subsequent extraction.

Extraction by the Cold Method

70 g of the air-dried leaves was soaked in 400 ml of n-hexane in a 500 ml beaker for nine (9) days. The mixture was transferred into a distillation flask for the recovery of n-hexane. The crude extract of the leaf was left in a beaker to further evaporate to dryness for one to two days.

Phytochemical Screening

Phytochemical tests were carried out on the crude extract following standard methods as described by Trease and Evans (1989).

Preparation of Reagents:

- **50% H_2SO_4:** 50 ml of distilled water was measured using a measuring cylinder and transferred into a 250 ml beaker. 50 ml of conc. H_2SO_4 of 2M was added to this portion with stirring. The solution was then placed in a water bath, allowed to cool, before it was transferred into a reagent bottle.
- **Mayer's reagent (potassium mercuric Iodide):** 10 g of potassium Iodide (KI) was measured and added to small quantity of distilled water in a 250 ml beaker was thoroughly stirred. To this, was added 10 g of HgI (Mercuric Iodide) with continuous stirring; the resulting solution was transferred into a 100 ml volumetric flask to which was again added some quantities of distilled water to 100 ml mark. This was transferred to a reagent bottle and reserved for analysis.
- **1% HCl:** 99 ml of distilled water was measured using a measuring cylinder and transferred to a 100 ml volumetric flask. Also, 1ml of conc. HCl was measured and carefully transferred into the solvent to form a solution which was then transferred into a reagent bottle.
- **5% $FeCl_3$:** 5 g of $FeCl_3$ was measured using a top load weighing balance and transferred to 250 ml beaker. To this, was added a little quantity of distilled water, and stirred to dissolve. It was then transferred into a 100 ml volumetric flask and made up to 100 ml mark for storage in a reagent bottle.

a. Test for Alkaloids

1 ml of 1% HCl was added to 3 ml of the dried extract in a test tube. Some portions of the extracts were further treated with few drops of Mayer's reagent (potassium mercuric Iodide). A creamy white precipitate was obtained, which indicated the presence of alkaloids in the extract.

b. Test for Phenols

0.5 cm^3 of the extract was mixed with 4 cm^3 of distilled water, and the mixture was heated and filtered. 3 cm^3 of neutral $FeCl_3$ solution was then added to the filtrate, and brown coloration was observed, indicating the presence of phenols.

c. Test for Terpeniods

To 0.5 ml of the extract was added 2 ml of chloroform, followed by a careful addition of 3 ml of conc. H_2S0_4 which formed a layer with a reddish- brown colouration at the interface, indicating the presence of terpenoids.

d. Test for Tannins:

2 drops of 5% $FeCl_3$ were added to 1ml of the extract. A blue green precipitate instead of a brown colouration was observed which suggested that other natural products other than tannins might be present.

e. Test for Glycosides:

5 ml of 50% H_2SO_4 was added to 5 ml of the extract. This was heated to boil in H_2O for 15 minutes, and allowed to cool. To this, was added about 5 ml of Fehlings solutions A and B (equal volume of A and B: 5 ml each) and boiled. A brick-red precipitate, indicating glycosides was formed.

f. Test for Saponins

5 ml of the extract was added to 10 ml of distilled water. It was shaken vigorously, and soapy-like foam was formed, indicating that saponins were present.

g. Test for Flavonoids

2 drops of conc. HCl and a few magnesium turnings were added to 1ml of the extract. A pink or red to purple colour that was expected was not observed, but a greenish colouration was observed, indicating the absence of flavonoids.

Sample Preparation for IR Analysis

The sample extract was further concentrated by subjecting it to evaporate any solvent that might still be associated with it. Thereafter, a little amount of the concentrated extract was placed between two polished flat potassium chloride (KCl) discs (cells) which were squeezed together in the appropriate sample holder. The holder with sample was mounted on the sample compartment of the FTIR spectrometer and scanned for transmittance within 4000 cm^{-1} to 400 cm^{-1} (mid IR region). The IR spectrum was printed with the individual peaks labelled with their corresponding wavelengths. Model of equipment used was; 1R prestige 21, manufactured by Shimadzu Corporation.

