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Research Article

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Study on Phenolic content, Antioxidant Activity and CHNS elemental analysis of *Amorphophallus sylvaticus*

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ABSTRACT: *Amorphophallus sylvaticus* (Roxb) (Araceae) seed acquired with different solvent extraction. The investigation aimed to carry out the Phenolic content, Antioxidant Activity and CHNS elemental analysis of different solvent extracts of the seed Amorphophallus sylvaticus (Roxb) (Araceae). The preliminary analysis revealed the elemental ratio of each of the light elements preserves individual information on the origin and history of organic natural compounds. Therefore, a multi-element ratio analysis is the most efficient means for the origin and authenticity assignment of food. Due to the extraordinary relative abundances of the elements hydrogen, carbon, nitrogen and sulfur in some biological material and to the need for individual sample preparations for H and S, their elemental ratio determination currently requires at least three independent procedures and approximately 1 h of work and CHNS elemental analyser it takes of all four elements in one sample within 20 min. The elemental composition was determined by CHNS analyzer and the elemental composition in the sample was the following: carbon (35.62%), hydrogen (7.45%), nitrogen (0.85%) and the sulphur is (0.51%). The analyser is able to combust samples with up to 100mg of organic material, sufficient to analyse samples with even unusual elemental ratios, in one run. The sensitivity of the device for the elemental ratio measurement of C and N corresponds to that of other systems. It is less by a factor of four for Hand by a factor of two for S, and the error ranges are identical to those of other systems.
Keywords: *Amorphophallus sylvaticus* (Roxb), CHNS, DPPH, Phenol content.

INTRODUCTION

India has a matchless position in the world, where a number of predictable ethnic systems of medicine viz., Siddha, Ayurveda, Unani, Yoga, Homeopathy, and Naturopathy are being used for the health care of people (1). Ayurvedic herbal arrangements based on the medicinal plants are used in present health care as nutritive supplements to avoid common bacterial diseases (2, 3, and 4). Consequently, many medicinal plants have been used by traditional medicine practitioners for the treatment of various diseases. Amongst the different evidence revealing that medicinal and culinary herbs have some common species, a diet rich in vegetables, fruits and Phytochemical which reduce the risk of cardiac and cancer diseases (5). Most of these plants were under consumed. Phytochemicals are biologically active chemical compounds found naturally in plants that protect against different ailments. They are non-nutritive compounds that contribute to flavor and colour (6, 7). Considering the importance of plants as a source of medicine, even today we have selected *Amorphophallus sylvaticus* (Roxb.), family- Araceae, which is in use for centuries in the treatment of dental ailments. However it is less explored plant for its varying activities. Hence an effort has been made here to investigate the potential uses of this plant.

Organic elemental ratio measurements have become the most important and most frequently applied method for origin and authenticity determinations of food commodities, fragrances (8-10) or flavourings (11), and other natural products (12). They have also been indispensable for the treatment and understanding of environmental and ecological questions (13), in food web and nutrition studies (14, 15) in archaeology (16), and in forensic sciences (17). All of these investigations demonstrate that it is advantageous, perhaps even obligatory, to perform the analyses, whenever possible, as a multi-element or multi compound/ multi-element ratio determination. As each of the bio elements is individually correlated to the conditions of biosynthesis and secondary treatments of natural compounds, a multi-element analysis will provide the greatest amount of independent information for the characterization and assignment of origin of the material in question. The source of adsorbed water and of bound oxygen and

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hydrogen in the biological material under investigation is always water; therefore, the corresponding ratios reflect the elemental signature of the local precipitation of the production area and so may be correlated to its latitude or its distance from the ocean.

In some cases, the elemental ratio data of the tissue water of an unknown biological sample may be indicative of its geographic origin but often the elemental characteristics of this water can be modulated by secondary external influences.

Therefore, it would be better and more reliable to measure the elemental ratios of the organically bound hydrogen or oxygen, which preserve the original information, and integrate it over a longer period. The carbon elemental ratio plant material is determined primarily by the photosynthesis type of the plant in question, but also by local and temporal climate conditions, under which it has grown (18), via food chains the elemental signature is correspondingly transferred to animal material. Nitrogen elemental ratios can give information on the primary nitrogen sources of plants such as the fertilisers used or, in case of animal material, on its primary protein source. Finally, sulfur elementals and elementals of heavier elements (e.g. Sr) are indicators for geological characteristics or for anthropogenic influences on the environment of the origin of the sample (e.g. Pb).

