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Evaluation of the Phytochemistry of Aqueous, Ethanolic and Methanolic Extracts of *Morus mesozygia* Linn. Stapf., Twig

Marcella Tari Joshua[1*], Edna O. Wachuku[1], N. Boisa[2] and Nsirim Nduka[1]

[1]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.
[2]Department of Chemistry, Rivers State University, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors NN, EOW and NB designed the study, wrote the protocol. Author MTJ wrote the draft of the manuscript, managed the analyses and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to phytochemically analyze the aqueous, ethanolic and methanolic twig extracts of the species *Morus mesozygia* Linn. Stapf.
Study Design: This is a cross-sectional study.
Place and Duration of Study: This study was carried out at the Plant Anatomy and Physiology Research Laboratory, University of Port Harcourt, between July, 2018 and November, 2018.
Methodology: *Morus mesozygia* linn twigs were collected and washed with distilled water, air dried for seven days and milled into fine powder. Maceration method was used to extract the powdered twig into a brownish paste using three different solvents; distilled water, ethanol and methanol. The different plant extracts were subjected to qualitative phytochemical screening for alkaloids, flavonoids, saponins, carbohydrates, tannins and anthraquinones. Quantitative phytochemical analysis was done using a Gas chromatography – Mass Spectroscopy machine.
Results: The results of this study showed that the powdered *Morus mesozygia* linn twigs contained flavonoids, saponins, carbohydrates, alkaloids, tannins, but not anthraquinones. The

**Corresponding author: E-mail: marcela..joshua4.3@yahoo.com;*

methanolic and aqueous twig extracts contained high amounts of alkaloids, flavonoids, saponins, carbohydrates and tannins, while the ethanolic extract also contained high amounts of the aforementioned phytochemicals in the same proportion, but had saponins in moderate amounts. It also showed that the methanolic twig extract had more carbohydrate than the other two extracts. The result of the GC-MS analysis showed that the three extracts contained complex compounds in varying amounts.

Conclusion: The qualitative and quantitative phytochemical analyses test results of *Morus mesozygia Linn Stapf.* revealed the presence of the substances like alkaloids, saponins, flavonoids, oils, phenolic compounds, tannins and some complex compounds discovered using GC-MS technique, in their varying concentrations for the three different extracts.

Keywords: Phytochemistry; aqueous; ethanolic; methanolic; Morus mesozygia linn. stapf.; twig.

1. INTRODUCTION

Natural plants are used as very good source of nutrition persistent food as well as source of various chemical constituents operative in curing various diseases which may demand as the biologically active constituents. Because of their fewer side effects, they are considered the potential resources of various bioactive compounds and are also easily available from the natural sources.

Plants such as vegetables, fruit, spices medicinal herbs, etc., have been used to cure many diseases since ancient time. Today in this modern world, even though synthetic drugs are readily available and highly effective in curing various diseases, there are people who still prefer using traditional folk medicines because of their less harmful effects. There is a wide diversity of compounds, especially secondary metabolites, found and isolated from plants and studies have shown that these compounds have anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral and many other activities to a greater or lesser extent, [1-2].

The *Morus mesozygia* is the only native species of the morus genus found in the forest as a small-sized tree in Tropical Africa amongst other species found in the temperate regions such as Hamalyan mulberry otherwise called *Morus serrata*, Red mulberry otherwise known as *Morus rubra*, White mulberry also called *Morus alba*, Fig mulberry otherwise called *Fiscua sycamorus* Indian mulberry also called *Morinda*, Chinese mulberry also called *Macluratris cuspidata* and the Paper mulberry also called *Brossonetia papyrifera*. [3].

