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PHYTOCHEMISTRY OF *CORCHORUS OLITORIUS* LEAF CULTIVATED IN CALABAR, CROSS RIVER STATE, NIGERIA

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ABSTRACT

Corchorus olitorius L. commonly known as Jute leaf is a member of the angiosperm family Tiliaceace that has a wide range of potential uses in food, medicine, research studies and biotechnology. Soil composition variations that exist in varying localities usually lead to a variance in a plant's phytochemical potentials; thus the phytochemistry of *C. olitorius L* cultivated in the South-South region of Nigeria was studied. This study entailed the phytochemical screening, proximate analysis, mineral constituents' analysis, chemical characterization, functional groups identification, acute toxicity testing and dose responses of *C. olitorius L* grown within the South-South region of Nigeria. The study results showed an abundance of reducing sugars and the presence of twenty-two (22) bioactive phytoconstituents with Cyclohexanediol and hydroxyl being the most abundant characterized chemical and functional group respectively. These results showed great variations with other prior indigenous studies of *C. olitorius L.*

Keywords: Fourier Transform Infra-Red, Gas Chromatography Mass Spectrometry, Phytochemicals, *C. Olitorius* Leaf.

I. INTRODUCTION

Corchorus olitorius L. commonly known as Jute leaf is a member of the angiosperm family Tiliaceace grown for its fibre and culinary purposes in the Middle Eastern, Asian and African countries. *Corchorus* was first described by the Swedish botanist, Carl Linnaeus, who derived the name from the Greek word Korkhorus, meaning "wild plant of uncertain identity" (MacDonald *et al.,* 2016). The *C. olitorius* plants are erect herbaceous plants that are 2-4 meters in height with leaves that are alternate and bear capsule-like fruits encapsulating about 25-40 seeds (Esmail Al-Suafi, 2016; Loumerem and Alercia, 2016; Helaly *et al.,* 2016). This plant is native to both the tropics and subtropics regions of the world and its edible leaves are served as traditional dishes in the Middle East, parts of Asia and Africa (Akoroda, 1988; Olawuyi *et al*., 2014). The leaves have a slimy texture when cooked and in Nigerian cuisines, amongst the Yorubas, it's known as Ewedu, in Hausa; Ayoyo, lgbo; Ahuara, Fula; Rama, Efik; Krinkrin and in English; Jew's mallow / Jute leaf (Folu *et al.,* 2009; Osawaru *et al.,* 2013).

These leaves have long been used as an important remedy in different cultures either by its several products such as the leaf juice, fried leaves' extract or whole green leaves as food or herbal drugs in different regions of the world. *Corchorus olitorius L.* usually produce a high amount of mucilaginous polysaccharides that are utilized as demulcent, diuretic, laxative, febrifuge, astringent, antiseptic and tonics for the treatments of different ailments such as fever, dysentery, liver disorders, cardiac problems, intestinal colic, skin diseases, atonic dyspepsia, mild jaundice and other disorders of the digestive system. In African cultures; they are often used as preparations for the treatment of gonorrhea, chronic cystitis, headaches, chickenpox, influenza, threatened miscarriages and ovarian cysts management amongst others. Its cold infusion is also used to rejuvenate appetite while its alcoholic extracts are used in preparations of skin cosmetics because of their moisturizing effects. Hence *C. olitorius* leaves serve a double purpose: as a food condiment and as a herbal drug (Ali *et al.,* 2013, Zakaria et al., 2005).

In general, *C. olitorius L* have been reported to have a lot of phytochemical metabolites such as polyphenols, cardiac glycosides, triterpenoids, ionones, flavonoids, coumarins, steroids and many other secondary metabolites. Phytochemicals are chemical compounds formed during a plant's normal metabolic process and are often referred to as secondary metabolites of which they are several classes including alkaloids, flavonoids, coumarins, steroids, glycosides, gum, phenol, tannins, terpenes and terpenoids. Phytochemical analyses are of

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paramount importance for the identification of new sources of therapeutically and industrially valuable compounds with medicinal significance and for the best and most judicious use of naturally available materials. Phytochemicals are known to work as immune modulators and may have biological efficacies such as anti-inflammatory, anticancer and antimicrobial activities. All these properties of phytochemicals are attributed to its effective antioxidative mechanisms against endogenously produced free radicals. *Corchorus olitorius* is a plant that has been found to contain a variety of bioactive phytochemicals with its leaves, stem, root, and seeds reported to contain: Mucilage, Flavonoids, Tannins, Alkaloids, Saponins, Phenols, Carotenoids, and Fiber (Islam *et al.,* 2013).

