GC-MS AND SPECTROPHOTOMETRIC QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF BIOACTIVE PHYTOCHEMICALS IN ETHYLACETATE EXTRACT OF LEAVES OF *Ficus exasperata* VAHL: A FURTHER EVIDENCE FOR ITS MEDICINAL DIVERSITY

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ABSTRACT

The GC-MS and phytochemical analysis of ethylacetate leaves extract of Ficus Exasperata Vahl was carried out. GC-MS Analysis revealed 23 constituents in which they were all identified. The major constituents were three(3) compounds with percentage peak areas of 23.20%(1- Nonadecane), 15.88%(1-Pentadecane) and 11.48%(Behenic alcohol), other constituents less than ten percentage peak area are 4.51%(1-Dodecanol, 3.56%(2,5-cyclohexadiene-1,4-dione,2,6-bis(1,1-d), 5.17%(Isopropyl myristate), 4.27%(n-hexadecanoic acid), 6.49%(Behenic alcohol), 4.91%(Oleic acid), 4.25%(propanoic acid, decyl ester), 2.89%(Octacosanol), 3.07%(Bis(2-ethylhexyl)phthalate while other constituents were less than 2%. Spectroscopic quantitative phytochemical analysis of the Ethylacetate Extract of Leaves of Ficus Exasperata was found to contain 16.18mg/g(Alkaloids), 3.78mg/g(Steroids), 85.13mg/g(Flavonoids) and 268.18mg/g(Phenolics).

Key words: *Ficus exasperate,* Ethylacetate extract, GC-MS, Phytochemicals, Spectroscopic.

INTRODUCTION

Natural products are naturally occurring compounds that are end products of secondary metabolism, often; they are unique compounds for particular organisms or classes of organisms, natural products have taken an important place health services all over the globe. However, the absence of scientific evaluation of medicinal plants may cause serious adverse effects.[1,2,3]

Most of the natural products isolated from medicinal plants are secondary metabolites which include alkaloids, tannins, flavonoids and phenols (Harvey, 2001).[4] Some of the products have nutritive value, anti-diabetic, anti-malarial, anti-fungal, anti-bacterial and against many other diseases[5].

Medical plants have been used extensively as a source for numerous active constituents for treating human diseases and they as well, have high content of therapeutic value.[6]

Ficus exasperate Vahl belong to the family Moraceae, commonly known as Sandpaper leaf

tree owing to the rough surface of the leaves, it is called "Borai" by the Hausa people, Epin by the Yorubas and "Ewi-epin" by the Igbos. It is increasingly being used for a number of ailments and hence, studies validating the traditional claims are on the increase. Available reports indicate that leaves of *F. exasperate* exhibit antiulcer, hypotensive, hypoglycemic, hypolipidemic, anti-inflammatory, anxiolytic, oxytocin inhibiting, anticonvulsant, antinociceptive, antipyretic, anti-microbial, anti candidal, insecticidal and pesticidal activities.[7]. *F. exasperate* a small tree well known on account of its very rough leaves being used as paper widely spread in all eco-regions of Nigeria, and it is mostly used for treatment of diabetes by the Hausa/Fulanis of Northern Nigeria.[8] The plant has been ethnobotanically reported to have diverse medicinal uses.

Plate I *F. exasperate* Vahl in its natural Habitat.

It is used in herbal medicine to treat cough, hemorrhoid and lowering high blood pressure[9], *F. exasperate* is used in various ethnomedicines for the treatment of pain, inflammatory diseases, wounds and abscesses.[10]

MATERIAL AND METHODS

Sample preparation and Extraction

The freshly collected plants leaves was separately cut into chips, and air-dried in the laboratory, grounded into powder using mortar and pestle, weighed and stored in polythene bags until needed.[11]

A portion (150g) of the ground plant leaves was percolated in 500 $cm^3$of methanol for two weeks and successively fractionated in petroleum ether, chloroform and ethyl acetate. The extracts were separately filtered and concentrated using a rotary evaporator at 45 ^{0}C. The marc was re-percolated with the recovered solvents for one week. The filtrates were drained, filtered, combined with respective ones each and concentrated using a rotary evaporator. Each extract was cooled, weighed and stored in the refrigerator until needed.[11]

Chemicals and Reagents

All chemicals and reagents were procured from certified suppliers and were of the highest analytical standard.

