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EVALUATION OF ANTIOXIDANT ACTIVITY, GC-MS AND FTIR PROFILING OF METHANOL STEM BARK EXTRACT OF *MORINDA CITRIFOLIA*

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ABSTRACT
This study was aimed at the elucidation of the bioactive compounds present in methanol extracts of *Morinda citrifolia* stem bark using Fourier transform infrared spectroscopic (FTIR) and Gas chromatography–mass spectrometry (GC-MS) techniques. The FTIR spectroscopic studies revealed the presence of these functional groups: amines, phenols, carboxylic acids, alcohols, alkenes, alkanes, and aldehydes in the extract. The GC-MS mass spectra of the identified compounds were compared with those of the National Institute of Standards and Technology database library. The results confirmed the presence of 25 compounds while 11 compounds had scientific backings about their biological activities as revealed in this present study. The presence of these bioactive compounds in the plant offers a platform for the traditional use of *M. citrifolia* in treatment of various diseases. However, the antioxidant screening of the bark extract should no activity as revealed in this study.

KEYWORD: Antioxidant, Functional groups, Methanol extract and *Morinda citrifolia.*

INTRODUCTION

Natural plants are used by a large proportion of the rural and urban population in Nigeria, owing to their availability and safer side effects. A medicinal plant is one that produces substances that can be used for therapeutic purposes or are precursors for the synthesis of useful drugs in one or more of its organs (Sofowora, 2008). Plants have long been used in traditional medicine, but there isn't enough scientific evidence to back them up (Sofowora, 2008). Over the years, nature has given many items for humanity, including the resources for the first therapeutic intervention (Nakanishi, 1999).

The use of synthetic drugs to treat diseases caused by oxidative stress, which has also contributed to an increase in the incidence of chronic inflammation, has become more common. Because most pharmaceutical companies rely on plant products for their raw materials, adverse drug reactions that are unique to individuals and cases of complicated side effects induced by these synthetic drugs have enabled clinical testing to be conducted in order to find alternative drugs from natural sources.

Natural products have seen a substantial resurgence in interest as a potential source of new medicines in recent years, both among academia and pharmaceutical companies. Natural ingredients have been used to produce a number of modern drugs (40 percent of all modern drugs in use). Throughout history, herbal medicine has been used to cure diseases by a wide range of people and cultures around the world. In most developed countries, this condition still exists, with two-thirds of the population lacking access to modern medicine.

Morinda citrifolia, a native plant, was historically used in clothing dyeing there (Setyani and Setyowati, 2018). It's also been used for colds, pneumonia, diabetics, anxiety, hypertension, and as an antidepressant and anxiolytic in the past (Kakad et al., 2015). “Indigenous people used noni blossoms in the preparation of native medicines, which were applied topically to treat conjunctivitis, styes, abscesses, irritated or red eyes, sore eyes, and so on,” according to Deng et al (2012). Additionally, the juice pressed from the blossoms was used alone to treat sore eyes. After childbirth, young blossoms were used to alleviate pain. The blossoms were used to treat kidney and bladder problems as well. The leaf of Morinda citrifolia is applied directly to the skin to treat ulcers and

minor infections (Sasikumar et al., 2012). Finally, heated noni leaves applied to the chest are believed to alleviate coughs, nausea, and colic in Malaysia. In Malaysia, the fruit is used as a shampoo and is said to be effective against head lice (Yashaswini et al., 2014). However, this present study aims at chemical composition and antioxidant capacities of stem bark extracts from *Morinda citrifolia*.

MATERIALS AND METHOD

Plant Collection

Fresh plant of *Morinda citrifolia* was collected from Osisioma, Abia State Nigeria. The plants were identified and authenticated by a Plant Taxonomist at Michael Okpara University of Agriculture Umudike and deposited in the herbarium of the same institution.

Extraction

The stem bark of *Morinda citrifolia* were powdered with a mechanical grinder and passed through Sieve no. 40. Powder of the plant samples was extracted with methanol by continuous Soxhlet extraction method. The excess solvent was removed by rotary vacuum evaporator; the remaining mass of extract was concentrated and dried.

