RESEARCH ARTICLE

European Journal of Agriculture and Food Sciences
www.ejfood.org

Phytochemical and Essential Oil Constituents of *Piper Umbellatum* Linn Leaves

Dikioye Emmanuel Peters, Sor-Aniabari Ruth Baradum, and Reginald Chibueze Ohiri

ABSTRACT

The study investigated the phytochemical and essential oil compositions of *Piper umbellatum* Linn leaves. The leaves were detached from the stalk and washed carefully under running tap water, air dried at room temperature (20-25℃) for 14 days. The dried leaves were ground into a fine powder using a grinding mill. Phytochemical and essential oils composition were analyzed using GC/MS. Phytochemical composition revealed higher concentrations of phenolic acids (434.95164 mg/100 g), tannin (326.79716 mg/100 g), flavonoids (263.37233 mg/100 g), and stilbenes (144.63450 mg/100 g). Essential oil composition revealed a total of forty compounds of which alpha-pinene (28.09%), beta-pinene (17.73%), beta-caryophyllene (15.21%), Nerolidol (11.72%), and Germacrene D (10.14%) were present in considerable amount. *P. umbellatum* Linn leaves are therefore rich in bioactive components that possess a wide range of biological activities.

Keywords: Essential Oil, Phenolic Acids, *Piper Umbellatum* Leaves, Phytochemicals.

Submitted: June 04, 2022

Published: October 20, 2022

ISSN: 2684-1827

DOI: 10.24018/ejfood.2022.4.5.517

D. E. Peters *
University of Port Harcourt, Port Harcourt, Nigeria.
(e-mail: petersdikioye@ymail.com)
S. A. R. Baradum
University of Port Harcourt, Port Harcourt, Nigeria.
(e-mail: baradumruth22@gmail.com)
R. C. Ohiri
University of Port Harcourt, Port Harcourt, Nigeria.
(e-mail: reginald.ohiri@uniport.edu.ng)

****Corresponding Author***

I. Introduction

Across the world, herbal medicines and phytonutrients or nutraceutical uses persistently accelerates speedily with many people now turning to these products for treatment of diverse health issues in different national healthcare settings [1]. Plants use as therapeutic medicines is an immemorial tradition, far aged than the existing sciences of medicine, pharmacology or chemistry. Its tradition is connected to both historical and prehistoric precedence, with its origins in era when medicine, magic, religion, and pharmacology are part of a single empirical discipline [2]. According to [3], medicines developed from plants are in comparison safer than their synthetic counterparts as a result rendering vast therapeutic benefits at an efficient treatment rate. It is generally thought that herbalism (traditional medicines) is the most primeval method of curing ailments [4]. Plants have been used as spices in food for seasoning, flavouring and as such enhance food taste, beverages and drug [5]. Plants possess the capacity to synthesize a large range of chemical compounds utilized to carry out significant biological functions and to defend against attack from predators suchlike microbes [2]. The occurrence of phytochemical compounds in plants mediate their effect in the human body through processes same as those already understood for the chemical compounds in orthodox drugs; thus herbal medicines to a great extent are not different from conventional drugs in terms of how they act [6]. Phytochemicals (bioactive compounds) in plant foods and herbs work with nutrient and dietary fibres to protect against disease [7].

Essential oils are highly concentrated, volatile substances synthesized as secondary metabolites by aromatic plants and are known by their strong odour. In the pharmaceutical and cosmetic industries they are utilized due to their fragrances, taste, antibacterial, antifungal, antiviral, analgesic, sedative, anti-inflammatory, spasmolytic and local anesthetic properties as well as insecticide activity [8].

P. umbellatum Linn is a member of the family Piperaceae and belongs to the order Piperalis. It is a perennial herbs or shrub that rises up to 4m height and vigorously branches off close to the base and are seen as undergrowth in evergreen rainforest and in river banks but few in moist or damp places [9]. *P. umbellatum* is commonly known as wild pepper, ebe-ahanbi in Bini, njam nja in Igbo and ewe-ofon, iwere, yawe and iyawe in Yoruba [10]. It is known as nyakorbadua in Ogoni, Khana Local Government Area of Rivers State where the plant sample was obtained. It originates from tropical America and is now widely naturalized in tropical rain forest in Africa, Japan and the Indian Ocean Islands [9]. *Piper umbellatum* linn has for long being used for therapeutic purposes and food in different parts of Nigeria. In Southern part of Nigeria, the leaves are normally utilized to make "Black and Pepper soups" or spicing, seasoning and/or flavoring agents for foods and soups. They are used singly, combined or mixed with other leafy vegetables to prepare traditional soups and medicines. As medicine, the leaves, roots and fruits have being used in the treatment of rheumatism, inflammatory tumors, stomach pains, ascites and anasarca; the fruits are expoit for diuretic and rubefacient purposes, and also for treating rheumatic pains. In adults the leaf infusion is employed as a cure for stomach pains,

anasarca, and ascites. The boiled leaves mixed with local palm kernel oil are used as a laxative by pregnant women. A decoction of the root in local dry gin (alcohol) is used for curing inflammation and rheumatism [10]. Since phytochemicals and volatile oils are recognized to add to the therapeutic properties of medicinal plants, it is thus crucial to identify the various phytochemicals and essential oils present in the plant and assess these medicinal plants scientifically.

