Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
www.plantsjournal.com
JMPS 2022; 10(2): 196-203

Received: 19-01-2022
Accepted: 21-02-2022

Odo Christopher Emeka
Biochemistry Department, Faculty of Science, University of Port Harcourt, PMB 5323, Port-Harcourt, Nigeria

Ogunka-Nnoka Charity
Biochemistry Department, Faculty of Science, University of Port Harcourt, PMB 5323, Port-Harcourt, Nigeria

Uwakwe Augustine A
Biochemistry Department, Faculty of Science, University of Port Harcourt, PMB 5323, Port-Harcourt, Nigeria

Corresponding Author:
Odo Christopher Emeka
Biochemistry Department, Faculty of Science, University of Port Harcourt, PMB 5323, Port-Harcourt, Nigeria

Evaluation of biologically active constituents of ethanol extracts of *H. verticillata* leave, *L. aestuans* leave and seeds of *L. aestuans*

Odo Christopher Emeka, Ogunka-Nnoka Charity and Uwakwe Augustine A

DOI: https://doi.org/10.22271/plants.2022.v10.i2c.1409

Abstract
There has been a great interest in the study of products of natural origin as an alternative and an adjunct to the chemically synthesized drugs for various therapeutic roles owing to the vast array of their medicinal activities and structural differences that abound in plants secondary metabolites. The preliminary analysis of the phytonutrients presents in the ethanol extracts of Hydrocotyle verticillata and Laportea aestuans showed the presence of saponins, various classes of alkaloids, Cardiac glycosides, Steroids, Phenolic chemicals, Tannins, Flavonoids, but Coumarins, Proteins and Quinines were lacking in both extracts. Noteworthy is that, tannins and alkaloids were predominant compared to other phytochemicals in the ethanol extract of Hydrocotyle verticillata leave. In the same vain, tannins and alkaloids were the more abundant phytochemicals in the Laportea aestuans leave extract. Analysis of the extract using combined chromatographic and spectrometric (GCMS) approach of Hydrocotyle verticillata leaves identified 3 major chemicals in descending order as 3. beta. 17.beta.-dihydroxyestr-4-ene [10.89%]; Hexadecanoic acid, ethyl ester [7.49%]; and 1-Methylbicyclo [3.2.1] octane [7.28%]. On the other hand, the 3 highest occurring chemical compounds identified in the ethanol extract of *L. aestuans* leaves are Diazoprogesterone [9.45%]; hexadecanoic acid, ethyl ester [8.29%]; and linoleic acid ethyl ester [7.31%]. It has been established that these chemical constituents were found to exert useful biological effects ranging from cellular, anticarcinogenic, anti-cholinergic effects, antioxidant, virus antagonists, anti-inflammatory as well as having profound effects on bacterial infections. Due to the numerous listed biological effects, the extracts could be used as an adjunct in a diverse number of diseases.

Keywords: Laportea aestuans, hydrocotyle verticillata, GC-MS, phytonutrients, biological effect

Introduction
Chemical substances found in plants of medical value, either as known extracts or pure substance results in large means for novel development of chemical substances such as drugs due to the unique availability inherent in phytochemicals owing to their diverse nature [1]. The frequently linked deleterious effects which that due to resistance to microorganisms in formulated drugs coupled with so much importance attached to their chemical diversity in screening processes has been instrumental to the huge demand for therapeutic chemical regime mostly seen in plants. Furthermore, numerous safe plants-derived phytochemicals have been unraveled and broadly used as effective alternatives with minor side-effects [2].
A vast number of biological roles have been accrued to the various chemical constituents ranging from their pain reducing effects to anticarcinogenic, anti-oxidant, in addition to their importance in healing wounds have been noted. Many trials at clinical levels have been conducted to unravel their Pharmacodynamics, kinetics with interactions among novel regimens and their compositions do calls for much careful evaluations. These trials have been crafted to guide against human health and equally give insight into answers to pertinent questions after due evaluation of their effects which might emanate and the various results deciphered prior to the extraction process before being applied to individual patients [3]. It has been stated in the latest World Health Organization reports, that not less than 20000 plants with biological roles do exists in not fewer than 91 countries.

The premier steps to utilize these biologically active plants constituents involves their extractions, followed by carrying out pharmacological Screening, to their Isolations and then being Characterized before the Toxicity evaluation and trial on the clinical basis [3, 4]. The west Indian plant wood nettle equally known as Laportea aestuans belonged to the Urticaceae or nestle family is an annual herb composed of about 22 species [5] and is a predominant old-world genus [6]. It has been discovered that 2 species from this family were reported in the new edition of Taiwan botanicals [7] but due to recent phylogenetic inventory, an additional species of weedy feature Laportea aestuans [L] chew were discovered equally in Taiwan.

On the morphological point of view, the genus Laportea aestuans have semblance to Laportea interrupta due to many features common to both plants such as the possession of same ovate leavy network and not minding the semblance in the 2 species, *L. aestuans* have been noted to show inflorescences which are absent in the other species while lateral branches seem to be grossly insufficient with its flowers showing some fasciculate at intervals going through the elongated peduncles.

The genus Laportea aestuans has been noted as an herbaceous plant with both geographical and ecological even distributions as 3 species of the plant has been found in Taiwan. Furthermore, L. bulbifera which is another species from the genus do grow along medium platforms located in the Chingshuishan province within the vicinity of Taipei region while L. interrupta shows more distribution along low elevations which is located in the Southern part [6]; and *L. aestuans* is noted to be a lowland species grow well in the central Taiwan province.