According to other studies; *C. olitorius L* are rich in vitamins (A, B, C and K), calcium, potassium, iron, dietary fibre and also have antioxidative activity with an α-tocopherol equivalence similar to vitamin E whilst its folate (B9 vitamin) concentration is substantially higher (0.03mg/L) than that of other folacin-rich plants (Li *et al.,* 2012). Furthermore, in research studies; *C. olitorius* extract markedly improved cardiac insufficiencies hence; it can possibly serve as a substitute for strophanthin. It also displayed great reduction in cell viability of the human hepatocellular carcinoma (HepG2) cells via DNA fragmentation and nuclear condensation–mediated apoptosis (Li *et al.,* 2012). This extract triggered the activation of procaspases-3 and 9 and caused the cleavage of downstream substrate, poly-ADP-ribose polymerase, followed by down regulation of the inhibitor of caspase-activated DNase signaling (Li *et al*., 2012). Its seed extracts exerts cytotoxic effects on the multiple myeloma-derived ARH-77 cells with IC_{50} values of 151 and 17 µg/mL, respectively (Iseri *et al*, 2013). Studies have demonstrated that extracts of the *C. olitorius L* have the potential to reduce total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides in experimental models (Airaodion *et al.,* 2019). The hypolipidemic effect of *C. olitorius* leaves has been attributed to its presence of flavonoids, tannins, and alkaloids, which have been found to inhibit the absorption of cholesterol from the gut and to increase the excretion of bile acids (Dewanjee *et al.,* 2013). In addition, *C. olitorius* leaves were found to have an effect on the hepatic enzymes involved in lipid metabolism, such as HMG-CoA reductase and lipoprotein lipase, which are responsible for the synthesis and degradation of cholesterol and triglycerides, respectively (Yabani *et al.,* 2018). The plant's fibers of *C. olitorius* have also been found to have potential applications in the field of biotechnology, as they have been used to make biodegradable plastics, bio-composites, and as a sorbent for heavy metal ions (Chipurura *et al*., 2011). These industrial and pharmacological activities of *C. olitorius* are commonly noted in its leaves compared to its other plant parts (Oboh *et al.,* 2009; Ragasa, 2010; Iseri *et al.,* 2013; Subasri *et al.,* 2015).

Overall*, Corchorus olitorius* is a valuable plant that has a wide range of potential uses in food, medicine and biotechnology, and still needs more research to reveal all its potentials. However, due to the variations that exist in the composition of a plant as result of its varying localities; the phytochemistry of *C. olitorius L* cultivated in the South-South region of Nigeria was studied.

Fig 1: Photograph of *C. olitorius* in a local farmland at Calabar, Cross River State, Nigeria

II. METHODOLOGY

Plant Collection

Corchorus olitorius leaves were purchased from Calabar Metropolis, authenticated and given herbarium voucher number of Herb/Bot/UCC/077 by a taxonomist in the Botany Department.

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Preparation of ethanol leaf extracts of *Corchorus olitorius*

C. olitorius leaves were shade dried for six weeks on table tops in the laboratory. Then, the dried leaves were pulverized into coarse powder and 180g of leaves powder divided into portions were macerated in 96% ethanol (650ml) for seventy-two hours. The mixture was filtered using Whatmans' filter paper No. 1 and oven-dried at 37^0C into a jelly substrate. The oven- dried filtrate was weighed and used in calculating the percentage yield as follows;

$$\frac{W3 - W1}{W2 - W1} \text{ X } 100$$

W_1 = weight of dry beaker

W_2 = weight of dried *C. olitorius* leaves before maceration + dry beaker

W_3 = weight of dried *C. olitorius* leaves after maceration + dry beaker

Thereafter, the ethanol leaf extract of *C. olitorius* was stored in air-tight fitted containers and kept in the laboratory refrigerator until the onset of the research when the extract was dissolved in 10% dimethysulphur oxide (DMSO).

Phytochemical Screening for compounds in *C. olitorius* leaves

Qualitative and quantitative analysis on *C. olitorius* leaf was conducted according to Trease and Evans (2002) and Sofowora (2008) methods. The results of the phytochemical screening are shown in Table 1.

Proximate analysis

The proximate compositions of fresh *C. olitorius* leaves were determined for carbohydrates, lipid, protein, ash, moisture, crude fiber and total energy using the American society of Testing and Materials method (ASTM, 2014).

Mineral constituents' analysis

Minerals in *C. olitorius* leaves were assessed using standard procedures streamlined by Achi *et al.* (2017). Atomic absorption spectrophotometer (AAS) was used in determining the following mineral constituents of *C. olitorius* leaves- Mg^{2+}, Fe^{3+}, Cu^{2+}, Pb, Ca^{2+} and P while the Flame photometer was used in determining K^+ and Na^+. The respective quantity for each mineral was determined from a known concentration gradient of similar salt solutions for each mineral.