II. Materials And Method

A. Sample Collection

Piper umbellatum Freshly harvested leaves were obtained from a garden both in Kaani and Boue; rural communities in Khana Local Government Area, Rivers State. The plant sample was identified and authenticated by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt Herbarium with voucher number UPH/P/182. The leaves were detached from the stalk and washed carefully under running tap water, air-dried for 14 days at room temperature (20-25 ℃). The dried leaves were grind into powder using a grinding mill and packaged for analysis.

B. Determination of phytochemical composition

The phytochemical composition was determined using GC/MS on a gas chromatographic Model: HP 6890 Powered with HP ChemStation Rev. A 09.01[1206] software equipped with 30 m × 0.25 mm × 0.25 μm AC-5 capillary column and a mass detector Model: FID.

1) Extraction of Tannins

Tannins extraction was conducted using the modified method reported by [11]. 0.2 grams of the powdered sample was weighed into a 50 ml borosilicate beaker and 20 ml of 50% methanol was added and paraffin was used to cover it. The mixture was positioned in a water bath at 80 ℃ for 1 hour and the content was prevented from lumping by stirring with a glass rod. The solution was filtered quantitatively with a double-layered Whatman No. 1 filter paper into a 100ml volumetric flask and rinsed well with 50% methanol and concentrated to 2 ml in the borosilicate vial for gas chromatography analysis.

2) Extraction of Alkaloids

Alkaloids were extracted following the modified method reported by [12] as described in: Antimicrobial diterpenoids alkaloids from Erythrophleum Suaveoleus (Bulletin of the Chemical Society of the Ethiopia). Exactly 5.0 grams of the pulverized sample was weighed and macerated in 25 ml hexane for about 72 hours. The extract was filtered and the residue collected and air-dried and later treated with 10% aqueous NH_3 macerated for 24 hours in $CHCl_3$. The filtration and evaporation was performed at low pressure, later the resultant crude extract was treated with 5% aqueous HCl of about 7.5 ml and aqueous NH_3 also was added to the aqueous phase to make it more alkaline and extracted thrice with $CHCl_3$. The $CHCl_3$ fraction was then washed with water. The extract obtained was dispensed into the round bottom flask of the rotator evaporator arrangement and separated by driving the solvent off the extract. Anhydrous sodium sulphate was then used to dehydrate the concentrated extract of water and the gas chromatography analysis was commenced.

3) Extraction of Flavonoids

This extraction was performed following the modified method reported by [13] in: Research Journal of Medical Sciences. Exactly 50 grams of the powdered sample was placed in a Stoppard flask and treated with ethanol till it was fully soaked. Every hour the flask was shook for the first 6 hours and afterward kept aside and shook after 24 hours. This process was reiterated for 3 days after that the extract was filtered. Using nitrogen stream, the extract was collected and evaporated to dryness. Exactly 0.5 grams of the concentrate was weighed into a 250 ml conical flask capacity and 100 ml of de-ionized water was added to it and heated for 10 minutes. The extract of the flavonoids was obtained by pouring 100 ml of the boiling methanol: water (70:300) onto the materials and the mixture was allowed to macerate for about 4 hours and subsequently filtered through No. 1 Whatman filter paper. The filtrate was derivatized in the gas chromatography for volatility and the mixture then concentrated to 2 ml in Agilent vial for gas chromatography.

4) Extraction of Glycosides

Glycoside extraction was conducted using the modified method reported by [14] in: Journal of Food Technology. 1.0 gram of the pulverized sample was extracted by sopping in 10 ml of 70% alcohol for two hours, after which it was filtered and concentrated. The initial solvent was then replaced with redistilled hexane and concentrated to 1 ml in the vial for gas chromatography analysis.

5) Extraction of Lignans

Lignan extraction was performed following the modified procedure described by [15] in: Identification of Antineoplastic and Neurotrophic Lignans in Medical Prairie Plants by Liquid Chromatography. Methanol was utilized to extract the organic constituents of the pulverized sample overnight with stirring. Suction filtration was used to remove the lignan and the filtrate shaken overnight with hexane and dichloromethane in the ratio 60:40. A separatory funnel was utilized to remove the aqueous layer. The resultant organic solvent was then washed with saturated sodium chloride and dried over sodium sulphate and the solvent rotary evaporated to obtain viscous dark oil. The sample of oil was then dissolved in acetone for GC and the extract concentrated to 1 ml in the vial for gas chromatography analysis.

6) Extraction of Phytosterols

The extraction and analysis of phytosterols was performed via the modified method of [16], [17]. 5 grams of the pulverized sample was weighed and placed in a Stoppard flask and treated with petroleum ether to fully soak the pulverized sample. Every hour the flask was shook for the first 6 hours and later kept aside and shook after 24 hours. This process was repeated for 3 days after that the extract was filtered and nitrogen stream was utilized to collect and evaporate the extract to dryness. To a screw-capped test tube, 0.5 gram of the extract from the sample was added and saponified at 95 ℃ for 30 minutes through the use of 3 ml of 10% KOH in ethanol to which 0.20 ml of benzene has been added to make sure there is miscibility. De-ionized water (3 ml) was added and 2 ml of hexane was used to extract the

non-saponifiable materials like sterols. 3 extractions were performed each with 2 ml of hexane for 1 hour, 30 minutes, and 39 minutes respectively to achieve full extraction of the sterols. The hexane was later concentrated to 2 ml in the Agilent vial for gas chromatography.

