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Analyses of bioactive compounds in fiddleheads of *Pteridium aquilinum* L. Kuhn collected from Khana, Southern Nigeria, using gas chromatography-flame ionization detector

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Abstract
Pteridium aquilinum fiddleheads are eaten as vegetable by the Ogonis of Nigeria, and decoctions taken as remedy for malaria. The analyses of bioactive compounds in extract from fiddleheads of *P. aqiulinum* from Khana, Southern Nigeria were done using GC-FID. Results revealed the present of quercetin-7-methyl ether, quercetin-3-o-rutinoside, quercetin-4-methyl ether, quercetin-3,7-dimethyl ether, kaempferol-3-0-rutinoside, kaemferol-4'-methyl ether, taxifolin-4'-methyl ether, taxifolin-7-methyl ether, aromadendrin-4'-methly ether, naringenin-4'-methyl ether, and eroidictyol-7,4'-dimethyl ether, as flavonoids, tannic acid, as the only tannin, digoxin, ouabain, salicin, amygdalin, arbutin, and digitoxin, as glycosides, avenacin A-1, and avenacin B-1 as saponin, buphanidrine, crinamidine, crinane-3α-ol, epoxy-3,7-dimethoxycrinane-11-one, oxoassoanine, dihydro-oxo-demethoxyhaemanthamine, 1-β, 2-β-epoxyambelline, 6-hydroxypowelline, undulatine, 6-hydroxyundulatine, powelline, augustamine, ambelline, 6-hydroxybuphanidrine, 9-octadecenamide, voacangine, mitraphylin, and akuammidine as alkaloids present in *P. aquilinum* extract. The results showed that *P. aquilinum* fiddleheads extract contained phytocconstituents with pharmacological potentials that makes it a good health promoting vegetable and a potential raw material for the production of supplements and novel drugs.

Keywords: Bioactive compounds, fiddleheads, pharmacological, phytochemicals, *Pteridium aquilinum*, vegetable

1. Introduction
Pteridium aquilinum L. Kuhn (bracken fern) is a large deciduous rhizomatous fern of the family dennstaedtiaceae. It is a cosmopolitan species with an almost world-wide distribution. *P. aquilinum* is one of the many members of the plant world that have practical and diverse use. It is most commonly used today as food for humans and herbal remedy in folk medicine [1]. The used of bracken fern as food in the middle Stone Age in Europe has been reported [2]. The fiddleheads and tender shoots of *P. aquilinum* are commonly consumed as food by humans [1]. Its anti-malarial, analgesic, anti-rheumatic, anti-parasitic and anti-bacterial properties have been reported [1-5]. It is also used as abortifacient agent in domestic animals [6], and in Cameroon, it is used for treatments of male infertility and piles [7, 8]. In Western Ghats, it is used to treat worms and diarrhoea, and for the treatment of inflammations of the gastric and mucous membrane of the intestines, and the decoctions of its rhizomes and fronds used to treat chronic disorders of the viscera and spleen [9, 10]. Bracken fern is an important dietary element. In Japan and Korea, it is consumed as a vegetable in a type of soup called "Warabe" [11], and in Khana, Ogoni in the Southern part of Nigeria, it is also eaten as vegetable in a special soup called "maalokebe". Decoctions of bracken fern are orally taken by the Ogonis as remedy for malaria, diabetes and stomach disturbances.
Plant bioactive compounds are phytochemicals that provide health benefits to humans and animals. The presence of phytochemical components in medicinal plants has been reported [12]. The principal biologically active plant chemicals include alkaloids, tannins, flavonoids, saponin, phytates, glycosides, phenols, anthocyanins and oxalates. Preliminary phytochemical screening of *P. aquilinum* fronds extract showed the presence of alkaloids, tannins, saponins, glycosides and flavonoids [4]. Also, the essential oil composition of fiddleheads of *P. aquilinum* has been reported [13]. Therefore, this study is aimed at the analyses of some phytochemical compositions of secondary metabolites in extract of *P. aquilinum* fiddleheads from Khana in Ogoni, South-South Nigeria, using gas chromatographic-flame ionization detector (GC-FID),

with a view of highlighting the possible use of the fiddleheads as food, sources of drugs, nutriceuticals, pharmaceuticals and food supplements. However, this study is quantitative; it has not being reported in literature, thus the need for this research.

2. Materials and Methods

2.1 Chemicals

The chemicals used for analyses were of GC analytical grade.