Gas Chromatography–Mass Spectrometry (GC-MS) ANALYSIS

Methanol extract of *M. citrofolia* were analyzed with the help of GC-MS analyzer (GC-MS-QP 2010 plus Shimadzu, Japan). The carrier gas helium (99.999 %) was used at a flow rate of 1 ml per min in split mode (10:1) v/v. Methanol and chloroform extracts (8 µl) were injected into the column at 250 °C injector temperature. Temperature of oven started at 70 °C and held for 5 min. It was then raised at the rate of 10 °C per min to 280 °C without holding. Analyze was allowed for 6 min at programmed rate of 5 °C per min. Temperature of ion sources was maintained at 200 °C. The injector temperature was set at 250 °C and detector temperature was set at 250 °C. The mass spectrum of compounds present in samples was obtained by electron ionization at 70 eV and detector operates in scan mode 50 to 600 Da atomic units. The MS Table was generated through ACQ mode scan within 0.5 seconds of scan interval at the speed of 666 and fragments from 30 to 350 Da was maintained. Total running was 21 minutes (Odo *et al*., 2017).

Identification of Compounds

Identification of the compounds were based on the molecular structure, molecular mass and Calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The spectrum of unknown components was compared with the spectrum of the known components stored in the NIST library.

FTIR Analysis

Buck scientific M530 USA FTIR was used for the analysis. This instrument was equipped with a detector of deuterated triglycine sulphate and beam splitter of potassium bromide. The software of the Gram A1 was used to obtain the spectra and to manipulate them. An approximately of 1.0g of samples, 0.5ml of nujol was added, they were mixed properly and placed on a the salt pellet. During measurement, FTIR spectra was obtained at frequency regions of 4,000 – 600 cm-1 and co-added at 32 scans and at 4 cm-1 resolution. FTIR spectra were displayed as transmitter values (Odo *et al*., 2017).

In-Vitro Antioxidant Assay

Free Radical (DPPH) Scavenging Test

This assay was carried out as described by Patel and Patel (2010). DPPH (1, 1 - diphenyl- 2- picrylhydrazil) solution (0.6 mM) was freshly prepared using Methanol. The reaction mixtures which contain 0.25 ml of various concentrations, 7.82, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 µg/ml of extract, 0.25 ml of 0.6 mMol DPPH and 2 ml of Methanol were incubated in the dark for thirty minutes at room temperature. Thereafter, absorbance of the samples were measured at 517nm using a Spectrophotometer. Ascorbic acid was used as a standard. A tube containing 0.25 ml of DPPH solution and 2.25 ml methanol served as a control. Assays were carried out in duplicates. Free radical scavenging activities of samples were obtained using the formula below.

DPPH scavenging activity =100 x (AC -AS)/AC).

AC = Absorbance of control.

AS = Absorbance of sample A graph of percentage inhibition against concentration was plotted and the concentration that caused 50% inhibition (IC50) was extrapolated using a regression analyses equation (Patel and Patel, 2010).

Ferric Reducing Antioxidant Power (FRAP)

FRAP assay was carried out following the method described by Habibur *et al* (2013). Two hundred and fifty microliter (0.25 ml) of various concentrations, 7.82, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 µg/ml of extract and fractions were mixed with 0.625 ml of phosphate buffer and 0.625 ml of 1% Potassium ferricyanide (K3FeCN6). The mixtures were heated at 50c for twenty minutes. Then, 0.625 ml of 10% trichloroacetic acid (TCA) was added and the mixtures were centrifuged at 3000 rpm for ten minutes. From the upper layer, 0.625 ml was pipetted and mixed with 0.625 ml of distilled water and 0.125 ml of 0.1% (w/v) ferric chloride (FeCl3) solution. Absorbance of the mixture were measured at 700 nm against air using a Spectrophotometer. Ascorbic acid was used as standards. Test were performed in duplicate and percentage inhibition was calculated using the formula below.

% Inhibition = (Absorbance of sample – Absorbance of blank) x 100.

A graph of percentage inhibition against concentration was plotted and the effective concentration (EC50) was extrapolated using a regression analyses equation.

RESULTS AND DISCUSSION

Table 1: Result For Dpph.

Sample	IC_{50} (ug/ml)
Stem Bark	617.9+165.75
Ascorbic acid	104. 64+28.21

Values are presented as mean+ standard deviation.

Table 2: Result for Frap.

Sample	EC_{50} (ug/ml)
Stem Bark	2257.61 + 550.00
Ascorbic acid	84.25+4.77

Values are presented as mean + standard deviation.

