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Phytochemical Screening of Aqueous, Ethanolic and Methanolic Extracts of *Morus mesozygia* Linn. Stapf., Leaves

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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: This study focused on the phytochemical screening of aqueous, ethanolic and methanolic leaf extracts on the species *Morus mesozygia linn*.
Study Design: This study was 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 leaves were collected and washed with distilled water, air dried for seven days and milled into fine powder. Maceration method was use to extract the powdered leaf 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: Results showed that the powdered *Morus mesozygia* linn leaves contained alkaloids, flavonoids, saponins, carbohydrates, tannins, but not anthraquinones. The methanolic and aqueous leaf extracts contained high amounts of alkaloids, flavonoids, saponins, carbohydrates and tannins,

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while the ethanolic extract also contained high amounts of the aforementioned phytochemicals in the same proportion, but had saponins in moderate amounts. The result of the GC-MS analysis showed that the three extracts contained complex compounds in varying amounts.
Conclusion: Phytochemical screening test of Morus *mesozygia* has revealed the presence of the substances like alkaloids, saponins, flavonoids, oils, phenolic compounds, tannins and some complex compounds discovered using GC-MS technique.

Keywords: Phytochemical screening; aqueous; ethanolic; methanolic; Morus mesozygia linn. stapf.; leaves.

1. INTRODUCTION

Plants parts such as; fruits, seed, bark, leaves, and so on, have been used to cure many diseases since ancient time because they are known to contained certain bioactive compounds called phytochemicals. 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 [1,3].

The African mulberry *(Morus mesozygia* Linn. *Stapf.)*., an herb, is also an African species of the Morus genus plant amongst its temperate species such *as Morus alba* has been reported by the western Yoruba tribes of the Nigerian people to have medicinal value that include treatments of ulcer, veneral diseases as well as certain stomach pains. This study focused on the phytochemical screening of aqueous, ethanolic and methanolic leaf extracts of the species *Morus mesozygia* linn*. Staph.*

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Morus mesozygia Linn. (family Moraceae) fresh leaves 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* leaves 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 leaves 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 leaves using distilled water, absolute ethanol and methanol

Nine hundred and sixty grams (960 g) of dried powdered *Morus mesozygia linn* leaves 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, 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 [4] 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 leaf extracts were kept in a 4°C refrigerator prior to phytochemical screening.

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

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 [5]

Into a clean test tube was 5 ml of the methanolic, ethanolic and aqueous extracts *Morus mesozygia linn Staph*. leaves 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 leaves 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 [6]

1 ml each of the three leave 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 [6]

5 mls each of the three leave 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 [7]

5 g each of the aqueous, ethanolic and methanolic leave 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 leave 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 [8]

10 g each of the three leave 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 [7]

10 g each of the three different leave 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 [9]

0.1 g each of the three leave 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 phytochemical screening of aqueous, ethanolic and methanolic extracts of *Morus mesozygia Linn. S.* leaf revealed the presence of some secondary metabolites such as alkaloids, steroids tannins and so on. Qualitative phytochemical analysis of *Morus mesozygia Linn. Stapf.* leaf powdered samples showed that the leaf extract contains alkaloids, flavonoids, carbohydrates, saponins and tannins, (Table 1).

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

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

Key: Absent (-), Present (+)

In a previous study by [10], the phytochemical profile of several pecies of several Morus species showed that the dry powdered leaf extract of members of the genus Morus are rich in alkaloids, flavonoids and polyphenols. Carbohydrates detected in the powdered leaf extract, were missing in the methanolic leaf extract, but all the other phytochemicals were present, but in high quantities (Table 2).

The result also showed that the ethanolic leaf extract of the plant contained alkaloids, flavonoids, tannins in high quantities, while saponins were present in moderate amount, (Table 3).

The aqueous extract of *Morus mesozygia Linn. Stapf.* was also found to contained alkaloids, flavonoids, saponins and tannins in high amounts, (Table 4).

The phytochemical compounds detected are known to have medicinal importance. Different phytochemical components have been linked with various bioactive or medicinal role they play, especially in the management of certain disease conditions, [11].

