COMPARATIVE SCREENING OF PHYTOCHEMICALS AND BIOACTIVE COMPOUNDS OF TREMA ORIENTALIS (LINN. BLUME) LEAF AND BARK EXTRACTS

Abstract:

Thisstudy compared the phytochemical constituents of the leaf with the Bark extracts of *T. orientalis*, using the same extraction solvents. The leaf and Barkof *T. orientalis* were harvested at Federal University of Technology, Akure forest, dried and pulverized into powder. Extract were prepared from the powdered plants using Methanol and N-hexane. The qualitative and quantitative phytochemical presents in the extracts were determined.The functional compounds of the leaf extract weredetermined by Fourier TransmissionInfrared Spectrometry (FT-IR). Percentage yield of Methanol was better than N-hexane for both plant parts. The phytochemicals revealed includes: Tannins, Saponins, Flavonoids, Steroids, terpenoids and cardiac glycosides. Steroids are present in Leaf extracts but absent in Bark extract, while Saponin is only present in methanol extract of Bark of the plant. Quantitative analysis revealed that terpenoids have the highest amount with 22.90 ± 0.03 mg/g in methanol extract and 28.09 ± 0.07 mg/g in N-hexane, compared with Bark extract that has 22.22 ± 0.09 mg/g in methanol extract and 23.38 ± 0.04 mg/g in N-hexane extracts. Higher quantity of phytochemicals are present in the leaf compared with the Bark of T. orientalisThe Fourier Transformed Infrared spectrometry analysis, FT-IR, unveiled the organic compounds available in the extracts, which are: aliphatic primary alcohol, secondary alcohol,aliphatic primary amine, alkane,alkene, carbon dioxide, deta-lactam, phenol, and halo compound. These results indicate that *T. orientalis*is promising in the choice of medicinal plant fortherapeutic research.

Keywords: Antibiotic resistance, *Trema orientalis*, Zone of inhibition, Medicinal plant, phytochemical, methanol, N-hexane, Fourier Transmission.

1.0 Introduction

Medicinal plants are a major source of compounds of therapeutic value; they contain different phytochemical compounds resulting in numerous pharmacological activities**[1, 12]**.Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans **[1, 2]**. They protect plants from disease and damage, and they contribute to the plant's color, aroma and flavor.They are essential to human's health globally. Many of these indigenous medicinal plants are used as spices and food and herbs. Most of the medicinal plants contain a number of chemical constituents such as flavonoids, alkaloids, tannins, saponins, steroids, terpenoids, carotenoids etc.[3, 4]. *Trema orientalis* is an evergreen tree which belongs to the family Ulmaceae. It has been used extensively in various ways. It has been applied in folk medicine in the treatment of respiratory, inflammatory, and helminthic diseases. Almost every part of the plant is used as medicine in various parts of Africa[5].*T. Orientalis*plant is used in various parts of Africa and Madagascar for medicinal purposes. The young leaves are eaten as spinach by the Zulus in South Africa, who also use the roots and stem bark as traditional medicine[6]. The fruit, leaves, bark, stem, twig and seeds are extensively used in traditional medicine [7].The root of *T. orientalis* plants is used in folk medicine for treatment of trauma, blood stasis, hematuria and bleeding of intestines and stomach. The stem bark decoctions are applied as vermifuge, also in the treatment of dysenteries. Infusion of the Stem bark and leaf decoction of the *T. orientalis* are used in treating fever, the decoction is gargled, drank or inhaled to relieve toothaches[3]. The stem and leaf are also reported to be effective against malaria, pain,muscle weakness and bone aches even venereal disease. Both, the stem bark and leaf decoctions can be used as a gargle, inhalation, drink, vapor bath for relieve of toothache [3, 8]. The leaves of *T. Orientalis* in combination with other plants are reported to treat Jaundice, bronchitis, pneumonia and pleurisy and cough[3].

These activities are presumed to be as a result of the phytochemicals that are inherent in the plant, hence the study, to identify and compare the phytochemicals present in the Methanol and N-Hexane extract of the leaf and Bark of *T. Orientalis*.