The *Morus mesozygia Linn. Stapf.* or African mulberry tree is planted using seedlings which grows to a height with a crown of 1.5 to 1.8 meters from the ground level and its specially raised with the assistance of well cultivated saplings and a stem girth of 10-13cm. It grows richly in the loamy soil and in a rainy area to tolerate drought to a period of about 8 to 10 months. Block formulations are erected for the plantations of these trees to a spacing of about 1.8 by 1.8m. After allowing the plant to prune to a height of 1.5 to 1.8m, the shoots are allowed to sprout out with a peak of 8 to 10 shoots at the crown after which the leaves are harvested mainly by leaf-picking during rainy seasons [4].

Fig. 1. Twigs of *Morus Mesozygia Linn. Stapf.*

Morus mesozygia Linn. Stapf. from the sweet mulberry plant [5], is from the genus' Morus' obtained from the Greek word "mouria" and of the flowering plants from the family name Moraceae, it is called 'Ewe aye' by the Yorubas in South West Nigeria [6], it was identified botanically as '*Morus Mesozygia Linn. Stapf*. The tree of the *Morus mesozygia Linn*. is average normally found in a fallow land with massive branch leaves that have with pronounced venation and sticky exudates. The decoctions have been used as baths and massaging body gels and enemas against rheumatism, lumbago, intercoastal pain, neuralgia, colic, stiffness, debility. In our modern times, the vegetative parts of the African mulberry tree (*Morus mesozygia Linn Stapf.)* is of essence as it seeks to acquire more pharmacological approach for treatment as modalities in the health care sector, the nutraceutical, food and cosmetics [7].

Today in this modern world, even though synthetic drugs are readily available and highly effective in curing various diseases, there are people who still prefer using traditional folk medicines because of their less harmful effects. There is a wide diversity of these compounds, especially secondary metabolites, found and isolated from plants. Several studies have shown that these compounds have anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral and many other activities to a greater or lesser extent [1,2]. Typical examples of these phytochemical compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides, stilbenes, tannins, nitrogen compounds (alkaloids, amines, betalains), terpenoids and some other endogenous metabolites [1,8].

For this research, the *Morus Mesozygia Linn*. *Stapf.* Twig was evaluated for the phytochemical composition of the extracts in three different solvent extractions of aqueous, methanol and ethanol, using Gas Chromatography: Mass Spectroscopy (GC-MS).

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Morus mesozygia Linn. (family Moraceae) fresh twigs samples were collected in the month of July, 2018 from an abandoned, fallow- farmland at Ile-Ife, Ilesha Road, Ile-Ife, Osun State, South-Western Nigeria and was authenticated by plant botanist, Dr. Oladele A.T.at the Department of Forestry and Wildlife Management, University of Port Harcourt with the herbarium voucher number (UPFH 0125) and was submitted at the department's herbarium.

2.1.1 Preparation of plant extract (cold maceration extraction method)

The *Morus mesozygia linn* twigs were washed with distilled water and air dried separately for seven days and milled into fine powder with the use of a milling machine, the powdered twigs produced a total weight of 2.90 kg, it was stored and labelled into an air tight container prior to use.

2.1.1.1 Extraction of powdered Morus mesozygia linn twigs using distilled water, absolute ethanol and methanol

Nine hundred and sixty grams (960 g) of dried powdered *Morus mesozygia linn* twigs was put into a clean beaker, five liters (5 L) of distilled water, ethanol and methanol separately and were suspended into the beaker, they were shaken severally on a shaker, and they were mixed properly and stored for 24hours. They were macerated and filtered through a muslin cloth and again filtered out through a Whatman's number one filter paper. The filtered extracts were concentrated (on low pressure) using the rotary evaporator equipment [8] after which they were dried on an evaporating dish at a temperature of 50°C to 60°C to a semi- solid form. A sticky semi-solid dark brownish substance was obtained. The extracts were stored in a well corked universal bottle. The twig extracts were kept in a 4°C refrigerator prior to phytochemical screening.

2.2 Phytochemical Qualitative and Quantitative Analysis of *Morus mesozygia Linn. Stapf* Twig

Phytochemical analysis was carried out at the Plant Anatomy and Physiology Research Laboratory, University of Port Harcourt.