Preparation of Extract

The ethylacetate extract of the leaves was analyzed using Gas Chromatography Mass

Spectroscopy for the identification of the phytochemical compounds present. A solvent blank analysis was first conducted using 1 µl of absolute ethylacatate. Then 1 µl of the reconstituted ethylacetate extract solution was employed for GC-MS analysis as previously described with modifications.[12]

ANALYSIS

Gas Chromatography Mass Spectroscopy

GC-MS analysis was carried out on a GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument; Schimadzu GCMS-QP2010, employing the following conditions:

Column Elite-1 fused silica capillary column (30×0.25 mm ID×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) as carrier gas at a constant flow of 1ml/ minute and a sample injection volume of 1 µl which was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minute, to 200°C, then 5°C/minute to 280°C, ending with a 9 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Total run time was 30 min. The compounds were then identified from the GC-MS peaks, using library data of the corresponding compounds. GC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library using NISP Search. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software.

Spectroscopic Phytochemical Analysis

Determination of Total Alkaloid Content

To a portion (1 cm^3) of the extract was added with 5 cm^3 of phosphate buffer (pH 4.7) and 5 cm^3 Bromo Crystal Green (BCG) solution and the mixture was shaken with 4 cm^3 of Chloroform. The extract was collected in a 10 cm^3 volumetric flask and is diluted to makeup the final volume with Chloroform. The blank was prepared as above but without the extract and the absorbance of the complex in chloroform was measured at 470 nm against the blank. Atropine was used as a standard to generate the atropine standard curve which was used to determine the atropine equivalence of the fraction

Blank solution: A portion, 5 cm^3 of pH 4.7 phosphate buffer and 5 cm^3 of BCG solution was mixed and then extracted with 5 cm^3 of chloroform. Extract was collected in 10 cm^3 volumetric flasks and then adjusted the volume to the mark with chloroform.

A standard curve of absorbance against concentration of Atropine was plotted and used for estimation of the Atropine equivalence (AE) of test sample.

This was determined according to the method of method.[13]

Determination of Flavonoids Content

The total flavonoid content of the plant extracts was determined using Aluminium Chloride colorimetric method. Quercetin was used as standard and the flavonoid content of the extracts was expressed as mg of quercetin equivalent /gm of dried extract.[14]

To a portion (1 cm^3) of the plant extract was taken in a test tube which is added with 2 cm^3 of 5% $NaNO_2$ and 3 cm^3 of $AlCl_3$ (10%) was added to this after 5 minutes, the reaction mixture was treated with 2 cm^3 of 1 M NaOH in another 5 minutes and the reaction mixture was made up to 10 cm^3 with water and the absorbance was measured at 510 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was taken. For the blank, the extracts were replaced with an equal volume of distilled water. The Flavonoids content in extracts was expressed in terms of Quercetin equivalents. A standard curve of absorbance against quercetin concentration was plotted, and used for estimation of the quercetin equivalence (QE) of test samples.

Estimation of Total Phenolic Content

Total phenolic content of samples was determined employing the method involving the use of Folin-Ciocalteu reagent (FCR) as oxidizing agent and with Gallic acid as standard.

Preparation of blank solution: In a 20 cm^3 volumetric flask 1.5 cm^3 Folin Ciocalteau reagent, 1 cm^3 distilled water and 4 ml 20% sodium carbonate was mixed in a 20 cm^3 volumetric flask.