Table 3: Compounds Identified by GC-MS.

S/N	Retention Time	Name of Compound	Molecular formula	Molecular weight	Area %
1	1.441	Adenosine, 4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]	$C_{20}H_{22}N_6O_6$	442	20.207
2	1.552	Ethanol	C_6H_6O	46	0.620
3	1.983	Ethanol, 1-methoxy-, benzoate	$C_{10}H_{12}O_3$	180	37.513
4.	2.559	Hydrocinnamic acid, benzyldimethylsilyl ester	$C_{18}H_{22}O_2Si$	298	0.316
5.	3.251	Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl	$C_{11}H_{14}N_2OS$	222	0.233
6	3.793	Benzo[h]quinoline, 2,4-dimethyl	$C_{15}H_{13}N$	207	2.971
7	4.654	1,3-Benzodioxole-5-carboxylic acid, methyl ester	$C_9H_8O_4$	180	5.415
8.	6.307	5-(p-Aminophenyl)-4-(p-tolyl)-2-thiazolamine	$C_{16}H_{15}N_3S$	281	0.123
9.	7.878	Benzofuran-6-ol-3-one, 2-(4-ethoxycarbonyl)benzylidene	$C_{18}H_{14}O_5$	310	5.130
10	9.502	Ergoline-8-methanol, 8,9-didehydro-6-methyl	$C_{16}H_{18}N_2O$	254	1.477
11	12.418	Octopamine, 3TMS derivative	$C_{17}H_{35}NO_2Si_3$	369	1.534
12	15.724	Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl	$C_{12}H_{35}O_5Si_6$	430	0.097
13	17.045	Bifenox	$C_{14}H_9Cl_2NO_5$	341	0.414
14	17.499	4-Tetradecanol	$C_{14}H_{30}O$	214	0.163
15	18.553	2-[(p-Trimethylsilyloxy)phenyl]-2-[(p-trimethylsilyloxyethylenoxy)phenyl]propane	$C_{23}H_{36}O_3Si_2$	416	0.244
16	18.692	5β,6β-Epoxy-7α-bromocholestan-3β-ol	$C_{27}H_{45}Br_6O_2$	480	0.226
17	20.165	l-Gala-l-ido-octose	$C_8H_{16}O_8$	240	0.432
18	20.392	Nonadecane	$C_{19}H_{40}$	268	2.556
19	21.864	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	0.774
20	26.119	3-Pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a,7a-epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester,[1aR(1aR*,2R*,3S*,4aR*,6S*,7S*,7aS*,8E,10R*,11R*,11aS*)]	$C_{32}H_{39}NO_{10}$	597	1.507
21	28.976	11α-Hydroxyprogesterone, trimethylsilyl ether, bis(O-methyloxime)	$C_{26}H_{14}N_2O_3Si$	460	2.555
22	30.228	Norcodeine, 2TMS derivative	$C_{23}H_{35}NO_3Si_2$	429	2.920
23	31.392	Glaucine	$C_{21}H_{25}NO_4$	355	4.270
24.	32.492	Dipyridamole	$C_{24}H_{40}N_3O_4$	504	4.357
25	33.533	Amodiaquine, 2TMS derivative	$C_{26}H_{38}ClN_3OSi_2$	499	3.938

Table 4: Bioactivity of Identified compounds in Morinda citrofolia Stem Bark.