7) Extraction of Phenols

Two stages of poly phenolic/phenolic compounds extraction procedures are followed for their effective removal:

First stage:

50 mg of the sample was weighed and extracted with 5 ml of 1M NaOH for sixteen hours on a shaker at ambient temperatures reported by [18], [19]. The sample was centrifuged (5000 x g) after extraction, rinsed with water, and centrifuged again. The upper layers (supernatants) were then combined and taken in a disposable glass test tube and heated for two hours at 90 ℃ to liberate the conjugated phenolic compounds as reported by [20]. The heated extract was allowed to cool and titrated with 4M HCl to a pH< 2.0, and de-ionized water was used to dilute to 10 ml, after which it was centrifuged to remove the precipitate. The supernatant was taken and set aside for the succeeding purification and the residue was further extracted in the second stage.

Second stage:

The residue obtained from the first stage above was extracted with 5 ml of 4M NaOH and heated to 160 ℃ in Teflon as stated by [19]. The mixture was cooled and filtered and the upper layer was collected. The residue was afterward washed with de-ionized water and the supernatants were combined and adjusted to pH< 2.0 with 4M HCl. The filtrates were taken for further purification.

Purification of phenolic acids:

5-15 ml aliquot of the various supernatants was passed through a conditioned Varian (Varian Association, Harbour City, CA) Bond Elut PPL (3 ml size with 200 mg packing) extraction solid-phase tube at ~5 ml min^{-1} attached to a Visiprep (Supelco, Bellefonte, PA). The resins were properly dried off by placing the tube under a vacuum (-60 kPa) afterward the phenolic acids were eluted with 1 ml of ethyl acetate into a gas chromatographic autosampler vials. The conditioning of the PPL tubes was done by first passing 2 ml of ethyl acetate followed by 2 ml water at a pH < 2.0. The standards of the phenolic acids used were purchased from Aldrich (Aldrich Chemical Co., Milwaukee, W1).

8) Derivatization Procedure (Silylation)

After the extraction process, the 2 ml of concentrated extract in the gas chromatography vial was derivatized by the addition of 20 microlitres of derivatizing agent BSTFA, (bis(trimethylsily) trifluoroacetamide). The silicone septum corked vial was then lowered into the water bath with a hanger for it to stand upright in the water bath with a magnetic stirrer at 45℃ for 10 minutes of derivatization.

9) Extraction of Saponins

Saponins extraction was conducted using the modified method of analytical Sciences reported by [21]. Saponins of the pulverized sample was extracted thrice with redistilled methanol and removed with 20 ml of the solvent with the help of the sonication. Under reduced pressure, the combined extracts were concentrated to syrup and suspended in water. After which petroleum ether, chloroform and 1-butanol was used to extract the suspension and successively saturated with water to give the respective extract after removing the solvent. The combined extract was then filtered and concentrated to 1 ml in the vial for gas chromatography analysis.

10) Extraction of Stilbenes

Stilbenes extraction was conducted following the method described by [22].

Sample preparation:

0.3 grams of the powdered sample was accurately weighed and placed in a round bottom flask and 50ml of methanol was added to it. The mixture was heated under reflux for 2 hours, cooled and the lost solvent was replenished with methanol. Afterward, it was filtered through 0.45 μm nylon membrane prior to analysis.

11) Extraction of Terpenes

Terpenes extraction was determined following the modified procedure of [23]. Terpenes constituents of the pulverized sample were extracted with redistilled chloroform. The terpenes were removed for 15 minutes with 10ml of the solvent. After this, the mixture was filtered and concentrated to 1 ml in the vial for gas chromatography analysis.

C. Determination Of Essential Oil Components

10 grams of an already pulverized sample was weighed into a well-corked bottle and 20 ml of dichloromethane was added to it. The bottle was shook vigorously and soaked for 5 days and the crude extract filtered into a quartz beaker and performed again. The combined aliquots obtained were positioned in a steam bath to concentrate to 5ml**.** The extract sealed with silicone was transferred to the head space vial and later positioned in the headspace jacket already connected to the gas chromatography system. The GC/MS analysis was performed on a gas chromatography HP 6890 Powered with HP Chem Station Rev. A 09 01[1206] Software.

III. Results and Discussion

A. Phytochemical Composition of Piper Umbellatum Leaves

The detected phytochemicals of the dried leaves of *P. umbellatum*, their retention times and concentrations are given in Table I. The result revealed the presence of terpenoids, tannins, sterols, stilbenes, saponins, phenolic acids, Lignans, glycosides, flavonoids, and alkaloids. The outcome revealed that phenolic acids (434.95164 mg/100 g), tannin (326.79716 mg/100 g), flavonoid (263.37233 mg/100 g) and stilbenes (144.63450 mg/100 g) were highest in concentration. The values of flavonoid, tannin and saponin were higher in the study compared to 0.087%, 0.213% and 0.011% respectively reported by [24] and lower than the values (1.123±0.001%, 0.474±0.001% and 0.421±0.001%) obtained by [25]. The phenol content compares favorably with the value (0.456±0.06%) reported by [25] and higher than the value (0.127%) reported by [24]. Phenols are antioxidants in humans and plants [26]. Phenol content present in plant tissues stimulates agents of leucocyte, protects cells from oxidative stress damage and also are effective antibody and best-used remedies for inflammatory