2. 0 Materials and methods

2.1 Collection and Preparation of Plant Materials:

Fresh Leaves and barks of *Trema orientalis* were harvested atFederal University of Technology, Akure, FUTA, forest. Authentication of the samples was done at the Department of Crop Science, FUTA. The leaves and barks were washed, cleaned, chopped into pieces and air dried for two and three weeks respectively. The dried plant parts were pulverized in a milling machine (Dietz motorren, 7311 Dettingen, Teck, West Germany).

2.2 Preparation of Plant Extract:

A 500g of ground samples of the plant were soaked in 3liters of methanol, and n-hexane, as solvents, for seventy-two hours, alongside with thorough stirring using stirrer. The soaked samples were filtered first with muslin cloth and then by No 1Whatman filter paper. The extract was concentrated by exposing at room temperature 27± 2^{o}C, leaving the crude extract behind. The crude extract was scraped into a cleaned small transparent container, and stored in the refrigerator with temperature -4^{0}C prior to use[9, 10, 11].

2.3 PHYTOCHEMICAL SCREENING

2.3.1 Qualitative analysis

2.3.1a. Alkaliod determination

A 0.5g of the extract was shaken with 50 mL of 1% aqueous HCl on a steam water bath, 1mL of the filtrate was treated with a few drops of Dragendorf reagent; blue black turbidity indicates the presence of Alkaloid.

2.3.1b. Saponin determination

A 0.5g of extract was shaken with distilled water in a test tube frothing which persist on warming was taken as preliminary evidence for the presence of saponins.

2.3.1c. Tannin determination

A 0.5g of the extract was thoroughly shaken with 100 mL of distilled water, filtered and ferric chloride reagent was added to the filtrate, a blue black green or blue green precipitate was taken as evidence for presence of tannin.

.2.3.1d. Flavonoid determination

A 0.5g of the extract was stirred with 20mL of dilute ammonia solution a yellow colouration was observed, the disappearance of the yellow colour after the addition of 1ml conc. H_2SO_4 indicate the presence of flavonoid.

2.3.1e. Steroid determination

A 20 mL of acetic anhydride was added to 0.5g of the extract and filtered, 2 mL of conc. H_2SO_4 was added to the filtrate. A colour change from violet to blue or green which indicates the presence of steroid.

2.3.1f. Terpenoid determination

A 0.5g of the extract was mixed with 20 mL of chloroform and filtered 3mL of conc. H_2SO_4 was added to the filtrate to form a layer. A reddish brown coloration at the interface indicates the presence of terpenoids.

2.3.1g. Cardiac glycosides

The followings were carried out to test for cardiac glycosides:

2.3.1h. Legal's test- The extract was dissolved in 5mL pyridine and a few drops of 2% sodium nitroprusside with few drops of 20% NaOH were added. A deep red colouration which faded to a brownish yellow indicates the presence of cardiac glycosides.

2.3.1i. Lieberman's test– Acetic anhydride (20mL) was added to 0.5g of the extract and filter, 2mL of conc. H_2SO_4 was added to the filtrate. There was a colour change from violet to blue or green which indicated the presence of steroids nucleous (i.e aglycone portion of the cardiac glycosides.)

2.3.1j. Salkowski's test- A 0.5g of the extract was mixed with 20mL of chloroform and filtered. Conc. H_2SO_4 (3mL)was added to the filtrate to form a layer. A reddish brown colour at the interface was observed which indicate the presence of steroidal ring.

2.3.1k. Keller- killiani's test- A 0.5g of the extract was dissolved in 2mL of glacial acetic acid containing one drop of ferric chloride solution. This was then under layer with 1mL of conc. H_2SO_4 a brown shown up at the interface indicated the presence of a deoxy sugar, characteristic of Cardiac glycosides.