S/N	Name of Compound	Activity	References…
1	Adenosine, 4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]	Unknown	_
2	Ethanol	Antifungal, Antibacterial	DrugBank (n. d)
3	Ethanol, 1-methoxy-, benzoate	Unknown	
4.	Hydrocinnamic acid, benzyldimethylsilyl ester	Antioxidant	Shanooba *et al* (2020)
5.	Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl	Analgesic, antihypertensive, anti-anginal	Brintha *et al* (2017)
6	Benzo[h]quinoline, 2,4-dimethyl	Anticancer	Yadav *et al*.(2016)
7	1,3-Benzodioxole-5-carboxylic acid, methyl ester	Unknown	
8.	5-(p-Aminophenyl)-4-(p-tolyl)-2-thiazolamine	Unknown	
9.	Benzofuran-6-ol-3-one, 2-(4-ethoxycarbonyl)benzylidene	Unknown	
10	Ergoline-8-methanol, 8,9-didehydro-6-methyl	Unknown	
11	Octopamine, 3TMS derivative	Unknown	
12	Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl	Antimicrobial, antiseptic, hair conditioning agent	Diabi *et al* (2021)
13	Bifenox	Unknown	
14	4-Tetradecanol	Unknown	
15	2-[(p-Trimethylsilyloxy)phenyl]-2-[(p-trimethylsilyloxyethylenoxy)phenyl]propane	Unknown	
16	5β,6β-Epoxy-7α-bromocholestan-3β-ol	Unknown	
17	l-Gala-l-ido-octose	Memory retention, prevents cognitive deficits	Nwaogwugwu *et al* (2019)
18	Nonadecane	Antioxidant, antifungal, antibacterial	Enema *et al* (2019)
19	7-Methyl-Z-tetradecen-1-ol acetate	Anti-inflammatory, anticancer, hepaprotective	Kishore *et al* (2020)
20	3-Pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a,7a-epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester,[1aR(1aR*,2R*,3S*,4aR*,6S*,7S*,7aS*,8E,10R*,11R*,11aS*)]	Unknown	
21	11α-Hydroxyprogesterone, trimethylsilyl ether, bis(O-methyloxime)	Unknown	
22	Norcodeine, 2TMS derivative	Unknown	
23	Glaucine	Anti-tissusive agent	Eric (2007)
24.	Dipyridamole	Antiplatelet agent	MedlinePlus (2017)
25	Amodiaquine, 2TMS derivative	Unknown	

Table 5: Ftir Bands Assignments.

Extracts	Group	Compound class
3847, 3693, 3557	O-H Stretching	Alcohol
3062, 3394	0-H Stretching	Allen, Amine, Carboxylic acid
2639, 2713, 2916	C-H Stretching	Alkene, alkane, aldehyde
2465, 2080, 2000	O=C=O Stretching N=C=S Stretching C=C=N Stretching	Carbon dioxide Isothiocyanate Ketenimine
1859, 1608	C=O Stretching C=C Stretching	anhydride Unsaturated ketone
1311	O-H bending	Phenol

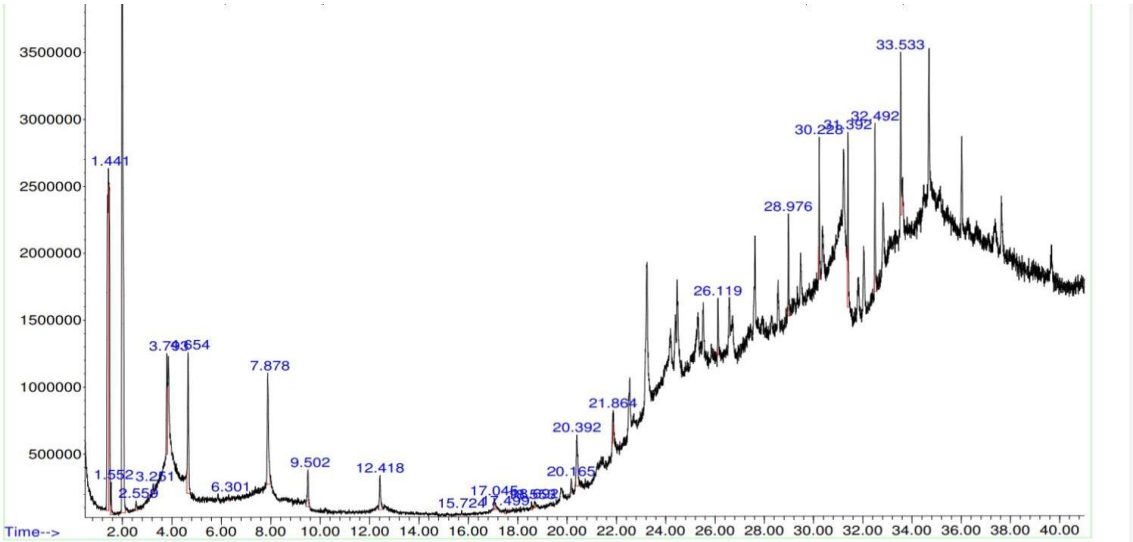

Fig 1: GC-MS Analysis Result.

