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Phytochemical Screening, GC-MS Analysis of the Methanolic Extract of Bracken Fern (*Pteridium aquilinum*)

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ABSTRACT

Bracken, a type of large fern belonging to the genus *Pteridium aqulinum* and family Dennstaedtiaceae, is found in various regions of the world and has been classified as carcinogenic to humans. It is also known for its anti-parasitic and anti-ascaris properties because of its ability to kill small intestinal ascaris roundworms in the body. This plant synthesize non nutritive phytochemicals with potential toxicity and health benefit. For instance, anti-nutrients, which are natural or synthetic compounds that interfere with the absorption of nutrients by binding to vitamins and minerals, preventing their uptake, or inhibiting enzymes. Literature on the phytochemical screening of bracken is limited; Thus, this research was carried out to access the anti-nutrient, phytochemical constituents and GC-profiling of methanol extract of bracken fern was carried out. Fresh plant samples were collected from Cece, in Lapai Local Government, of Niger State and samples were identified by certified botanist. The results of the study shows that the percentage yield of the methanolic extract of stem was higher than that of the leave. It also shows the presence of terpenoids in both extracts but absence of tanins. Stem is rich in flavonoid and alkaloid while leave is rich in saponin and phenolic compound. Both leaves and stem contains higher content of anti nutrients in the range (mg/kg) cyanide (5.197 - 3.063), oxalate (2.112 - 2.843) and phytate (0.951 - 0.798). GC-MS analysis reveals that presence of bioactive components. The major constituents were 1,3 butadiene (19.60 %) for leave, spiro[4.5]decan-2-one at 33.90 and 12.08 % for leave and stem extract respectively. Anthracene and its derivatives were present in major amount in the leave and stem extract. These are physiologically important bioactive molecules with anti-inflammatory, antibiotic, anticancer and antimalarial properties that can be isolated for treatment. In addition to other industrial applications such as used as an antioxidant in polymers, production of rubber and lubricants.

Keywords: Bracken, Pteridium Aqulinum, Antimicrobial, Anti-Nutrient, Methanol Extract

INTRODUCTION

Bracken, a type of large fern belonging to the genus *Pteridium aqulinum* and family Dennstaedtiaceae, is found in various regions of the world (Malik et al., 2023). It is characterized by its large, divided leaves. This plant has an alternating generation system, with large plants producing spores and small plants producing sex cells (Potter & Baird, 2000). Bracken fern is one of the most widely distributed plants on the planet, except in polar and desert regions, and thrives in moorland habitats. (Marrs and Watt, 2006). In the past, it was considered to be a single species, but recent studies have identified around ten different species within the genus. Although some fiddleheads, or immature fronds are consumed, bracken is suspected of causing human cancers and has been classified as 'possibly carcinogenic to humans' by the WHO/IARC. (Fletcher *et al.,* 2011). Exposure

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to the potentially harmful compounds can occur through the consumption of traditional medicine, such as traditional Chinese medicine, or ingesting contaminated milk, meat, spores, or drinking water, which is common in certain regions of Asia and North and South America (Rasmussen, 2021).

Bracken has long been recognized for its potential pharmaceutical uses and its toxicity. Furthermore, bracken has been used medicinally as an anthelminthic agent (Marrs and Watt,

2006). Due to anti-parasitic and anti-ascaris properties, it is used as a biocid to kill small intestinal ascaris roundworms in the body. Also, mixture of bracken and olive oil has been used as a treatment of wound in livestock (Marrs & Watt, 2006). Phytochemicals are non-nutritive plant compounds with potential health benefits. Epidemiological studies show potential cancer prevention benefits, but clinical trials lack evidence (Ji & Tae-Jin, 2023). Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients (Guldiken et al., 2018). They protect plants from disease and damage, and also contribute to the plant's colour, aroma and flavor (Ji, & Tae-Jin, 2023). Recently, it has been clearly shown that they also have roles in the protection of human health, when their dietary intake is significant (Guldiken et al., 2018). Till date over 4,500 phytochemicals have been reported and are classified on the basis of their protective functions, and physical and chemical characteristics. (Ji & Tae-Jin, 2023).

