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Phytochemical Screening, GC-MS And FTIR Analysis of Ethanol Extract of *Piliostigma thonningii (*schum Milne—Redth*)* Leaf

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Abstract *The present investigation was carried out to investigate the phytoconstituent of piliostigma thonningii leaf through phytochemical screening GC-MS analysis and Fourier Transform Infrared Spectroscopy (FTIR). Cold extraction method was used to extract the organic constituent of the plant leaf using ethanol as a solvent. The result obtained indicated a yield of 4.6 g (3.06 %). Preliminary phytochemical screening revealed the presence of flavonoid, terpenoid, cardiac glycosides, tannins, phytosterols, phlobatannins and alkaloid. The GC-MS analyses of ethanol leaf extracts showed the presence of 64 components based on separation of individual peaks through the column with respect to retention time (R_t) and area under the respective peaks. The prominent molecular functional vibration of chemical groups was also determined. The peak at 3250.2 cm^{-1} was assigned to hydroxyl vibration in alcohol while the band at 2918.5cm^{-1}, 2847.7 cm^{-1}, and 1461.1 cm^{-1} were attributed to the presence of alkanes.*

Key words: *Phytochemical identification, piliostigma thonningii leaf, screening, GCMS, FTIR*

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1.0 Introduction

Studies have shown that many plants have chemical components and biological activities. The most important of these bioactive constituents of plant are alkaloids, flavonoids, terpenoid, steroids, tannins, and saponins (Cody, 1985). Flavonoids are the commonest and widely distributed plant's phenolic compounds, occurring virtually in all plant parts, particularly the photosynthesizing plant cells (Koes, *et al*, 2005). *Piliostigma* and other species in the genus have been reported to have a wide range of usefulness to mankind ranging from food to medicinal purposes (Ibewuike *et al.*, 2010). The medicinal uses include treatments of loose stool in teething children, wound dressing, ulcers, worms' infestation, arrest of bleeding, inflammations, bacterial infections, gonorrhea, stomach ache, headache, etc. (Burkhil, 2010, Ozolua *et al.*, 2012). The roots and twigs have been used locally in the treatments of dysentery, fever, respiratory ailments, snake bites, hookworm and skin infections while the leaf extract has been found to be useful for the treatment of malaria (Kwaji *et al.*, 2010). In spite of the numerous traditional benefits of this plant, researches is relatively scanty on the its active components and most of the studies carried out on the plants uses classical analytical methods, The use of instrumental methods such as gas chromatography mass spectrophotometer and Fourier Transformed Infra-Red (FTIR) techniques can be better than classical analytical methods because of the high sensitivity and precision that can be derived instrumental methods. Therefore, the present study seek to screened ethanol extract of *piliostigma thonningii* leaf for their phytochemical constituents and to know the functional groups (using FTIR) in the extract as well as chemical constituents that can pass through a chromatography column (using GCMS).

2.0 Materials and Methods

2.1 Plant collection and extraction

Samples of *piliostigma thonningii* leaf were collected from Oboroke, Ihima village in Okehi LGA of Kogi State. The samples were identified by a Botanist in the herbarium of Federal University Lokoja where it was given voucher number 0130. The identified samples were dried to constant weight after which it was grounded to powered form. 150 g of the powdered sample was macerated using 400 ml of ethanol. After 72 hours of soaking in ethanol, the solution was decanted and filtered using Whatman No. 1 filter paper. Rotary evaporator was used to extract the organic portion of the extract that was used for further analysis.

2.2 Phytochemical screening

This screening was carried out for identification of carbohydrates, tannins, cardiac glycoside, alkaloids, saponins, phytosterol, phlobatannins, glycosides, flavonoids, steroid, resins and terpenoid. The method reported elsewhere was adopted for the screening (Edeoga *et* al., 2005; Njoku and Obi, 2009,=Yadav and Agarwala, 2011, Ayoola *et al*., 2008, Siddiqui *et al*., 2009, Egwaikhide and Gimba, 2007,Roopashree *et al*.,2008, and Chuckwu *et al*., 2012).