Chemical characterization of *C. olitorius* leaf

This was conducted in two stages; in the first stage, Gas chromatography-Mass spectrometry (SHIMADZU GCMS - QP2010) was used in characterizing the chemical compounds in *C. olitorius* leaf extract in procedures used by Yakubu *et al.,* (2017). Thereafter, the Fourier transform infrared (FTIR) spectroscopy (SHIMADZU FTIR-8400S) was used to determine the molecular 'fingerprint' of the sample using Ashokkumar, and Ramaswamy, (2014) procedure.

Acute toxicity testing (LD50) of *C. olitorius*

The protocol as detailed in Organization of Economic Co-operation and Development (OECD) technique of determining the acute toxicity of a plant extract that was further modified by Chinedu *et. al.,* (2013) was used. In this method, the LD50 determination was conducted in three sequential stages; with the outcome of each stage determining the next step.

Confirmatory test-

When mortality occurred at groups 2 and 3 at stage 3; a confirmatory test was conducted in order to validate that *C. olitorius* extract was the actual cause of death. This confirmatory test involved the re- administration of the least 'lethal' dose that caused the mortality of the initial rat to two new rats. The occurrence of the deaths in the two new rats at the dose served as a confirmation and validation of the previous test according to Chinedu *et. al.,* (2013) method.

$$\text{Thus: } LD50 = \frac{M0 + M1}{2}$$

M_0 = highest 'test compound dose' that caused no mortality in the experimental animals

M_1 = lowest 'test compound dose' that caused mortality in the experimental animals

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Dose responses of ethanol leaf extract of *C. olitorius*

A 100mg/kg, 200 mg/kg, 400 mg/kg, 600 mg/kg, 800 mg/kg and 1000mg/kg of *C. olitorius* ethanol leaf extract was administered to six female rats for duration of 14 days in order to ascertain the best study doses of *C. olitorius L* for research studies. Their serum estrogen concentrations were the determinant used as the dose response control and the corresponding graph was plotted as seen in Fig 4.

III. RESULTS AND DISCUSSION

Percentage yield of ethanol leaf extract of *C. olitorius.*

The percentage yield of 180g of macerated *C. olitorius* leaves yielded 7.89g of ethanol leaf extract; giving a percentage yield of 4.34 %.

Phytochemical analysis of *C. olitorius* leaves

The Qualitative analysis of *C. olitorius* leaves tested positive for flavonoids, glycosides, reducing sugars, mucilage, saponins amongst others. Its quantitative analysis had mucilage, reducing sugars, saponins, polyphenols and flavonoids as the phytochemicals with the highest concentration as shown in Table 1.

Reducing sugar content at 150.26 mg/100g and mucilage content at 154.59mg/100g were the most abundant phytochemicals in the *C. olitorus* leaf. This high mucilage content of *C. olitorius* leaves' has been attributed for its gelatinous nature in extractive solvents (Airaodion *et al.,* 2019). Mucilage is an edible polymeric polysaccharide that has high water binding capacity and is responsible for the gelatinous nature of *C.olitorius* leaf (Ahmed *et al.,* 2014; Chowdhury *et al.,* 2017; Tosif et al., 2021). Biologically, mucilage has also been shown to be quite effective in weight loss due to its efficacy in binding to intestinal cholesterol and inhibiting their absorption (Ameri *et al.,* 2015; Ibrahim *et al.,* 2016).

Flavonoids, saponins and polyphenols were also significantly present in *C. olitorius*; in which flavonoid content was 50.46 mg / 100g. Chemically, flavonoids are classified into flavones, flavonols, flavanones, flavanonol, Isoflavones and flavan-3-ols whilst flavonoids such as quercetin, kaempferol, and rutin have been found in *C.olitorius L* in prior studies (Biswal, 2014; Günalan *et al*., 2018). Other phytochemicals such as tannins, glycosides, oxalate, phytates and alkaloids were also found in the *C.olitorius* leaves while alkaloids such as corchorine, corchorinol and corchorinine have been found in *C. olitorius* leaves in other studies (Islam, 2010; Grijalva et al., 2020).

Acute toxicity testing (LD50) of ethanol leaf extract of *C. olitorius*

The lethal dose (LD50) of ELEOCO was at 3500mg/kg dose against most published LD50 doses at over 5000 mg/kg. The probable cause of deaths could be as a result of its high cardiac glycosides content that has been reported by Hassan *et al.,* (2019) to cause mortality especially when the end points involves a lot of breathing difficulties for hours before death.

