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GC-MS ANALYSIS AND NUTRIENT EVALUATION OF RARE, ENDEMIC AND THREATENED SPECIES- *Aponogeton appendiculatus* V. BRUGGEN OF SOUTH INDIA

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ABSTRACT

***Aponogeton appendicualtus* V. Bruggen is an aquatic, endemic species of Southern Western Ghats and belongs to the family of Aponogetonaceae. The present study aims to determine the bioactive constituents from leaf parts by GC-MS and nutrient value assessment of its tuber by chemical analysis. The GCMS analysis of ethanol crude leaf extract revealed the presence of 36 bioactive compounds with significant biological activities. The major chemical constituent in the crude leaf extract is determined as phytol with peak area 14.812%. The other major observed chemical bioactive component are γ-Sitosterol (9.763%), n-Hexadecanoic acid (7.390%), Hexadeconic acid, ethyl ester (6.270%). Nutrient analysis of the tuber of *Aponogeton appendiculatus* showed carbohydrate is the major nutrient (77±0.71%) and has rich crude protein (11.74±0.37%), low crude fat content (0.94±0.1%) and good crude fiber (2.45±0.08%). These nutrients and phyto components present in the plant indicated the beneficial aspects of the plant.**

KEYWORDS: *Aponogeton appendicualtus,* GCMS, Nutrient Analysis, Phytol

Aponogeton appendiculatus V. Bruggen (Aponogetonaceae) is a rare, endemic and threatened aquatic plant species of Southern Western Ghats and included in IUCN Red List (2011), occurs mostly in brackish water. It is a perennial and its edible root tuber was part of diet in certain parts of Kerala and two years old tubers are preferred by local farmers as subsistence of food. Biochemically, the tubers are composed of proteins, fats, carbohydrates and minerals and are good source of energy (Sridhar & Bhat, 2007).

Aponogeton appendiculatus is a folkloric valued medicinal plant. The folkloric medicinal plants are rich sources of secondary metabolites and are responsible for various therapeutic activities. In recent years, gas chromatography and mass spectrum (GC-MS) has been applied unambiguously to identify the structures of different phyto constituents from the plant extracts with great success. The genus *Aponogeton* is reported to have medicinal properties as anti-diabetic, anti-inflammatory, anti-microbial activities, used for treating stomach disorder, reviving the digestive system (Les *et al*.,2005). Literature reports that leaf paste consumed along with the hot water used to treat cuts and wounds (Britto *et a*l., 2007). *Aponogeton appendiculatus* is still a major source of food for forest-based communities like Paniya in Wayanad and this plant serve as a 'life saving' plant group during periods of food scarcity (Ratheesh Narayanan *et al.,* 2011). However, no information is available on chemical profiling in ethanol extract of leaf part and limited information about the nutritional composition of the root tubers available in India. Therefore the present study has been planned to identify bioactive compounds from crude leaf ethanol extracts and also estimate the concentration of minerals and essential life nutrients in tuber of *A. appendiculatus.* To the best of our knowledge, this is the prime report investigating the phyto components and nutrient value of *A. appendicualtus*.

MATERIALS AND METHODS

Collection of plant material: Fresh leaves and tubers of *A. appendiculatus* were collected from Karupadanna (Latitude -10°15'27.72' N and Longitude -76° 12' 8.23'E), Thrissur District, during August 2015 to November 2016.

Preparation of sample powder and leaf extract : The fresh leaves and tubers were washed with distilled water to remove mud. Materials are dried under shade and made fine powder by using mechanical grinder. 50 gm of leaf powder mixed with 500ml ethanol solvent for 18 hours, thus plant extracts were prepared by using soxhlet apparatus. All the extracts were collected, condensed and stored in a vial for further studies (Alade and Irobi, 1993). Gas chromatographically analysis was made by Joachim and Hubschmann, 2008. The chemical components from the ethanol extract of plant leaves were identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library (2011) to relative retention indices. The following methods were applied for analyzing various nutrients in the tuber. The nitrogen content in sample tuber powder was estimated by the Kjeldahl's method (AOAC. 2000). The protein percentage was calculated by multiplying nitrogen percent with a factor 6.25. Soxhlet extraction technique using petroleum ether (40-50 C) was used to evaluate the crude