2.2 Collection of plant materials

Fresh fiddleheads of *P. aquilinum* were collected from Wii-Luere farmland in Nyogor-Beeri, Khana local government area of Rivers state, Nigeria. The plant was identified and authenticated by Dr. N.L. Edwin-Wosu of the Department of Plant Science and Biotechnology, University of Port Harcourt in Rivers state, Nigeria, where voucher specimen is deposited (UPHV-1032). The fresh fiddleheads were washed in clean water, dried and cut into smaller pieces. The cut pieces were then air-dried under shade for 10 days and pulverized into powder form using electric blender (Bruders BL-133), and stored in a refrigerator in an air-tight container at 4ºC until required for use.

2.3 Gas chromatography-flame ionization detector (GC-FID) analysis.

Analyses of phytochemicals in extract from fiddleheads of *P. aquilinum* were done with Gas Chromatography-Flame Ionization Detector (GC-FID) fitted with Hewlett Packard HP 6890 gas chromatography and flame ionization detector (FID, 320ºC) that is powered by HP Chemstation Rev. A 90.01(1206) software (SEM Ltd, Istanbul, Turkey).

2.3.1 Extraction and analysis of flavonoids

1g of the pulverized sample was placed in a test tube and 10 ml of methanol added and then placed in a water bath regulated at 60ºC for 15 minutes. The mixture was then filtered into 50 ml pre-cleaned borosilicate beaker with filter paper (Whatman no.1). It was later concentrated to 1.0 ml with rotatory evaporator (Stuart scientific, UK). Flavonoid was analyzed by gas chromatography (GC) using HP INNOWaX capillary column (30 m X 0.25 mm X0.25 µm film thickness) with nitrogen as the carrier gas. Initial oven temperature was 50ºC and was increased to 80ºC/min for 20 minutes, maintained for 4 minutes and to 120ºC/min. for 4 minutes, and maintained for another 4 minutes. Injector temperature was 250ºC and the injected volume 0.2 µl, while the split ratio used was 20:1. The compositions were obtained from electronic integration using flame ionization detector (FID, 320ºC) and components confirmed by comparison of the retention times with commercially available standard (NIST).

2.3.2 Extraction and analysis of tannins

500 mg of the pulverized sample was placed in a 100 ml plastic bottle. 50 ml of distilled water was added and shaken for 1hour with mechanical shaker. The mixture was filtered with filter paper (Whatman no.1) into a 50 ml volumetric flask and made up to the mark with distilled water. Then 5ml of the filtrate was pipetted into a test tube and mixed with 3 ml of 0.1 M $Fecl_3$ in 0.IN HCl and 0.008 M potassium ferrocyanide. The recovered tannin was made available for gas chromatography analysis using HP 5 capillary column (30 m x 0.25 mm x 0.25µm film thickness) and nitrogen as the carrier gas. Initial oven temperature was 120ºC, which was increased by 10ºC/min for 20 mins. Injector temperature was 250ºC and 0.2 µl injected, with a split ratio of 20:1. Relative composition of tannin was obtained from electronic integration using flame ionization detector (FID, 320ºC) and compared with the commercially available standard (NIST).

2.3.3 Extraction and analysis of glycosides

1g of the pulverized sample was placed in a cleaned borosilicate beaker and 10 ml of mixture of ethanol and water (7:3) was added and allowed to stand on the laboratory bench for 2 hours. The mixture was filtered with filter paper (Whatman no. 1) and the extract obtained washed with lead acetate, and sodium hydrogen phosphate added. The extract was concentrated to the 1ml. Glycosides were analyzed with AC-5 capillary column (30 m x 0.25 mm x 0.25µm film thickness) using nitrogen as the carrier gas. Initial temperature was 70ºC for 5 minutes and increased by 12ºC/min for 20 minutes. Injector temperature was 250ºC and 0.2 µl of sample was injected. The spilt ratio used was 20:1. The relative compositions were obtained from electronic integration using flame ionization detector (FID, 320ºC) and a commercially available standard (NIST) used for confirmation of components.