Table 1: Qualitative and Quantitative analysis of Phytochemicals and antinutrients in *C. olitorius* leaf

Phytocompounds	Qualitative assessment	Quantitative assessment
Alkaloids	++	20.41± 0.06
Glycosides	+	9.6± 0.01
Coumarins	++	46.01± 0.52
Flavonoids	+++	50.46± 0.33
Mucilage	++++	154.59 ± 0.06
Oxalate	+	4.1± 0.05
Phytate	+	8.7± 0.05
Polyphenol	+++	60.72± 0.16
Reducing sugars	++++	150.26 ± 0.42
Saponins	+++	50.68 ± 0.37
Steroids	-	0.0

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Tannins	+	5.2 ± 0.04
Terpenoid	+	7.8 ± 0.12

Legend: The table presents the quantity of each phytochemical (mg) in 100g of *C. olitorius* leaves powder, expressed as Mean ± SD of triplicate determinations. Mildly Present (+), Present (++), Strongly present (+++), Very strongly present (++++), Absent (-)

Proximate analysis of *C. olitorius* leaves

The proximate composition of *Corchorus olitorius* leaf powder consisted of carbohydrates, lipids, protein, ash, moisture and fiber, with a caloric energy value of 250.28 KJ as shown in Table 2. *C. olitorius* leaves in this study had an abundance of carbohydrates with a resultant nutritional value of 56.1g which denotes the high nutritional importance of *C. olitorius* leaf as an edible food. Furthermore, *C. olitorius* leaf fibres have been documented in their usage as surgical dressings (Isuosuo *et al.,* 2019). The moisture and fibre contents of *C. olitorius* in this present study were higher than that obtained by Isuosuo *et al.* (2019), which can be attributed to the climatic conditions of Cross-river state. However, high moisture content of a plant has been associated with a decreased shelf life due to their susceptibility to microbial growth (Al-Snafi, 2006). The protein and ash contents of *C. olitorius* leaf in this study were similar to that of Isuosuo *et al.* (2019), and the presence of high ash content has been attributed to abundance of vitamins (Isuosuo *et al.,* 2019).

Table 2: Proximate Analysis of *Corchorus olitorius* leaf

Compounds	Quantity
Carbohydrate (%)	56.10 ± 3.59
Lipid (%)	4.20 ± 0.48
Protein (%)	15.75 ± 0.84
Ash (%)	10.05 ± 0.09
Moisture (%)	50.10 ± 2.81
Fibre (%)	18.06 ± 0.36
Energy (KJ)	250.28

Legend: The table presents the percentage content of each nutritional constituent in a 100g of *C. olitorius* leaf powder. Expressed as Mean ± SD of triplicate determinations

Minerals composition of *C. olitorius* leaves

The mineral constituents of *C. olitorius* leaf in this study had a greater level of potassium, iron and calcium. As shown in Table 3. This result was similar to already published articles on *C. olitorius* leaf that states its high potassium and iron contents (Ameri *et al.,* 2015).

Table 3: Mineral compositions of *C. olitorius* leaves

MINERAL	QUANTITY (g)
Potassium	42.54 ± 0.1
Iron	14.14 ± 0.1
Calcium	13.17 ± 0.1
Phosphorus	6.89 ± 0.2
Magnesium	2.33 ± 0.1
Chloride	1.96 ± 0.1
Sodium	1.75 ± 0.1
Aluminium	0.33 ± 0.1

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Cadmium	0.09 ± 0.1
Lead	0.02 ± 0.2

Legend: Table shows the amount of various minerals present in 100g of *C. olitorius* leaves powder. The mineral compositions were done in triplicates and the values presented as Mean ± SD (standard deviation).

Gas chromatography- Mass spectrometry of ethanol leaf extract of *C. olitorius*

GC-MS chromatogram of ethanol leaf extract of *C. olitorius* (ELEOCO) showed the presence of twenty-two (22) bioactive metabolites as seen in Table 4. The most abundant of these were 1,2- Cyclohexanediol, Phenol, Octadecanoic acid, Octadecenoic acids at peaks 13, 12, 21 and 22 respectively as shown in Fig. 1 and illustrated in Fig 2. Some of these compounds have been researched upon on their biological and pharmacological effects in the human body. For instance, Mensink (2005) and Hunter *et al.,* (2010) reports that octadecenoic acid commonly known as stearic acid decreases serum LDL cholesterol and increases serum HDL cholesterol levels; which is good in the avoidance of cardiovascular diseases. Also, in another study conducted by Kelly *et al.,* (2001) in healthy males; it was observed that a stearic rich diet reduced the plasma lipid concentrations by 22%. Octadecenoic acid commonly known as oleic acid also facilitates rapid weight loss (Hunter *et al.,* 2010; Schaefer and Nock, 2019). For the most abundant chemical constituent of *C. olitorius*; 1,2- cyclohexanediol, there is little or no literature studies about it. Nonetheless, its chemical structure bears a striking resemblance to another bioactive compound called *inositol* (cyclohexanehexol); in which both of these compounds are carbocyclic sugars with attached hydroxyl groups. Inositol have been well-researched upon and studies have shown that it is effective in the management of PCOS where it acts by increasing insulin sensitivity downstream its signaling pathway, thus leading to an improved ovarian functions (Gateva *et al.,* 2018).