2.2.1 Pytochemical qualitative analysis of flavonoids [9]

Into a clean test tube was 5 ml of the methanolic, ethanolic and aqueous extracts *Morus mesozygia linn Staph*. twigs separately pipetted with the further addition of 5 ml of 10% of dilute ammonia solution into each tube. To the test sample, was the careful addition of 1ml of

concentrated sulphuric acid a yellowish coloration of the solution was observed which indicated the presence of flavonoid in the test sample.

To indicate the presence of the severity of flavonoids, the below symbols were used:

+ → Mildly present
++ → Moderately present
+++ → Highly present

2.2.2 Phytochemical qualitative analysis of alkaloids using wagner's reagent [5]

5 ml of the three twigs extracts were pipetted into three dry clean test tubes. 3 mls in drops of Wagner's reagent was introduced into each test tube. Homogenity of the mixture was ensured as the test tubes were shaken thoroughly. A precipitate of the mixture was observed which indicated the presence of alkaloids. The severity of alkaloids was represented as described below:

+ → Mildly present
++ → Moderately present
+++ → Highly present

2.2.3 Phytochemical qualitative analysis of tannins using folin-denis's reagent [10]

1 ml each of the three twig extracts was pipetted into three clean test tubes. Into the test sample in the test tubes was a drop of sodium carbonate solution added, likewise was two drops of Folin's Denis reagents added into the mixtures. The mixture in the test tubes were kept on standing for ten minutes for total colour development. A bluish colour of the mixtures indicated the presence of tannis. The severity of the presence of tannis was indicated with the symbol as shown below:

+ → Mildly present
++ → Moderately present
+++ → Highly present

2.2.4 Phytochemical qualitative analysis of saponins using frothing's test [10]

5 mls each of the three twig extracts was boiled in 20 mls of distilled water in a water bath, after which it was then filtered. 10 ml of the filtered was mixed with 5 ml distilled water, shaken vigorously for the appearance of a stable persistent froth. The froth formed was mixed with 3 drops of olive oil for each tube, which was again shaken vigorously for uniformity and the three tubes were observed for the formation of an emulsion. The concentration of the emulsion formed to show the presence of saponins was recorded with its severity as:

+ → Mildly present
++ → Moderately present
+++ → Highly present

2.3 Phytochemical Quantitative Analysis of Alkaloids

2.3.1 Phytochemical quantitative analysis of alkaloids [11]

5 g each of the aqueous, ethanolic and methanolic twig extracts of the sample was weighed and dispensed into three different 250 ml beaker, to which 200 mls of 10% acetic acetic in ethanol was added to each tube. The mixture was covered and allowed to stand for 4 hours after which the filterate that was filtered through a Whatman's number 541 filter paper was concentrated on a water bath. To a one quarter of each of the twig extract sample of the original volume collected was the addition of concentrated ammonium hydroxide which was added in a drop wise volume to the filtrate which showed a complete precipitation process. The entire mixture of the solution was left on standing to settle while the precipitate formed was washed with ammonium hydroxide and again filtered. The residue on the filter paper was dried and weighed and calculated thus:

Weight of Alkaloid = Weight of filter paper + residue – Weight of empty filter paper

Therefore, percentage yield of Alkaloid, = weight of filter paper + residue – weight of empty filter paper/ Weight of sample x 100

2.3.2 Phytochemical Quantitative Analysis of Flavonoids [12]

10 g each of the three twig extracts was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The complete portion of this mixture was filtered through a Whatman's number 42 filter paper. The filtrates obtained were then transferred into three crucibles and then subjected to a water bath for them to evaporate into dryness and further air dried in an air oven, cooled to room temperature in a desiccator and weighed in an analytical balance.