2.3.4 Extraction and analysis of saponins

Pulverized sample was defatted by petroleum ether for 3 hours at 40ºC. After filtering with filter paper (Whatman no. 1), the filtrate was used for extraction using methanol as solvent for 3 hours with moderate heat. The methanol extract was concentrated and re-extracted with methanol and acetone in a ratio of 1:5. The precipitate obtained was oven dried (corsair heating oven), which turned to a whitish amorphous powder after drying. It was then eluted on a silica gel column (230 - 400 mesh) with chloroform-methanol-water mixture in a 7:3:1 proportion. The first fraction collected was air dried at room temperature and the residue obtained treated as pure saponins. The saponins obtained was dissolved in methanol for gas chromatography analysis using a DB-225MS capillary column (30 m x 0.25 mm x 0.25µm film thickness), with nitrogen as carrier gas. Initial oven temperature was 50ºC and was increased by 8ºC/min for 20 minutes, maintained for 4 minutes and by 12ºC/min for another 4 minutes, and maintained for more 4 minutes. Injector temperature was 250ºC and the injected volume 0.2 µl, while the split ratio was 20:1. The compositions were obtained from electronic integration using flame ionization detector (FID, 320ºC) and comparison done with commercially available standard (NIST).

2.3.5 Extraction and analysis of alkaloids

250 ml of boiled distilled water was added to 30 g of powdered *P. aquilinum* fiddleheads in a beaker and allowed to be soaked for 30 minutes before filtration with filter paper (Whatman no. 1). The filtrate was acidified with acetic acid at pH 4.0, and 30 ml each of petroleum spirit and chloroform were added. Later, the acidic solution was made alkaline with 25% aqueous ammonia at pH 9.0. The mixture was later extracted with chloroform three times and concentrated to 1.0 ml with water bath. The extract was dissolved in methanol for gas chromatography using a DB-5MS capillary column (30 m x 0.25 mm x 0.25 µm film thicknesses) and nitrogen as carrier gas. Initial oven temperature was 60ºC for 5 minutes, which was increased by 10ºC/min for 20 minutes and by 15ºC for 4 minutes. Injector temperature was 250ºC and the volume injected was 0.2 µl, with a split ratio of 20:1. Compositions of the alkaloid were obtained from electronic integration using

flame ionization detector (FID, 32°C) which were compared with a commercially available standard (NIST).

3. Results and Discussions

Analytical procedure for different phytochemicals from *P. aquilinum* fiddleheads using GC-FID showed presence of 11 flavonoids, one tannin, 6 glycosides, 2 saponins and 18 alkaloids. Results revealed that the fiddleheads extract is rich in flavonoids (178778.2902 mg/100g), followed by tannins (77884.6 mg/100g), glycosides (41709.08539 mg/100g), saponins (6283.55506 mg/100g), and alkaloids (786.21576 mg/100g). The flavonoids detected include quercetin-7-methyl ether (33.220% by weight), quercetin-3-O-rutinoside (21.812%), quercetin-4-methyl ether (16.630%), quercetin-7,4'-dimethyl ether (9.692%), kaempferol-3-0-rutinoside (8.714%), kaemferol-4'-methyl ether (6.292%), taxifolin-4'-methyl ether (1.730%), taxifolin-7-methyl ether (0.890%), aromadendrin-4'-methly ether (0.506%), naringenin-4'-methyl ether (0.375%) and low in eroidictyol-7,4'-dimethyl ether (0.139%).

Table 1: The composition (mg/kg) of flavonoids in *P. aqiulinum* fiddleheads extract

Name of compounds	Ret time (min)	Amount (mg/kg)
Kaempferol-3-0-rutinoside	13.779	15578.9
Quercetin-3-0-rutinoside	15.088	38995.2
Aromadendrin-4'-methly ether	16.622	904.53488
Quercetin-4-methyl ether	17.451	29731.5
Taxifolin-7-methyl ether	18.448	1590.19077
Taxifolin-4'-methyl ether	19.563	3092.87082
Quercetin-7-methly ether	20.544	59391.2
Naringenin-4'-methyl ether	21.478	670.90525
Kaempferol-4'- methyl ether	22.638	11248.8
Eroidictyol-7,4'-dimethyl ether	23.229	247.98846
Quercetin-7,4'-dimethyl ether	24.194	17326.2
Totals		178778.2902