The plant Laportea aestuans from notable publications is a species with numerous medicinal features which has been used in the treatment of renal ailments in ruminant animals in Trinidad and Tobago as well as to attenuate post-partum cycle in addition to placental removal [8, 9]. On epidemiological point of view, it has been shown that consumption of the plant in linked to the reduction of the damaging effects of reactive oxygen species in human study [10]. These reactive species get through our body by way of metabolic pathways within the body tissues and equally from outside factors including xenobiotics, food and environmental hazards [11].

Thus, the plants, Laportea aestuans that abound in our locality, particularly in the Southern part of Nigeria, have been reported by several studies, to be active in ameliorating some physiological and genetic derangement including blood disorders as used in folk medicine [6, 7, 12]. It equally finds applications in traditional medicine across the West African states where the extracts are made use of in the cure and management of ulcer [7]; menstrual bleeding, chest problems, and anti-inflammation [6]. It has been noted that epidemiological studies have shown that the administration of the plant extracts leads to the inhibition of the damaging effects occasioned by reactive oxygen species in the human body [10, 13]. It has equally been noted that these reactive species which includes oxygen superoxide radical [O_2-]; Hydroxyl radical [OH^-]; as well as the well-known carcinogen such as 7,12-dimethylbenz[α] anthracene that get through to humans leads to cellular damage, occurrence of neurodegenerative and genetic diseases as we well as ageing [13].

Hydrocotyle verticillata belongs to the plant family known as the Apiaceae and made up of about 100 different species that are found in almost all the tropical and temperate areas of the world [14]. Whorled pennywort as it is also called, is a flowering plant which is native to North and South America, Western Africa as well as the West Indies. It is characterized by its half-dollar like leaves which can grow into compact bushes of green.

It is widely used as a foreground plant and it grows well in marshy, boggy and wet places. The plant have been noted to be quick in growth and in many instances do out-grow and over-populate the field if it is not trimmed. Trimming the plant frequently helps in its efficient growth CO_2 utilization and these could give rise to a carpet-like features around the field where it is located. Across the warm areas of Americas and Africa, Hydrocotyle verticillata happens to be a known plant and it prefers moist soils and grow well in shallow water bodies [14].

It is a medicinal plants which are made use of in the treatment of different kinds of ailments due to its lesser deleterious effects [14] coupled with the cost implication when one considers individuals from sub-Saharan Africa with poor per capital income and low gross domestic products. The quest to look more in phytochemical as a means to alleviate different illness stems from their wider distribution, less toxic, cheap and easy to prepare among the local populace hence its role as a medicinal plant. These plants secondary metabolites have been found to be of great sources of novel therapeutic regime. Hydrocotyle verticillata has been mistakenly identified to be Gotu kola [Centella asiatica] by local folks of Iligan city, Butuan city, and in Nige Delta area of Nigeria but efforts of taxonomists have shown that there has been a reclassification necessitating the plant to be moved to another genera. The plant is one of the numerous species of the genus Hydrocotyle with localization around South and North America as well as in the Niger Delta, Nigeria. The assumption is that the plant is effective in the treatment of various ailments including low blood level, lung inflammation, hematological disorders, asthma, Splenomegaly associated to C. asiatica [13]. It has been equally discovered that toxic effect determination by the use of Brine shrimp lethality study carried out on the extract of the plant proved that the plant extract possesses profound biological agents [17].

The various phytochemicals that abound in these plants may impart their beneficial effects mainly by acting antagonistic to the effects of the carcinogen, thus reversing the effect of the genetic mutation or methylation that gave rise to the leukemic cells. Considering the numerous reports on the possible beneficial and synergistic potentials of phytochemicals that are found in *Hydrocotyle verticillata* and *Laportea aestuans*, this research will set out to verify and further develop a yet relatively cheap and locally available natural therapy for leukemias in male and female Wistar rats experimentally induced with this condition.

Over the years, research efforts have given rise to much insight into the chemistry of natural products that abound in plants such as phytonutrients and these has been associated to the vast array of conspicuous biological roles that were inherent in these plants extracts with stunning and varying components of medicinal activities. The birth of novel and sensitive biological techniques aimed at detecting and characterizing these extracts have let to great improvement in the management of several illnesses due to the ease with which these phytonutrients are isolated, purified, and subsequently characterized structurally to give the active components inherent in the plants extracts.

This research is focused on the investigation on the plant chemical constituents in ethanol extracts of Hydrocotyle

verticillata, Laportea aestuans leaves and seeds of Laportea aestuans using combined chromatography and spectrometry as in GCMS.

The present study aims to identify and quantify the various phytonutrients inherent in the ethanol extracts of both plants using known and novel techniques. In applying the techniques of GCMS analysis, the area composition depicts the percentage quantity of the various chemical components isolated in the study.

Materials and Methods

Study Ethical Approval

The research ethical approval was requested and granted by the University of Port Harcourt Research Management and Development (with reference number: UPH/CEREMAD/REC/04, dated August 1, 2021), University of Port Harcourt, Nigeria. All animals were handled according to the recommendations of the dictates and guidelines of the United of America's National Institute of Health and the National Research council guidelines on the use of experimental animals.

Collection of Plants and identification

The plants, *Hydrocotyle verticillata* and *Laportea aestuans* were collected from the field of Bonny Island, Rivers State Nigeria and was identified as *Hydrocotyle verticillata and Laportea eastuans* by a taxonomist with the department of Plants science and biotechnology, University of Port Harcourt and assigned a voucher samples identification for both plants and deposited at the herbarium.

Preparation of crude extract

Whole plant of *H. verticillata and L. aestuans* were collected, sorted and washed to remove dirt, weighed and air dried for about 4 weeks until the weight was constant and then pulverized using mill machine at the Biochemistry Department of the Faculty of Science, University of Port Harcourt. The pulverized samples (1000g) of each of the two plants were kept in an air tight container for further analysis.