The calculation used to obtain the quantified flavonoid included:

Weight of Flavonoids = Weight of Beaker x residue – weight of empty beaker

$$\% \text{ flavonoids} = \frac{\text{weight of flavonoid} \times 100}{\text{Weight of sample} \quad 1}$$

2.3.3 Phytochemical quantitative analysis of saponins [11]

10 g each of the three different twig extracts was weighed and transferred into three different 250 ml conical flask. 20% in 100mls each of aqueous ethanol solution was added to the samples. The samples were subjected to heat on a water bath with series of stirring on a temperature maintained at 55°C for 4 hours. The mixtures were then filtered while the residues were re-extracted with 20% of a 200 ml in portion of ethanol. The combined extracts were evaporated to 40 ml over a temperature of 90°C over a water bath. The aqueous layer that was recovered during the process was kept while the ether layer was discarded. The recovered aqueous layer was purified with 60 ml n-butanol. The combined n-butanol extracts were washed twice with 10 ml of 55% aqueous solution of sodium chloride. The left-over solution was heated in a water bath and further left to air dry in an evaporator where its weight was obtained with the use of this formula:

Weight of Saponin = weight of flask x residue – weight of empty flask

$$\% \text{ Saponin} = \frac{\text{weight of saponin residue} \times 100}{\text{Weight of sample} \quad 1}$$

2.3.4 Phytochemical quantitative analysis of tannins [13]

0.1 g each of the three twig extracts was weighed on a weighing scale and transferred into three 250 ml conical flasks. 100 ml of distilled water was added into the samples and boiled for 1 hour. The samples were allowed to cool at room temperature and diluted with 50 ml of distilled water. 1ml each of the diluent was pipetted into three test tubes and 2 to 5 mls of Folin-Denis's reagent was added with 1 ml of 17% sodium carbonate.

A blank test was prepared with 1 ml distilled water and the reagents as earlier stated. The bluish colour formed in the test sample was read spectrophotometrically at 750 nm wavelength using blank to calibrate the spectrophotometer.

0.1 g of the tannic acid was dissolved into 100 ml dissolved water to prepare the standard concentration to enable the dilutions of the working standards of choice to be plotted against the concentration.

A linear graph that passed through the margin was obtained. The concentrations of tannin in the three samples were extrapolated from the standard graph.

3. RESULTS AND DISCUSSION

The results of the phytochemical screening of aqueous, ethanolic and methanolic extracts of *Morus mesozygia* Linn. S. twigs, showed that they contain some secondary metabolites such as tannins alkaloids, steroids and so on, present in varying proportions, as shown in Tables 1-5 for qualitative, Tables 6-10 for quantitative and Figs. 2, 3 and 4 for mass spectrum respectively for aqueous, ethanolic and methanolic extracts. Qualitative phytochemical analysis of *Morus mesozygia* Linn. Stapf. Twigs powdered samples also showed that the Twigs extract contains flavonoids, carbohydrates, alkaloids, saponins and tannins. *Morus mesozygia* Linn. Stapf. Twigs extracts revealed highest flavonoid yield of methanolic twig (22.99%) followed by ethanolic twig (18.10%) then aqueous twig (12.71%) (Table 9). Flavonoids have been reported to be anti-oxidative in a mechanism that describes their scavenging properties of reactive oxygen species against lipid peroxidation by increase in MDA concentrations. This is in consonance with the reports of [14].

Table 1. Summary of the preliminary phytochemical analysis of *Morus mesozygia Linn. Stapf.* twigs dried powdered samples

Compound Classes	Test	Observation	Inference
Alkaloids	Wagner's test	Reddish brown color	+
Flavonoids	Harborne's test	Yellowish color	+
Carbohydrates	Fehling's test	Brick Red colour	+
Saponins	Frothing's test	Frothing head formed	+
Tannins	Folin-Denis's test	Bluish colour	+
Anthraquinnones		No colouration	-

Key: Absent (-), Present (+)

Table 2. Summary of the qualitative phytochemical analysis of *Morus mesozygia Linn. Stapf.* methanolic twig extracts

Compound Classes	Test	Observation	Inference
Alkaloids	Wagner's test	Reddish brown color	+++
Flavonoids	Harborne's test	yellowish color	+++
Saponins	Frothing's test	Frothing head formed	+++
Tannins	Folin-Denis's test	Bluish colour	++
Anthraquinnones		No colouration	-