III. Results And Discussion

The physical characteristics of the extract, the percentage yield and results of phytochemical screening are shown in Table 1, while the IR spectrum is presented as Figure 1.

Table 1: Physical characteristics of the n-hexane extract, the percentage yield and results of phytochemical screening

Extract	Weight	Colour	Nature	% yield
Leaf	8.2 g	Dark-green	Oily	10.25% w/w

Phytochemical Screening			
S/N		Observed Colour	Results
1.	Phenols	Brown	+
2.	Alkaloids	Creamy-White ppt	+
3.	Flavonoids	Green	-
4.	Saponins	No Observed Colour, Soapy-Like Foam Formed	+
5.	Glycosides	Brick-Red	+
6.	Tannins	Blue-Green ppt	-
7.	Terpenoids	Reddish-Brown	+

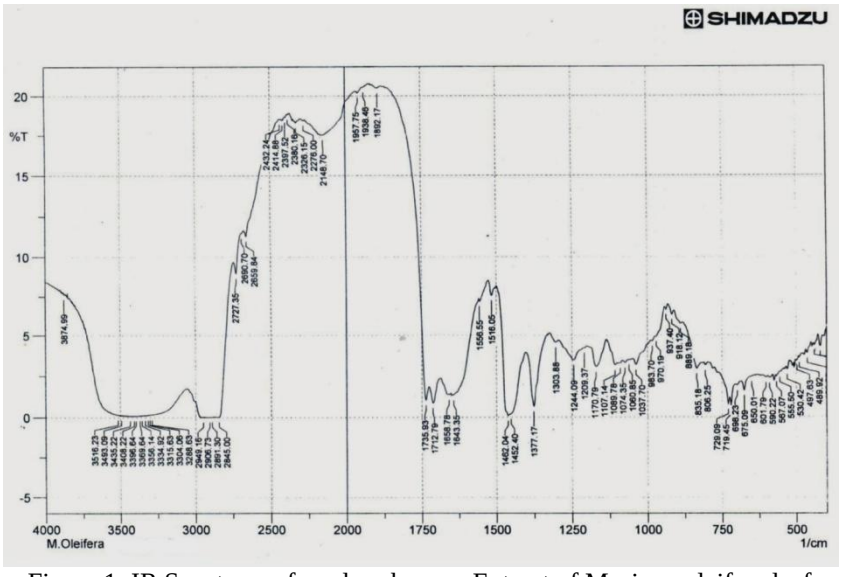

Figure 1: IR Spectrum of crude n-hexane Extract of Moringa oleifera leaf

The phytochemical screening of the crude n-hexane extract of Moringa oleifera leaf, revealed the presence of saponins, terpenoids, phenols alkaloids, glycosides, while flavonoids and tannins were absent. Plants are found to be sources of many chemical compounds, most of which account for their various uses by man. The presence of phytochemicals which include, terpenoids, alkaloids, tannins, phenols, glycosides, as has also been observed by the present study is in agreement with the findings of Egereonu and Nduchukwu (2006), who in their study reported that Crassula argentea and Bryophyllum pinnatum contain tannins, phenols, glycosides, alkaloids, terpeniods as their phytochemicals.

