ISOLATION, CHEMICAL COMPOSITION, CHARACTERIZATION AND ANTI-BACTERIAL ACTIVITY OF ACRIDINE DIGLYCOSIDE FROM MORINGA OLIFERA

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Abstract. *Moringa olifera* (drumstick tree) is a medicinal plant commonly used in phytomedicine to cure and prevent diseases in Nigeria. The ethanolic extract of the leave sample showed alkaloid {(9.66 ± 0.20) %}, phenols {(0.75 ± 0.22) %}, flavonoids {(6.86 ± 0.20) %}, saponines {(8.46 ± 0.10) %}, and tannins {(1.10 ± 0.20) %}. The plant also contains nutritional nutrients such as ash {(6.08 ± 0.02) %}, protein {(23.65 ± 0.12)%}, carbohydrates {(57.01 ± 0.01)%}, fats {(2.43 ± 0.01) %}, crude fibre {(6.02 ± 0.13)%}, moisture {(9.50 ± 0.10)%} and energy {(3276 ± 0.14)%}. Antibacterial studies showed that the plant leave successfully inhibited staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis and Klebisiella pneumonia. Acridine diglycoside was elucidated using Bruker NMR spectroscopy in combination with FT-IR NATRIC and HREIMS (m/z) mass spectral data. This result authenticates the use of the plant leave in the treatment of infections, tumors and as an anti-inflammatory agent

Introduction

Moringa oleifera, the most widely cultivated species of moringa plants is a multipurpose tree cultivated throughout the tropics [1]. *M.olifera*, commonly known as drumstick tree, horseradish tree or benzoil tree [2] is used in herbal medicine and can also be used for water purification and hand washing [3]. *M. oleifera* is used as an alternative source for nutritional supplements and growth promoters in some countries [4]. Apart from nutritional benefits, *M. Oleifera* has been reported to be used for the treatment of rheumatism, ascites, infection, hiccough influenza and internal abscess [5].

The leaf extract is capable of reducing hyperglycaemia, cholesterol lowering [9], and dyslipidemia [6]. Important medicinal properties of the *M. olifera* which have been recently reported include antipyretic, antiepileptic, anti-inflammatory, anti-ulcerative [7], anti-hypertensive [8], anti-oxidant [10], anti- diabetic, hepatoprotective [11], anti-bacterial and anti-fungal activities [10]. In addition, *M. oleifera* seed possesses water purifying power [12,13]. The plant species have long been recognized by folk medicine practitioners as having value in the treatment of tumours [14]. The edible *Moringa* leaves contain essential provitamins, including ascorbic acid and carotenoids [15]. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, and also possesses anti-tumour agents [16].

MATERIAL AND METHODS

Plant materials. Matured leaves sample were harvested in a garden at Umezeala Nsu in Ehime Mbano of Imo State on December 30th 2014. Authentication of the plant was done at taxonomy section of Forestry Department of Michael Okpara University of Agriculture Umudike Nigeria.

Extraction and isolation of the plant sample was done by drying the leaves at room temperature for two weeks. The leaves were grounded to powder and stored in an amber bottle. Phytochemicals and proximate analysis were done with some of the powdered leave sample, while the rest were percolated with 2L of ethanol for four days. The extract was filtered and concentrated with rota at 35°C-40°C.

Proximate analysis. Moisture content of the sample was determined using the method described by AOAC [17]. One gram of sample in pre-weighed crucible was placed in an oven (105°C) for 24 h, cooled, and reweighed. The percentage moisture was calculated as follows: Moisture (%) = $\frac{w_2 - w_3}{w_2 - w_1} \times 100$ (w_1 = weight of the crucible, w_2 = weight of the crucible and sample after drying, and w_3 = weight of the crucible and the sample after cooling in airtight desiccators).

The ash content was determined using a weighed (1.0g) sample which was subjected to dry ashing in a well cleaned porcelain crucible at 550°C in a muffle furnace for 2 hours. The ash percentage was calculated as follows: Ash (%) = $\frac{w_2 - w_3}{w_2 - w_1} \times 100$. Where, w_1 = weight of the crucible, w_2 is the weight of the crucible and sample ash at 550°C, and w_3 = weight of the crucible and the sample ash after cooling in airtight desiccators [18].

Crude protein was determined using the micro-Kjeldahl method as reported by Fahey [18]. The crude protein was calculated by multiplying nitrogen with the conversion factor of 6.25 [$P\% = TN \times 6.25$]. Fat content was determined using the method described by Folch, and Stanley [19]. Homogenized tissue (10g) was progressively added to small amounts of a chloroform/methanol 2:1 (v/v) mixture (up to 200 ml), with vigorous shaking, and then the extraction was carried on for a further 2 hours, using an electromagnetic stirrer. The mixture was filtered and the filter was rewashed with fresh solvent and pressed. Fifty millilitres of 0.88% potassium chloride were added and the mixture was shaken. The aqueous layer (upper) was removed by aspiration and the washing procedure was repeated. The extract was then dried by adding anhydrous sodium sulphate, which was filtered again before the solvent was removed using a rotary evaporator. The extract was then placed in desiccators overnight and weighed.