Key: (+) →Mildly Present
(++) →Moderately Present
(+++) → Highly Present
(-) → Absent

Table 3. Summary of the qualitative phytochemical analysis of *Morus mesozygia Linn. Stapf.* ethanolic twig extracts

Compound Classes	Test	Observation	Inference
Alkaloids	Wagner's test	Reddish brown color	+++
Flavonoids	Harborne's test	yellowish color	+++
Saponins	Frothing test	Frothing head formed	+++
Tannins	Folin-Denis's test	Bluish colour	++
Anthraquinnones		No colouration	-

Key: (+) →Mildly Present
(++) →Moderately Present
(+++) → Highly Present
(-) → Absent

Table 4. Summary of the qualitative phytochemical analysis of *Morus mesozygia Linn. Stapf.* aqueous twig extracts

Compound Classes	Test	Observation	Inference
Alkaloids	Wagner's test	Reddish brown color	+++
Flavonoids	Harborne's test	yellowish color	+
Saponins	Frothing test	Frothing head formed	+++
Tannins	Folin-Denis's test	Bluish colour	++
Anthraquinnones		No colouration	-

Key: (+) →Mildly Present
(++) →Moderately Present
(+++) → Highly Present
(-) → Absent

Table 5. Summary of the qualitative phytochemical analysis of carbohydrates in *Morus mesozygia Linn. Stapf.* methanolic, ethanolic, aqueous twigs extracts

Carbohydrates in Sample	Test	Observation	Inference
Aqueous Twigs	Fehling's test	Reddish brown color	++
Ethanolic Twigs	Fehling's test		+++
Methanolic Twigs	Fehling's test		+

Key: (+) →Mildly Present
(++) →Moderately Present
(+++) → Highly Present
(-) → Absent

Table 6. Gas chromatography-mass spectrometry analysis of aqueous twig extracts of *Morus mesozygia Linn. S.* (African Mulberry)

RT	Component	Formula	MW	%
2.956	5,8,11-Heptadecatrien-1-ol	$C_{17}H_{30}O$	250	34.1 42
7.167	4H-Thiopyranol(4^1,3^1, 4,5)	$C_{11}H_{14}N_4OS$	250	17.592
8.308	Oxirane	$C_8H_{17}NO_2$	128	12.406
8.870	8-Aminocaprylic acid	$C_8H_{17}NO_2$	159	8.364
7.108	1,4-Naphthoquinone	$C_{12}H_{10}O_6$	250	3.678

Table 7. Gas chromatography-mass spectrometry analysis of ethanolic twig extracts of *Morus mesozygia Linn. S.* (African Mulberry)

RT	Component	Formula	MW	%
7.939	2H-Azonin-2-One	$C_8H_{15}NO_2$	157	16.39
7.830	2-Hydroxy-3-(thiophen-2-yl)	$C_{12}H_{10}O_4S$	250	16.37
7.570	Pyrimidine,5-Bromo-2,4-	$C_6H_7BrN_2S_2$	251	11.93
	bis (methylthio)	C_7H_{14}	98.1	9.888
8.174	Isopropylcyclobutane			
8.006	2-Propynoic acid	$C_4H_4O_2$	84.0	9.756

Table 8. Gas chromatography-mass spectrometry analysis of methanolic twig extracts of *Morus mesozygia Linn.S.* (African Mulberry)

RT		Formula	MW	%
11.798	Phenol	$C_{10}H_{10}O$	162	16.479
21.814	Styryl-urea	$C_9H_{10}N_2O$	162.1	12.512
25.186	Undecanoic acid	$C_{11}H_{22}O_2$	186.2	5.523
25.756	Benzene,(2-nitroethenyl)	$C_8H_7NO_2$	149.1	4.456

Table 9. Summary of the quanitative phytochemical analysis of *Morus mesozygia Linn. Stapf*., in methanolic, ethanolic, aqueous twig extract