The organic elemental ratio analysis of an organic sample implies its conversion into simple gases by the Dumas combustion procedure using an elemental analyser and the subsequent mass spectrometric determination on the elemental ologues of the product gas molecules. Normally, after the combustion of the sample, the product gas molecules are separated by gas chromatographic or cryogenic procedures. Water and SO2 are (chemically) removed and only N2 and CO2 are used for the elemental ratio measurements of nitrogen and carbon elementals, respectively. The elemental ratio analysis of sulfur as a minor element in biological material is normally carried out as a single measurement, using larger samples. Hydrogen and oxygen elemental analyses are performed on H2 and CO, respectively, and a separate pyrolytic sample preparation must be applied for each element. Thus, the conventional multi-element elemental analysis of a compound including oxygen demands up to four independent weighing, conversion and measurement processes – a time- and labour consuming procedure.

METERIALS AND METHODS

COLLECTION OF SOURCE

The specimen of *Amorphophallus sylvaticus* (Roxb.) were collected from Pullaveli forest, Dindigul. The *Amorphophallus sylvaticus* seeds were processed and crushed into powder and the extract was collected by using different solvents.

PREPARATION OF EXTRACT'S

The seed powder of *Amorphophallus sylvaticus* (Roxb.) were subjected to extraction various solvents (Ethanol, Methanol and Aqueous) in increasing order of polarity. The prepared extracts were then subjected to preliminary phytochemical analysis. The plant seeds were dried in shade, separated and made to dry powder. A weighed quantity (10 gm each) of the powder was subjected to continuous cold extraction in separate apparatus with solvents such as ethanol, methanol, water and hexane respectively for 24 hours in room temperature. The extracts were evaporated under reduced heat using water bath until all the solvent have been removed to obtain concentrated extract.

CHNS INSTRUMENT CONDITION

Elemental analyses of total nitrogen and carbon (and sulfur) is performed to provide carbonate and organic carbon and to get some idea of the composition of the organic matter (i.e., to distinguish between marine and terrigenous sources, based on total organic carbon/total nitrogen [C/N] ratios).

The total nitrogen, carbon, and sulfur are determined using a CHNS analyzer, model FLASH 2000 CHNS/O Analyzers Thermo Fisher Scientific Instruments. For the CHNS analysis, freeze-dried and crushed samples are weighed (5-10 mg) and mixed with an oxidizer (vanadium pentoxide [V2O5]) in a tin capsule, which is then combusted in a reactor at 1000°C. The sample and container melt, and the tin promotes a violent reaction (flash combustion) in a temporarily enriched oxygen atmosphere. The combustion products CO2, SO2, and NO2 are carried by a constant flow of carrier gas (helium) that passes through a glass column packed with an oxidation catalyst of tungsten trioxide (WO3) and a copper reducer, both kept at 1000°C. At this temperature, the nitrogen oxide is reduced to N2. The N2, CO2, and SO2 are then transported by the helium to, and separated by, a 2-m-long packed column (Poropak Q/S 50/80 mesh) and quantified with a TCD (set at 290°C.)

The chromatographic responses are calibrated against preanalyzed standards, and the CHNS elemental contents are reported in weight percent. Eager 200 software is used for running the equipment, storing the data, and for post run analysis.

Organic C/N ratios can be used to help identify the origin of the organic matter in sediments. (Shipboard Scientific Party, 1998). C/N ratios of 5-8 indicate unaltered algal organic matter, whereas C/N ratios of 25-35 indicate fresh land-derived organic matter. (19, 20). Interpretation of C/N ratios needs to be done with caution. Low C/N values in sediment containing low organic carbon may be biased by the tendency of clay to absorb ammonium ions generated during the degradation of organic matter (21). Preferential loss of nitrogen-rich, proteinaceous matter can elevate the C/N ratios of algal organic matter during settling to the seafloor (22).