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fat contents of the samples (Pearson *et al*., 1981). Crude fiber was estimated as per standard procedure stated in methods of analysis (AOAC. 2000). The contents of carbohydrate of the samples were estimated by difference method (Onyeike *et al*., 1995). Moisture content determined by using moisture analyzer precise Xm 120 (AOAC. 2000). Ascorbic acid content was extracted and estimated as per the method given by Sadasivam and Manickam (1992). Phosphorus and Calcium were estimated colori-metrically (Dickman & Bray, 1940). Gross energy value determined by the method of Martin & Coolidge, 1978. Total ash content was estimated by AOAC. 1990. Statistical analysis: Three determinations were carried out for each parameters of nutrient evaluation of *Aponogeton appendiculatus* tuber. All values are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

In present study indicated total 36 bioactive chemical components were identified in ethanol crude extract of leaf of *A. appendicualtus*. Identification of the compound was confirmed based on the peak area, molecular weight, molecular formula and retention time. (table 1). The identified compounds posses many biological properties. Derivatives and therapeutic activities of major compounds are given in table 2. From GC-MS analysis, the most abundant bioactive component found in the leaf was Phytol with peak area 14.812%. Inoue *et al.*, 2005, reported that phytol has antibacterial activities against *Staphylococcus aureus* by damaging its cell membrane. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamin E and K_1. It is used along with simple sugar or corn syrup as a hardener in candies. The other identified major chemical bioactive component are γ-Sitosterol (9.763%), n-Hexadecanoic acid (7.390%), Hexadeconic acid, ethyl ester (6.270%), 2-Pentyne-1,4-diol, 4-methyl-1-(2-thienyl)-(4.95%), Cycloartanol (3.46%), Campesterol (3.062%), 1,2-Benzenedicarboxylic acid, disooctyl ester (2.89%), 9,12,15-Octadecatrienoic acid, 2,3-bis [(trimethylsilyl) oxy] propyl ester, (Z,Z,Z)- (2.63%) and other chemical constituents are represented below 2% peak area.

Among the major identified chemical compound in the ethanolic crude extracts, 4 are steroid compounds, 3 are ester compounds, 2 are fatty acid, and one compound of each terpene alcohol and fatty acid ester. The γ-Sitosterol, Stigmasterol, Cycloartanol compounds have antioxidant, antimicrobial, antidiabetic and anticancerous property (Jegadeeswari *et al.,* 2012). Hexadecanoic acid, ethylester, n-Hexadecanoic acid and tetradeconic acid can be an anti-oxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities. 1,2-Benzenedicarboxylic acid, diisooctyl ester, 3 -Cyclopentyl propionicacid, 2 – dimethylaminoethylester, showed anti-microbial, and anti-fouling properties. 9, 12, 15–Octadecatrienoic acid, 2, 3 bis [(trimethylsilyl) oxy] propyl ester, (Z, Z, Z) has cancer preventive, anti-oxidant, antidiabetic, anti-inflammatory, nematicide, and anti–arthritic properties. (Sermakkani *et al.*, 2012).