Extraction of the crude plant extracts

The fine powder each of *Hydrocotyle verticillata and Laportea aestuans* weighing about 500g was subjected to successive solvent extraction using absolute ethanol in a volume ratio of 1:4 which is 250g powder dissolved in 1000ml of absolute ethanol, soaked for 72hrs, macerated, filtered using filter paper and concentrated/dried in a rotary evaporator in reduced pressure to obtain the dried extract of both plants. The recovered extracts were 50g of *L. aestuans*, and 38g of H. verticillata respectively.

Preliminary Phytochemical analysis of the plants extracts

The methods of Trease and Evans (1983) [15] was used to determine the qualitative phytochemical to detect the presence of Tannins, Saponins, Alkaloids, Flavonoids, phenols, and Glycosides.

GC-MS technique was utilized for the analysis of the ethanolic extracts of both plant for the study. Gas chromatography (GC) from Agilient Technologies United States of America, with model number 7890(B) was coupled to a mass spectrometer (MS) equally from Agilient Technologies United States of America, with model number 5975(B). The procedure adopted for the GC-MS analysis of both ethanolic extracts for this study is as described.

The quantitative analysis of the ethanolic extract of the 2 plants was done using a GCMS instrument from Agilent Technologies, United States. This was done using a GC apparatus coupled to a 5975C; VLMSD mass spectrometer of an injector 7683B device. The system 9091-413; 325^{o}C HP-5 column using helium as a carrier gas at the flow rate of 3.3245ml/min. The gas spectrometric oven temperature was firstly primed at 50^{o}C [1 minute hold] and finally taken to 300^{o}C [5-minute hold] at the rate of 80^{o}C/min with the trial temperature of 37.23^{o}C. The GC column heater was programmed at 250^{o}C in a split less mode while the atmospheric pressure was put at 10.153psi having an average velocity of 66.45cm/sec having a hold up interval of 0.75245 minutes. The mass spectrometric instrument was programmed in the electron impact mode [EI] at 70eV with percentage composition obtained from an electronic integration measurement using flame ionization detector [FID] primed at 250^{o}C.

The analysis of the results of the quantitative Phytochemical determination was carried out by examining the database of the National Institute, Standard and Technology [NIST], which is made up of about 65000 structural patterns [16]. The spectrum obtained of the unknown component was matched with that from a known constituent found on the database library. Attempt was made to establish the names of the compounds, structure, molecular formular, and molecular weights of each of the identified components in each of the ethanolic extracts respectively.

Results

Table 1: Phytochemical screening of ethanol extract of Hydrocotyle verticillate

S/N	Phytochemicals	Observations	Inference	Degree
1	Saponnins	Stable froth	Present	+
2	Tannins	Formation of blue black, green blue, or green precipitate	Present	+++
3	Flavonoids	Intense yellow to colorless	Present	+
4	Alkaloids	Creamy precipitate, Orange precipitate, Brown precipitate	Present	++
5	Cardiac glycosides	Greenish-Blue	Present	+
6	Phenolic compounds	Violet color	Present	++
7	Steroids	Purple color which changes to blue or green	Present	+++
8	Coumarin	No yellow color	Absent	-
9	Quinines	No blue-green or red color	Absent	_
10	Proteins	No red precipitate	Absent	_

Table 2: Phytochemical screening of ethanol extract of Laportea aestuans

S/N	Metabolite	Observations	Inference	Degree
1	Saponnins	Stable froth	Present	+
2	Tannins	Formation of blue black, green blue, or green precipitate	Present	+++
3	Flavonoids	Intense yellow to colorless	Present	++
4	Alkaloids	Creamy precipitate, Orange precipitate, Brown precipitate	Present	+++
5	Cardiac glycosides	Greenish-Blue	Present	+
6	Phenolic compounds	Violet color	Present	+
7	Steroids	Purple color which changes to blue or green	Present	++
8	Coumarin	No yellow color	Absent	-
9	Quinines	No blue-green or red color	Absent	_
10	Proteins	No red precipitate	Absent	_

Table 3: GC-MS analysis of the chemical compounds present in ethanolic extracts of *Hydrocotyle verticillata* leaves.