disease [27]. Flavonoids promote particular taste of food [28]. Flavonoids have been described to have antioxidant effects and are able to inhibit the initiation, promotion and progression of tumors, and reduce coronary heart disease [29]. Saponins are said to react with rich cholesterol membrane of cancer cells thereby reducing their growth and viability [30]. Saponins possess the property of precipitating and coagulating red blood cells [31]. Sapogenins have been described to function majorly in the deactivation of viruses suchlike the inhibition of HIV-1 virus replication possibly through the suppression of its protease activity [32]. Some saponins characteristics include foam formation in aqueous solution, bitterness, cholesterol binding properties and hemolytic activity [33], [34]. Saponins present in medicinal plants are liable for most biological effects that are connected to cell growth and division in humans and have an inflammatory inhibitory effect [35]-[37]. The presence of tannins in herbs are helpful due to their astringent properties [38], as a result are used for the treatment of internal disorders such as dysentery and diarrhea [39]. Tannins are also known to be used to preclude cancer and for treatment of inflamed or ulcerated tissues [39]-[41]. Alkaloids have been detailed to have a wide-ranging pharmaceutical activity including anti-malaria, anti-asthma and anti-cancer properties [42]. They also have vasodilatory, analgesic and antibacterial properties [43]-[45]. [46] reported that stilbenes possess anti-inflammatory activities in their capability to inhibit the arachidonic acid pathway leading to prostaglandins formation which activates carcinogenesis and stimulates the growth of cancer cells by suppressing the activity of hydro peroxidase of cyclooxygenase. This progression of carcinogenesis is slowed down by stilbenes in a dose-dependent manner hence inhibiting the development of preneoplastic leisions. [47] also reported that stilbenes inhibit the synthesis and duplication of DNA and proliferation of lymphocyte during immunosuppressive therapies. Thus the outcome of this study evidence that the leaves of *P. umbellatum* exhibits pharmacological and biochemical properties.

TABLE I: PHYTOCHEMICAL COMPOSITION OF PIPER UMBELLATUM LEAVES

Phytochemicals	Type	Retention time (min)	Concentration (mg/100)
Terpenoids	Transphytol	19.518	4.14e-1
	Taraxerol	19.675	1.66e-3
	Terpinolene	20.535	3.71e-1
	Alpha-Amyrin	21.131	1.82e-1
	Beta-Amyrin	21.820	5.42e-1
	Lupeol	23.229	5.99e-2
	Bauerenol acetate	24.728	1.50e-3
Total terpenoids	–	–	1.57
Tannin	Tanic acid	19.516	326.80
Sterols	Cholesterol	19.481	3.29e-4
	Cholestanol	20.390	4.66e-5
	Ergosterol	21.513	1.84e-3
	Campesterol	22.380	3.79e-1
	Stig-masterol	23.212	8.21e-1
	Savenasterol	23.720	3.49e-1
	Sitosterol	25.250	3.82
Total sterol	–	–	5.37
Stilbenes	5-b-D-glucosuloxy-3-hydroxyl-tr-stilbene-1	12.737	4.21e-2
	Pinosylvin 3-O-??-D-glucopyranoside	14.150	5.04e-2
	Pinosylvin	15.396	1.89e-2
	Resveratrol	16.037	119.31
	Piceid	18.053	9.92
	Astringin	18.934	15.30
Total stilbenes	–	–	144.63
Saponins	Hispigenin	17.547	3.61e-2
	Solagenin	18.589	3.79e-4
	Diosgenin	19.516	9.88e-5
	Tigogenin	20.110	5.71e-4
	Neochlorogenin	20.471	3.33
	Hecogenin	21.819	6.82e-5
	Sapogenin	22.599	36.20
	Tribuloin	23.116	7.54e-4
	Yanogenin	23.967	3.59e-4
	Conyzorgin	24.997	3.85e-5
	Saponine	26.345	15.55
Total saponins	–	–	55.11
Phenolic acids	Salicyclic acid	9.689	2.79e-1
	Protocatechuic acid	10.516	8.45e-1
	p-Coumaric acid	11.366	89.77
	Vanillic acid	12.441	5.53e-2
	p-Hydroxybenzoic acid	12.829	3.32
	Gallic acid	13.824	21.30
	Ferulic acid	14.967	17.87
	Syringic acid	16.449	112.80

TABLE I: PHYTOCHEMICAL COMPOSITION OF PIPER UMBELLATUM LEAVES (CONT.)