An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobials encompass a wide range of substances that combat microorganisms like bacteria, fungi, and viruses. These agents can act by inhibiting microbial growth (microbiostatic) or by directly killing them (microbiocidal) (Presterl et al., 2019). Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients. Nutrition studies focus on anti-nutrients commonly found in food sources and beverages. (Awulachew, 2022). Anti-nutrients may take the form of drugs, chemicals that naturally occur in food sources, proteins, or overconsumption of nutrients themselves. Anti-nutrients may act by binding to vitamins and minerals, preventing their uptake, or inhibiting enzymes.

The Chemical Constituents of Bracken

Ptaquiloside: Ptaquiloside, a prominent illudane-type glycoside, is found in various ferns, but its toxicology and health effects have been most extensively studied in bracken ferns. These large perennial ferns have a global distribution, and farm animals tend to browse on them only during food scarcity, as they are neither nutrient-rich nor very palatable. Bracken has been known to cause several diseases in farm animals, including Bovine Enzootic Haematuria, Acute Bracken Poisoning, and Bright Blindness. Cattle and buffaloes in paddocks or open grazing areas with bracken are affected by the first two syndromes, while the latter is commonly encountered in free-ranging sheep on bracken-infested land. These diseases are commonly reported in countries such as Brazil, Venezuela, Australia, New Zealand, Italy, Spain, Portugal, England, and Scotland (Marrs and Watt, 2006; Vetter, 2010; O'Connor et al., 2019). Bracken and ptaquiloside have also been included on the WHO/IARC urgency list of potentially carcinogenic compounds/products to be re-evaluated (WHO/IARC, 2021.) There is a considerable variation in the distribution of illudane glycosides within and between bracken species. The content of ptaquiloside in spores

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and roots is considered low compared to what is found in the fronds. (Kisielius *et al.,* 2019; Rasmussen *et al.,* 2015).

Pterosins and Pterosides: Pterosins are a large group of compounds, which are naturally occurring in different plant ferns such as bracken (Mohammad *et al.,* 2016). Pterosins are sesquiterpenoids and their structures are derivatives of 1-indanone skeletons. Ptaquiloside is readily hydrolyzed to form the non-carcinogenic aromatic indanonepterosin B (Nagao *et al.,* 1989). Recently, (Mohammad *et al.,* 2016) identified a range of pterosins and pterosides in the bracken rhizomes indicating a more complex ptaquiloside-chemistry. Pterosin B has been reported to be present in rather high concentrations (>2100 mg/g) in bracken fronds and rhizomes (Rasmussen, 2008; Mohammad *et al.,* 2016). Quantification of pterosins in the living tissue is difficult, as e.g. pterosin B may form in substantial amounts during extraction and sample pre-treatment. In particular, drying temperature of the biomass has a profound effect on formation of pterosins, in particular pterosin B (Caceres-Pena *et al.,* 2013). Ptaquiloside hydrolysis to pterosin B is strongly pH dependent (Ayala-Luis *et al.,* 2006). This has been used by many researchers in developing analytical methods for ptaquiloside based on a 1:1 conversion into pterosin B, e.g. for analysis of ptaquiloside and ptaquiloside residues in plants, environmental samples, meat and milk (e.g. Zaccone *et al.,* 2014). Glycosidase enzymes can also cause PTA degradation. PtB becomes the end product of the above-mentioned reactions, but other intermediates exist: Ptaquilosin and the so-called Bracken Dienone (O'Connor *et al.,* 2019). PtB is present at similar levels as PTA in bracken fronds and can be used an indicator of an earlier presence of PTA in the compound (Rasmussen and Pedersen, 2017). Ptaquilosin and Bracken Dienone are believed to be the ultimate carcinogens (alkylating metabolites) and are highly unstable under acid and neutral pH. They are only found in neutral to alkaline matrices such as milk (Aranha *et al.,* 2019).