Name of compound	Retention time (RT) (Minutes)	Molecular formula	Molecular weight (g/mol)	Peak Area (%)
Azelaic acid, monoethyl ester	11.887	$C_{11}H_{20}O_4$	216.27	2.25%
Diethyl azelate	12.197	$C_{13}H_{24}O_4$	244.33	2.74%
n-Hexadecanoic acid	14.854	$C_{16}H_{32}O_2$	256.40	3.45%
Hexadecanoic acid, ethyl ester	15.159	$C_{18}H_{36}O_2$	284.48	7.49%
9,12-Octadecadienoic acid (Z,Z)-	16.216	$C_{18}H_{32}O_2$	280.40	2.93%
9-Octadecenoic acid	16.287	$C_{18}H_{34}O_2$	282.50	3.41%
Linoleic acid ethyl ester	16.473	$C_{20}H_{34}D_2O_2$	310.51	6.90%
Ethyl Oleate	16.540	$C_{20}H_{38}O_2$	310.51	5.65%
Octadecanoic acid, ethyl ester	16.763	$C_{20}H_{40}O_2$	312.50	3.05%
3.alpha.,17.beta.-dihydroxyestr-4- ene	18.268	$C_{18}H_{28}O_2$	276.21	7.06%
Cyclohexane, 1,2-diethenyl-4-(1-methylethylidene)-	18.444	$C_{13}H_{20}$	176.30	1.42%
Thunbergol	18.749	$C_{20}H_{34}0$	290.50	3.13%
Methyl 6,9,12-hexadecatrienoate	18.968	$C_{17}H_{28}O_2$	264.40	2.77%
Methyl 6,9,12-hexadecatrienoate	19.097	$C_{17}H_{28}O_2$	264.40	2.41%
1(3H)-Isobenzofuranone, 3,3-dimethyl-	19.163	$C_{10}H_{10}O_2$	162.18	4.21%
Caryophyllene oxide	19.340	$C_{15}H_{24}O$	220.35	5.29%
cis-1,3,3-trimethylbicyclo exane-1-carboxaldehyde	19.397	$C_{10}H_{16}O$	152.23	6.27%
1-Methylbicyclo[3.2.1]octane	19.616	C_9H_{16}	124.22	7.28%
3.beta.,17.beta.-dihydroxyestr-4-ene	19.711	$C_{18}H_{28}O_2$	276.41	10.89%
7-Oxabicyclo[4.1.0]heptane, 1-meth yl-4-(2-methyloxiranyl)-	19.811	$C_{10}H_{16}O_2$	168.23	2.96%
5-(4-Methoxy-phenylcarbamoyl)-1H-i midazole-4-carboxylic acid, ethyl ester	19.987	$C_{30}H_{40}N_2O_6$	524.60	3.62%
3.beta.,17.beta.-dihydroxyestr-4-ene	20.044	$C_{18}H_{28}O_2$	276.41	2.91%
2,2,6-Trimethyl-1-(2-methyl-cyclobut-2-enyl)-hepta-4,6-dien-3-one	20.068	$C_{15}H_{22}O$	218.33	1.60%
Bicyclo[5.2.0]nonane, 4-methylene- 2,8,8-trimethyl-2-vinyl-	20.297	$C_{15}H_{24}$	204.35	0.31%

Table 4: GC-MS analysis of the chemical compounds present in ethanolic extracts of *Laportea aestuans leaves*

Name of compound	Retention time (RT) (Minutes)	Molecular formula	Molecular weight (g/mol)	Peak Area (%)
Azelaic acid, monoethyl ester	11.882	$C_{11}H_{20}O_4$	216.27	2.366%
Diethyl azelate	12.201	$C_{11}H_{24}O_4$	244.33	2.991%
n-Hexadecanoic acid	14.854	$C_{16}H_{32}O_2$	256.40	3.649%
Hexadecanoic acid, ethyl ester	15.158	$C_{18}H_{36}O_2$	284.48	8.288%
9,12-Octadecadienoic acid (Z,Z)-	16.211	$C_{18}H_{32}O_2$	282.40	2.663%
9-Octadecenoic acid, (E)-	16.282	$C_{18}H_{32}O_2$	282.50	3.267%
Linoleic acid ethyl ester	16.473	$C_{20}H_{34}D_{20}O_2$	310.51	7.313%
Ethyl Oleate	16.544	$C_{20}H_{38}O_2$	310.51	6.044%
Octadecanoic acid, ethyl ester	16.768	$C_{20}H_{40}O_2$	312.50	3.282%
3.alpha.,17.beta.-dihydroxyestr-4-ene	18.263	$C_{18}H_{28}O_2$	276.21	6.310%
Aromandendrene	18.449	$C_{15}H_{24}$	204.35	1.311%
Thunbergol	18.749	$C_{20}H_{34}O$	290.50	3.076%
5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	18.968	$C_{21}H_{34}O$	318.49	2.848%
Benzoic acid, 2,4,6-trimethyl-, 2, 4,6-trimethylphenyl ester	19.163	$C_{19}H_{22}O_2$	282.40	4.082%
Kauran-18-al, 17-(acetyloxy)-, (4. beta.)-	19.306	$C_{22}H_{34}O_3$	346.50	2.911%
8a(2H)-Phenanthrenol, 7-ethenyldodecahydro-1,1,4a,7-tetramethyl-, acetate, [4as-(4a.alpha.,4b.beta.,7. beta.,8a.alpha.,10a.beta.)]-	19.397	$C_{22}H_{36}O_2$	332.50	5.951%)
9,12-Tetradecadien-1-ol, acetate, (Z,E)-	19.611	$C_{16}H_{28}O_2$	252.39	6.571%
Diazoprogesterone	19.697	$C_{21}H_{30}O_2$	314.50	9.447%
7-Propylidene-bicyclo[4.1.0]heptan	19.811	$C_{10}H_{16}$	136.23	2.775%
3-Oxatricyclo[3.2.1.0(2,4)]octane, (1.alpha.,2.beta.,4.beta.,5.alpha.)	19.982	$C_7H_8O_3$	140.14	5.220%
Androstane-3,17-diol, 17-methyl-, (3. beta.,5. alpha.,17. beta.)-	20.039	$C_{19}H_{32}O_2$	292.50	3.651%
7-Propylidene-bicyclo[4.1.0]heptan	20.068	$C_{10}H_{16}$	136.23	5.327%
Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	20.292	$C_{15}H_{24}$	204.35	0.655%

Table 5.: GC-MS analysis of the chemical compounds present in ethanolic extracts of seed of *Laportea aestuans.*