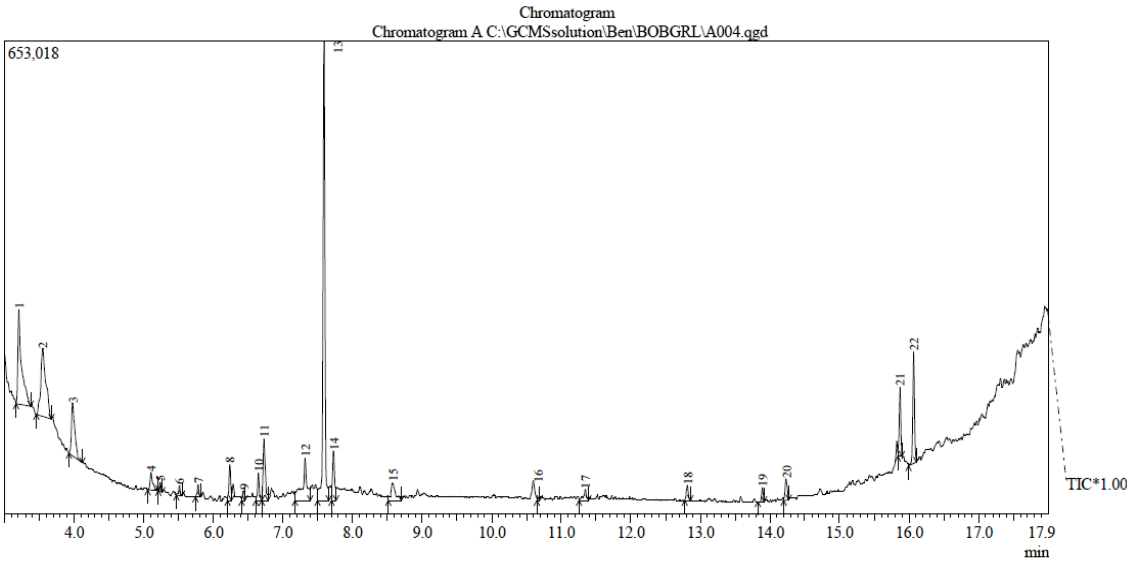

Fig 2: Chromatogram of ethanol leaf extract of *C. olitorius* showing the presence of its twenty-two (22) chemical constituents represented as peaks in varying retention time.

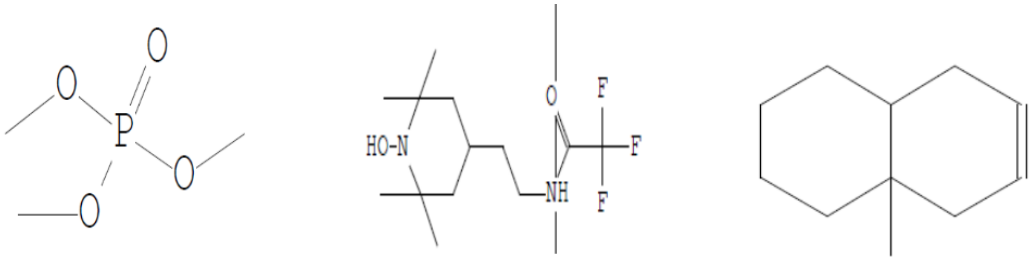

Fig 2a: Phosphoric acid *Fig 2b:* 2,2,2-Trifluoro-N-acetamide *Fig 2c*: Naphthalene

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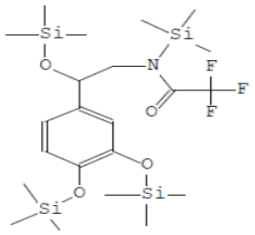