Ret Time = Retention Time

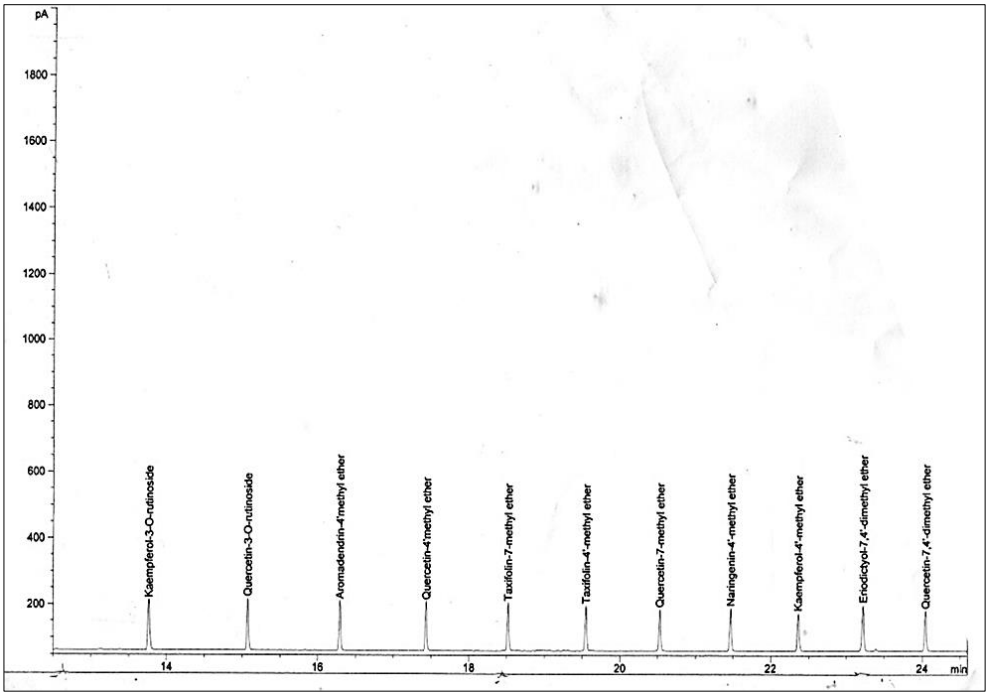

Fig 1: GC-FID chromatogram of flavonoid compositions in *P. aquilinum* extract

Tannic acid (77884.6 mg/kg) was obtained as the only tannins presence in the fiddleheads extract of *P. aquilinum* (table 2.) The GC-FID separation of saponin showed the presence of two saponins, with high content of avenacin A-1 (59.129% by weight) and avenacin B-1 (40.871%) fiddleheads extract (table 2).

Table 2: The composition (mg/kg) of tannins and saponins in *P. aqiulinum* fiddleheads extract

Name of compounds	Ret time (min)	Amount (mg/kg)
Tannin		
Tannic acid	19.571	77884.6
Saponin		
Avenacin A-1	23.427	3715.40710
Avenacin B-1	24.789	2568.14796
Totals		6283.55506

Ret Time = Retention Time

Six glycosides were detected in *P. aquilinum* fiddleheads extract. Digoxin (45.886%), ouabain (16.037%), salicin (14.687%), amygdalin (11.839%), arbutin (8.604%) and digitoxin (0.0000295%) were the glycosides detected to be presence in fiddleheads extract of *P. aquilinum* (table 3).