Phytochemicals	Type	Retention time (min)	Concentration (mg/100)
	Syringic acid	16.449	112.80
	Caffeic acid	17.696	44.00
	Sinapinic	18.775	75.93
	Ellagic acid	21.307	3.94e-1
	Chlorogenic acid	22.501	68.39
Total phenolic acids	–	–	434.95
Lignans	(9E, 12E, 15E)-9, 12, 15-Octadecatrien-1-ol	14.155	4.50e-6
	Apigenin-4',7-dimethy ether	16.459	1.09e-5
	Dehydrobietic acid	18.050	1.32e-4
	Sesamin	18.931	1.34
	Sesamolin	19.516	7.75e-1
	Retusin	19.686	1.65e-2
	Dihydrocubalin	20.193	7.38e-3
	Galgravin	21.503	1.48e-1
	Kadsurin	22.599	4.72
	Sakuranin	23.967	1.87e-1
Total lignans	–	–	7.19
Glycosides	Scillarenin	14.503	16.67
	Patuloside A	17.698	22.21
	Salicin	18.773	7.57e-5
	Artemetin	19.105	1.90
	Amygdalin	19.484	4.43e-1
	Ouabain	20.446	6.62e-4
	Dhurrin	21.200	3.35e-5
	Prunasin	21.522	9.45e-6
	Cucurbitin	21.798	2.52e-5
	Digitoxin	21.984	9.38e-1
	Digoxin	22.569	3.39e-1
	Lotaustralin	24.025	1.02e-4
Total glycosides	–	–	42.50
flavonoids	(+)-Catechin	13.747	23.15
	Reveratrol	15.164	4.16e-6
	Apigenin	16.145	35.60
	Naringenin	16.981	6.31e-1
	Biochanin	17.165	1.02e-5
	Luteolin	17.672	3.76e-1
	Kaemferol	18.060	51.49
	(-)-Epicatechin	19.526	4.06e-1
	(-)-Epigallocatechin	20.599	7.13e-2
	Gallocatechin	21.777	4.32e-3
	Quercetin	22.608	130.09
	(-)-Epicatechin-3-gallate	22.858	3.30e-1
	(-)-Epigallocatechin-3-gallate	23.476	4.02e-4
	Isorhamnetin	23.978	5.28e-6
	Robinetin	24.189	6.92e-6
	Myricetin	24.796	8.18
	Baicalein	25.622	6.08e-6
	Nobiletin	26.065	9.66e-6
	Baicalin	26.300	3.08e-6
	Tageretin	26.503	2.49e-6
	Artemetin	26.852	3.47e-6
	Silymarin	27.000	1.49e-6
	Naringin	27.372	1.40e-6
	Quercitrin	27.646	3.00e-5
	Rutin	28.048	18.05
	Hesperidin	28.391	3.51e-7
Total flavonoids	–	–	268.37
Phytochemicals	Type	Retention time (min)	Concentration (mg/100g)
Alkaloids	Dopamine	10.959	4.23e-1
	Pellitorine	11.361	4.01e-2
	Piperine	12.412	4.23e-1
	Capsaicin	13.794	4.77e-1
	Cinchonidine	14.466	7.60e-5
	Piplartine	15.111	3.02e-1
	Echinatine	16.010	4.53e-5
	Buphanidine	16.572	2.95e-5
	Cepharadione A	17.384	8.93e-2
Total alkaloids	–	–	1.75

European Journal of Agriculture and Food Sciences
www.ejfood.org

B. Essential Oil Composition of Piper Umbellatum Leaves

The essential oil composition of *Piper umbellatum* leaves yielded forty compounds which amounted to 100% of the total essential oil content. The various compounds detected in the dried leaves of *Piper umbellatum*, their retention times and percentage compositions are given on (Table II). The major identified essential oil constituents of the leaves of *P. umbellatum* on GC/MS analysis were alpha- pinene (28.09%), beta-pinene (17.73%), beta-caryophyllene (15.21%), Nerolidol (11.72%) and Germacrene D (10.14%). The percent composition of alpha-pinene was higher than the value (12.80%) reported by [48] for *Piper capense* (wild pepper) leaves. Beta-pinene was higher than the value (10.80%) reported by [48]. Alpha and beta-pinenes are two monoterpenoid hydrocarbon isomers found in nature and have been detailed to have antimicrobial, anticancer, anti-inflammatory, and anti-allergic properties [49]. They are components of renal and hepatic drugs [50]. Beta-caryophyllene and nerolidol percent compositions were lower than 28.2% and 16.5% respectively as reported by [48]. Beta-caryohyllene (BCP) is a natural bicyclic sesquiterpene unveiled by [51] to possess poly-pharmacological properties and its therapeutic potential has been linked many diseases such as neuropathic pains, ulcerative colitis, liver disease, cancer, cerebral ischemia, neurodegenerative and neuropsychiatric diseases. Nerolidol is a naturally occurring sesquiterpenoic alcohol present in two geometric isomeric forms (cis and trans) reported to possess antimicrobial, antioxidant, anti-parasitic, anti-inflammatory, skin penetration, skin repellant, anti-nociceptive and anticancer properties [52]. Germacrene D is a hydrocarbon commonly found as plant constituents which is regarded to be a key intermediate in the biosynthesis of many sesquiterpenoids [53]-[55]. Germacrene D has been described to possess antimicrobial properties [56].

IV. Conclusion

The study thus disclosed that *P. umbellatum* are rich in bioactive components that possess wide range of biological activities such as anti-cancer, anti-inflammatory, anti-parasitic, analgesic, antioxidant, antimicrobial amongst others.