2.3.2 QUANTITATIVE ANALYSIS

2.3.2a. Tannin determination

About 0.2g of finely ground sample was weighed into a 50mL sample bottle, 10mL of 70% aqueous acetone was added and properly covered and shaken for 2hours at 30 ^{0}C. Each solution was then centrifuged and the supernatant stored in ice. A 0.2mL of each solution was pipetted into the test tube and 0.8mL of distilled water was added. Standard tannin acid solutions were prepared from a 0.5mg/mL of the stock and the solution made up to 1mL with distilled water. A 0.5mL of Folin ciocateau reagent followed by 2.5mL of 20% $Na_2CO_3$was added to the solutions. The solutions were vortexed and allow to incubate for 40minutes at room temperature, its absorbance was read at 725nm against a reagent blank concentration of the same solution from a standard tannic acid curve was prepared.

2.3.2b. Determination of saponin

A 2g of the sample was weighed into a 250mL beaker and 100mL of Isobutyl alcohol or (But-2-ol) was added. The mixture was taken to the shaker to vortex the mixture for 5hours so as to ensure uniform mixing. The mixture was then filtered with No 1 Whatman filter paper into 100mL beaker holding 20mL of 40% saturated solution of magnesium carbonate ($MgCO_3$). The mixture gotten was filtered through No 1 Whatman filter paper. 1mL of the colourless solution was pipette into 50mL volumetric flask, 2mL of 5% iron (iii) chloride ($FeCl_3$) solution was added and made up to the mark with distil water. It was allowed to stand for 30minutes and the absorbance was read against the blank at 380nm.

2.3.2c. Determination of cardiac glycosides

The procedure described by [**13**] was used. About 10mL of the extract was pipetted into a 250mLconical flask. Not less than50mL chloroform was added and shaken on vortex mixer for 1hour. The mixture was filtered into 100mL conical flask. Exactly 10mL of pyridine and 2mL of 29% of sodium nitroprusside were added and shaken thoroughly for 10minutes. An amount of 3ml of 20% NaOH was added to develop a brownish yellow colour, glycosides standard (Digitoxin). A concentration which range from 0 – 50mg/mL were prepared from stock solution whereafter the absorbance was read at 510nm.

2.3.2d. Determination of terpenoid

The procedure described by [**13**] was used. An amount of 0.5g of finely grounded sample was weighed into a 50mL conical flask 20ml of chloroform: methanol (2:1) was added, the mixture was shaken thoroughly and allowed to stand for 15minutes at room temp. The suspension was centrifuged at 3000 r/pm, the supernatant was discarded and the precipitate was re-washed with 20MLchloroform: methanol (2:1) and then re-centrifuged again.The precipitate was dissolved in 40ml of 10% SDS solution. About 1mL of 0.01M ferric chloride was added and allowed to stand for 30minutes before taken the absorbance at 510 nm.

2.3.2e. Determination of steroid

A quantitative determination of steroid was determined by weighing a 5g of the finely powdered sample into 100 mL conical flask and 50 mL of pyridine was added to it, and vortexed for 30minutes at room temperature, 3ml of 250 mg/mL metallic copper powder or copper (1) oxide was added and incubated for 1hour in the dark and the absorbance was measured at 350nm against reagent blank[14**]**.

2.4 Fourier – Transform Infrared Spectrophotometer (FTIR) of fraction

The functional groups were identified by interpreting the infrared absorption spectrum. Infra-red analysis was performed using infra-red spectrophotometer (Perkin-Elmer spectrum bx) at the Multi-Disciplinary Central Research Laboratory, Federal University of Technology, Akure (FUTA). An aliquot portion of purified extract was placed on fused sodium chloride (NaCl) cell. It was cautiously dropped on cell clamped loosely and fixed on the infra-red beam. The infra-red data was compared to the IR manual [9, 11].

3. 0 Results and Discussion

3.1 Percentage yield of *Trema orientalis* leaf and Bark extract by solvents of extraction

Table 1 presentsthe amount of crude extract of leaves and barks obtained from 500g of the powdered sample. The amount of recovered extracts and percentage yield by both methanol and n-hexane were 8g, and 1.6%, 6g and 1.2% respectively for the leaves sample. An amount of 50g and 10%, 1g and 0.2% by both methanol and n-hexane were recovered for the bark sample respectively. From this study, methanol extracted more effectively than N-hexane. [14] reported methanol to extract favorably compared with N-hexane.