For example, alkaloids have been reported as powerful poison and many alkaloids and flavonoids derived from medicinal plants show biological activities like, anti-inflammatory [11], antimalarial [12] antimicrobial [13], cytotoxicity, antispasmodic and pharmacological effects [14] and antiproliferative potentials [15]. The results also showed that the phenolic content of the plant contained alkaloids, flavonoids, tannins, saponins and CHO in different quantities, (Table 6).

The GC-MS analysis of this study demonstrated differences in chemical components of the aqueous, methanolic and ethanolic solvent extract types of the leaves of *MMLS*.

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

Compound Classes	Test	Observation	Inference
Alkaloids	Wagner's Reagent	Reddish brown color	+++
Flavonoids	Shinoda's test	yellowish color	+++
Saponins	Frothing test	Frothing head formed	+++
Tannins	Folin-Denis's reagent	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 leaves extracts

Compound Classes	Test	Observation	Inference
Alkaloids	Wagner's Reagent	Reddish brown color	+++
Flavonoids	Shinoda's test	yellowish color	+++
Saponins	Frothing test	Frothing head formed	++
Tannins	Folin-Denis's reagent	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 leaves extracts

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

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

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

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

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

Table 6. Phenolic content of dried powdered samples of *Morus mesozygia Linn. Stapf.*, (African Mulberry) leaves

S/N	Sample Identity	Alkaloids (%)	Flavonoids (%)	Tannin (%)	Saponin (%)	CHO %
1.	Dried powdered Leaf	2.72	2.80	2.36	16.3	22.00

Fig. 1. Graph of gas chromatography-mass spectrometry analysis of aqueous leaf extracts of *Morus mesozygia Linn.S*

The results revealed 'Twenty-two (22) chemical components from the Methanolic leaf extracts having the highest numbers of chemical components from which the top five with the highest percentage of components identified included: 4-Methyl-1-3-(-3-Nitrophenyl)- 6-Phenyl (24.8%), 3(2H)-Pyridazinone-6-Methyl (11.45%), 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol also known as Phytol (9.1%), 9,12,15-Octadecatrienoic acid (8.6%) (Tables 8 & 9 and Figs. 2 and 3).

Followed by Ethanolic leaf extract with Eighteen (18) components in which four with the highest percentage components included: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol also called phytol (41.61%), 9,12,15-Octadecatrienal (13.4%), Squalene (8.35%), Trans-Farnesol (3.39%) (Table 8) then the Aqueous extract with Six (6)

components which included:1,6,10,14-Hexadecatetraen-3-ol (32.21%), 2,3-Butanediol (21.50%), propanoic acid (21.50%), Phytol (12.15%)(Table 7).

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

RT	Component	Formula	MW	%
36.729	729 1,6,10,14 Hexadecatetraen-3ol	$C_{20}H_{34}O$	290.48	32.24
3.593	2,3-Butanediol	$C_4H_{10}C_{l2}0_2$	90.12	21.331
3.442	Propanoic acid	$C_6H_{12}C_{l2}0_2S$	215.1	21.150
7.175	Phytol	$C_{20}H_{40}O$	296	12.511