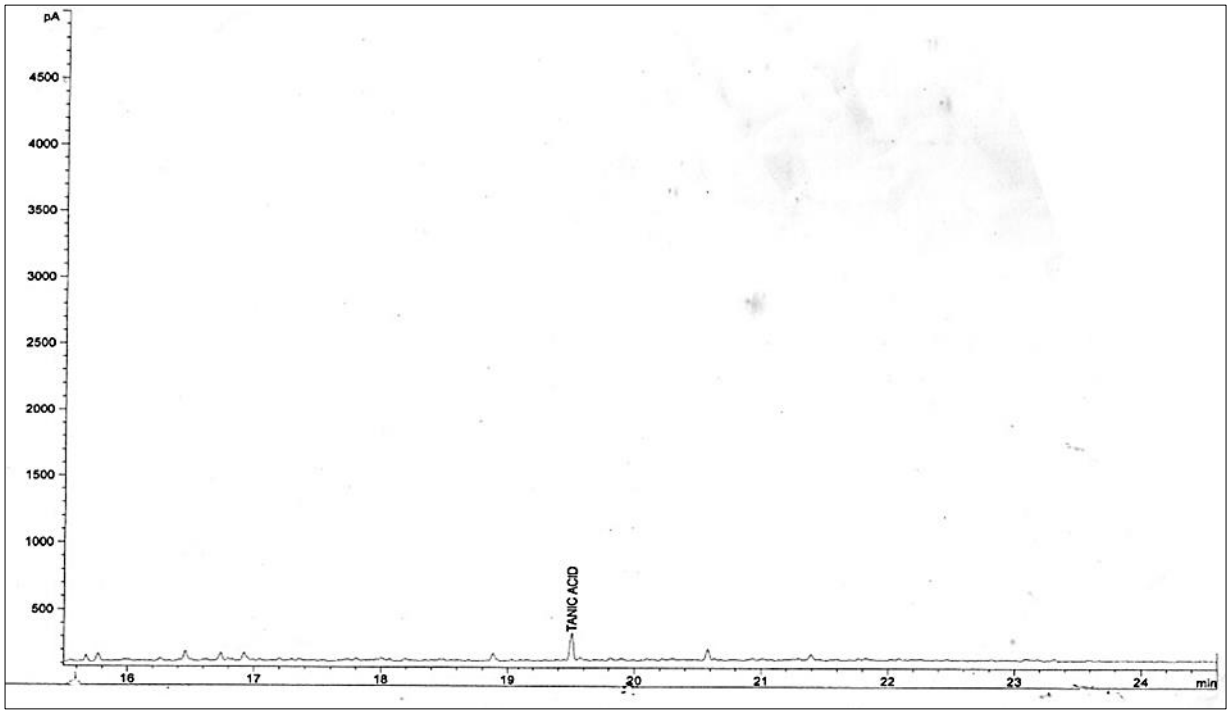

Fig 2: GC-FID chromatogram of tannin composition in *P. aquilinum* extract

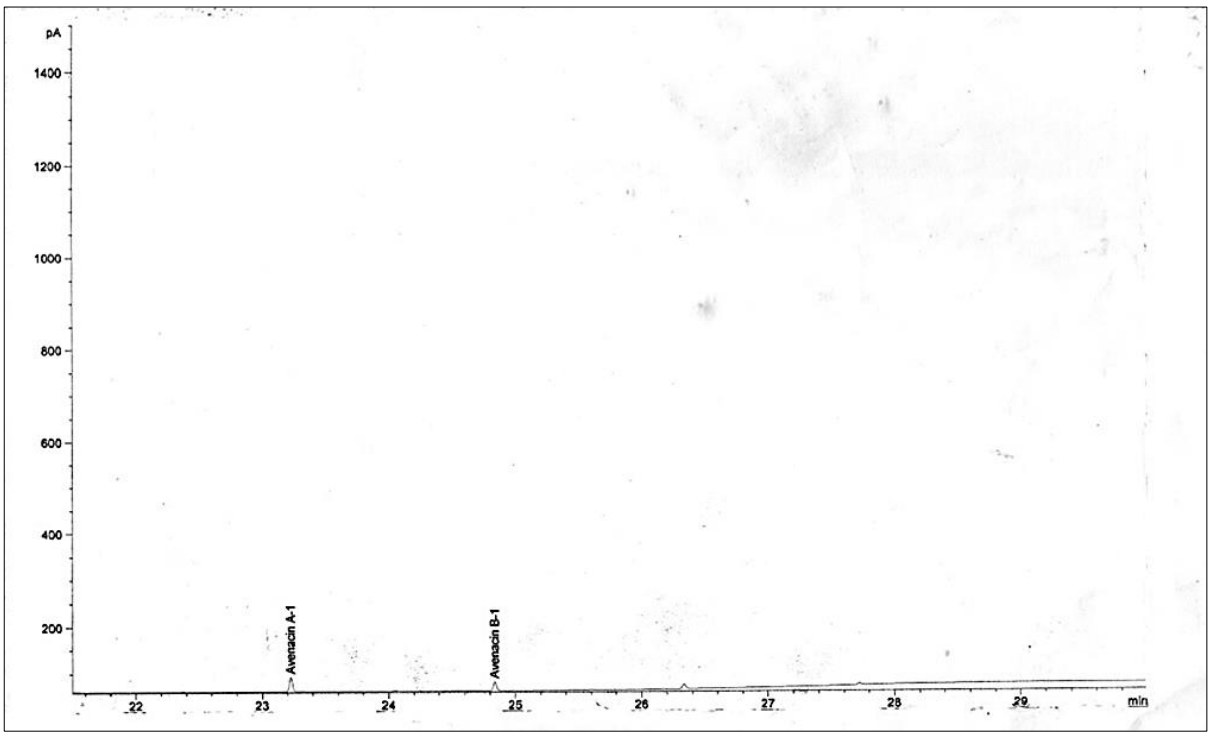

Fig 3: GC-FID chromatogram of saponin compositions in *P. aquilinum* extract

Table 3: The composition (mg/kg) of glycosides of *P. aqiulinum* fiddleheads extract

Name of Compounds	Ret time (min)	Amount (mg/100g)
Arbutin	17.345	3588.66576
Salicin	18.818	6125.86191
Amygdalin	19.459	4938.04477
Ouabain	20.501	6688.89062
Digitoxin	21.342	1228.92233
Digoxin	23.049	19138.7
Totals		41709.08539