Table 1: Percentage yield of *Tremaorientalis* leaf and Bark extract by solvents of extraction

Plant part	Solvents	Powdered sample(g)	Extract recovered(g)	Percentage yield (%)
Leaf	Methanol	500	8	1.6
	N-hexane	500	6	1.2
Bark	Methanol	500	50	10
	N-hexane	500	1	0.2

3.2Phytochemical Analysis of *Trema orientalis* leaf and Bark

The phytochemical analysis of the extracts of *T. orientalis*is revealed in Table 2. The secondary metabolites present in the leaf and bark extracts are tannin, flavonoid, steroid, terpenoid, and cardiac glycoside(Cardenolides); while alkaloid and Phlobatannins are absent in all the extracts. Saponin was present in only the Bark methanol extracts and Steroids are only present in the Leaf extracts. Previous studies on *T. orientalis* showed similar phytoconstituents[15, 16.]
Table 3 show the quantitative analysis of leaf and bark extracts of *T. orientalis*. The screening exercise unveiled that terpenoid is the most abundant phytochemical compounds with higher

concentration in the Bark of the plant with 22.90 ± 0.03 mg/g from methanol extract and 28.09± 0.07 mg/g from N-hexane extract compared to the Leaf part with 22.22 ± 0.09 mg/g from methanol extract and 23.38 ± 0.04 mg/g from N-hexane extracts. Phytochemicals have pharmacological properties, for example, antibacterial, antipyretic, cell reinforcement, anticonvulsant, antiplasmodial, antiepileptic activityantidiabetic and pain relieving properties [3, 17].Terpenoids are known to have extreme aromatic aromatic qualities. They play a role in traditional herbal sonedies and are may have Antibacterial,Antineoplastic and other Pharmaceuticalfunctions[18].It has been reported that flavonoids are free radical scavengers that preventoxidative cell damage, and have strong anticancer activities [19]. Flavonoids are also known to have biologicalliver toxins, tumors, viruses and other microbes. The presence of Tannins in medicinal plants is reported to enhance their use for the treatmentof intestinal disorders such as diarrhoea and dysentery[15].

Steroids have been reported to have antibacterial properties also implicated in compounds as sex hormonesand Cardiac glycosides are important class of naturally occurring drugs whose actions helps in thetreatment of congestive heart failure [20, 21].

Table 2: Qualitative phytochemical Analysis of *Trema orientalis* leaf and Bark

Phytochemicals	LEAF		BARK	
	MET	NH	MET	NH
Saponin	-	-	+	-
Tannin	+	+	+	+
Phlobatannin	-	-	-	-
Flavonoid	+	+	+	+
Steroid	+	+	-	-
Terpenoid	+	+	+	+
Alkaloid	-	-	-	-
Cardiac glycoside				
Legal test	+	+	+	+
Keller killiani's test	+	+	+	+
Salkwoski test	+	+	+	+
Lieberman test	+	+	+	-

Key: + (present), - (absent)
MET- Methanol extract, NH- N-Hexane extract

Table 3: Quantitative phytochemical Analysis of*Trema orientalis* leaf and Bark extract

	Leaf		Bark	
Phytochemicals	MET (mg/g)	NH (mg/g)	MET (mg/g)	NH (mg/g)
Saponin	-	-	7.94± 0.05	-
Tannin	2.98 ± 0.12	6.63± 0.14	4.15± 0.06	4.64± 0.07
Phlobatannin	-	-	-	-
Flavonoid	3.74 ± 0.13	7.21± 0.10	0.39± 0.08	0.71± 0.11
Steroid	10.94± 0.06	13.83± 0.03	-	-
Terpenoid	22.22± 0.09	23.38 ± 0.04	22.90± 0.03	28.09± 0.07
Alkaloid	-	-	-	-
Glycoside	4.74± 0. 08	7.33± 0.11	2.32± 0.09	8.05± 0. 014

