Nutraceutical Constituents of *Casuarina Equisetifolia* Leaves and Fruits

Muibat Olabisi Bello*, Temitope Azeezat, Yekeen, , and Endurance Oghenekevwe Aneke

Abstract— The levels of some phytochemicals in the leaves and fruits of *Casuarina equisetifolia* were quantified. Results revealed that both the leaves and fruits contained valuable nutrients such as crude fibre (21.85±0.20, 16.62±0.33g/100g) and ascorbic acid (8.24±0.15, 8.15±0.19 mg/100g). Nutritive elements in mg/g were Fe (264.00, 225.30), Mn (225.90, 120.90), K (235.50, 246.30), Zn (16.50, 13.20) in the leaves and fruits respectively. The levels of antinutrients in the samples were low to be of health threat. The fatty acids profile showed that the parts contain health benefitting unsaturated fatty acids; linoleic acid (12.08%, 36.24%) and oleic acid (11.81%, 35.05%) while the saturated fatty acids are stearic acid (2.95%, 1.31%) and palmitic acid (2.87%, 6.20%) respectively. The different levels of nutrients in the leaves and fruits support the medicinal use of the plant, and in addition, unveiled the possibility of the parts acting as potential sources of food nutrients and nutraceuticals.

Keywords— *Casuarina equisetifolia*, fatty acids, phytochemicals, nutraceuticals

I. Introduction

THE goal of achieving an optimal state of nutrition and preventing micronutrients deficiencies is becoming a global challenge with the reliance on only few plant species to meet the nutrient requirements of man. Several underutilized plant species have been investigated by [1], [2], [3], [4], [5] for the level of nutrient and anti-nutrients in plant species in an effort to search for those with nutraceutical potentials. As part of ongoing screening processes for plant parts with health benefits, leaves and fruits of *Casuarina equisetifolia* were investigated. The plant *Casuarina equisetifolia* Forst belongs to the family *Casuarinaceae* [6]. It is a widespread seashore tree commonly known as Ironwood, Beefwood and Whistling pine and it's often planted as a wind break. Its fruit is tiny, cone-like, rough, brownish color, usually 2cm long (figure 1). The fruit naturally splits open after its dried and sometimes after it falls off the tree. This tree is often planted for Coastal reclamation, erosion control and pulp for making paper, timber for fuel [7]. The plant is a source of biologically active compounds such as catechin, ellagic acid, gallic acid, quercetin, and lupeol, which are anti-oxidants [8], coumaroyl triterpenes [9] and d-gallocatechin [10]. The plant is also known to store tannin [11] and proline [12] as well as being a nitrogen fixing plant. *C. equisetifolia* is used as astringents [13] and to treat diarrhea, dysentery, headache, fever, cough, ulcers and toothache [14]. Extracts of leaves exhibit anticancer properties and seeds are anthelmintic, antispasmodic and antidiabetic [15]. In view of the fact that the plant parts have been widely exploited for its medicinal values, this study focused on investigating its proximate constituents, level of mineral elements and fatty acids profile.

Muibat Olabisi Bello,Temitope Azeezat, Yekeen, and Endurance Oghenekevwe Aneke are with Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria.

email id: *mobello@lautech.edu.ng, temitopeyekeen2007@yahoo.com, eoaneke@lautech.edu.ng

II. Materials and Methods.

A. Sample Collection and Preparation

The leaves and fruits of *Casuarina equisetifolia* were collected in front of Ghana house at the Agricultural Economics and Extension Department, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso. It was identified and authenticated by Dr. A.T.J. Ogunkunle of the Pure and Applied Biology Department, LAUTECH, Ogbomoso. The samples were dried under laboratory shade and ground to fine powder and stored in airtight container prior to analyses. All analyses were carried out in triplicates.

Fig. 1: Parts of *Casuarina equisetifolia*

B. Proximate Analysis.

Procedures of Association of Official Analytical Chemists [16] were adopted for the determinations. Moisture content was determined by heating 2.0 g of each sample to a constant weight in a crucible placed in an oven maintained at 105^0C. Ash was determined by the incineration of 1.0 g dried defatted samples placed in a muffle furnace maintained at 550^0C for 5hours, Crude fat was obtained by exhaustively extracting 5.0 g of the dried sample in a Soxhlet apparatus using petroleum ether (40-60^0C) as the extractant. Crude protein (% total nitrogen x 6.25) was determined by the Kjedhal method, using

2.0 g of dried, defatted samples. Carbohydrate content was determined by difference.