Crude fibre was determined using 1.0g of sample (W_2) which was transferred directly into a filter bag and sealed with a heat sealer. Sample and blank bags were immersed in enough amount petroleum ether for 10 minutes to extract fat content from samples. All bags were air dried and transferred to a Ankom 2000 Fiber Analyzer using H_2SO_4 and NaOH and the crude fibre was calculated according to the following equation: % Crude fibre = $100 \times \frac{(W_3 - (W_1 \times C_1))}{W_2}$ Where: W_1 = Bag tare weight W_2 = Sample Weight W_3 = Weight of Organic matter (Loss of weight on ignition of bag and fibre) C_1 = Ash corrected blank bag factor (running average of loss of weight on ignition of blank bag/original blank bag) [20].

Carbohydrate content was determined by difference, that is, addition of all the percentages of moisture, fat, crude protein, ash, and crude fibre were subtracted from 100%, while the sample energy value was estimated (in KCal/g) by multiplying the percentages of protein, lipid, and carbohydrate [21].

Phytochemical evaluation. The *M. olifera* plant leave sample was evaluated for alkaloids and phenols according to Harbon [22]. While flavonoids were evaluated as described by Boham [23]. The method described by Van-burden and Robinson was adopted for the determination of tannins [24]. Saponines were determined as reported by Obadoni [25].

FRACTIONAL SEPARATION OF THE COMPOUNDS

The concentrated ethanol was dried to obtain dark green gummy crude extract and a portion was partitioned between chloroform and water to obtain chloroform and water fractions. The dried chloroform fraction was partitioned between methanol and petroleum ether. The possible number of compounds in each fraction was determined using TLC. Methanol fraction showed the highest zone of inhibition in antibacterial activity and was subjected to column chromatography over silica gel Merck grade (60-120 mesh). The labelled eluted fractions were analysed with TLC and fraction M6

which is a dark green oil gave one spot with R_f value of 0.74. The pure dark green oil was further analysed and found to be acridine diglycoside using IR Perkin Elmer model 760. The mass was determined with HREIMS positive ion mode using Bruker multinuclear NMR experiments of ^1H and ^{13}C .

EVALUATION OF ANTIBACTERIAL ACTIVITY OF *M. OLEIFERA* LEAVES

Preparation of the inoculums. The standard clinical isolated organisms of *Pseudomonas aeruginosa* (P. Aeruginosa), *Staphylococcus aureus* (Staph), *Escherichia coli* (E-coli), *klebsiella pneumonia* (Kleb) and *Proteus mirabilis* (Proteus) were obtained from Federal Medical Centre, Owerri and the analysis was carried at Department of Medical Science Laboratory Imo state University. The strains of the organisms were propagated on nutrient agar plates and maintained at 4°C . The isolates were sub-cultured in nutrient broth at 37°C for 8h prior to antibacterial testing.

Antibacterial sensitivity testing of compounds. Agar well diffusion technique was used to determine the antibacterial activity of the fractions of the leave sample. Sensitivity test of the agar plates were by inoculating with 0.1ml of an overnight culture of each bacteria strain (equivalent to 10^8 CFU/ml⁻¹). The inoculated agar plates were allowed to dry and were appropriately labelled. Using a plastic cork borer of 6mm in diameter uniformed wells was bored in the inoculated nutrient agar. With a micropipette, 300 μl of 20mg/ml of each fraction of the leave sample was delivered into each well. The plates were left on the bench for 30 minutes to allow the compound to diffuse into the agar. Thereafter, the plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for inhibition zones around the wells. The diameters of the zones were measured with metre rule to the nearest whole millimetre [26].

RESULTS AND DISCUSSION

The results of proximate analyses (in %) as shown in table 1 indicates that the *Moringa oleifera* leaves contains carbohydrate (57.01 ± 0.01), protein (23.65 ± 0.12), fats (2.43 ± 0.01), moisture (9.50 ± 0.10), crude fibre (6.20 ± 0.13), ash (6.08 ± 0.02) and energy (3276.34 ± 0.14) revealing an excellent source of nutrition and natural energy which can be used as a good nutritional supplements.

Table 1: Proximate composition of *M.Oleifere* leaves

Nutrient	Composition[%]
Ash	6.08 ± 0.02
Protein	23.65 ± 0.12
Carbohydrate	57.01 ± 0.01
Fats	2.43 ± 0.01
Crude Fibre	6.02 ± 0.13
Moisture	9.50 ± 0.10
Energy	3276 ± 0.14

Value=SD of triplicate values

Table 2: Phytochemical analysis of *M.Oleifere* leaves.