Table 3 represents the result of nutritional values of *Aponogeton appendiculatus*. The carbohydrate (77±0.71%) as the principal component in the tuber. The total caloric value (274.89± 0.07 %) and carbohydrate value are positively significant. This high carbohydrate value may also be attributed low content of moisture. Moisture content, which plays an important role in determining the shelf-life (Webb, 1985) varied from 7.1 to 11.6% in rice. Here, the Percentage of moisture content in the sample is 7.6±0.08**.** Low content of moisture of the sample tuber indicates longer shelf life and its percentage found within safe limits as in rice it was below 11%. The nutritional quality depends on the protein content of the tuber (11.74±0.37%). This is the second major component of sample next to carbohydrate. RDA Based safe level intake of nutrients per Day for adults is 5.5 % (Dr. B. Sesikeran, 2010). This high rate of protein component in the tuber is very essentially as proteins form the basic unit of building blocks and tissue repairs in the body (G.Nirmala Devi *et al.,*2015).The percentage of crude fat (0.94±0.1) is relatively low. The total ash content indicates an idea of the mineral present in the food sample. The result of total ash shows the percentage of 2.45±0.08. Fiber rich foods help to promote proper bowel functioning and reduce the risk of developing intestinal disorders. When it compare with crude fiber *(*0.007% in 1 gm) of *Aponogeton undulates* (Q.R. Islam, 1996) the tuber of *A. appeniculatus* plant possess significantly higher crude fiber (2.45±0.8%). However, the values are below the RDA for fiber in children and lactating mothers, which are 19-25, and 29% respectively (Ishida et al. 2000). Earlier reports (Fleck 1976), revealed that calcium, magnesium, phosphorus, manganese in conjunction with vitamins A, C, D, and protein are involved in bone formation. The root tuber of *A. appendicualtus* can important source of Ascorbic acid (0.003 ±0.001%) calcium (0.84±0.05%) and phosphorus (0.7±0.06 %). The ethanol crude extracts of aerial leaf part of *Aponogeton appendiculatus* possesss various potent bioactive components.

Table 1: Identified chemical components of the crude ethanol leaf extract of *Aponogeton appendiculatus*. V. Bruggen

Sl. No	RT	Name of the Compound	Molecular Formula	Molecular Weight g/mol	Peak Area (%)
1	3.038	Tetraethyl silicate	$SiC_8H_{20}O_4$	208.325	0.881
2	12.186	Benzoic acid	$C_7H_6O_2$	122.12	0.469
3	18.652	Naphthalene	$C_{10}H_8$	128.170	0.556
4	20.671	3-Oxo-4-phenylbutyronitrile	$C_{10}H_9NO$	159.184	1.328
5	21.010	2-Isopropylidene-5-methylhex-4-enal	$C_{10}H_{18}O$	152.233	0.598
6	24.612	Benzyl oxy tridecanoic acid	$C_{20}H_{24}O_2$	320.466	0.353
7	28.046	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	180.243	0.283
8	28.883	Dodecanoic acid	$C_{12}H_{24}O_2$	200.317	0.717
9	29.628	13-Oxadispiro[5.0.5.1]tridecan-1-one	$C_{12}H_{18}O_2$	194.274	0.470
10	31.731	1,6,6-Trimethyl-7-(3-oxobut-1-enyl)-3,8-dioxatricyclo[5.1.0.0(2,4)]octan-5-one	$C_{13}H_{16}$	236.264	1.043
11	33.396	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.370	1.358
12	33.542	2-Pentyne-1,4-diol, 4-methyl-1-(2-thienyl)-	$C_{10}H_{12}O_2S$	196.27	4.954
13	33.933	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$	222.280	1.099
14	34.998	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.319
15	37.358	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	0.573
16	37.562	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.424	7.390
17	37.711	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl	$C_9H_9F_3O_2$	206.161	2.361
18	38.159	Hexadecanoic acid, ethyl ester	$C_{16}H_{33}NO$	284.477	6.270
19	40.187	2(3H)-Furanone, 5-dodecyldihydro	$C_{16}H_{30}O_2$	254.408	0.661
20	40.330	Phytol	$C_{20}H_{40}O$	296.531	14.81
21	41.245	Ethyl 9.cis.,11.trans.-octadecadienoate	$C_{18}H_{31}O_2$	279.437	0.955
22	41.370	Ethyl Oleate	$C_{20}H_{38}O$	310.514	0.901
23	41.560	Hexadecanamide	$C_{16}H_{33}NO$	255.439	0.889
24	43.417	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}NO_2$	213.316	1.571
25	44.280	1,10-Cycloeicosanedione	$C_{20}H_{36}O_2$	308.498	1.626
26	46.197	Cyclopentadecanone	$C_{15}H_{28}O$	224.382	0.951
27	46.836	1H-Indene, 1-hexadecyl-2,3-dihydro	$C_{25}H_{42}$	342.601	1.044
28	47.131	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330.502	0.885
29	47.411	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	2.890
30	55.117	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	496.8704	2.638
31	58.170	Campesterol	$C_{28}H_{48}O$	400.69	3.062%
32	58.766	Stigmasterol	$C_{29}H_{48}O$	412.6908	2.777%
33	60.269	γ-Sitosterol	$C_{29}H_{50}O$	414.7067	9.763%
34	61.219	Cycloartanol	$C_{30}H_{52}$	412.734	3.642%
35	62.434	Calconcarboxylic acid	$C_{21}H_{14}N_2O_7S$	438.41006	1.489%
36	62.709	Stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	410.67494	1.216%