Key: MET (methanol), - (absent), NH (n-hexane)

3.5 The Fourier Transmission Infrared Spectrometry of methanol and n-hexane leaf extracts.

Figures1 and 2shows the graphical representationsof the infra-red analysis of the functional groups of *T.orientalis*methanol leaf extract and N-hexane leaf extract respectively with their different peaks. The wavelength measured represents functional group and the compound names are presented in Tables 4 and 5.The FT-IR reveals the organic compounds available present in the extracts. The peculiar compounds in both extracts include: aliphatic primary alcohol, secondary alcohol, aliphatic primary amine, alkane, alkene, carbon dioxide, deta-lactam, phenol, and halo compound. These functional groups promote the activity of the leaf. As OH group has the ability of forming hydrogen bonding capacity, presence of OH group particularly in methanol extract probably indicates the higher potential of methanol extract towards inhibitory activity against microorganisms **[10].**These phytochemicals are important markers in the identification of medicinalplants[22] The presence of a phenol ring, alkane group, 0-H Amines, suggest the presence of the possible compounds, flavonoids, Saponins, and Phenolic compound in the extracts [23, 24].

Table 4: FTIR spectra of figure 1

S/No	Wave number (cm^{-1})	Group	Compound
1	3390	O-H	Aliphatic primary Amines
2	2930	C-H	Alkanes
3	2850	C-H	(Alkanes)
4	2330	O=C=O	CO_2
5	1650	C=O	δ-lactam
6	1465	C-C	Alkanes
7	1360	C-O	(Phenol)
8	1250	C-N	Amine
9	1088	C-O	Primary alcohol
10	940	C=C	Alkenes
11	710	C-Cl	Alkene

Table 5: FTIR Spectra of figure 2

S/No	Wave number (cm^{-1})	Group	Compound
1	3380	O-H	Aliphatic primary Amines
2	2930	C-H	Alkane
	1650	C=O	δ-lactam
3	1480	C-C	Alkane
4	1360	C-O	Phenols
5	1250	C-N	Amines
6	1096	C-O	Secondary alcohol
7	940	C=C	Alkene
8	660	C-Br	Halo compounds