Name of compound	Retention time (RT) (Minutes)	Molecular formula	Molecular weight (g/mol)	Peak Area (%)
9,12-Octadecadienoic acid (Z,Z)-	16.197	$C_{18}H_{32}O_2$	280.4	1.95%
9-Octadecenoic acid	16.263	$C_{18}H_{34}O_2$	282.5	3.04%
(+)-Sesamin	17.244	$C_{20}H_{18}O_6$	354.35	10.17%
Dispiro[2.1.2.4]undecane, 8-methylene	18.244	$C_{12}H_{18}$	162.27	9.00%
5-Cholestene-3-ol, 24-methyl-	19.320	$C_{28}H_{48}O$	400.7	10.06%
Stigmasterol	19.630	$C_{29}H_{48}O$	412.69	18.61%
MDMA methylene homolog	19.759	$C_{12}H_{17}NO_2$	207.27	5.98%
Cyclohexane, 1,5-diethenyl-3-methyl-2-methylene-, (1. alpha.,3. alpha. ,5.alpha.)-	20.016	$C_{12}H_{18}$	162.27	6.51%
Aromandendrene	20.044	$C_{15}H_{24}$	204.35	4.32%
.gamma.-Sitosterol	20.121	$C_{29}H_{50}O$	414.7	30.35%

Table 6a: Biological effects of chemical compounds presents in ethanol extract of Hydrocotyle verticillata, Laportea aestuans leaves and seed of Laportea aestuans.

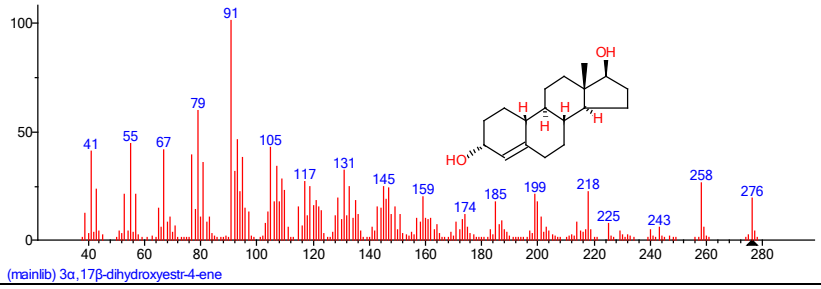
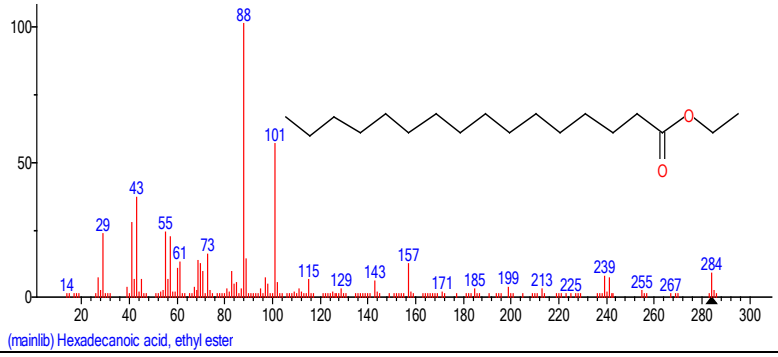
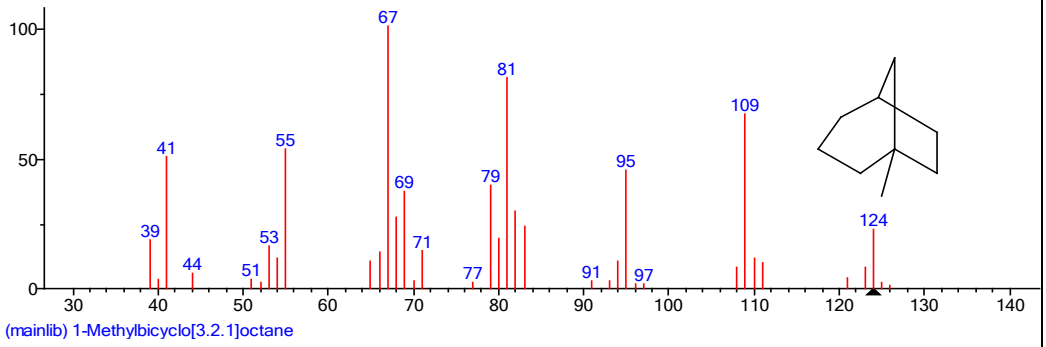
Name of compound	Class of compound	**Biological effects
Azelaic acid, monoethyl ester	Ester	Anti-inflammatory, anti-oxidant, and bactericidal effects.
Diethyl azelate	ester	Anti-inflammatory, and treatment of insulin resistance
n-Hexadecanoic acid	Fatty acid	An anti-inflammatory, anti-oxidant, and anti-cancer effects.
Hexadecanoic acid, ethyl ester	Fatty acid ethyl ester	Anti-inflammatory and anti-oxidant effects
9,12-Octadecadienoic acid (Z,Z)-	Fatty acid	Treatment of hyperlipoidemia and atherosclerosis.
9-Octadecenoic acid	Unsaturated fatty acid	Antioxidant and antimicrobial properties.
Linoleic acid ethyl ester	Ester	Exhibit antioxidant activities
Ethyl Oleate	Ester	Anti-proliferative and anti-cytotoxic effect
Octadecanoic acid, ethyl ester	Ester	Anti-oxidant and anti-inflammatory effects
3.alpha.,17. beta.-dihydroxyestr-4- ene	Steroid	As a synthetic anabolic steroid used as a dietary supplement by athletes to enhance performance.
Cyclohexane, 1,2-diethenyl-4-(1-methylethylidene)-	Terpene (diterpene)	Anti-microbial, anti-oxidant and cytotoxic effects
Thunbergol	Oxygenated diterpene (ODT)	Anti-microbial, anti-fungal, anti-oxidant and anti-cytotoxic effect
Methyl 6,9,12-hexadecatrienoate	Fatty acid methyl ester	Lipid metabolism regulator, Anti-secretory, Anti-inflammatory, reductant, Anti-helminthic, and Anti-infective effects.
1(3H)-Isobenzofuranone, 3,3-dimethyl-	Alkaloid	Neuroprotective, anti-oxidant, and cytotoxic effects
Caryophyllene oxide	Terpene	Analgesic, anti-inflammatory, antidepressant, anti-diabetic, neuroprotective, anti-convulsant, anti-proliferative, vasorelaxant, anti-pyretic, anti-ulcer, and analgesic effects.