2.3 GC-MS analysis

The GC-MS of ethanol extract of *Piliostigma Thonningii* leaf was conducted using Agilent equipment 7890 A, designed by Mass Hunter to identify the volatile compounds from the plant parts. The equipment consists of inert capillary tube having a dimension of 30 mm × 0.25 mm ID × 0.25 µm film; helium as a carrier gas flowing at 1.0 mL/min. The operating temperature of the injector was 250 °C, while the temperature of the oven was increased gradually from 50 °C to 300°C at a rate of 10 minutes/°C. The NIST library data was employed to identify the components from the peak areas.

2.4 FTIR analysis

Fourier transform infrared spectrophotometer (FTIR) is one of the most powerful tools for identifying the types of chemical bonds (functional groups) present in compounds. Dried powders of the ethanol leaf extract were used for FTIR analysis. carbohydrate saponins glycoside and resin were absent.

Tannins is a major constituent of *Piliostigma thonningii* leaf. It has a documented history of been useful for the treatment of bacterial infection of the KBr was used in the preparation of the sample. The molecular functional vibration of chemical groups present in the sample was recorded with Happ-Genzel FT-IR.

3.0 Results and Discussion

The result of the maceration showed that the ethanol extract yielded 4.6 g (3.06%) as shown in Table 1

Table 1: Amount of extract from *Piliostigma thonningii* leaf

Solvent	Weight of the Extract	Percent Yield (%)
Ethanol	4.6 g	3.06 %

3.1 Phytochemical screening

The preliminary phytochemical screening of ethanol extract of *Piliostigma thonningii* leaf carried out to identify phytochemicals including carbohydrates, steroids, flavonoids, tannins, alkaloids, cardiac glycosides, phlobatannins, phytosterols and saponins yielded results that are recorded in Table 2.

Table 2: Phytochemical analysis of the *Piliostigma thonningii* leaf extract

S/N	Phytochemical	Ethanol leaf extract
1	Flavonoid	+ve
2	Terpenoids	+ve
3	Cardiac glycosides	+ve
4	Tannins	+ve
5	Steroid	
	a. Mayer's test	+ve
	b.Wagner's test	-ve
6	Saponins	-ve
7	Phlobatannins	+ve
8	Phytosterols	+ve
9	Alkaloids	
	a. Mayer's test	+ve
	b. Wagner's test	+ve
10	Carbohydrates	-ve
11	Glycosides	-ve
12	Resins	-ve

**** +ve = present, -ve = absent**

The results obtained (Table 2) revealed the presence of flavonoid, terpenoid, cardiac glycosides, tannins, phytosterols, phlobatannins and alkaloid. However, bladder. Also, some flavonoids have anti-tumor, anti- bacteria and anti-fungal properties, they are used in domestic veterinary medicine, particularly in form of ointment for treating dermal diseases (Trease and Evans, 2010).

Fig. 1 shows the GCMS of ethanol extract of *Piliostigma thonningii* leaf while Table 3 presents information deduced from the spectrum. The results of GC-MS analyses of ethanol extract of the *Piliostigma thonningii* leaf showed the presence of 64 components with various retention time and concentrations.