C. Determination of Mineral Elements.

The sample was digested with nitric: perchloric acid (30ml) in ratio 2:1. The digestate was analysed in triplicate for its elemental composition using Atomic Absorption Spectrophotometer (Series GE711430 V1.27).

Fatty acid Composition and Analysis: A 100 mg oil sample was saponified, neutralized and methylated. The fatty acid methyl ester (FAME) was separated by a Perkin Elmer Autosampler XL gas chromatography (GC) equipped with a flame ionization detector and integrator. The fatty acids were identified by comparing their retention times with those of standards and the content of fatty acids was expressed as percentage of total fatty acids.

D. Quantification of Some Phytochemicals

Quantification of tannins, alkaloids, saponins, oxalates and glycosides was carried out according to standard methods described by [17].

III. Results And Discussion

The proximate composition of both the leaves and fruits of *Casuarina equisetifolia* were reported in Table I. The moisture content of the leaf (8.24±0.35%) and fruit (8.15±0.19%) is low when compared to 55% in *Elaeis guineensis Jacq* leaf [18] and 8.90/100g in *Moringa oleifer*a seed [19]. Foods with high moisture contents are prone to easy microbial spoilage and subsequent short shelf life [20], [21]. Moderate moisture content of ≤ 12mg/g is preferred for shelf stability of food on long storage [22]. The low moisture content of *C. equisetifolia* would hinder the growth of microorganisms and the storage life would be high. The plant parts contain higher ash content compared to herbal leaves of *Mucuna utilis* with ash content of 0.11% [23] and 0.25% of *Prunus armeniaca* seed [24]. The high ash content is an indication that the plants may be good sources of minerals. Proteins are one of the building blocks of the body tissue and also serve as fuel sources. It has been reported that protein-calories malnutrition deficiencies is a major factor responsible in nutritional pathology [24]. Protein content of the leaf (6.61±0.67%) was higher than in the fruit (3.07±0.62%). This is higher than 2.22% in *Prunus armeniaca* seed [25] but lower than protein content of *Vitex doninana* leaf [24]. The crude fibre content in the studied plant parts was higher compared to 7.09±0.11% in *Moringa oleifera* leaves [26] and 3.60% in bean seed [27]. Dietary fibre has been reported to lower the risk of coronary heart disease, hypertension, constipation, diabetes, and colon and breast cancer [28], [29]. Thus *C. equisetifolia* could be valuable sources of dietary fibre in human nutrition as a result of its relatively high fibre content. Crude fat of the plant parts were low but this values are however higher than 2% in *Elaeis guineensis* leaves [30] and 3.15% in *Prunus armeniaca* seed [25]. Carbohydrates play several vital roles in living organisms. They can be oxidized to yield energy, their polymers act as energy storage molecules and their derivatives are found in a number of biological molecules including coenzymes and the nucleic acids [31]. The carbohydrate content of the plant parts were high (leaf; 56.64% and fruit; 65.99%) when compared to 16.59% in *Vitex glandifolia* fruit [32].

Ascorbic acid (Vitamin C) is an antioxidant which block some of the damage caused by free-radicals; substances that damage the DNA. It also helps to strengthen the immune system. The ascorbic acid level was high compared to *Piper guinenses* leaves [33]. A very small daily intake of this vitamin for an adult is required to avoid deficiency disease; scurvy. Even in small amounts it can protect indispensable molecules in the body, such as proteins, lipids (fats), carbohydrates, and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants [34].

Table I
Proximate Composition And Ascorbic Acid Content Of *C. Equisetifolia* Leaf And Fruit (%)

Parameters	Leaf	Fruit
Moisture content	8.24±0.16	8.15±0.19
Crude Protein	6.61±0.67	3.07±0.62
Crude fibre	21.85±0.2	16.62±0.33
Crude fat	4.42±0.20	3.82±0.2
Ash	2.25±0.28	2.36±0.21
Carbohydrate	56.64±0.35	65.99±0.17
Ascorbic mg/100g	34.51±0.11	51.45±0.4

Values are means ±standard deviation of triplicate analyses

Table II
Level Of Some Mineral Elements In *C. Equisetifolia* Leaf And Fruit (Mg/G)

Elements	Leaf	Fruit
Cu	12.00±0.31	17.40±0.25
Zn	16.50±0.15	13.20±0.12
Fe	264.00±1.52	225.30±0.25
Mn	225.90±1.07	120.90±1.02
K	235.50±0.81	246.30±0.31