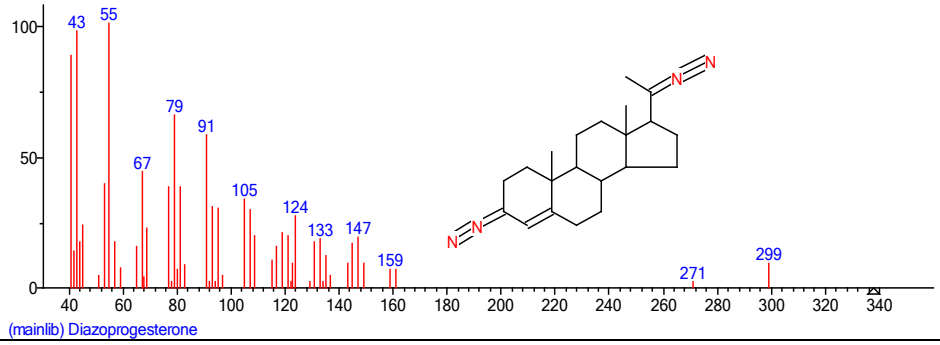
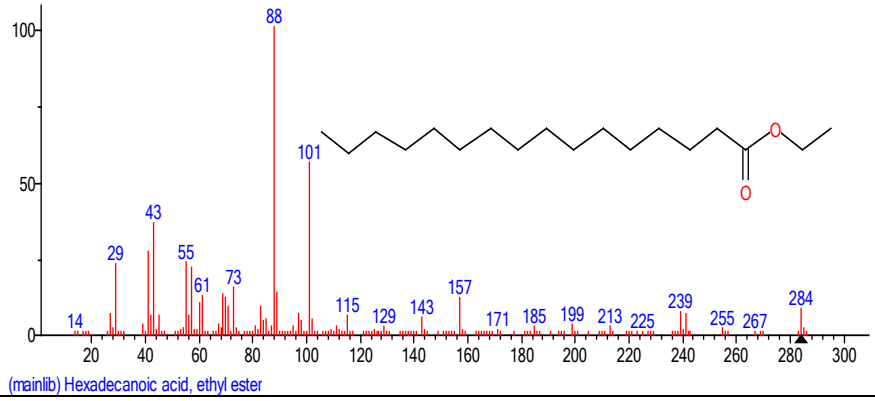
**Biological effects: Dr. Duke's Phytochemical and Ethnobotanical database [17].

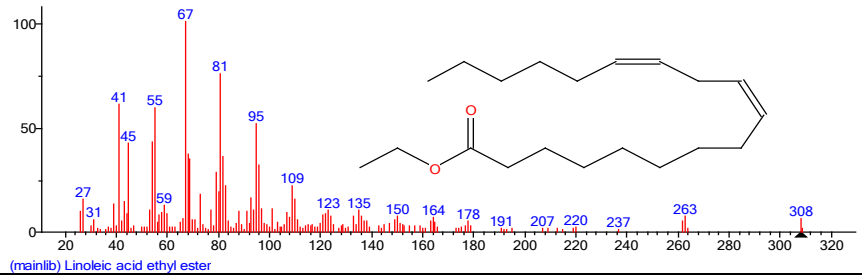
Table 6b: Biological effects of chemical compounds presents in ethanol extract of Hydrocotyle verticillata, Laportea aestuans leaves and seed of Laportea aestuans.

Name of compound	Class of compound	**Biological effects
3-Oxatricyclo[3.2.1.0(2,4)]octane, (1.alpha.,2.beta.,4.beta.,5.alpha.)-	Alkaloid	Anti-oxidant, anti-proliferative, and anti-microbial effects
Androstane-3,17-diol, 17-methyl-, (3.beta.,5.alpha.,17.beta.)-	Terpene	Anti-proliferative, anti-depressant, and stress-relieving effects.
Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	steroid	Anti-inflammatory and anti-bacterial effects
Benzene, 1,2,4-trimethyl- (pseudocumene)	Terpene (Sesquiterpenes)	Used in dyes, and pharmaceuticals as neurotoxin
1,3,7-Octatriene, 3,7-dimethyl (terpenes)/alpha ocimene	Terpene	Anti-fungal effect
Estragole	Terpene	Anti-microbial, anti-cytotoxic and genotoxicity effects.
(R)-1-Methyl-4-(6-methylhept-5-en- 2-yl)cyclohexa-1,4-diene (β-curcumene)	Phenolic compound	Anti-inflammatory, anti-bacterial, anti-fungal, and anti-neoplastic effects
cis-.alpha.-Bisabolene (terpenes)	Terpene	Anti-inflammatory effect
Pentadecanal-	Terpene	Anti-inflammatory, anti-fungal, anti-bacterial, and anti-neoplastic effects.
Bis(2-ethylhexyl) phthalate	Aldehyde	Apoptosis inhibitor, an androstane receptor agonist and a plasticizer.
1-Propene, 1-chloro-2-methyl-	Carboxylic ester	Anti-oxidant effect
Furan, 2,3-dihydro-4-methyl-	Alkaloid	Anti-oxidant and anti-microbial effects
Nonanoic acid, methyl ester	Methyl ester	Anti-microbial, and cytotoxic effect
3-Penten-2-one, 3,4-dimethyl-, semicarbazone	Methyl ester	Anti-fungal, and cytotoxic effect
Undecylenic acid	Flavonoid	Anti-fungal, and anti-oxidant effects
Undecanoic acid, 2-methyl-	Medium chain fatty acid	Anti-fungal, and anti-oxidant effects.
trans-3-oxabicyclo[4.4.0]decane	Methyl ester	Anti-oxidant, anti-inflammatory, and anti-viral effects
Neophytadiene	Alkaloid	Anti-bacterial, Carminative, Gastrin inhibitor, Anti-ulcerative, Histamine release inhibitor, Anti-protozoan, and anti-parasitic effects.