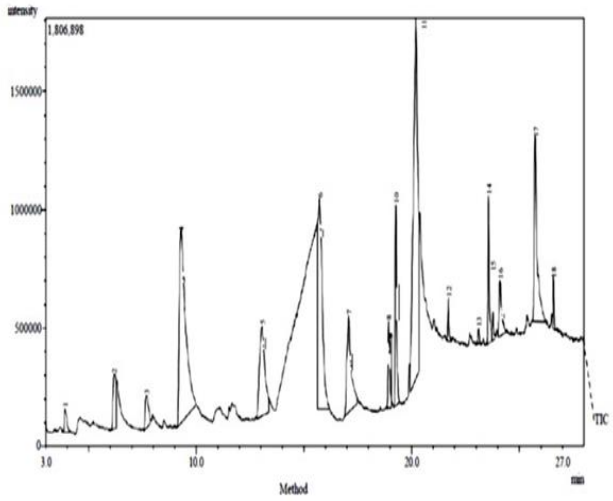

Fig. 1: Chromatogram od ethanol extract of *Piliostigma thonningii* leaf

The mass spectra of these compounds were matched with the spectra of known compounds listed in NIST08.LIB spectral databases/ libraries. Most of the components presented in the leaf extract have been already reported to exhibit different biological activities

These bioactive constituents are closely linked to human growth and general health. Oleic acid, methyl ester present in the ethanol extract of the leaf, are bioactive compounds that may be responsible for the anticancer and antimycoplasmal activity of the plant, Nonadecane is a volatile heterocyclic hydrocarbon which has been reported to possess antioxidant effect (Radhamani and Britto, 2013). 11-octadecenoic acid, methyl ester selectively inhibits eukaryotic DNA polymerase activities in vitro (Radhamani and Britto, 2013). 9, 12-octadecadienoic acid, methyl ester is a polyenoic fatty acid which has been reported to have hepatoprotective, antihistaminic, hypocholesterolemic and antieczemic effect (Radhamani and Britto, 2013). 11- octadecenoic acid, methyl ester is a stearic acid which has been studied to possess antiviral, antibacterial and antioxidant activites (Prajna *et al.*,2016). Hexadecanoic acid is a palmitic acid which has been scientifically studied to have antioxidant, anti-inflammatory, hypocholesterolemic and antidiabetic activities (Prajna *et al.*, 2016).

Table 3: Chemical composition of the ethanol leaf extract.

S/N	Compounds	Retention time (minute)	Molecular weight (g/mol)	Area (%)	Retention index
1	cis-3-Undecene	3.892	154	0.91	1123
2	trans-3-Undecene	3.892	154	0.91	1123
3	Dec-1-ene	3.892	140	0.91	1005
4	3-Tridecene	3.892	182	0.91	1312
5	Isoamylacetate	6.175	130	2.61	820
6	Pentylacetate	6.175	130	2.61	884
7	o-methyl-benzaldehyde	7.675	120	1.61	1095
8	Methylenebicyclo[3,2,0]hept -3-en-2-yn	7.675	120	1.61	962
9	2,3-Dihydrobenzofuran	7.675	120	1.61	1036
10	m-methylbenzaldehyde	7.675	120	1.61	1095
11	1,3-Benzenediol	9.275	110	19.02	1122
12	1,4-Benzendiol	9.275	110	19.02	1122
13	4-methylenecyclohexanone	9.275	110	19.02	957
14	Alpha-methylglycoside	13.058	194	7.55	1714
15	alpha-l-Galecta pyranosidemethyl-6-deoxy	13.058	178	7.55	1471
16	3-o-methyl-d-glucose	15.725	194	14.50	1647