Fig 2d: Tetrakis norepinephrine

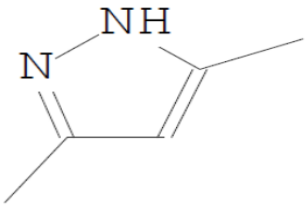

Fig 2e: 1H-Pyrazole

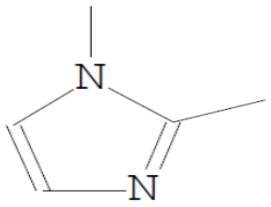

Fig 2f: 1H-Imidazole

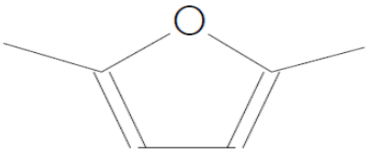

Fig 2g: Furan

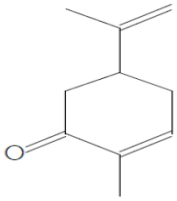

Fig 2h: 2-Cyclohexen-1-one

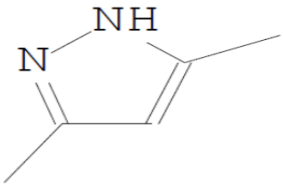

Fig 2i: 1H-Pyrazole

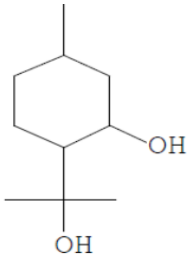

Fig 2j: p-Methane-3, 8-diol

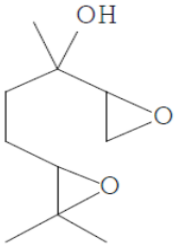

Fig 2k: Epoxy-linalooloxide

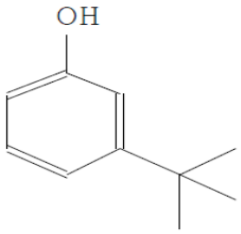

Fig 2l: Phenol

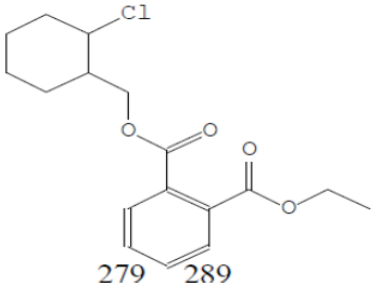

Fig 2p: Phthalic acid

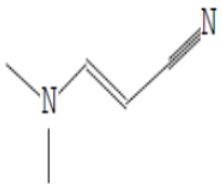

Fig 2q: 3-Dimethylaminoacrylonitrile

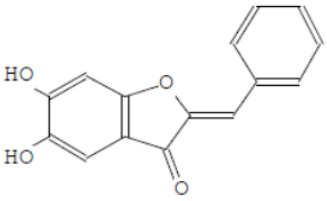

Fig 2r: Benzofuran-5, 6-diol-3-one

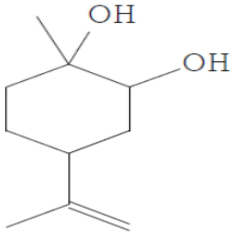

Fig 2m: 1, 2-Cycloheanediol

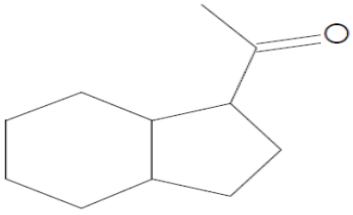

Fig 2n: Ethanone

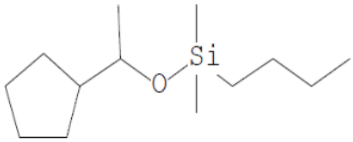

Fig 2o: Butylsilyoxy-1-cyclopentylethane

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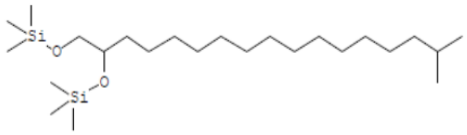

Fig 2s: 16-Methyl-heptadecane-diol

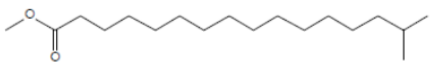

Fig 2t: Structure of Hexadecanoic acid

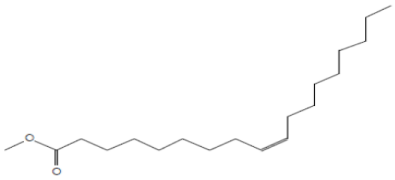

Fig 2u: 9-Octadecenoic acid

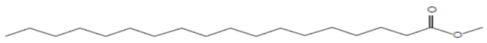

Fig 2v: Structure of Octadecanoic acid

Fig 2: Chemical structures of the phytochemical constituents in *C. olitorius* leaves detected in GC-MS chromatogram

Table 4: Phytoconstituents detected in the ethanolic leaf extract of *C olitorius* using GC-MS