TABLE II: Essential Oil Composition Of Piper Umbellatum Leaves

S/N	Essential oils	Retention Time (min)	Percentage composition (%)
1	alpha-Pinene	10.702	28.07
2	beta-Pinene	11.374	17.73
3	Benzyl alcohol	11.987	0.03
4	Cis Ocimene	12.902	0.03
5	Myrcene	12.955	0.01
6	Allo Ocimene	13.204	0.02
7	alpha-Thujene	14.161	0.01
8	gama-Terpinene	14.362	0.03
9	Geijerene	14.930	0.03
10	Fenchone	15.474	0.03
11	Neral	15.748	0.02
12	Geranial	15.923	0.02
13	Isoartemisia	16.503	0.01
14	1, 8-Cineole	16.612	0.03
15	Geraniol	17.193	0.31
16	Nerol	17.441	0.02
17	beta- Caryophyllene	17.709	15.21
18	Linalool	18.025	0.01
19	Borneol	18.417	0.03
20	alpha-Terpineol	18.693	0.01
21	Terpinen-4-ol	18.790	0.01
22	Pregeijerene	19.370	0.02
23	Thymyl methl ether	19.688	0.02
24	Ascaridole	20.087	0.02
25	Linalyl acetate	20.818	0.02
26	Ethyl Cinnamate	21.427	0.03
27	Borneol acetate	21.637	0.04
28	Linalyl acetate	21.724	0.01
29	beta-Bisabolene	21.929	0.02
30	trans-alpha-Bergamotene	22.931	0.03
31	gama-Cardinene	23.250	2.74
32	beta-Cardiene	23.356	2.04
33	Bicyclogermacrene	24.690	6.59
34	alpha-Copane	24.733	0.01
35	Germacrene	25.781	10.14
36	Safrole	26.751	2.31
37	Elemicin	27.104	0.01
38	Benzyl Benzoate	27.790	0.02
39	alpha-Humulene	28.053	2.52
40	Nerolidol	29.336	11.72
Total	–	–	100

REFERENCES

[1] WHO. World Health Organization Guidelines on safety monitoring of herbal medicine in pharmacovigilance systems. Geneva, Swizerland, 2004:15-17.

[2] R. Zaree, M. Farhadi, Z. Mohammdzadeh, and G. R. Goudarz. (2013). Extraction and comparison of alkaloids in different organs during different phonological periods of *Nitraria schoberi*. *Ann. Biol. Res.*, 4(2):130-135.

[3] K. T. Kareem, S. O. Kareem, O. J. Adeyemo, and R. K. Egberongbe. *In vitro* antimicrobial properties of *Bridelia ferruginea* on some clinical isolates. *Agr. Bio. J. N. Am*., 2010; 3(1):418–419.

[4] J. Evans. (2005). Mistletoe good for more than kisses. *J. Am. Bot. Council*. 68:50–59.

[5] N. P. Manandhar. Substitute spice in Nepal. *J. Herbs, Spices and Med. Plants*, 1995;3:7-77.

[6] L.C. Tapsell, I. Hemphill, and L. Coblac. Health benefits of herbs and spices .The past, the present and the future. *Aust. Med. J*., 2006;185:54–24.

[7] A. A. Akindahunsi, and S. O. Salawu. Phytochemical screening and nutrient-anti-nutrient composition of selected green leafy vegetables. *Afr. J. Biotechnol*. [Online], 2005;4:497–501.

[8] L. S. F. Gushiken, A. L. Rozza, A. J. Vieira, F. P. Beserra, and C. H. Pellizon. Essential Oils and their use in skin wound healing. *Nat. Prod. Res Rev*., 2016;3, 501-513.

[9] V. Nunez, V. Castro, R. Nurillo, L. A. Ponce-soto, I. Merfoot, and B. Lomonte. Inhibitory effects of *Piper umbellatum* and *Piper peltatum* extracts towards myotoxic phospholipase A2 from Bothrops snake venoms: isolation of 4-nerolidylcatchol as active principle. *Phytochemistry*, 2005;66 (9):1017-1025.

[10] B. C. Ndukwu, Ben-Nwadibia. Ethnomedicinal Aspect of Plants Used as Spices in the Niger Delta Area of Nigeria. E*thnobotanical leaflets*, [Online] 2005:1. Retrieved from https://0pensiuc.lib.siu.edu/ebl/vol2005/iss1/10.

[11] T. Swain, Tannins and Lignins. *Hebivores: their interaction with Secondary Metabolites*, G. A. Rosenthal, and D. H. Janzen Eds. New York: Academic Press, 1979, pp. 657-682.

[12] F. N. Ngounou, R. N. Manfouo1, L. A. Tapondjou2, D. Lontsi, V. Kuete, V. Penlap, F. X. Etoa, M. A. L. Dubois, and B. L. Sondengam. Antimicrobial Diterpenoid alkaloids from *Erythrophleum suaveolens* (guill. & perr.) Brenan. *Bull. Chem. Soc. Ethiop.*, 2005;19(2): 221-226.

[13] H. Millogo-Kone, M. Lompo, F. Kini, S. Asimi, I. P. Guissou, and O. Nacoulma. Evaluation of Flavonoids and Total Phenolic Content of Stem and Leaves of *Parkin biglobosa* (Jacq.) Benth. (Mimosaceae)-Free Radical Scavenging and Antimicrobial Activities. *Res. J. Med. Sci.*, 2009;3(2): 70-74.

[14] O. O. Oluwaniyi, and S. A. Ibiyemi. A Study of the Extractability of Thevetia Glycosides with Alcohol Mixture. *J. Food Technol*., 2007;5(2): 147-151.

[15] M. C. James, C. Knoy, K. Kindscher, R. C. D. Brown, and S. Niemann. Identification of Antineoplastic and Neurotrophic Lignans in Medicinal Prairie Plants by Liquid Chromatography Electron Impact Mass Spectrometry (LC/EI/MS). Rockhurst University, Kansas City. 2006, pp. 1-3.

[16] AOAC International. AOAC Official Method 994.10: Cholesterol in Foods. In Official Methods of Analysis of the Association of Official Analytical Chemists, 17th edition, Arlington, VA, 2000.