A portion (1 cm^3) of test sample solution and the various concentrations of the gallic acid standards were placed in different test tubes. To each of the test tubes, 1 ml of distilled water and 1.5 cm^3 Folin Ciocalteu's reagent was added, the mixture was covered with aluminium foil and allowed to incubate at room temperature for 5 minutes. Afterwards, 4 cm^3 of 20% (w/w) Na_2CO_3 was added to each of the test tube, the mixtures were agitated and placed in a water bath at a temperature of 40 ^{O}C for 30 minutes. The test tubes were placed in ice water to quench the reaction. The absorbance of the test samples and standards at 765 nm using UV/VIS spectrophotometer against blank was measured.

Determination of Total Steroids Content

A portion (20mg) of AD-E was suspended in chloroform, covered and heated at 60 ^{0}C for 30 minutes in water bath with shaking. The suspension was filtered.

The resultant marc was thereafter, extracted with 20 cm^3 of chloroform and filtered. The volume of

the combined filtrate was adjusted to 50 cm^3 with same solvent (chloroform).

To 10 cm^3 volumetric flasks, 5 cm^3 of combined filtrate was transferred and 2 cm^3 of Liebermann-Burchard (LB) reagent was added. The volume was adjusted with chloroform. The absorbance was measured using a UV/Vis spectrophotometer 5 min after the addition of the reagent LB at 625 nm wavelength.

Preparation of blank:

In 10 cm^3 volumetric flask 5 cm^3 of chloroform, 2 cm^3 of LB was added and adjusted the volume to the 10 cm^3 mark with chloroform. It was allowed to stand for 5 minutes and the absorbance was measured at 625 nm.

RESULTS AND DISCUSSION

Total Ion Chromatogram (TIC) of Ethylacetate leaves extract of *Ficus exasperate*.

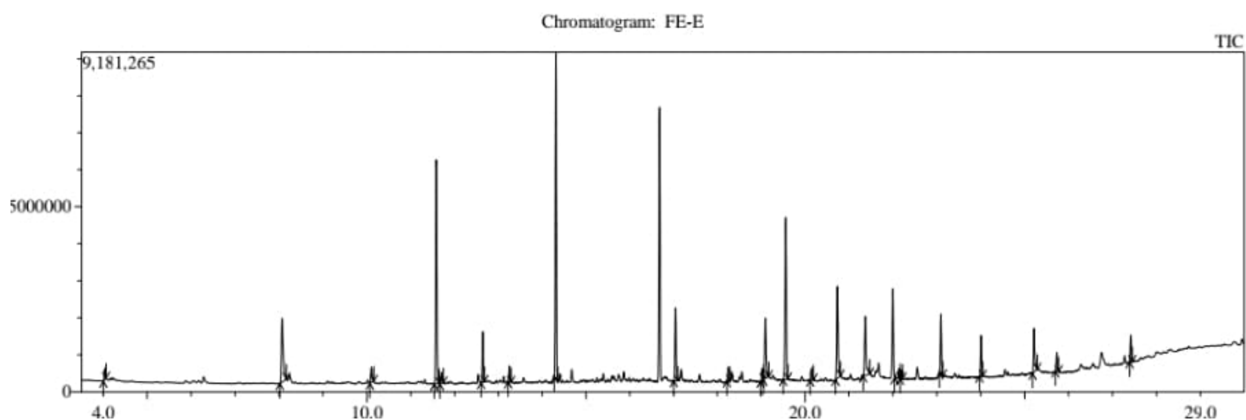

Table I: Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	4.030	429770	0.60	304305	0.80	1.41	1-Decene
2	8.069	5062684	7.05	1717607	4.51	2.95	1-Dodecanol
3	10.105	863685	1.20	406068	1.07	2.13	Tridecane, 6-methyl-
4	11.581	11151287	15.53	6043116	15.88	1.85	1-Pentadecene
5	11.700	537510	0.75	299888	0.79	1.79	Tetradecane
6	12.640	2202321	3.07	1354662	3.56	1.63	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-d
7	13.249	693251	0.97	429091	1.13	1.62	2,4-Di-tert-butylphenol
8	14.310	13472983	18.77	8827926	23.20	1.53	1-Nonadecene
9	17.047	3562988	4.96	1967375	5.17	1.81	Isopropyl myristate
10	18.251	760399	1.06	386472	1.02	1.97	(2,3,5,6-Tetrafluorophenyl)methyl 3-(2,2-dic
11	19.027	590938	0.82	311290	0.82	1.90	Dibutyl phthalate