S/N	RT (min)	Phytoconstituent IUPAC name	Molecular Formula	Molecular weight	Area (%)
1	3.208	Phosphoric acid	C3H9O4P	140	11.71
2	3.546	2,2,2-Trifluoro-N-acetamide	CI3H23F3N2O2	296	11.11
3	3.979	Naphthalene	C11H18	150	6.08
4	5.109	Tetrakis norepinephrine	C22H42F3NO4Si4	553	1.45
5	5.236	1H-Pyrazole	C5H8N2	96	0.42
6	5.513	1H-Imidazole	C5H8N2	96	0.59
7	5.781	Furan	C6H8O	96	0.64
8	6.235	2-Cyclohexen-1-one	C10H140	150	2.13
9	6.438	1H-Pyrazole	C5H8N2	96	0.68
10	6.650	p-Methane-3,8-diol	C10H20O2	172	1.82
11	6.732	Epoxy-linalooloxide	C10H18O3	186	4.21
12	7.317	Phenol	C10H14O	150	6.67
13	7.589	1,2-Cyclohexanediol	C10H18O2	170	31.63
14	7.729	Ethanone	C11H18O	166	3.66
15	8.578	1-Butylsilyloxy-1-cyclopentylethane	C13H28OSi	228	2.71
16	10.667	Phthalic acid	C17H21CIO4	324	0.17
17	11.344	3-Dimethylaminoacrylnitrile	C5H8N2	96	1.06
18	12.814	Benzofuran-5,6-diol-3-one	C15H10O4	254	1.09
19	13.893	16-Methyl-heptadecane-1,2-diol	C24H54O2Si2	430	0.77
20	14.234	Hexadecanoic acid	C18H36O2	284	1.36
21	15.866	9-Octadecenoic acid	C19H36O2	296	3.47
22	16.067	Octadecanoic acid	C19H38O2	298	6.56

Fourier transform infrared spectroscopy of ethanol leaf extract of *C. olitorius*

FTIR is a biomedical technique based on the identification of functional groups within a compound when such groups vibrate under the frequency of different light wavelengths; thus, creating molecular fingerprints of a

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drug compound. The FTIR spectra is plotted as the vibrations of the various functional groups (% transmission) within a sample against varying light frequencies (cm-1); which is unique for each pure compound. The FTIR analysis *of C. olitorius* leaf extract was carried by FTIR 8400S Shimadzu in the spectral region between 4000 and 750 cm−1. The analysis identified the chemical bonds and functional groups present in an ethanol extract of *C. olitorius* leaves. The spectra had twelve (12) peaks from 648.1 – 3873.19 which according to its standard analysis table comprises of C =C, (alkene), N-H (primary amine), COOH (carboxylic acid), S-H (thiol), C=C=N (ketenimine), C-H (aromatic), C==O (conjugated ketone), C-Br (halo), O-H (alcohol) functional groups seen in Figure 3 and Table 5. The FTIR spectra detected potential variations in the functional group diversity amongst *C. olitorius* leaf even within the same locality. FTIR spectroscopic analysis for *C. olitorius* leaf extract which identified twelve absorption peaks corresponding to the vibrations of alkene, amine, carboxylic acid, thiol, ketenimine, aromatic, ketone, halo and alcohol functional groups further demonstrates the highly lipophilic nature of *C. olitorius* leaf (Fig. 3). Functional groups are important physicochemical properties of a compound because they pre-determine the characteristic chemical reactivity of the compound and play a major role in formation of molecules such as DNA, lipids, proteins and carbohydrates.