Ret Time = Retention Time

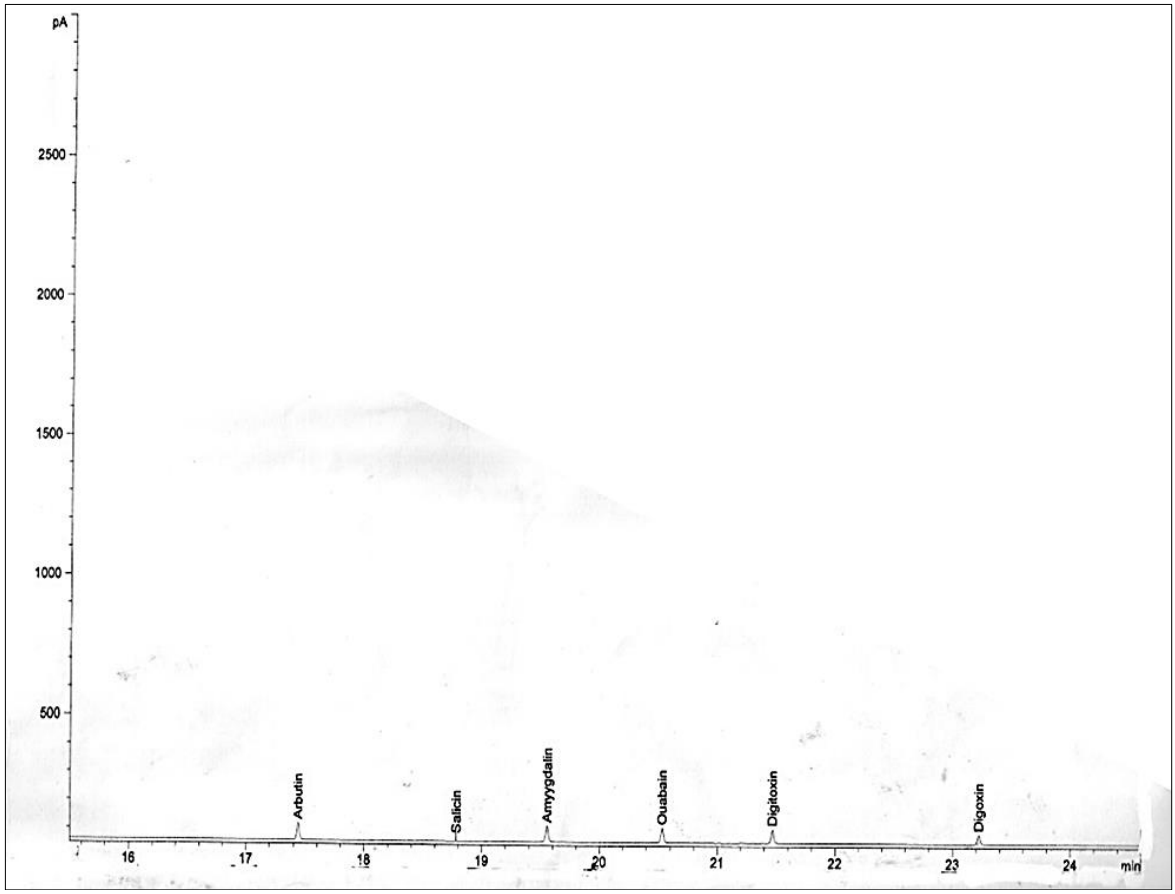

Fig 4: GC-FID chromatogram of cardiac glycoside compositions in *P. aquilinum* extract

The alkaloids composition of fiddleheads extract of *P. aquilinum* showed that the extract is rich in buphanidrine with 20.899% by weight, followed by crinamidine (13.664%), crinane-3α-ol (11.989%), epoxy-3,7-dimethoxycrinane-11-one (10.453%), oxoassoanine (6.873%), dihydro-oxo-demethoxyhaemanthamine (6.771%), 1-β, 2-β-epoxyambelline (6.495%), 6-hydroxypowelline (5.990%), undulatine (4.300%), 6-hydroxyundulatine (2.423%), powelline (1.841%), augustamine (1.678%), ambelline (1.534%), 6-hydroxybuphanidrine (1.523%), 9-octadecenamide (1.356%), voacangine (0.852%), mitraphylin (0.724%) and akuammidine (0.646%) as the minor constituent (Table 4).