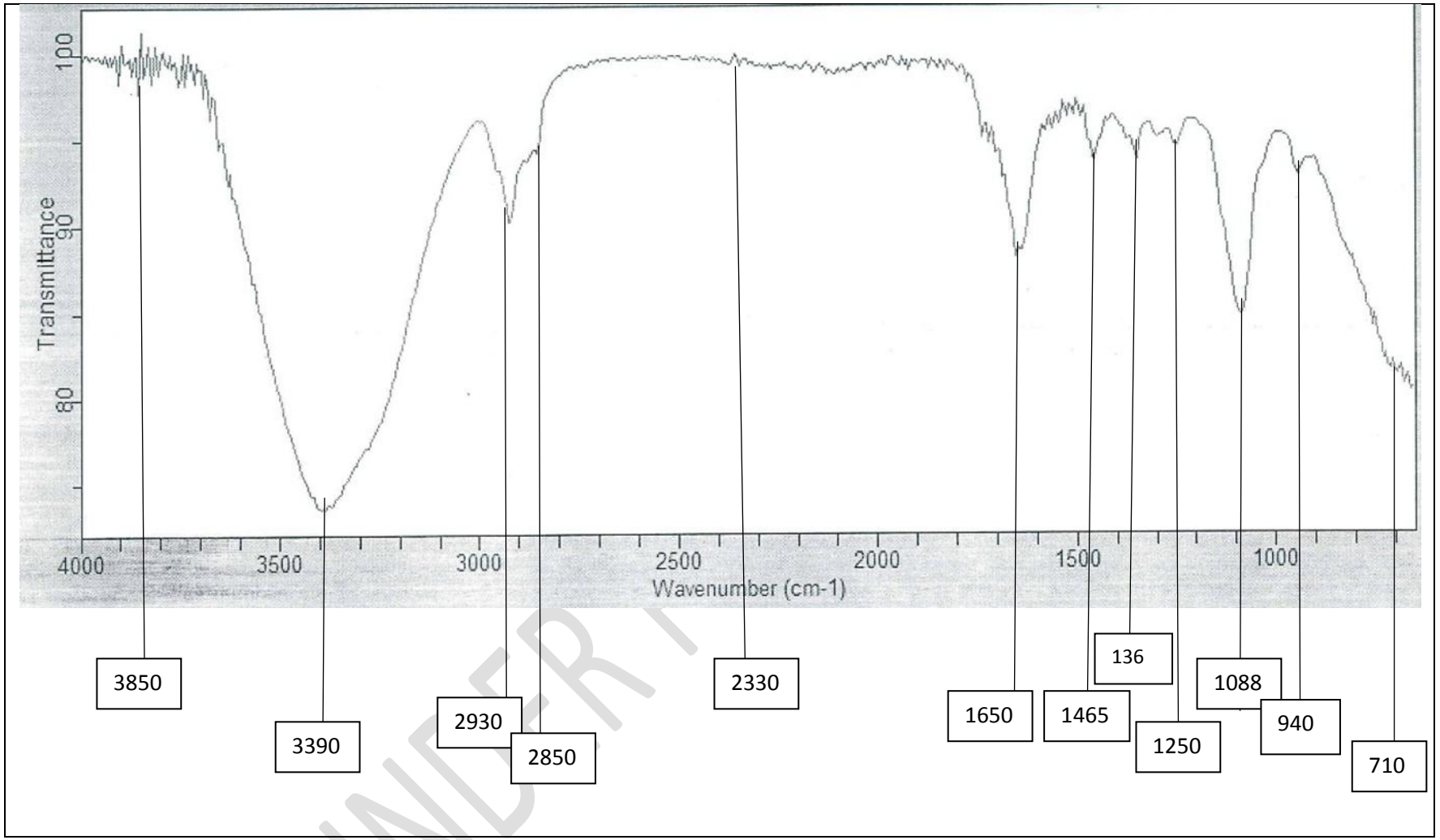

Figure 1: **The Fourier Transmission Infrared Spectrophometry of methanol leaf extract .**