Table 7.0 The 3-most abundant chemical substances from ethanol extract of Laportea eastuans and Hydrocotyle verticillata.

Names	Chemical substances	Structures
Hydrocotyle verticillata	3. beta., 17. beta.-dihydroxyestr-4-ene (10.89%)	
	Hexadecanoic acid, ethyl ester (7.49%)	
	1-Methylbicyclo [3.2.1] octane (7.28%)	

Names	Chemical substances	Structures
Laportea eastuans	Diazoprogesterone (9.45%)	
	Hexadecanoic acid, ethyl ester (8.29%)	

	Linoleic acid ethyl ester (7.31%)	

Tables 1.0 and 2.0 shows the outcome of the qualitative screening of the ethanolic extracts of Hydrocotyle verticillata and Laportea aestuans. The result shows that flavonoids, alkaloids, cardiac glycosides, phenol and steroids were seen in both plants extracts with absence of proteins, quinines and coumarin respectively.

Noteworthy is that, tannins and alkaloids were predominant (with +++ each) compared to other phytochemicals present in the *Hydrocotyle verticillata* leave extract. In the same vein, tannins and alkaloids were the more abundant phytochemicals in the *Laportea eastuans* leave extract.

Tables 3.0 and 4.0 show the chemical compounds present in ethanolic extracts of *Hydrocotyle verticillata* leaves and *Laportea eastuans* leaves respectively. The names of the compounds, retention times (RT), peak areas (percentage), molecular formula and molecular weights of the identified components in GC-MS analysis were all shown.

The 3 most abundant compounds captured in the ethanolic extract of *H. verticillata* leaves (as shown in Tables 1.3) in descending order of magnitude includes 3.beta.,17.beta.-dihydroxyestr-4-ene (10.89%), Hexadecanoic acid, ethyl ester (7.49%) and 1-Methylbicyclo[3.2.1] octane (7.28%). On the other hand, the 3-highest occurring compounds identified in the extract of *L. aestuans* leaves (as shown in Table 4.0) includes Diazoprogesterone (9.45%), hexadecanoic acid, ethyl ester (28%), and Linoleic acid ethyl ester (7.31%).

Tables 6.0a and 6.0b shows the chemical classes and their earlier reported biological effects of the identified chemical compounds from the extracts of the two plants, H. verticillata and *L. aestuans* which ranges from cytotoxic, anti-cholinergic effects to anti-viral, antioxidant properties, as well as anti-inflammatory effects respectively.

Discussions

Complementary/alternative or herbal medicine with their known curative potencies, bioavailability, and reduced side effects has led to popular demands on them. More often than not, these popular demands on complementary/alternative or herbal medicine are attributable to their anecdotal or proven biological roles in vast number of disease conditions [18]. It is therefore suggestive to say that the comprehensive scientific investigations of the anti-leukemic potentials of some herbs, Laportea aestuans and Hydrocotyle verticillata, from our locale considering the huge anecdotal claims on them may be of great benefits.

The phytochemical screening of the 2 plants extracts showed the presence of saponins, tannins, flavonoids, alkaloids, cardiac glycosides, phenolic compounds, and steroids in both plants. Phytonutrients are described as a group of plant derived compounds and reports suggests that they may account for many of the beneficial effects attributed to vegetable, fruits and numerous plant-based products and diets [19-20]. There is no doubt that, the investigated plants may possess immense therapeutic potentials.

The screening of phytochemicals is beneficial, particularly on frequently consumed plant products [19], so by applying the methods of gas chromatographic and spectrometric approach, the research have been designed to decipher and attempt to identify and quantitatively determined the chemical compounds present in Hydrocotyle verticillata leave and Laportea aestuans. A vast array of chemical compounds were identified in the ethanolic extract of Hydrocotyle *verticillata* and *Laportea eastuans* leaves, *Laportea eastuans* seeds, as well as in the ethanol and dichloromethane extracted essential oils of *Hydrocotyle verticillata* and *Laportea eastuans* following GC-MS analyses.

Some of the most abundant chemical compounds identified in the ethanolic extracts and essential oils of both *H. verticillata* and *Laportea eastuans* leaves include linoleic acid ethyl ester (common the two plants), Hexadecanoic acid, ethyl ester (common to the two plants), 1-Methylbicyclo [3.2.1] octane (present only in ethanolic leaf extract of *H. verticillata*) and phytol (found only in dichloromethane extracted essential oil of *Laportea eastuans*). Linoleic acid ethyl ester is known to exhibit antioxidant activities; hexadecanoic acid (ethyl ester) is reported to have some biological roles such as anti-inflammatory and anti-oxidant effects. The 1-Methylbicyclo [3.2.1] octane belongs to the cycloalkane family and have been identified to possess antiviral, anticancer, antioxidant, hypocholesterolemic, anaphylactic, neurostimulant, pesticidal, and insectidical properties. And finally, the compound phytol, is of the phynol family and possess antimicrobial, anti-inflammatory, anticancer, and anti-diuretic properties. The following findings of the present study reveals that the extracts of both plants may to a large extent possess similar biological effects and could thus, account for rationale for their likely anecdotal applications. This position is supported by the earlier report of Bekinbo *et al*., (2020) [21] who stated that, similarity in the compositions of active ingredients of different plants may confer similar therapeutic properties on similar medical conditions.