Phytochemical Screening of Bracken

Phytochemical screening of bracken fern (*Pteridium aquilinum*) has revealed the presence of numerous active compounds (Kobayashi & Koshimizu, 2014), including phenolics, terpenoids, flavonoids, tannins, saponins, alkaloids, and phytoecdysteroids. Phenolics and flavonoids are the most abundant compounds, and are thought to be responsible for the plants antioxidant activity. Other components, such as saponins, alkaloids, and phytoecdysteroids, have been suggested to have biological activities such as antimicrobial, cytotoxic, and/or anti-inflammatory properties. Additionally, ptaquiloside, a carcinogenic sesquiterpenes, has been isolated from bracken fern, and its presence may be responsible for some of the plants toxic effects (Hilal *et al.,* 2014).

Anti-nutrient Activities of Bracken

Bracken ferns has been studied for its anti-nutrient activity. In laboratory studies, pterosins B has been found to have anti-nutrient activity against a range of animals, including rats, mice, chickens, and pigs. The anti-nutrient activity of pterosin B has been attributed to its ability to inhibit the absorption of essential nutrients, such as proteins, carbohydrates, and fats, as well as its ability to interfere with the utilization of vitamins and minerals. Additionally, pterosins B has been found to have antioxidant activity, which may help protect against oxidative stress and cell damage.

It has been established that bracken fern is carcinogenic. However, literature on the study of bracken is limited; thus, this research was carried out to access the anti-nutrient, phytochemical constituents and GC-profiling

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of methanolic extract of bracken fern for presence of physiologically and industrially important compounds in bracken fern.

MATERIALS AND METHODS

Sample collection and Preparation of Plant Extract

Fresh plant were collected from Cece, under Lapai Local Government, of Niger State. The samples were identified in the Department of Biology, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria. The collected samples were dried under shade at room temperature, subsequently pulverized using motar and pestle separately and further sieved to obtain a coarse powder. 25 g of each sample was collected extracted in 250 ml of methanol for 48 hours. The solution was decanted, and then filtered with Whatman 0.45 mm filter paper. The filtrate was concentrated using simple distillation apparatus. The extract were then screened qualitatively and quantitatively for the presence of phytochemicals.

Preliminary Phytochemical Screening

The Phytochemical Screening Of The Methanolic extract of both the stem and leave of the bracken were determined. The phytochemical components analyze include saponins, tannin, flavonoid, phenolic compounds, alkaloid and terpenoids. This carryout using standard procedures as described by (Albari *et al.,* 2014).

Gas Chromatography-Mass Spectrometry Analysis

The Gas Chromatography-Mass Spectrometry analysis of the sample extracts was carried out by a method as described by (Lawerence & Paul, 2019) using an Agilent chromatography hypherated to a mass spectrophotometer (Model number: 7890A GC system, 5675C Inert MSD with triple –Axis detector) with an auto injector (10ul syringe) using helium as a carrier gas. Capillary column with specifications: length; 30m, internal diameter 0.2 um, thickness; 250 um, treated with phenyl methyl silox was used for all chromatographic separation. Ms Solution software provided by supplier was used to control the system and to acquire the data; Identification of the compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from NIST library (NISTII).

RESULTS AND DISCUSSION

Table 1 shows the percentage yield of methanolic extract of leave and stem samples. The methanolic extract of the stem shows the highest percentage yield than the leave. This is because, stem back are more soluble in methanol than water.

Table 1: Percentage yield

Plant extract	Solvent	Wt. of Dry sample (g)	Wt. of Extract (g)	% Yield
Leaves	Methanol	25	0.781	3.124
Stem	Methanol	25	1.585	6.34

Percentage yield = weight of extract*100/weight of the dry sample

Phytochemical Constituents

Bracken fern have both been qualitatively and quantitatively studied for their

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phytoconstituents and the result of both extract were more or less the same. Methanolic extract of bracken of the leave and the stem contain similar phytochemical which include terpernoid, but lack only tannin among the phytochemicals tested. Table 2 shows that various phytochemicals have been found to possess a wide range of activities, which helps in protection against chronic diseases. For instance, alkaloid, flavonoid, saponins and tannins have hypoglycemic and anti-inflammatory activites (Fadilaturahmah, et al., 2022; Sandhiutami et al., 2022). Terpenoids have also been shown to decrease blood sugar level while saponins demonstrate hypocholesterolemic and antidiabetic properties (Rahal et al., 2014).). Alkaloid are highly reactive substances with biological activities even at low doses. Many alkaloid have remarkable effects on the central nervous system and gastrointestinal tract. The anti-oxidants of *pteridium aquilinum* effects is attributed to the presence of alkaloids, flavonoids and saponins (O Pokhrel et al., 2015). Janos (2010) reported some of the flavonoids present in bracken include kraempferol, quercetin and apigenin with combined concemtration range of 10 – 25 mg/g while phenoloids such as cinnamic and benzoic acids.