IV. Discussion Of IR Data

The major peaks identifiable in Figure.1 are described as follows:

1. A broad band around 3500 and 3000 cm^{-1} which are associated with phenols and alcohols with hydrogen bond -OH stretch, which usually appears between 3500-3200 cm^{-1}
2. A peak between 3500- 3100 cm^{-1} which could also be due to N-H stretch from a primary or secondary amine or amide in which R could be alkyl or aryl.
3. A similar band around 3000- 2800 cm^{-1} due to C-H stretch from a hydrocarbon.
4. Weak bands between 2750-2500 cm^{-1} are due to a carbonyl C=O in form of H-C=O stretch at 2725. 35 cm^{-1} which often appears as shoulder-type peaks to the right of alkyl C-H stretches.
5. Between 1750 and 1625 cm^{-1}, aliphatic C=O bonds due to aliphatic ketones or esters are identifiable.
6. In the finger print region, there are peaks around 1500-1375cm^{-1}

- There appears to be –OH band at 1462 .04 cm^{-1} and N–O symmetric stretch from an aliphatic nitrogen containing organic compound
- There is also a C=C-H "oop" from 900-675.09 cm^{-1} pointing to C-C stretching and C=C bonds in aromatic rings

7. The absorption at 1244. 40 cm^{-1} due to a C-O stretch from an alcohol or phenol.
8. The peak 1170.09 cm^{-1} between 1250 and 1125 cm^{-1} due to an Ar-O bond in an aromatic ring.

In any sample where hydrogen bonding occurs, the number and strength of intermolecular interaction varies greatly within the sample, causing bonds in the sample to be particularly broad (Coates 2000, Marcus, 2013). This has been evident in the spectrum of Moringa oleifera leaf. When intermolecular interactions are weak, the numbers of chemical environments are small, and narrow infrared bonds are observed.

Most of the frequencies are group frequencies which tell the presence or absence of specific functional groups in a sample. The peaks therefore, between 3500-3200 cm^{-1}, 3500- 3100 cm^{-1}, 2750-250 cm^{-1} and the oop at 900 – 675 cm^{-1} are diagnostic marker for the presence of OH, NH, C=O and C=C functions respectively.

The C-H stretch associated with H-C=O usually differed a little in frequency because of the inductive effect of oxygen attached to the carbon atom also attached to the hydrogen atom, thereby making it weaker. Thus, in the spectrum of the leaf of Moringa oleifera the absorption at 2727.35 cm^{-1} is slightly lower than theoretically known 2750 cm^{-1}

A C-O stretch at 1244.40 cm^{-1} may be used to confirm the presence of – OH group in phenol or alcohol which usually appears between 3500-3200 cm^{-1}. The broad band, therefore, in this region of the spectrum is diagnostic of the presence an OH function.

The results of IR analysis (nos. 5-8) also reveal that, the components of Moringa oleifera leaf could be aliphatic or aromatic. It may therefore be inferred that aromatic or aliphatic alcohols or phenols, amine, ketones, esters and some nitrogen containing compounds are some of the constituents of the leaf of Moringa oleifera. However, because of the fingerprint region which has a pattern that is specific for every molecule, the presence of OH function and N-O stretch, suggest that aromatic or aliphatic phenols or alcohols and nitrogen containing molecules are major components of the Moringa oleifera leaf that was studied. However, because the spectrum is from a mixture, the fingerprint region cannot be particularly assigned to any specific molecule.

The characterization of n-hexane extract of Moringa oleifera leaf reveals the presence of C=O, C-O, C=C etc band stretchings, suggesting that components of Moringa oleifera leaf may be aromatic or aliphatic. This is also in agreement with the opinion of Bajia (2007), who conducted a fluorescence spectroscopic study of a coagulating protein extracted from Moringa oleifera seeds

Also, in the IR spectrum, the bands at 3000-2800 cm^{-1} which are due to C-H stretch from a hydrocarbon corresponds to the findings of Bajia (2007), on the same study.

V. Conclusion

The present study thus concludes that the n-hexane extract of Moringa oleifera leaf possesses phytochemicals, such as alkaloids, phenols, glycosides, terpenoids etc, that are of high therapeutic value. The infrared characterization revealed the presence of C=O, C=C, C-O, N-O etc bond stretchings. Further chemical studies on Moringa oleifera plant as a whole and medicinal plant generally are therefore encouraged with polar solvents to explore the inexhaustible benefits to society.

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