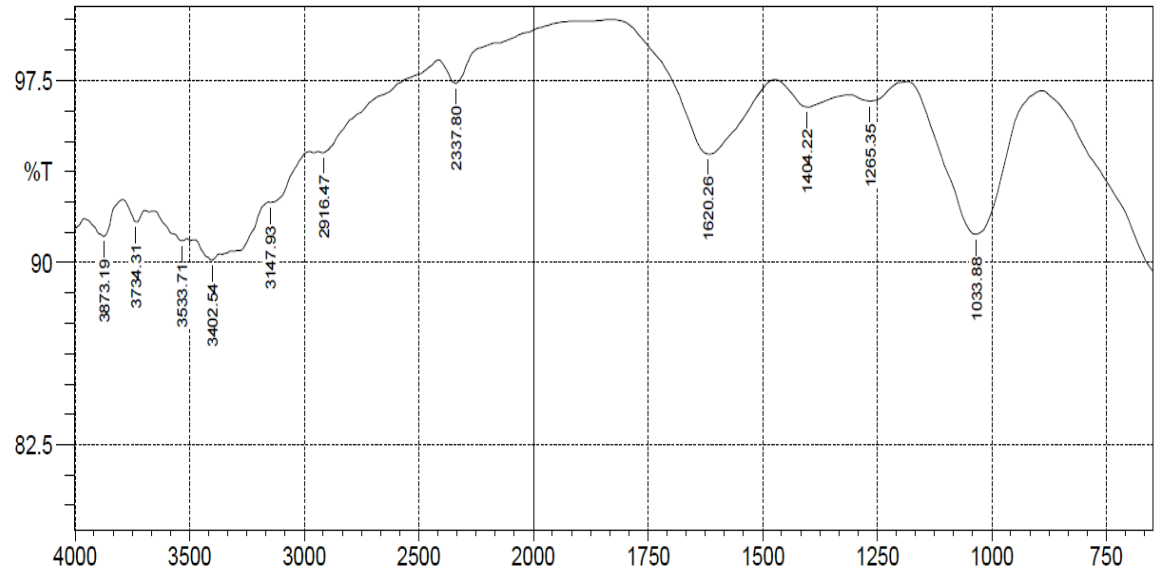

Fig 3: FTIR spectra of ethanolic extract of *C. olitorius* leaves

Table 5: FTIR peak values of ethanol leaf extract of *C. olitorius*

No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area Corr.	Area
1	648.1	89.631	0	895	648.1	6.819	0.646
2	1033.88	91.165	6.083	1188.19	895	7.363	3.823
3	1265.35	96.651	0.465	1311.64	1188.19	1.658	0.126
4	1404.22	96.409	0.867	1473.66	1311.64	2.275	0.299
5	1620.26	94.463	4.099	1828.58	1473.66	4.608	2.698
6	2337.8	97.374	1.204	2414.96	1898.02	2.453	0.509
7	2916.47	94.515	0.226	2939.61	2414.96	7.877	0.386

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8	3147.93	92.47	0.115	0.086	3155.65	2978.19	5.242
9	3402.54	90.101	0.431	0.129	3479.7	3371.68	4.732
10	3533.71	90.894	0.298	0.147	3649.44	3510.56	5.462
11	3734.31	91.676	0.662	0.146	3788.32	3695.73	3.337
12	3873.19	91.074	1.135	0.385	3965.78	3788.32	6.651

Dose responses of ethanol leaf extract of *C. olitorius*

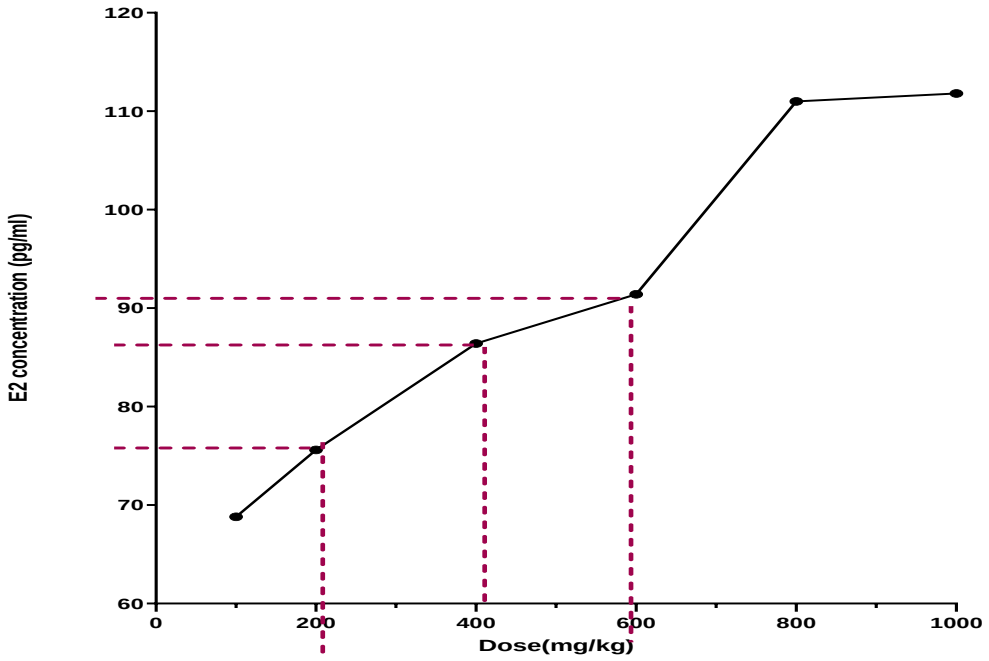

Fig 4: Dose response curve of ethanol leaf extract of *C. olitorius* on Wistar rats' estrogen serum levels after a duration of 14 days. The three median doses were selected as the research study doses. E2= Estrogen

IV. CONCLUSION

The results of this study showed that *C. olitorius* leaves cultivated in the Southern region of Nigeria has an abundance of reducing sugars, cardiac glycosides, coumarins and flavonoids. Its GC-MS chromatogram indicated the presence of twenty-two (22) bioactive metabolites; with 1, 2- Cyclohexanediol, Phenol, Octadecanoic acid, Octadecenoic acids as the most abundant metabolites. The chemical characterization and functional groups of *C. olitorius* leaves yielded an abundance of Cyclohexanediol and hydroxyl group respectively whilst its mineral analysis yielded a high concentration of the elements; potassium and iron. The dose-response curve showed effects of ELEOCO having a minimum dose of 100 mg/kg and a maximum dose of 1000 mg/kg; with its 800 mg/kg dose having no statistical significance ($p > 0.05$) to the 1000 mg/kg dose.

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