Table 2: Results of qualitative analysis of Bracken fern (Stem and Leave) extract

Tests	Leave	Stem
Saponin	+	-
Tanin	-	-
Flavonoid	-	+
Phenolic compound	+	-
Alkaloid	-	+
Terpenoid	+	+

Anti-Nutrient Result

Pteridophytes are the most primitive vascular plants, found in living and fossil conditions. Generally animal avoids feeding on pteridophytic plants, it indicate that these plant may have some kind of toxicity. Anti-nutritients are natural or synthetic substances, which adversely affect health and growth of human, and animals both. In present investigation pteridophytic plants, *pteridium aaquilinum* was studied for the anti-nutritional contents. Result (Table 3) shows that the stem and leave samples contain high levels of cyanide. Both leave and stem contain high content of cyanide (5.197 ± 0.050 mg/kg and 3.063 ± 0.025 mg/kg), followed by oxalate content contain (2.112 ± 0.062 mg and 2.843 ± 0.286 g) and phytate content contain (0.951 ± 0.005 % and 0.798 ± 0.092 %) respectively.

Table 3: Results of quantitative analysis of anti-nutrients of Bracken fern extract from leave and stem

Sample	Cyanide	Oxalate	Phytate
Stem	3.063 ± 0.025	2.843 ± 0.286	0.951 ± 0.005
Leaves	5.197 ± 0.050	2.112 ± 0.062	0.798 ± 0.092

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The GC analysis shows the presence of more compounds in the stem extract than the leave extract. This agrees with data of percentage yield of methanol extract obtained for this study. The results (Table 4 and 5) for GC analysis of methanolic extract suggest the presence of physiologically important bioactive molecules such as pyridine, clofibrate, oxipurinol and spiro[4.5]decan-2-one. Pyridine serves as a scaffold for biologically active compounds with anticancer and antimalarial properties while spiro[4.5]decan-2-one is used in drug formulations for treating of cerebral and nervous disorders. It is also used as an intermediate in the synthesis of other drugs, such as anti-inflammatory agents and antibiotics (Alrooqi et al. 2021). Clofibrate lowers blood glucose levels diabetic patients and reduce cellular damage caused by reactive oxygen species (ROS) in hepatoma cells. (Twomey & Bloom, 1979; Chen et al., 2017). Oxipurinol is suggested to treat allopurinol-intolerant hyperuricaemia and congestive heart failure by improving cardiac muscle work efficiency and reducing oxygen demand (Hailemichael et al., 2022). 1,3-butadiene, a classified human carcinogen is also present in significant amount in extract and can have adverse health effects upon long-term exposure. Other chemicals present in extract have others industrial applications. For instance phenol, 3,5-bis(1,1-dimethyl) is used as an antioxidant in polymers and lubricants while 1,3-Butadiene is used for the production of styrene-butadiene rubber (SBR), which is used in car tires and other products. Dehydrocohumulic acid is contributes to the bitter taste and aroma characteristics some plants. 2-Ethylanthracene is used for the production of dyes, antioxidants, and pharmaceuticals.