Minerals are considered to be important in human nutrition [35]. Table II shows the mineral composition of both the leaves and fruits of *Casuarina equisetifolia* in (mg/g). The copper contents of the plant parts were very high compared to the required 2 m/g daily requirement. Copper is a component of several enzymes needed for proper metabolism. Diagnosed deficiency is rare but when it becomes deficient, it may lead to anemia, impaired immunity and bone diseases. Copper and zinc compete for gastrointestinal transport, therefore, an excess of one can theoretically produce a deficiency of the other. There is no recommended daily allowance (RDA) for copper, and excess copper in the body will lead to convulsion in children [36], [37]. Zinc content was 16.5±0.00 mg/g in leaf *and* 13.20±0.00 mg/g in fruit; all much higher than the RDA of 3 mg/g [38]. Zinc is involved in RNA and DNA synthesis, which influences cell division, repair and growth. Accordingly, zinc may help to prevent growth of abnormal cells associated with cancer. Zinc has been used to enhance wound healing and to prevent or treat impaired acuity of taste, smell and night vision. Lack of zinc in the body causes rapid egesting on the surface of wound which may delay quick healing [22]. The iron content of *C.equisetifolia* leaf and fruit were high compared to 15.00 mg/g RDA. Iron is an important element

required by the body to prevent diseases such as anemia. For the formation of hemoglobin, normal functioning of central nervous system and in the oxidation of carbohydrate, proteins and fats [39]. Iron is reported to be very important in normal functioning of central nervous system and in the oxidation of carbohydrate, protein and fats. In circulating red blood cells, iron is a major component of haemoglobin, which transports the respiratory gases; oxygen and carbon dioxide, to the appropriate channels [38]. Manganese (Mn) is one of the important essential elements required in carbohydrates metabolism. It is required by the body for prevention and treatment of weak bones (osteoporosis) and anemia. It is required in very little quantity and its deficiency rarely occurs [40]. The levels of Mn in both the leaf and the fruit of *C. equisetifolia* are 225.90±3.55 and 120.90±1.90 respectively. The potassium contents of the leaf and fruit of *C. equisetifolia* are 235.50±0.00 and 246.30±0.00 respectively which were many times higher than the recommended 18.0 mg/g daily requirement. The WHO recommended intake of potassium per day is 2000 mg for adult and 1600 mg for children. Potassium is involved in the regulation of water balance in the body, maintenance of proper heartbeat, contraction of muscles and conduction of nerve impulses [41]. Potassium regulates water balance, heart rhythm, muscles contraction and nerve-signal conduction. Potassium also influence glucose and lipid metabolism. Increase intake of potassium can lower blood pressure and may help prevent strokes. The presence of phytochemicals (Table III) in the leaves and fruits of *C. equisetifolia* suggests possible medicinal applications. The importance of tannin, steroid, alkaloid, flavonoid and glycoside in various antibiotics used in treating common pathogenic strains has recently been reported by [42]. *C. equisetifolia* contains tannins, used topically to treat acne because of their natural astringent properties [43] and alkaloids which are nitrogen containing naturally occurring compound, commonly found to have antimicrobial properties due to their ability to intercalate with DNA of the microorganism [44]

TABLE III
PHYTOCHEMICAL SCREENING OF *C. EQUISTIFOLIA* LEAF A FRUIT

Parameters	Leaf	Fruit
Tannins	+	+
Steroids	+	+
Alkaloids	+	+
Flavonoids	+	+
Glycosides	+	+

Key: + = present

The plant also contains flavonoids which are used as an ingredient in supplements, beverages or foods. Alkaloid is used in the production of analgesic in the pharmaceutical industries, owing to its analgesic properties [45]. *C. equisetifolia* may be used as analgesic by rural communities due to the presence of alkaloids in it. The plant also contains steroids which help in decreasing inflammation and suppresses the body's immune system.

TABLE IV
LEVEL OF ANTI-NUTRIENT COMPONENTS OF *C. EQUISETIFOLIA* LEAF AND FRUIT

Parameters	Leaf	Fruit
Tannin (mg/g)	13.72±2.46	7.54±1.21
Saponin (g/100g)	1.37±0.03	0.69±0.03
Alkaloid (g/100g)	0.98±0.22	0.96±0.03
Phytate (mg/g)	0.81±0.41	0.92±0.41
Oxalate (g/100g)	0.93±0.41	0.95±0.41