Phytochemical	Percentage composition [%]
Alkaliod	9.66 ± 0.20
Phenols	0.75 ± 0.22
Flavonioids	6.86 ± 0.20
Saponine	8.46 ± 0.10
Tannins	1.10 ± 0.20

Value=SD of triplicate values

The result of phytochemical analysis of *M.oleifera* leaves is presented in table 2. The high percentage of alkaloids, flavonoids and saponini contents of *M.Oleifera* supports the use of the plant leaves for the treatment of bacterial infections, allergies, inflammation, tumour, viruses and cancer [27].

Table 3: Anti-bacterial Activities of *M.Oleifera* with 300 μ l of 20mg/ml.

Zone of Inhibition of the fractions in [mm]

Fractions	P.Aeruginosa	Staph	E-Coli	Kleb	Proteus
Chloroform	4	6	12	4	-
Ethanol	14	14	14	14	14
Methanol	12	16	14	16	12
Pet-ether	4	14	4	4	4

Value=SD of triplicate values

Results of anti-bacterial activities of *M.oleifera* is presented in table 3. The anti-bacterial activity showed that the ethanol and methanol fractions showed higher zone of inhibition (in mm) than other fractions. The result supports the use of *M.oleifera* for the treatment of wound [28], which justifies the traditional use of *M.oleifera* in herbal treatments [3].

Table 4: NMR assignment Analysis of *M.oleifera*

Assignment	$\delta^{13}\text{C}$	Multiplicity	$\delta^1\text{H}$	Integral Value
1	116.69	S	7.25	1
2	135.25	T	-	-
3	116.68	S	7.50	1
4	115.20	S	7.06	1
5	187.01	D	-	-
6	117.00	S	7.00	1
7	135.20	T	-	-
8	115.21	S	7.75	1
9	86.72	Q	-	-
10	-	-	-	-
11	42.05	S	1.47	3
12	80.28	D	1.75	1
13	80.30	M	1.50	1
14	45.02	D	0.90	3
15	112.21	S	2.25	1
16	112.76	S	2.02	3
A	140.08	S	-	-
B	172.87	D	-	-
C	172.89	S	-	-
D	140.02	D	-	-
Sugar	92.45-102.21	-	2.40-5.50	-

The column chromatography gave dark green oil with R_f of 0.74 on TLC. Its mass 613 was determined by HREIMS of (612.54 m/z) and calculated for $\text{C}_{31}\text{H}_{39}\text{N}_2\text{O}_{11}$.

The IR spectrum exhibited absorptions (in cm^{-1}) at 3358.79 (NH stretching), 2919.12 (C-H stretching), 2158.85 (N-C = N) and 1711.51 (C-O-Ar)

Table 4 is the NMR analysis of *M.oleifera*. The H^1 NMR spectrum of compound I showed aromatic proton at (δH 7.06, 7.50, 7.25, 7.00 and 7.75) with integral values of one each. At (δH 1.75 and 1.50) are methylene protons with integral value of one each and methyl protons at (δH 0.90 and 1.47), methyl proton with nitrogen linkage at (δH 2.02). Table 4 indicates that ^{13}C NMR showed signals of aromatic C-H carbon at (δC 115.20_(s), 116.68_(s), 116.69_(s), 117.00_(s), 115.21_(s), aromatic

quaternary carbon at (δC . 135.23_(t), 172.87_(d), 86.72_(q), 172.89_(s), 187.01_(d), 135.20_(t). C-H with nitrogen linkage at (δC . 112.21_(s), Methylene carbon (C=C) at (δC 80.30_(m) and 80.28_(d), Saturated methyl carbon at (δC . 42.5_(s) and 45.02_(d) and Methyl carbon with N-linkage at (δC . 112.76_(s). Quaternary carbon with Het. N-linkage at (δC : 140.08_(s) and 140.02_(d)) The data in table 4 and the elucidated structure (compd 1) below showed that the compound is an Acridine diglycoside (compd 1).

compound 1: Acridine dervative

The presence of acridine derivatives which acts as anti-bacterial, anti-parasitic and anti-cancer agents [29], supports the use of *M. Oleifera* in the treatment of bacterial, parasitic infection [10], cancer and viral attacks [7,14]. The isolated compound Acridine diglycoside can be used by pharmaceutical firms for drug formulation.

Conclusion

This work showed that *M. Oleifera* is an all-purpose plant. The leaves have very high nutritional value and a good source of protein, fiber, carbohydrate, fats and energy. *M. oleifera* is very important for its many impressive ranges of medicinal uses. Antibacterial studies of the plant leave proved that it could successfully inhibit the growth of staphylococcus aureus, escherichia coli, pseudomonas aeruginosa, proteus mirabilis and klebsiella pneumonia. The isolated compound Acridine diglycoside, which was elucidated with Bruker NMR spectroscopy in combination with FT-IR NATRIC and HREIMS (m/z) mass spectral data could be used by pharmaceutical by firms for drug formulation

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