[17] AOAC International. AOAC Official Method 970.51: Fats (animal) in vegetable fats and oils (determination of cholesterol*)*. In Official Methods of Analysis of the Association of Official Analytical Chemists. 17th edition, Arlington, VA, 2005.

[18] W. T. Kelley, D. L. Coffey, & T. C. Mueller. Liquid chromatographic determination of phenolic acids in soil. *AOAC International*, 1994l;77: 805-809.

[19] G. J. Provan, L. Scobbie, and A. Chesson. Determination of phenolic acids in plant cell walls by microwave digestion. *Sci. Food and Agric*., 1994;64: 63-65.

[20] D. Whitehead, C. H. Dibb, and R. D. Hartley. Bound phenolic compounds in water extracts of soils, plant roots, and leaf litter. *Soil Biol. Biochem.*, 1983;15:133-136.

[21] M. Guo, L. Zhang, and Z. Liu. Analysis of Saponins from Leaves of *Aralia elata* by Liquid Chromatography and Multi-stage Tandem Mass Spectrometry. *Anal. Sci*., 2009;25: 754.

[22] S. Li, D. Zhang, L. Yang, Y. Li, and X. Zhu. HPLC Quantitative Analysis of Main Stilbenes and Flavones in Different Parts of *Matteuccia struthiopteris*. *J. Chem.*, 2013: 1- 6.

[23] A. Ortan, M. L. Popescu, A. L. Gaita, C. Dinu-Pirvu, and G. H. Campeanu. Contributions to the pharmacognostical study on *Anethum graveolens*, Dill (Apiaceae). *Rom. Biotechn. Lett.*, 2009;14(2): 4342-4348.

[24] L. A. Ejei-Okeke, T. N. Akujobi, C. Odum, Okafor, and J. A. Eke. Evaluating the Nutrient and Phytochemical Content and Acceptability of Wide Scent Leaves (*Piper umbellatum*-linn) From Nigeria. *World Educators Forum*, 2017;9(1): 1-12.

[25] E. M. Isikhuemen, B. O. Ogbomwan, and I. U. Efenudu. Evaluation of Phytochemical and Mineral Constituents *Piper guineense* Schum. & Thonn. And *Piper umbellatum* Linn: Implications for Ethomedicine. *Eur. J. Med. Plants*, 2020;31(1): 84-97.

[26] C. J. Dillard, and J. B. German. Review Phytochemicals: nutraceuticals and human health. *J. Sci. Food Agric.*, 2000;80: 1744-1756.

[27] N. Husain, and S. Gupta. A critical study on chemistry and distribution of phenolic compounds in plants and their Role in human health. *J. Environ. Sci. Toxicol. Food Technol.*, 2015;1(3): 57-60.

[28] F. D. Dakoro. Plant Flavonoids: Biological Molecules for Useful Exploitation *Aust. J. Plant Physiol*. [Online], 1995;22:87-99.

[29] M. G. L. Herlog, E. J. M. Feskens, C. H. Hollman, M. B. and Katan, D. Kromhout. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. *Lan*., 1993;342:1007-1011.

[30] R. R. Roa, R. M. Babu, and M. R. V. Roa. Saponins as anti-carcinogens. *J. Nutr*., 1995;125: 717-724.

[31] R. N. S. Yadav, and M. Agarwala. Phytochemical analysis of some medicinal plants. *J. Phytol*., 2011;3(12): 10-14.

[32] F. Mengoni, M. Lichtner, L. Battinelli, M. Marzi, C. M. Mastroianni, V. Vullo, and G. Mazzantle G. In vitro anti-HIV activtity of oleanolic acid on infected human mononuclear cells. *Planta Med*., 2002;68: 111-114.

[33] O. A. Sodipo, J. A. Akiniyi, and J. U. Ogunbamosu. Studies o certain characteristics of extracts of bark of *Pansinystalia macruceras (K schemp) pierre exbeille. Global J. Pure Appl. Sci*., 2000;6: 83-87.

[34] D. E. Okwu. Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. *J. Sustain. Agric. Environ*., 2004;6 (1): 30-37.

[35] M. J. Just, M. C. Recio, R. M. Giner, S. Manez, A. R. Billa, and J. L. Rios. Anti-inflammatory activity of unusual lupine saponins from *Burpleurum Fruticescens. Plant Med*., 1998;64: 04-407.

[36] J. Liu, J. Tian, M. Haas, J. I. Shapiro, A. Askari, and Z. Xie. Ouabain interaction with cardiac Na^+/K^+-ATPase initiates signal cascades independent of changes in intracellular Na^+ and Ca^{2+} concentrations. *J. Biol. Chem*., 2000;275(36): 27838-27844.

[37] D. E. Okwu, and I. N. Emenike. Evaluation of the phytonutrients and vitamins contents of citrus fruits. *Int. J. Mol. Med. Adv. Sci.*, 2006;2: 1-6.

[38] I. B. Harbone. *Introduction to Ecological Biochemistry*, 3rd Edition. London: Academic Press, 1988, pp. 10-15.

[39] A. M. Bajia. Effect of Natural extract of pineapple on disstibility, performance traits and nitrogen balance of broiler chicks. *Aust. J. Basic & Appl. Sci*., 2001;5 (20): 10-30.