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EVALUATION OF ANTIOXIDANT ACTIVITY BY DPPH RADICAL SCAVENGING METHOD

The molecule of 1,1-diphenyl-2-picrylhydrazyl (1,1-diphenyl-2- picryl hydrazyl; DPPH:1) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerism, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in methanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced anther form with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present). Representing the DPPH radical by DPPH and the donor molecule by AH, the primary reaction is: DPPH + AH = DPPH-H + A {1} Where DPPH-H is the reduced form and A is free radical produced in this first step. This latter radical will then undergo further reactions, which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorized) by one molecule of the reluctant. The reaction {1} is therefore intend to provide the link with the reactions taking place in an oxidizing system, such as the autoxidation of a lipid or other unsaturated substance; the DPPH molecule is thus intended to represent the free radicals formed in the system whose activity is to be suppressed by the substance AH. The substrate concentrations used should for definiteness, be those that would be in the reaction cuvette in the absence of any DPPH. Alternatively, the amount (moles) of substrate added to the reaction vessel may be used. Three (ml) of standard antioxidant after proper dilution with methanol has been prepared in six tubes. Then 1ml of DPPH (200 µmol/L) added to achieve a standard serial chain of 2.5, 5, 7.5, 10, 12.5, 15 µmol/L studied antioxidant. The tubes were shaked and kept in dark until the end of the reaction. The measures were taking at the maximum wavelength and at the laboratory temperature. The remaining percentage of DPPH (%DPPH) for different antioxidants in different concentrations was determined. All measurements of free radical scavenging activity were performed in triplicate and standard deviation was calculated. The relationship between measured sample and decreasing amount of (%DPPH• rem) have been determined. The ability to scavenge the DPPH radical was calculated using the following equation (1):

$\%DPPH\bullet \text{ rem} = (A_F/A_{DPPH\bullet})*100$ Equation (1)

Where A_{DPPH} is the absorbance of control, and AF is the absorbance of the sample after addition of antioxidant and complete reaction, and %DPPH rem is the remaining percentage of DPPH. The total free radical-scavenging capacity of standard antioxidants were evaluated by measuring the disappearance of the stabilized 2, 2-diphenyl-1-picrylhydrazyl artificial free radical (DPPH) by measuring the absorbance at 517 nm according to Espin et al method (23). The resulted were expressed as parameter that has been introduced for the interpretation of the results from the DPPH method, is the "efficient concentration" or EC_{50} value (otherwise called the IC_{50} value), which is the concentration of antioxidant that causes 50% loss of the DPPH activity (absorbance). This parameter was apparently introduced by Brand-Williams and his colleagues (24, 25). This parameter has the drawback that the higher the antioxidant activity ability of free radical scavenging activity), the lower is the value of EC_{50}. This is a disadvantage particularly when results are presented graphically as a bar chart (26) even if the same data are also available in numerical form (27). Because that we had determined the antioxidant activity from the equation: $(1/EC_{50})$.

DETERMINATION OF TOTAL PHENOLICS CONTENT

Total phenolic content of methanol extract of *Amorphophallus sylvaticus* seed extract was determined with Folin Ciocalteu method (28). The Folin–Ciocalteu (F–C) reagent is sensitive to reducing compounds, polyphenols and thus produces a blue colour complex. The F-C assay relies on the transfer of reducing equivalents (electrons), in the alkaline medium, from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes, manifested in the formation of blue colour complexes that are determined on a UV-visible spectrophotometer (Shimadzu 1800-UV Spectrophotometer) by monitoring the absorbance at 765 nm (29, 30). Gallic acid was used as the reference compound for comparison and values are evaluated as the mg equivalent of gallic acid per g of extract. Briefly, a mixture containing 0.1 g of extract, 0.8 ml of deionised water and 0.1 ml of Folin-Ciocalteu reagent was first incubated at room temperature for 3 min. After adding 0.3 ml of Na2CO3 (20% w/v), the mixture was further incubated at room temperature for 30 min. To obtain a calibration curve, various concentration of gallic acid solutions (0.05, 0.04, 0.03, 0.02, 0.01, 0.008, 0.005 and 0.001 mg/ml) were prepared. Appropriate volume of sodium carbonate solution was added in each flask and the final volume was adjusted with distilled water. Measurements were carried out after 1 h at 765 nm on a UV-visible spectrometer against the reagent blank. The calibration curve of concentration against the absorbance was plotted. 1 mL of stock solution of extracts was transferred in a 25 mL flask; similar procedure (vide supra) was adopted for the preparation of calibration curve. With the help of the calibration curve, the phenolic concentration of extracts was determined.