S/N	Sample Identity	Alkaloids (%)	Flavonoids (%)	Tannins (%)	Saponin (%)
1	Aqueous Twigs Extract	1.49	6.22	3.01	1.49
2.	Ethanolic Twigs Extract	2.55	18.10	3.36	20.31
3.	Methanolic Twigs Extract	0.98	22.99	2.99	20.50

Table 10. Quantitative analysis of carbohydrate phytochemicals content of methanolic, ethanolic and aqueous twig extracts of *Morus mesozygia Linn. Stapf*., (African Mulberry)

S/N	Sample Identity	CHO (%)
1.	Aqueous Twig Extracts	6.20
2.	Ethanolic Twig Extracts	18.22
3.	Methanolic Twig Extracts	7.39

Flavonoids is reported to act as anti-diabetic in a mechanism that works synergetically with its anti-oxidative properties that is beneficial to the central consumption of glucose and prevention of the glucose transporter activity from the intestine. The anti-diabetic effect exhibited may also be due to the presence of 5,8,11-Heptadecatrien-1-ol (Table 6), obtained from the GC-MS analysis of *Morus mesozygia* Linn. Stapf. Twigs. As phytol is reported to as a branched chain acyclic diterpene that are anti-diabetic, an heterodimer found in chlorophyll part of a plant of a gerannylgeraniol, that once metabolized in humans and mammals, gets converted into its natural precursor form of rexinoid, a substance that when in the liver gets into phytanic acid to ameliorate insulin insensitivity that stimulates muscle beta-oxidation and further enhances the uptake of glucose into cell membranes thereby suppressing the action of hepatic glucose production and inhibiting the action of TNF-α.

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File       :C:\msdchem\1\data\TWIG WATER.D
Operator   :
Acquired   : 17 Oct 2018  13:55     using AcqMethod SCAN.M
Instrument :    GCMS
Sample Name: TWIG WATER
Misc Info  :
Vial Number: 1
```

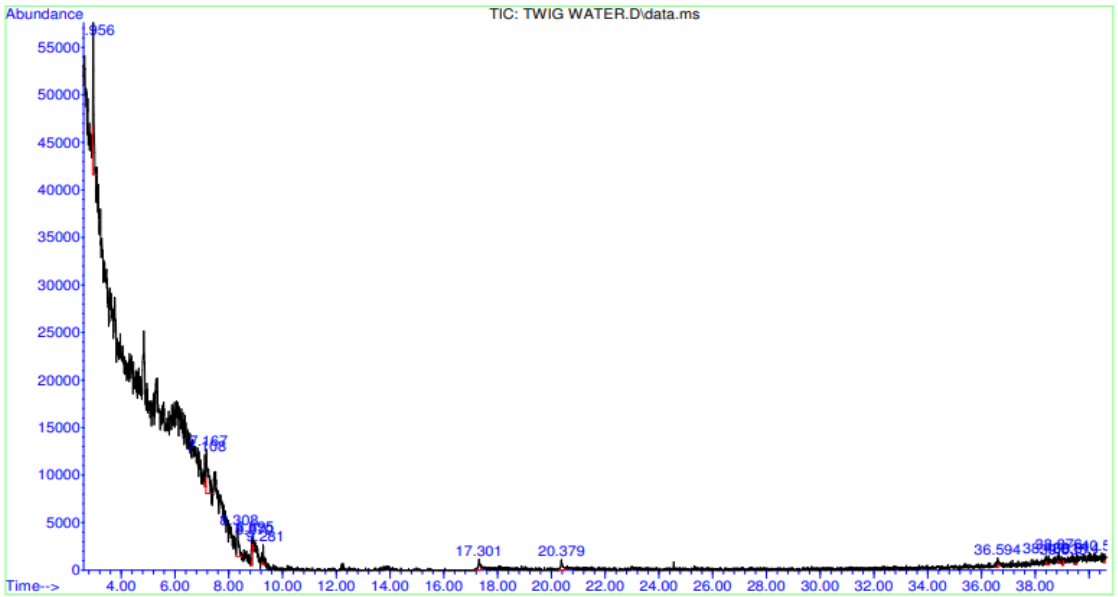

Fig. 2. Mass spectrum of aqueous extract of *Morus mesozygia* Linn. Stapf. Twig