[40] H. Li, Z. Wang, and Y. Liu. Reviews in the studies on tannins activity of cancer prevention and anticancer. *Zhong-Yao-Cai*, 2003;26(6): 444-448.

[41] M. F. Adegboye, D. A. Akinpelu, and A. Okoh. The bioactive and phytochemical properties of *Garcinia kola* (*Heckel*) seed extract on some pathogens. *Afr. J. Biotechnol.*, 2008;7 (21): 3934-3938.

[42] P. Kittakoop, C. Mahidol, and S. Ruchirawat. Alkaloids as important scaffolds in therapeutic drugs for the treatment of cancer, tuberculosis and smoking cessation. *Curr. Top. Med. Chem.*, 2014;14(2): 239-252.

[43] S. S. Raymond, S. J. Jonathan, and M. J. Watkins-Pitchford. *The essence of Analgesia and Analgesics.* Cambridge University Press, 2010, pp. 82-90.

[44] P. Russo, A. Frutaci, A. Del Bufalo, M. Fini, and A. Cesario. Multi target drugs of plants origin acting on "Alzhemer's disease". *Curr Med. Chem.*, 2013;20(13): 168-193.

[45] T. P. Cushnie, B. Cushnie, and A. J. Lamb. Alkaloids: An overview of their bacterial, antibiotic-enhancing and antivirulence activities. *Int. J. Antimicrob. Agents*, 2014;44(5): 377-386.

[46] J. Dai, and R. J. Mumper. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 2010;15(10): 7313-7352.

[47] B. Uttara, A. V. Singh, P. Zamboni, and R. T. Mahajan. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr. Neuropharmacol.*, 2009;7(1): 65-74.

[48] F. Tchoumbougnang, P. M. J. Dongmo, M. L. Sameza, N. Fombotioh N. A. V. Wouatsa, P. H. A. Zollo, and C. I. Menut. Comparative essential oils composition and insecticidal effect of different tissues of *Piper capense* L., *Piper guineense* Schum. et Thonn., *Piper nigrum* L. and *Piper umbellatum* L. grown in Cameroon. *Afr. J. Biotechnol.*, 2009;8 (3): 424-431.

[49] B. Salehi, S. Upadhyay, E. I. Orhan, A. K. Jugran, L. D. S. Jayaweera, D. A. Dias, F. Sharopov, Y. Taheri, N. Martins, N. Baghalpour, W. C. Cho, J. Sharifi-Rad. Therapeutic Potential of α- and β-Pinene: A Miracle Gift of Nature. *Biomolecules*, 2019;9(11): 738.

[50] D. Sybilska, J. Kowalczyk, M. Asztemborska, R. J. Ochocka, and H. Lamparczyk.Chromatographic studies of the enantiomeric composition of some therapeutic compositions applied in the treatment of liver and kidney diseases. *J. Chromatogr. A.*, 1994;665 (1): 67–73.

[51] C. Sharma, J. M. Al Kaabi, S. M. Nurulain, S. N. Goyal, M. A. Kamal, and S. Shreesh Ojha. Polypharmacological Properties and Therapeutic Potential of β-Caryophyllene: A Dietary Phytocannabinoid of Pharmaceutical Promise. *Curr. Pharm. Des.*, 2016;22(21): 1-28.

[52] W. Chan, L. T. Tan, K. Chan, L. Lee, and B. Goh. Nerolidol: A Sesquiterpene Alcohol with Multi-Faceted Pharmacological and Biological Activities. *Molecules*, 2016;21: 529.

[53] N. Bulow, and W. A. Konig. The role of germacrene D as a precursor in sesquiterpene biosynthesis: investigations of acid catalyzed, photochemically and thermally induced rearrangements. *Phytochem.*, 2000;55: 141-168.

[54] P. Steliopoulos, M. Wust, K. Adamb, A. Mosandla. Biosynthesis of the sesquiterpene germacrene D in *Solidago canadensis*: ^{13}C and ^{2}H labeling studies. *Phytochem.*, 2002;60: 13-20.

[55] I. Prosser, I. G. Altug, A. L. Phillips, W. A. Konig, H. J. Bouwmeester, and M. H. Beale. Enantiospecific (+) and (-) -Germacrene D synthases, cloneed from goldenrod, reveal a functionally active variant of the universal isoprenoid-biosynthesis aspartate-rich motif. *Archives of Biochemistry and Biophysics*, 2004;432: 136-144.

[56] L. F. Da Silva Junior, S. O. Balogun, R. G. de Oliveira, A. Domazo and D. T. O. Martins. *Piper umbellatum* L: A medicinal plant with gastric-ulcer protective and ulcer healing effects in experimental rodent models. *Journal of ethnopharmacol*, 2016;4(192): 123-131.

Dr. Dikioye Emmanuel Peters is a Senior Lecturer in Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria and holds a B.Sc. (Biochemistry), M.Sc. (Nutrition and Toxicology) and PhD (Medical Biochemistry) degrees from University of Port Harcourt.

He had supervised 1 PhD, 13 M.Sc., 5 PGD and over 60 Undergraduate students. Students currently under his supervision are 3 PhD, 6 M.Sc., 2 PGD and 14 Undergraduate students. He has published over 45 original research articles in local and international journals. He is currently researching on Fertility enhancing, immunomodulatory, anti- inflammatory, anti-pyretic and analgesic potentials of some plant extracts on animal model.

Dr. Peters is a member of American Association for Science and Technology (AASCIT).