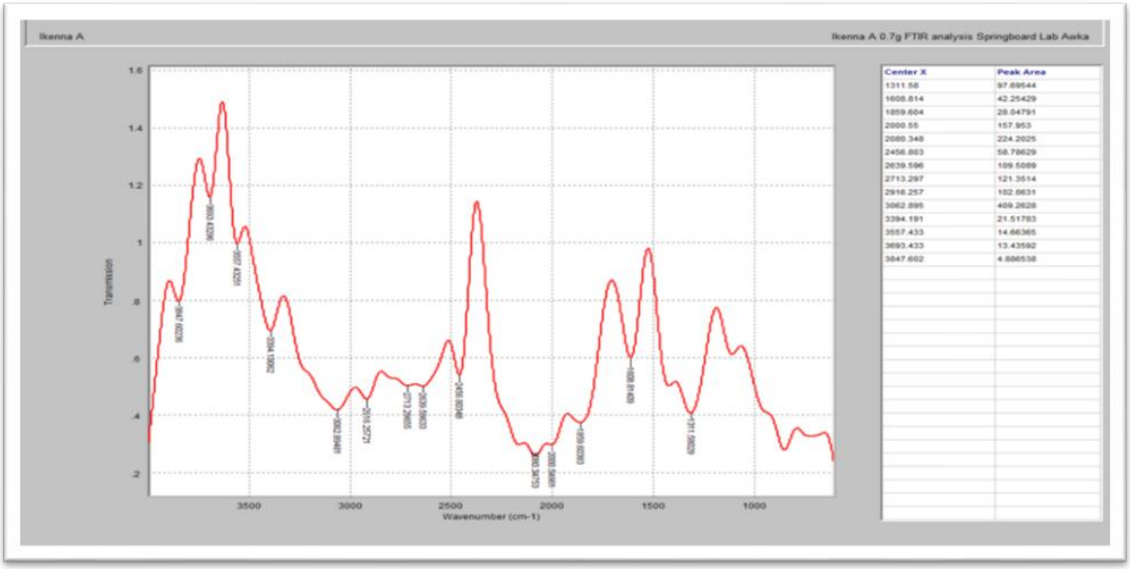

Fig 2: FTIR Analysis Result.

The functional groups present in *M.citrofolia* stem bark are; Alcohols, alkene, carboxylic acid, Amine, alkane, aldehyde, carbondioxide, Isothiocyanate, ketenimine, anhydride, unsaturated ketone and phenol. They were confirmed by the FTIR Spectra which revealed the presence of these groups; O-H, O=C=O, N=C=S, C=C=N, C=O and C=C and these compounds present was in line with the study of Anbazahan *et al* (2014) showing that they are responsible for the plant's biological activities.

The study identified 25 compounds from the bark extract, showing the most abundant from the plant which is Ethanol, 1-methoxy-, benzoate (37.513%) but its activity is still unknown. Following that compound is Adenosine, 4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl] (20.207%) which has a suspected activity of antioxidant and anticancer upon receiving the report of Mona *et al* (2015). However, its activity is still unclear and currently under investigation. Nevertheless, 11 out of 25 identified compounds showed some wide range of pharamcological activities according to various scientific reports which includes: antioxidant, antifungal, antibacterial, anticancer, analgesic, antihypertensive, antianginal, antiseptic, antitissusive, anti-inflammatory, antiplatelet and Hepaprotective activities.

Antioxidants plays a beneficial role in combating oxidative stress which could result into aging, disorder and various other kind of diseases due to the reaction of oxygen in the body. The DPPH study for the extracts revealed that bark extract of *Morinda citrofolia* exhibited no antioxidant activity on comparison to standard (Ascorbic acid). Additionally, the FRAP result also revealed that the extract could not reduce the potency and suppress radical formation to prevent oxidative damage on comparing to the standard. Hence, the stem

bark of *Morinda citrofolia* can be said to have no antioxidant activity.

CONCLUSION

Morinda citrofolia is endowed with a lot of bioactive compounds with known medicinal application. Hence, the wide use of the plant in traditional medicine in treating various diseases. Further research should be carried out to examine pharmacological activities of the other plant parts especially in treatment of oxidative stress isolating these compounds to identify a compound. With the development of new technologies some of the agents which failed earlier clinical studies are stimulating renewed interest. The current study was aimed to characterize and carry out in-vitro antioxidant assay on the stem bark extract.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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