Values are mean ± standard deviation of duplicate analyses

Table IV shows the level of antinutrients; saponin, alkaloid, phytates and oxalate were observed to be significantly low except for tannin which had a higher value in the leaf (13.72±2.46) than (7.54±1.21) in the fruit. This is considered low when compared to 21.19±0.25% reported in *Moringa oleifera* leaves [46]. Tannins are naturally occurring plants polyphenols which main characteristic is to bind and precipitate proteins, thereby interfering with their bioavailability [47]. Tannins in plants act as antioxidants which helps the body against cardiovascular diseases and certain forms of cancer. Saponins have been shown to possess both beneficial (cholesterol lowering) and deleterious properties [48, 49, 50, 51]. Although some saponins have been shown to be highly toxic under experimental conditions, acute poisoning is relatively rare both in animals and man [48], [52]. Saponins are naturally oily glycosides occurring in wide variety of plant. When eaten, they are nonpoisonous to warm blooded animals but are poisonous when injected into the blood stream [53]. So their consumption may likely not to cause adverse effect due to the presence of saponins. The saponin contents of *Cauarina equisetifolia* were low compared to 2.67±0.28% in *Ficus asperifolia* leaves [54]. Alkaloids are used in the production of analgesic in the pharmaceutical industries, due to its analgesic properties [45]. This implies that the plant parts may be used as analgesic by the rural communities due to the presence of alkaloids in it.

Antinutrients are such natural or synthetic compounds that interfere with the absorption of nutrients. One common example is phytate, which forms insoluble complexes with calcium, zinc, iron, and copper [55].

TABLE V
FATTY ACIDS PROFILE OF *CASUARINA EQUISETIFOLIA* LEAF AND FRUIT OIL (%)

Fatty acid	Leaf	Fruit
SATURATED		
Caproic (6:0)	0.17	0.24
Caprylic (8:0)	2.49	1.26
Capric (10:0)	3.57	0.96
Lauric (12:0)	1.17	1.67
Myristic (14:0)	3.53	2.43
Palmitic (16:0)	2.87	6.20
Margaric (17:0)	0.17	0.03
Stearic (18:0)	2.95	1.31
Behenic (22:0)	0.02	0.05
Lignoceric (24:0)	0.00	0.05
Total	16.94	14.2
MONOUNSATURATED		
Palmitoleic (16:1)	0.19	0.12
Oleic (18:1)	11.81	35.05
Erucic (22:1)	0.12	0.04
Total	12.12	35.21
POLYUNSATURATED		
Linoleic (18:2)	12.08	36.24
Linolenic (18:3)	0.04	0.14
Arachidonic (20:4)	0.05	0.05
Total	12.17	36.43

The low content of phytates and oxalate in the plant parts makes it safe for mineral utilization in the body and also for safe consumption in diet. An oxalate diet limits the ingestion of oxalate to 40 – 50mg a day. Higher oxalate content contains more than 10mg per serving, while low content has less than 2mg per serving. The minimum amounts of phytic acid to cause negative effect on iron and zinc absorptions are 10 – 50mg per meal [56]. In view of the aforementioned, the phytates and oxalate contents of the plant parts pose no danger in diet, as [57] reported a safe or normal range of 4 – 9mg/100g.

Sixteen fatty acids were identified in both the leaf and fruit oils of *C.equisetifolia* (Table V). The leaves had 16.94% saturated fatty acids, 12.12% monounsaturated fatty acids and 12.17% polyunsaturated fatty acids while the fruits had 14.2% saturated fatty acids, 35.21% monounsaturated fatty acids and 36.43% poly unsaturated fatty acids. Among the saturated fatty acids, both plant parts contained a higher proportion of palmitic acid (2.87% and 6.20%). Both the leaf and fruit were found to contain high concentration of certain unsaturated fatty acids, linoleic (12.08% and 36.24%) and oleic (11.81% and 35.05%).

Linoleic acid is an important essential fatty acid required for growth, physiological functions and maintenance. The presence of high level of linoleic acid in the plant part could beneficial as dietary supplements as it has been linked to help in weight management, in the prevention of cancers, asthma, high blood pressure, cardiovascular diseases, gastro intestinal and immune systems dysfunction [58].

Oleic acid which has the highest value in the fruit is an omega-9 fatty acid, which is considered to be one of the healthier sources of fat in the diet. It helps to lower cholesterol level, high blood pressure, chest pain and also helps in the production of antioxidants [59].

IV. Conclusion

The study revealed that the leaves and fruits of *Casuarina equisetifolia* can serve as constituents of human diet supplying the body with minerals, anti- oxidant ascorbic acid and dietary fibre. The parts are also rich in bioactive nutraceutical such as oleic and linoleic acids and thus have good potentials of serving as nutritional supplements.

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