Table 4: GC – MS Aromatic Mode of analysis on Methanolic Extract of BFL

S/no	Rt (min)	Compound	MF	Area (%)
1.	62.545	Benzoic acid	C_6H_5COOH	1.03
2.	67.113	Anthracene, 9-propyl	$C_{17}H_{16}$	1.90
3.	69.093	6-Chloro-2-methylquinoxaline	$C_9H_7ClN_2$	3.94
4.	70.717	Pyridine	C_5H_5N	1.25
5.	71.717	2-methylsulfonyl-thiophene-3-carboxylic Acid	$C_6H_60_4S_2$	3.32
6.	71.986	Spiro [4.5] decan-2-one	$C_{10}H_{16}O$	33.90
7.	73.001	10-methyldodecan-4-olide	$C_{13}H_{24}O_2$	7.23
8.	74.524	1, 3-Butadiene	$(CH_2=CH-CH=CH)_2$	19.60
9.	76.706	(3H) Pyrazole, 3,5-diphenyl-3-methyl	$C_{16}H_{14}N_2$	3.93
10.	77.265	Phenol, 3,5-bis(1,1-dimethyl)-	$C_{14}H_{22}O$	14.08
11.	77.925	3,3-Diphenyl-5-Methyl-3H-pyrazole	$C_{16}H_{14}N_2$	3.73
12.	78.990	Phenanthrene, 9-ethyl	$C_{16}H_{14}$	

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Table 5: GC-MS Aromatic Mode of Analysis on Methanolic Extract of BFS

S/N	Rt (min)	Compound	MF	Area (%)
1.	13.970	Oxypurinol	$C_5H_4N_4O_2$	5.25
2.	20.416	Phenol, 2,4-bis (1,1 dimethyl ethyl)	$C_{17}H_{30}OSi$	0.88
3.	24.274	Phenol, 2,5-bis(1,1dimethyl)		$C_{14}H_{22}O$ 20.84
4.	32.598	Dehydro – cohumulic Acid	$C_{14}H_{18}O_3$	1.20
5.	37.826	10-Methylanthracene-9-carboxaldehyde	$C_{16}H_{12}O$	2.04
6.	40.313	1-(4-Methoxyl-biphenyl-4-yl)-ethanol	$C_{15}H_{16}O_2$	1.60
7.	43.714	5,6-Dimethyl-1-(propynyl)-1H-Benzimidazole	$C_{12}H_{12}N_2$	3.04
8.	45.389	Cyclobuta[a]dibenzo[c,f]cyclohepta-3,6-diene	$C_{17}H_{16}$	3.38
9.	47.419	Germane, dichlorobis (1-Methylethyl)-	$C_6H_{14}Cl_2Ge$	1.45
10.	48.231	3,5-Hexadiene-2-One	C_6H_8O	0.09
11.	49.145	Clofibrate	$C_{12}H_{15}ClO_3$	1.28
12.	51.853	1-Amino-2-hydromethyl	$C_{15}H_{11}NO_3$	1.02
13.	53.764	6-Nitro-2P-tolyl-2H-Indazole	$C_{13}H_9N_3O_2$	2.08
14.	55.388	Acetimide	CH_3CONH_2	2.54
15.	57.063	9,9–Diazidofluorene	$C_{13}H_8N_6$	0.94
16.	69.651	2,5-Cyclohexadiene-1,4-dione,2-bromo -3,5,6-trimethyl	$C_9H_9BrO_2$	.10
17.	72.138	Spiro[4.5] decan-2-One	$C_{10}H_{16}O$	12.08
18.	73.965	Anthracene, 2-ethyl	$C_{16}H_{14}$	28.40
19.	74.879	Phenanthrene, 9-ethyl	$C_{16}H_{14}$	9.99

CONCLUSION

The percentage yield of the stem is higher than that of the leave, this suggests that the stem contains compounds that are more soluble in methanol than contained in the leave. Physiochemical studies revealed the presence of terpenoids in both extracts but absence of tanins. In addition, stem is rich in flavonoid and alkaloid while leave is rich in saponin and phenolic compound. Both leaves and stem contains higher content of cyanide (5.197 mg/kg) and (3.063 mg/kg) respectively. GC-MS analysis of the ethanolic extract of *pteridium aquilinum* (L) Kuhn reveals that presence of physiologically and industrially important compounds. The major constituents were spiro[4.5]decan-2-one at 33.90 and 12.08 % for leave and stem extract respectively. 1,3 butadiene (19.60 %). Anthracene and its derivatives were present in major amount in the leave and stem extract. Anthracene and its derivatives were present in major amount in the leave and stem extract. These are physiologically important bioactive molecules with anti-inflammatory, antibiotic, anticancer and antimalarial properties can be isolated and use for treatment. In addition to other industrial applications such as used as an antioxidant in polymers, production of rubber and lubricants.

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