Table 2: Major derivative compounds and their therapeutic activities of ethanol crude leaf extract of *Aponogeton appendiculatus* V. Bruggen

Sl. No.	Compound	Derivative	Therapeutic activities	Peak area (%)
1	Phytol	Diterpene alcohol	Anti-microbial, Cancer preventive, Anti-diabetic, Anti-diuretic, Anti-inflammatory Used as precursor for the vitamin E and vitamin K1	14.8
2	γ-Sitosterol	Steroid	Anti- diabetic activity Anti-cancer Anti-oxidant	9.76
3	n-Hexadecanoic acid	Fatty acid	Anti-oxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Anti-androgenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor	7.39
4	Hexadecanoic acid, ethyl ester	Ester	Anti-oxidant, Flavor Hypocholesterolemic Nematicide, Pesticide, Lubricant, Anti-androgenic, Hemolytic, 5-Alpha reductase inhibitor	6.27
5	Cycloartanol	Steroid	Produced significant hypoglycemic effect via β-cell protective effects , Inhibitory effects on glucose-absorption speed, A modulation of liver enzymes	3.64
6	Campesterol	Steroid	Cholesterol lowering ,Anti-carcinogenic effect	3.06
7	1,2-Benzenedicarboxylic acid, diisooctyl ester	Ester	Anti-microbial, anti-fouling	2.89
8	Stigmasterol	Steroid	Anti-cancer Anti-oxidant, Anti-inflammatory, Anti-arthritic, Anti HIV reverse transcriptase	2.77
9	9,12,15-Octadecatrienoic acid, 2,3-bis [(trimethylsilyl) oxy] propyl ester, (Z,Z,Z)-	Fatty acid ester	Anti-oxidant, Anti-diabetic, Anti-inflammatory Cancer preventive, Nematicide, Anti-arthritic	2.63
10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Anti-inflammatory, Anti-oxidant, Anti-microbial	2.31
11	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	Ester	Anti-microbial, antifouling	1.57
12	Tetradecanoic acid	Fatty acid	Used in cosmetic	1.35

Table 3: Proximate and mineral composition of *Aponogeton appendicualtus* V.Bruggen

Sl. No.	Components (%)	Concentration of percentage/1gm
1	Moisture	7.6±0.08
2	Crude proteins	11.74±0.37
3	Crude fiber	2.45±0.08
4	Crude fat	0.94±0.1
5	Total Ash	2.40±0.77
6	Carbohydrate	77±0.71
7	Ascorbic acid	0.003±0.001
8	Phosphorus	0.7±0.06
9	Calcium	0.84±0.05
10	Calorie value (Kcal/100g)	274.89±0.07

Mean ± Standard Deviation of three replicates

After evaluation of all pharmacological activity the phytocomponents of this plant was supportive to identify new drugs. Further studies are needed to explore the potential bioactive compounds responsible for the biological activities. Nutritional evaluation data reveals that tubers of *A. appendicualtus* were found to be an impending source of carbohydrate, protein and energy. Low crude fat content is observed from the present study is an exciting feature for people to include this as a main ingredient of daily diet. After anti-nutritional evaluation it acting as an alternative food source.

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