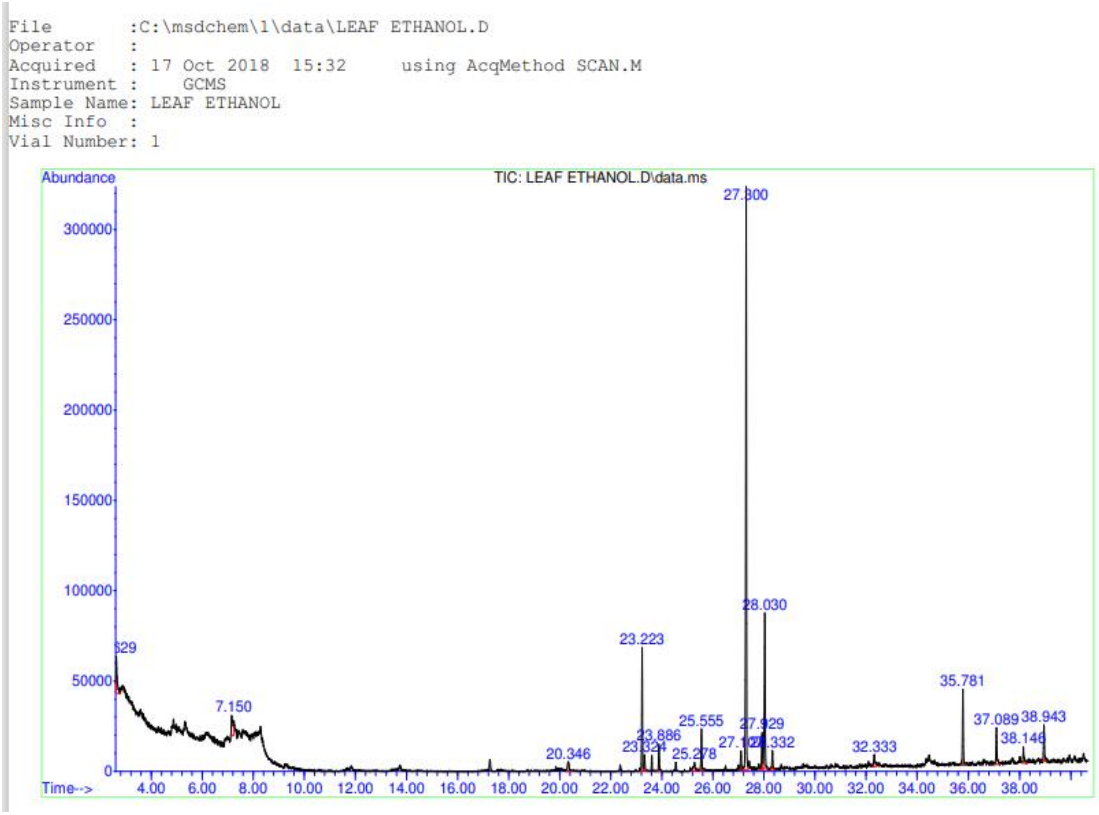

Fig. 2. Graph of gas chromatography-mass spectrometry analysis of ethanol leaf extracts of *Morus mesozygia Linn.S*

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

RT	Component	Formula	MW	%
27.107	3.7.11.15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5	41.613
28.030	9,12,15- Octadecatrienal	$C_{18}H_{30}O$	262.4	13.404
23.346	Neophytadiene	$C_{20}H_{38}$	278.5	8.253
35.781	Squalene	$C_{30}H_5O$	410.7	5.456
37.089	Trans-Farnesol	$C_{15}H_{26}O$	222.37	3.394

Table 9. Gas chromatography-mass spectrometry analysis of methanolic leaf extracts of *Morus mesozygia Linn. S.* (African Mulberry)

RT	Component	Formula	MW	%
7.159	4-Methyl-3-(3-nitrophenyl)-6-phenyl-5,6-dihydro-4H-(1,2,4,5)	$C_{15}H_{14}N4O_3$	298	24.800
17.267	3(2H)-Pyridazinone,6-Methyl	$C_5H_6N_2O$	110.11	11.454
8.274	Methoxyacetic acid	$C_{11}H_{22}O_3$	202.29	9.125
27.26	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53	8.622
27.098	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.46	7.439

Fig. 3. Graph of gas chromatography-mass spectrometry analysis of methanol leaf extracts of *Morus mesozygia Linn.S*

Table 10. Summary of the quantitative phytochemical analysis of *Morus mesozygia Linn.* Stapf., in methanolic, ethanolic, aqueous leaves and methanolic, ethanolic, aqueous extracts

S/N	Sample Identity	Alkaloids (%)	Flavonoids (%)	Tannins (%)	Saponin (%)
1.	Aqeous Leaves Extract	1.21	12.71	4.84	24.70
2.	Ethanolic Leaves extract	2.69	25.83	9.29	51.71
3.	Methanolic Leaves Extract	6.05	21.72	4.51	23.90

4. CONCLUSION

Phytochemical screening test of Morus *mesozygia* has revealed the presence of the substances like alkaloids, saponins, flavonoids, oils, phenolic compounds, tannins and some complex compounds discovered using GC-MS technique. Phytochemical compounds found in leaf extracts of the plant indicates its potential as an important source of medicine and also to improve the health of its users as a result of the presence of various compounds that are vital for good health.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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