```
File       :C:\msdchem\1\data\TWIG ETHANOL.D
Operator   :
Acquired   : 17 Oct 2018  13:06     using AcqMethod SCAN.M
Instrument :    GCMS
Sample Name: TWIG ETHANOL
Misc Info  :
Vial Number: 1
```

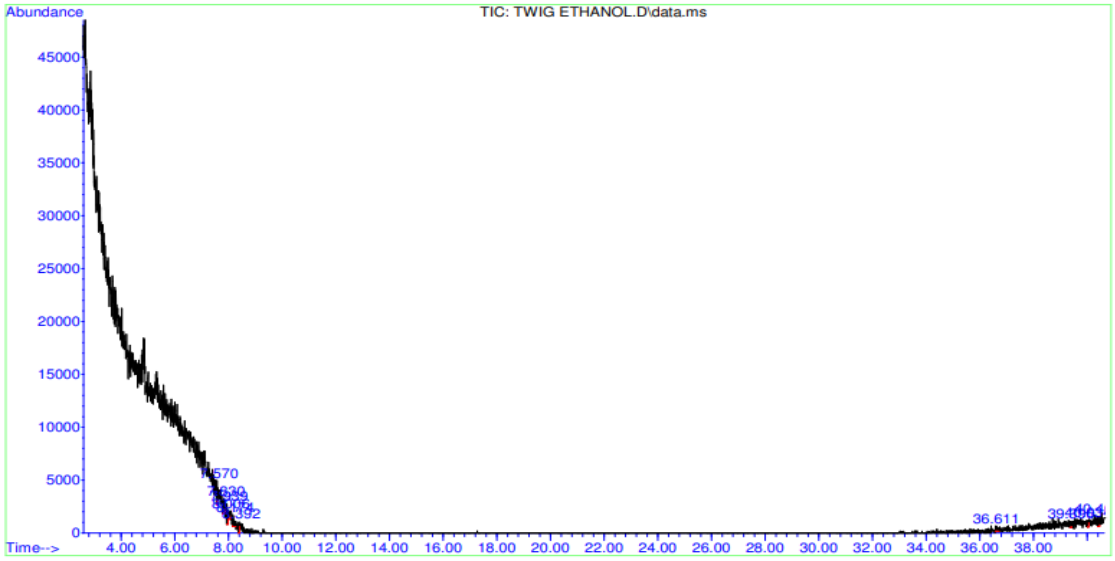

Fig. 3. Mass spectrum of ethanol extract of *Morus mesozygia* Linn. Stapf. twig

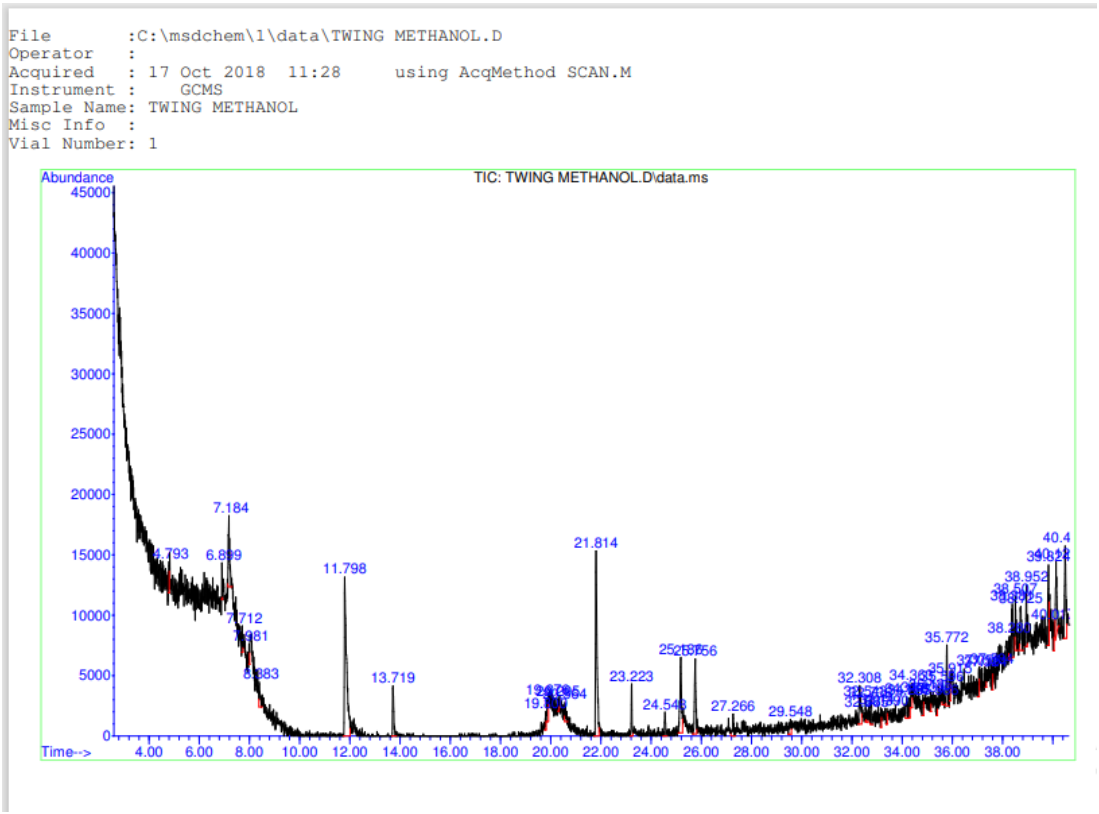

Fig. 4: Mass spectrum of methanol extract of *Morus mesozygia* Linn. Stapf. twig

Squalene and flavonoids are reported to have a suppressive action on TNF-α which enables glucose functioning in the cells. The anti-inflammatory exhibited by the ethanolic twig extract might have prevented endothelial apoptosis on the pancreatic beta islets integrity of the streptozotocin induced diabetic rat model, the action in mechanism is one in which squalene may have inhibited amylase as well as α-glycosidase, preventing the absorption of glucose in the intestine by sodium dependent glucose transporter likewise causing a stimulative effect in insulin secretion thus initiating a resultant effect of a reduction of glucose in the hepatic system. These findings are similar to the reports of [15] who reported a decrease in FBG, but was not consistent with the reports of [16].

This hepato-protective effect of aqueous twig extracts may be due to the presence of flavonoids (6.22%, Table 9). Flavonoid on hepato-protection has been reported to activate liver cells linked to gluconeogenic metabolism. This study is similar with the findings of [17].

Tannins have been reported to be an astringent, nephron-protective in a mechanism of action that has the tannins pulling cells together of the gallic esters of glucose in a manner that seem to extract the 'unwanted from the wanted'. However, this was similar to the reports of [18] that stated significant reductions in urea levels.

Tannins and flavonoids have been reported to be astringents that scavenge the unwanted presence of reactive oxygen species that are responsible for oxidative stress and inflammatory response on endothelial cells and apoptosis. This is similar to the reports of [19].

4. CONCLUSION

The qualitative and quantitative phytochemical analyses test results of *Morus mesozygia* revealed the presence of the substances like alkaloids, saponins, flavonoids, oils, phenolic compounds, tannins and some complex compounds discovered using GC-MS technique, in their varying concentrations for the three different extracts. More so, the methanolic twig extract revealed an increase of flavonoids reported to be anti-diabetic compared to that of the ethanolic and aqueous extracts of the twig part of Morus *mesozygia* Linn Stapf.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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