Table 4: The composition (mg/kg) of alkaloids in fiddleheads extract of *P. aqiulinum*

Name of compounds	Ret time (min)	Amount (mg/kg)
9-Octadecenamide	10.584	10.65964
Dihydro-oxo-demethoxyhaemanthamine	12.175	53.23113
Augustamine	13.625	13.19471
Oxoassoanine	15.086	54.03688
Crinane-3α-ol	16.473	94.25554
Buphanidrine	17.357	164.31128
Powelline	18.760	14.39730
Undulatine	19.338	33.806667
Ambelline	20.541	12.06384
6-Hydroxybuphanidrine	21.008	11.97238
6-Hydroxypowelline	22.191	47.09292
Crinamidine	23.147	107.42688
6-Hydroxyundulatine	23.963	19.05268
1-β, 2-β-Epoxyambelline	24.802	51.06240
Epoxy-3,7-dimethoxycrinane-11-one	25.825	82.18475
Akuammidine	26.609	5.07932
Mitraphylin	27.273	5.69136
Voacangine	27.622	6.69608
Total		786.21576

Ret Time = Retention Time

Information on phytochemcal compositions of plant materials are needed generally for the development of pharmacologically bioactive drugs that will be used in the treatments of diseases of various kinds. Table 1 revealed that *P. aquilinum* fiddleheads is richer in flavonoids, which are known to exhibit antioxidant, anti-inflammatory, antibacterial,

antiviral, vasodilatory, antiulcer, hepatoprotective, free radical scavenging, and antimalarial properties, and for treatment of neurodegerative diseases[14-18]. They are reported also to inhibit variety of enzymes including aromatase activity of cytochrome $P_{-450,}$ hydrolases, hyalouronidase, cAmp-phosphodiesterase, lipase, kinase and α-glucosidase, and to exert membrane-stabilizing effect[19, 20]. Keampferol-3-o-rutinoside which is present in *P. aquilinum* fiddleheads extract is anti-hypertensive in action[21], and quercetin-3-o-rutinoside, an important therapeutic substance used to treat blood circulatory disorders, atherosclerosis; and to reduce blood pressure and to stimulate vitamin C utilization [22], while aromadendrin-4'-methyl ether is reported to possess antibacterial, anticandidal, radical scavenging and chelating activities[23]. Also, eriodictyol-7,4'-dimethyl ether and naringenin-4'-methyl ether have been shown to exhibit antibacterial activity[24].