12	19.100	3734095	5.20	1624151	4.27	2.30	n-Hexadecanoic acid
13	19.564	8940135	12.45	4369082	11.48	2.05	Behenic alcohol
14	20.152	701542	0.98	321208	0.84	2.18	9-Tricosene, (Z)-
15	20.739	5120445	7.13	2471531	6.49	2.07	Behenic alcohol
16	21.371	3965076	5.52	1595334	4.19	2.49	Oleic Acid
17	22.138	596178	0.83	264707	0.70	2.25	Acetic acid n-octadecyl ester
18	22.191	413338	0.58	251992	0.66	1.64	Phytol
19	23.097	2670923	3.72	1615448	4.25	1.65	Propanoic acid, decyl ester
20	24.007	1870716	2.61	1101210	2.89	1.70	Octacosanol
21	25.215	2386584	3.32	1169938	3.07	2.04	Bis(2-ethylhexyl) phthalate
22	25.734	916012	1.28	508993	1.34	1.80	1-Hexacosanol
23	27.414	1151987	1.60	712458	1.87	1.62	Squalene

Spectroscopic Quantitative Phytochmical Analysis

Table II: Total amount of Phytochemical in FEE

Plant fractions	Alkaloids mg/g	Steroids mg/g	Flavonoids mg/g	Phenols mg/g
FEE	16.18	3.78	85.13	268.18

GC-MS Analysis

Using GC-MS Analysis, 25 compounds have been elucidated. The major constituents were three(3) compounds with percentage peak areas of 23.20%(1- Nonadecane), 15.88%(1-Pentadecane) and 11.48%(Behenic alcohol), other constituents less than ten percentage peak area are 4.51%(1-Dodecanol, 3.56%(2,5-cyclohexadiene-1,4-dione,2,6-bis(1,1-d), 5.17%(Isopropyl myristate), 4.27%(n-hexadecanoic acid), 6.49%(Behenic alcohol), 4.91%(Oleic acid), 4.25%(propanoic acid, decyl ester), 2.89%(Octacosanol), 3.07%(Bis(2-ethylhexyl)phthalate while other constituents were less than 2% as in table I. The bioactive constituents identified include unsaturated hydrocarbon, acids, alcohols and esters which are bioactive compounds which medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For

example, Alkaloids protects against chronic disease. Saponins protect against hypercholesterolemia and antibiotic properties.[15] Steroids and triterpenoids show the analgesic properties. The Steroids and saponins exhibits central nervous system activities.[16] Flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities.[17]

Spectroscopic Quantitative Phytochemical Analysis

The presence of various secondary class metabolites identified which are bioactive constituents puts these results in line with GC-MS analysis that was carried out, the phytochemical analysis showed that it contains secondary metabolites compounds group: alkaloids(16.18mg/g), flavonoids(85.13mg/g), phenols(268.18mg/g) and steroids(3.78mg/g). They are noted to be defense chemical compounds of plants produced in the plant tissue.[18] The plant could thus be used for the management of various healthy conditions associated with the metabolites screened.

CONCLUSION

The GC-MS analysis revealed 25 compounds in which all were identified with three major peaks height of 15.88%(1-pentadecene), 23.20%(1-nonadecene) and 11.48%(behenic alcohol) while the others were less than 10%. This signifies the presence of bioactive constituents.

The spectroscopic quantitative analysis of ethylacetate leaf extract of *Ficus exasperate*, FEE reveals the presence of medicinally valued bio active components like, flavonoids, steroids, alkaloids and phenolic compounds. Quantitative estimation leaf contained higher content of flavonoids(85.13mg/g) and phenolic(268.18mg/g) compounds with lesser number of alkaloids(16.18mg/g) and steroids(3.78mg/g). The presence of these bioactive constituents will be very helpful for the manufacturing of new drugs for treatment of various diseases.

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