17	3-Methylmoannoside	15.725	194	14.50	1714
18	alpha-Methylmannufuranoside	15.725	194	14.50	1667
19	beta-d-Methylmannofuranoside	15.725	194	14.50	1667
20	4-o-methylmannose	15.725	194	14.50	1714
21	Hexadecanoic acid	17.075	256	6.44	1968
22	Nonadecanoic acid	17.075	298	6.44	2266
23	Nonadecane	17.075	158	6.44	1272
24	9,12-Octeadecadienoic acid, methyl ester, (E,E)	18.925	294	1.41	2093
25	Methyl(11E,14E)-11,14-icosadienoate	18.925	322	1.41	2292
26	13-tetradece-11-yn-1-ol	18.925	208	1.41	1663
27	7,10-Hwxadecadienoic acid, methyl ester	18.925	266	1.41	1894
28	11-octadecynoic acid, methyl ester	18.925	294	1.41	2095
29	11-octadecenoic acid, methyl ester	18.992	296	1.09	2085
30	15-Tetracosenoic acid, methyl ester	18.992	380	1.09	2682
31	13-Docosenoic acid, methyl ester	18.992	352	1.09	2483
32	Methyl-11-(3-pentyl-2-oxiranyl)undecanoate	18.992	312	1.09	2129
33	10-octadecenoic acid. Methyl ester	18.992	296	1.09	2085
34	Phytol	19.267	296	4.28	2045
35	(2E)-2-Methyl-2-nonene-1-ol	19.267	156	4.28	1243
36	3,7-Dimethyl-1,7-octadien-6-ol	19.267	154	4.28	1071
37	cis-Oleic acid	20.192	282	24.85	2175
38	Erucic acid	20.192	338	24.85	2572
39	(11Z)-11-Hexaecenoic acid	20.192	254	24.85	1976
40	Z-8-Methyl-9-tetradecenoic acid	20.192	240	24.85	1813
41	1,2-Cyclobutanediol	21.700	144	0.72	1335
42	Epoxycyclooctane	21.700	126	0.72	970
43	10-Undecenal	21.700	168	0.72	1293
44	trans-2-Decenol	21.700	156	0.72	1266
45	1,4-Dimethyloctyltrifluoroacetate	21.700	282	0.72	1286
46	2-Octylcycloprpene-1-heptanol	23.108	266	0.29	2056
47	Linoleic acid chloride	23.108	298	0.29	2139
48	13-Tetradec-11-yn-1-ol	23.108	208	0.29	1663

49	(6Z,9Z)-6,9-pentadecadien-1-ol	23.108	224	0.29	1771
50	7-Tetradecenal	23.108	210	0.29	1609
51	1,5-Cyclododecadiene	23.567	164	3.12	1403
52	4-Vinyl-1-cyclooctene	23.567	136	3.12	1092
53	Cyclododecyne	23.567	164	3.12	0
54	N-butylacetamide	23.783	115	0.67	1018
55	N-isopentylacetamide	23.783	129	0.67	1053
56	N,N-Diethylguanidine	23.783	115	0.67	769
57	Allantoic acid	23.783	176	0.67	1927
58	2-Methylmalonamide	23.783	116	0.67	1174
59	Palmitic acid, beta-monoglyceride	24.108	330	2.10	2498
60	2-Hydroxy-1-(hydroxymethyl)ethylpentadecanoate	24.108	316	2.10	2399
61	Hexadecanoic acid-2,3-dihydroxypropyl ester	24.108	330	2.10	2482
62	Stearic acid hydrazide	24.108	298	2.10	2454
63	Z,E-3,13-octadien-1-ol	25.742	266	8.12	2069
64	Spinacene	26.575	410	0.78	2914

3.2 FT-IR analysis

The ethanol extract was scanned to identified functional groups using FT-IR spectrophotometer. The FTIR spectrum of ethanol extract of leaf is shown in Fig. 2.