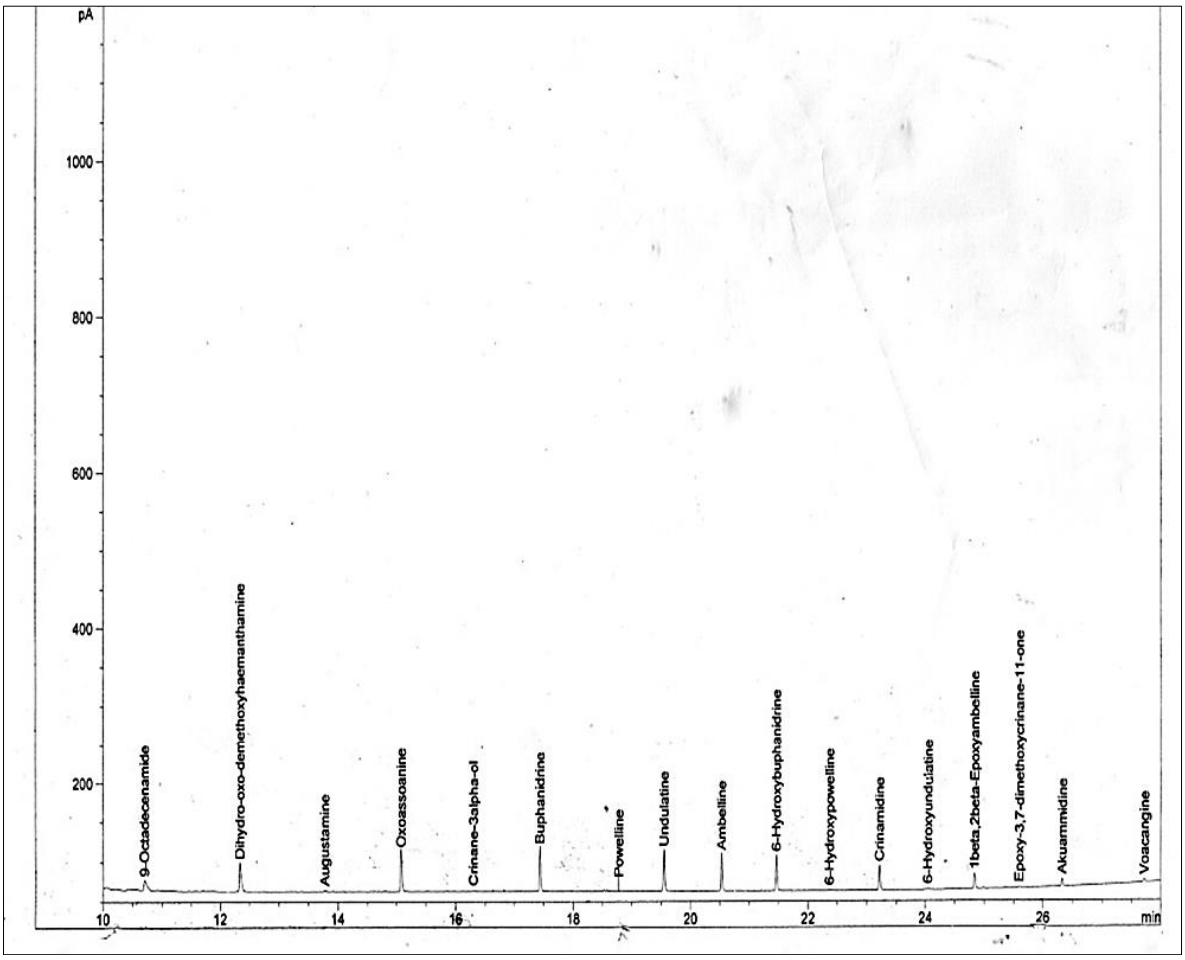

Fig 5: GC-FID chromatogram of alkaloid compositions in *P. aquilinum* extract

Tannins are physiological important phytochemicals with proven antioxidants, antidiarrheal, anti-haemorrhoid, antimalarial and wound healing activities [25-27]. The antioxidant and radical scavenging activities of tannic acid have been reported [28]. Avenacins, present in *P. aquilinum* fiddleheads extract have been shown to exhibits membrane-stabilizing activity [21], which makes saponins suitable candidates for the repair of inflamed membranes [29]. They are also known as potential antifungal compounds [30]. Cardiac glycosides possessed broad range of pharmacological properties. Arbutin detected in the extract is used to inhibit melanin biosynthesis and thus may be useful in treating skin hyperpigmentation [31]. Also, its antioxidant, anti-inflammatory, and antitumour properties have been reported [32-34]. Salicin has been shown to exhibit antibacterial activity [35], and also to inhibit cyclooxygenase (COX) activity and thus a useful anticancer agent [36]. Ouabain, also present in extract of *P. aquilinum* fiddleheads is a cardiotonic steroid [37]. It is used also to regulate Na^+/K^+-ATPase activity, and to treat heart insufficiency [38]. Amygdalin possess antipyretic, antitussive, anticancer, antiatherogenic, anti-inflammatory, immune regulatory and thirst quenching effects [39, 40]. Cardiac glycosides such as digitoxin and ouabain have been shown to exhibit antitumour activities [41]. They are also very valuable in the treatment of chronic cardiac insufficiency [42, 43].

Many alkaloids have demonstrated important pharmacological effects including antihypertensive, anticancer, and antimalarial [45], analgesic, antispasmodic and bactericidal [29, 45] activities. Some alkaloids have antioxidant activities [46], while others are utilized as local stimulants, including the stimulation of central nervous system (CNS) [47], and in treating erectile dysfunction [48]. 9-octadecenamide, a phytochemical detected in *P. aquilinum* fiddleheads extract is used for treating mood and sleep disorders, and cannabinoid-regulated depression [59], while haemanthamine derived compounds are well known for their antimalarial activities [50]. Oxoassoanine and crinamidine which are phytoconstituents present in *P. aquilinum* fiddleheads extract have been reported to exhibit acetylcholnesterase inhibitory activities [51, 52]. The antimicrobial activities of buphanidrine, undulatine, ambelline and akuammidine have been reported [53, 54], and akuammidine is said to possess antipyretic and anti-inflammatory activities [55, 56]. Voacangine, another bioactive compound in the extract of *P. aquilinum* fiddleheads has been

shown to possess filaricidal activity [57]. *P. aquilinum* fiddleheads extract contained phytocconstituents with proven multi-functional pharmacological potentials. This makes *P. aquilinum* fiddleheads a very good source of health promoting vegetable and therefore, could be a potential raw material for the production of supplements and novel drugs.

4. Acknowledgement

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