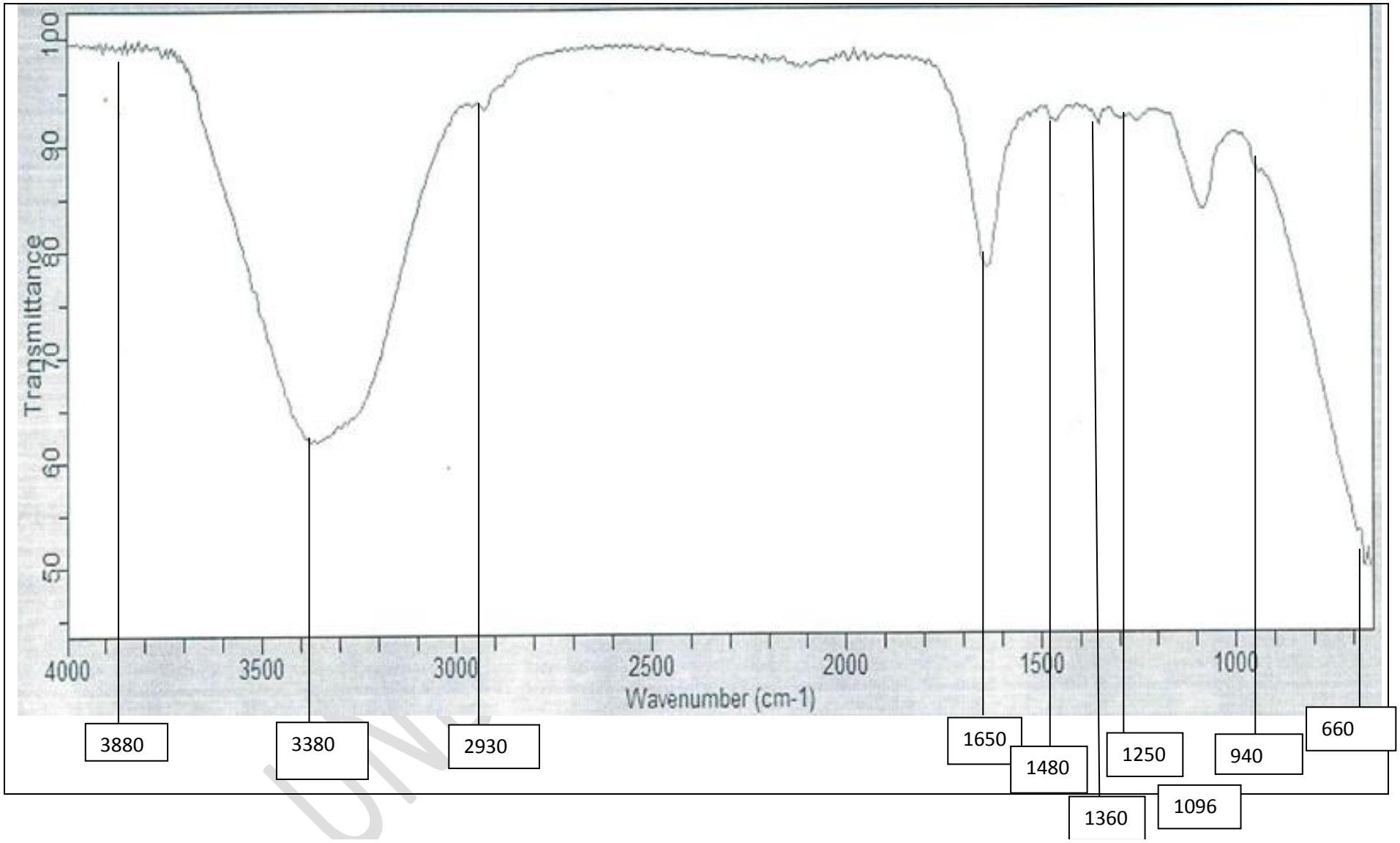

Figure 2: The Fourier Transmission Infrared Spectrometry of N-hexane leaf extract

CONCLUSION

The study revealed the presence of essential phytochemicals Tannins, Cardiac glycosides, Flavonoids, and terpenoids in all the extracts. Terpenoid is more abundant in the methanol and N-Hexane extracts of Leaf and Bark of *T. orientalis*. The bioactive compounds in the leaf of *T. orietalis* are Amines, Alkanes and Alkenes. The presence of these essential phytochemicals validates *T. orientalis* as an important medicinal plant.