Conclusions

In respect of the results obtained from this study, it is evident that the ethanol extracts of Laportea aestuans and Hydrocotyle verticillata contains phytonutrients made up of tannins, alkaloids, steroids, phenolic compounds and the study equally showed that the 3 most abundant chemical compounds identified in a descending order includes 3.beta., 17.beta.-dihydroxyestr-4-ene (10.89%), Hexadecanoic acid, ethyl ester (7.49%) and 1-Methylbicyclo [3.2.1] octane (7.28%). On the other hand, the 3 most abundant chemical compounds identified in the extract of *L. eastuans* leaves includes Diazoprogesterone (9.45%), Hexadecanoic acid, ethyl ester (8.29%) and Linoleic acid ethyl ester (7.31%) and these chemical compounds possesses anti-leukemic, anti-bacterial, anti-oxidant, anti-inflammatory and anti-viral properties and could be applied in research involving these areas of study.

Conflicts of Interests

The authors hereby confirms that this article contents have no conflicts of interest.

Acknowledgment

The authors appreciate the supports from FIIRO Oshodi, Lagos Nigeria as well as Biochemistry Department, University of Port Harcourt for their assistants during the course of this study.

References

1. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of concept'. Journal of Ethnopharmacology. 2006;106:290-302.
2. Rout SP, Choudary KA, Kar, DM, Das LOPAMUDRA, Jain A. Plants in traditional medicinal system-future source of new drugs. International Journal of Pharmacy and Pharmaceutical Sciences. 2009;1(1):1-23.
3. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of traditional, complementary and alternative medicines. 2011;8(1):1-10.
4. Amsath A. Isolation technique of bioactive molecules from plants and their applications. International Journal of Current Tropical Medicine and Health Research. 2013;1(1):1-4.
5. Chew WL. A monograph of Laportea (Urticaceae). Gard. Bull. Singapore. 1969;25(1):111-178.
6. Simaremare ES, Holle E, Gunawan E, Yabansabra YR, Octavia F, Pratiwi RD. Toxicity, Antioxidant, Analgesic and Anti-inflamantory of Ethanol Extracts of Laportea aestuans (Linn.) Chew, Journal of Chemical and Pharmaceutical Research. 2018;10(5):16-23.
7. Okereke S, Chukwuma N, Chidi I, Chukwudoruo CS. Evaluation of Anti-ulcer Activity of Laportea aestuans (Linn) Leaf Extract on Aspirin-induced ulcer in Male Rats; Nature and Science. 2015;13(11):33-41.
8. Lans CA. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. Journal of Ethnobiology and Ethnomedicine. 2006;2:45.
9. Lans CA. Ethnomedicines used in Trinidad and Tobago for reproductive problems. Journal of Ethnobiology and Ethnomedicine. 2007;3:13.
10. Morrison JF, Twumasi SK. Comparative studies on the *in vitro* antioxidant properties of methanolic and hydro-ethanolic leafy extracts from eight edible leafy vegetables of Ghana. African Journal of Biotechnology. 2010;9(32):5177-5184.
11. Miller RA, Britigan BE. Role of oxidants in microbial pathophysiology, Clinical Microbiology Review. 1997;10(1):1-18. Doi: 10.1128/CMR.10.1.1.
12. Akanni EO, Adedeji AL, Adedosu OT, Olaniran OI, Oloke JK. Chemopreventive and anti-leukemic effects of ethanol extracts of Moringa oleifera leaves on wistar rats bearing benzene induced leukemia. Current pharmaceutical biotechnology. 2014;15(6):563-568.
13. Oloyede GK, Oyelola MS. Chrysen-2-ol derivative from west indian wood nettle Laportea aestuans (l.) Chew inhibits oxidation and microbial growth *in vitro*; Experimental and Clinical Sciences. Journal 2013;12:894-906 – issn 1611-2156.
14. Airy-Shaw, HK. A dictionary of the flowering plants and ferns by J. C. Willis (ed. 8). 1897 (Reprinted 1980), Cambridge University Press, England.
15. Trease GE, Evans WC. Pharmacognosy. 12th Ed. London: Bailliere Tindal, 1983, 622.
16. NIST [National Institute of Standards and Technology] Chemistry Web Book, SRD 69. Search for species data by chemical name: https://webbook.nist.gov/chemistry/name-ser/, 2019, 22.
17. Duke J. Handbook of Medicinal Herbs. Boca Raton, FL: CRC Press, 1985, 101-1.
18. Del Prete A, Scalera A, Iadevaia MD, Miranda A, Zulli C, Gaeta L, *et al*. Herbal products: benefits, limits, and applications in chronic liver disease. Evidence-Based Complementary and Alternative Medicine 2012.
19. Altemimi A, Lakhssassi N, Baharlouei A. Watson DG, Lightfoot DA. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants (Basel, Switzerland). 2017;6(4):1-23.
20. Omoregie ES, Oikeh EI. Comparative Studies on the Phytochemical Composition, Phenolic Content and Antioxidant Activities of Methanol Leaf Extracts of *Spondias mombin* and *Polyathia longifolia*. Jordan Journal of Biological Sciences. 2015;8(2):45-149.
21. Bekinbo MT, Amah-Tariah FS, Dapper DV. Comparative GC-MS determination of bioactive constituents of the methanolic extracts of *Curcuma longa* rhizome and *Spondias mombin* leaves. Journal of Medicinal Plants Studies. 2020;8(1):01-06.