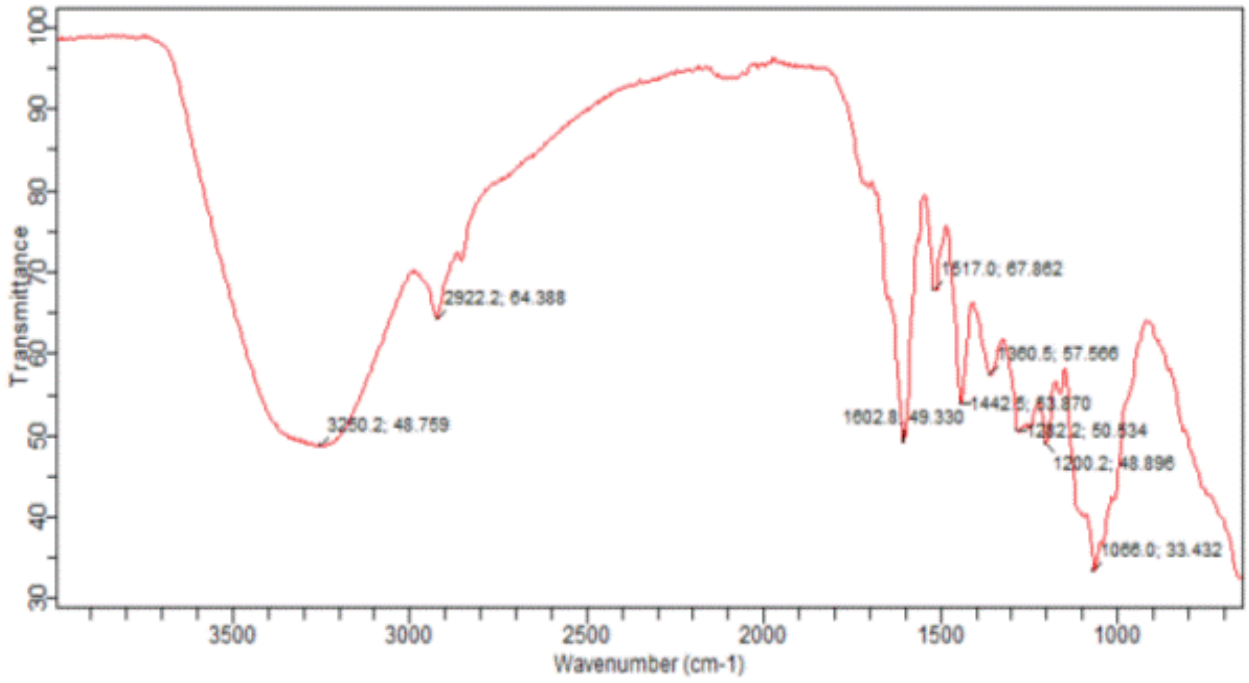

Fig. 2: FT-IR spectrum of ethanol extract of *pilliostigma thonningii* leaf

The prominent molecular functional vibration of chemical groups present in the sample are recorded in Table 4. The peak at 3250.2 cm^{-1} could be due to the presence of alcohol while the band at 2918.5 cm^{-1}, 2847.7 cm^{-1}, and 1461.1 cm^{-1} is due to the presence of alkanes. The functional groups were analyzed and the wavelengths ranged between 1028.7cm^{-1} an 3410.5 $cm^{-1.}$ The peak at 3250.2cm^{-1} is due to the presence of alcohol. These band may likely be due to the presence of 1, 2-Cyclobutanediol, Phytol or 2-Octylcycloprpene-1-heptanol present in the GCMS result. The band at 2918.5 cm^{-1}, 2847.7 cm^{-1}, and epoxycyclooctane and Nonadecane observed in the GCMS result 1461.1 cm^{-1} is due to the presence of alkanes.

Table 4: Peaks and functional groups of IR absorption by ethanol extract of *pilliostigma thonningii*

Peak values (cm^{-1})	Bond	Functional groups	Frequency Range (cm^{-1})
3250.2	O-H	Alcohol	3300-3500
2918.5	C-H stretching	Alkanes	2850-2970
2847.7	C-H Stretching	Alkanes	2850-2970
1461.1	C-H Bending	Alkanes	1340-1470

4.0 Conclusion

From the results and findings of this work, ethanol extract of *pilliostigma thonningii* leaf has phytochemical constituents have been reported to exhibit some medicinal values including flavonoid, terpenoid, cardiac glycosides,tannins, phytosterols, phlobatannins and alkaloid. The information revealed by phytochemical screening, GCMS and the FTIR analysis may be a useful guide for further bioassay and confirmed medicinal and other applications of the plant.

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