REFERNECES

1. Accra, Ghana: The Adventist Press; 1992. Ghana Herbal Pharmacopeia; pp. 141–43.
2. **Akendengue, J.** Entheogenic drugs, their plant sources and history. *Journal of Ethnopharmacology*.**1992**; 37: 165**.**
3. **Adinortey MB, Galyuon IK, Asamoah NO. Trema orientalis Linn. Blume: A potential for prospecting for drugs for various uses. Pharmacognosy reviews. 2013 Jan;7(13):67.**
4. **Edoga HO, Okwu DE, Mbaebie B O.** Phytochemicals constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*. **2005;** 4(7):685-688.
5. Yanes CV. Germination of a pioneer tree from Equatorial Africa. Turrialba. 2007; 27:301–2.
6. Coates PK. 1st ed. Cape Town, Johannesburg: C. Struik Publishers CapeTown, Johannesberg; 1977. Trees of Southern Africa.
7. Iwu MM. Boca Raton, Florida: CRC Press Inc; 1993. Handbook of African Medicinal Plants; pp. 251–2.
8. **Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A.** Agroforestrydatabase: A tree reference and selection guide.**2009;** version 4.0. http://www.worldagroforestry.org/af/treedb.
9. **Zheng W, Wang SY.** Oxygen radical absorption capacity of phenolics in blueberries, cranberries, chokeberries and lingonberries. *Journal of Agricultural Food Chemistry*.**2003;** 51:502-509.
10. **Junaid SA, Olabode AO, Onwuliri FC, Okworiu A E, Agina SE.** The Antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. *African Journal of Biotechnology*.**2006;** 5(22): 2315-2321
11. **Ashokkumar R, Ramaswamy M. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants *International Journal of Current Microbiology Applied Science*.2014; 3(1): 395-406.**
12. **Sofowora EA.** Medicinal plants: Traditional medicine in Africa (3rd edition).Spectrum Books Limited, Ibadan, (Nigeria). **2008;** Pp 199-204.
13. **Aruoma OI**. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutation Research*.**2003;** 523 (524):9-20.
14. Tanimowo WO, Osiyemi O A, Ashidi JS. Antibacterial and phytochemical activity of Anthocleista djalonensis (A. Chev). *Imperial Journal of Pharmacology and Toxicology*.2011; 21-28.
15. Akin-Osanaiye BC, Ahmad R. Phytochemical analysis, anti-microbial screening and antioxidant activity of the seed of trema orientalis. Academic Journal of Science. 2014; Vol 3(1):211–217.
16. Parvez A, Shaheen SM. *A phytochemical and pharmacological.Review on Trema:A*

Potential Medicinal Plant.2019; (February 2020).

17. Geetha KM, Vishnnupriya K, Fareshteh J, Murugan V. Preliminary Phytochemical Investigations and Antiepileptic Activity Of *Trema orientalis* (Linn.) *International Journal of Pharmaceutical Science and Research.*2019**;***Vol. 10(8): 3957-3962.*

18. Yamunadevi MEG, Wesely JM. P. studies on the terpenoids of medicinally important plant A. lanata L. using H. A. P. J. of T. B. S.-S. (n.d.). No Title. *Biomedicine. S220- S225*1. Accra, Ghana: The Adventist Press; 1992. Ghana Herbal Pharmacopeia.2011; pp. 141–43.

19. Okechukwu PU, Okwesili FN, Parker E J, Abubakar B, Emmanuel CO, Christian EO. Phytochemical and acute toxicity studies of Moringa oleifera ethanol leaf extract. *International Journal of Life Science BiotechNology and Pharma Research*. 2013; *2*(2), 66-71.

20. Adjileye RA, Amoussa AMO, Lagnika L. Trema orientalis L. and Dialium guineense Wild. used to manage hypertension in Benin: Phytochemical study and antioxidant activity. *Journal of Medicinal Plants Studies*.2019; *7(3)*,(3), 43–48.

21. Gabriel AF, Omoniyi AO, Ezeani SC. (2016). *Scientific Approach on the Antimicrobial Potentials of Bioactive Phytochemicals of Trema Orientalis Leaves and Stalk*. 2016;*III*(12), 12972–12981.

22. Lestyo WY, Retnaningtyas Nuri, Hilmia L.Analysis of Flavonoid in Medicinal Plant Extract Using Infrared Spectroscopy and Chemometrics. Journal of Analytical Methods in Chemistry. 2016; Article ID 4696803 | 6 pages | https://doi.org/10.1155/2016/4696803

23. Onifade A K, Agunloye OO. Antibacterial Assessment of Crude and Fractionated Extracts of *Vernonia amygdalina* Leaf against Multiple Antibiotic Resistant Bacteria of Wound Infection . *Asian Journal of Research in Medical and Pharmaceutical Sciences.2019; 7(1): 1-12.*

24. James SA, Omwirhiren EM, Ladan Z, Alhassan N, Mohammed SN. Spectroscopic characterization of the antidiabetic properties of partially purified ethanolic extract of *Hibiscus Cannabinus*, *Vernonia Amygdalina, Murraya Koenigii* and *Telfairia Occidentalis.* Journal of Pharmacognosy and Phytochemistry. 2018;7(2):1508-1519.