RESULTS AND DISCUSSION

DETERMINATION OF ANTIOXIDANT ACTIVITY

The antioxidant activity of *Amorphophallus sylvaticus* (Roxb) (Araceae) seed was determined by DPPH (2, 2- Diphenyl-1-picryl hydrazyl) radical scavenging activity. The antioxidant activity of the seed extracted sample in different solvents was calculated (Table 1). The seed extracted sample showed different antioxidant activity in different solvents, which showed that the solvent has effect on inhibition of DPPH. The solvent play a role in the reaction between concentration of antioxidants in a sample and %

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inhibition of DPPH. It affects the kinetics of reaction (31, 32, and 33). The antioxidant activity of a material is affected by the type and polarity of the solvent, which is used for extraction (34, 35).

The antioxidant activity also depends upon those compounds, which show the antioxidant activity. These compounds are polar in nature and they inhibit the different % of DPPH in different solvent extracts (36). Different types of plants show different type of antioxidant potential. The *Amorphophallus sylvaticus* (Roxb) (Araceae) seed extracts showed 81.21 % antioxidant activity in methanol extract (Table 1).

Table.1 % inhibition of DPPH in different solvent extracts

% inhibition of DPPH in different solvents			
Time	**Ethanol**	**Methanol**	**Aqueous**
0	100	100	100
10	70.4 ±0.42	81.21 ±0.22	83.10 ±0. 21
20	57.93 ±0.60	50.63 ±0.43	59.23 ±0.55
30	50.80 ±0.12	55.16 ±1.11	53.83 ±0.33
40	34.46 ±0.30	41.33 ±0.98	44.10 ±0.12
50	10.70 ±0.20	13.40 ±0.76	14.96 ±0.10

TOTAL PHENOLIC CONTENT

The concentration-absorbance calibration curve for 8 sequentially and separately prepared stock standards of gallic acid solution is illustrated in Figure 1. The measured absorbance values at 765 nm for the indicated concentration of gallic acid solutions are in the range of 0.08 to 1.16. Within this range of concentrations (0.001 to 0.05 mg/ml), the calibration curve of gallic acid has clearly exhibited linearity (Figure 1). The antioxidant activities of several natural polyphenol compounds present in seed extracted sample has been reported (37). Moreover, the polyphenolic compounds have exhibited inhibitory effects on mutagenesis and carcinogenesis in humans (38). Total phenol content in the methanolic extract of *Amorphophallus sylvaticus*, using the calibration curve, was found to be 30.0 mg gallic acid equivalents/g dry weight of extract.

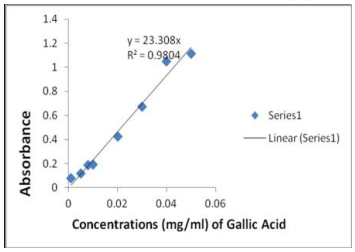

Figure 1. Standard calibration curve of gallic acid for the determination of total phenolic content.

Table.2 **TOTAL PHENOLIC CONTENT** of *Amorphophallus sylvaticus* (Roxb) (Araceae) seed extracts

% inhibition of DPPH in different solvents			
S.No	Ethanol(mg/g)	Methanol (mg/g)	Aqueous (mg/g)
1.	20.4 ±0.40	30.00 ±0.20	13.11 ±0. 11

ELEMENTAL ANALYSIS

Elemental analysis is done to find out the percent composition of different elements in a compound. The elements which were found in the analysis were carbon, hydrogen, nitrogen and sulphur. Nitrogen and sulphur are macronutrients and are very important for the growth of plants. The presence of different elements in a plant material is not only useful for plants but also the source of essential elements for those who consume it. The higher percent of carbon and hydrogen indicates that plant is a source of carbohydrates and hydrocarbons. The higher percent of nitrogen and sulphur indicates that plant is also a source of proteins and vitamins, which are very important for health (39). The results show that the necessary percent of elements is present in *Amorphophallus sylvaticus* (Roxb) (Araceae) seed extracts.

Table.3 Elemental composition of *Amorphophallus sylvaticus* (Roxb) (Araceae) seeds

Elements Percentage composition		
S.No	Elements	Result (%)
1.	Carbon	55.62
2.	Hydrogen	8.568
3.	Nitrogen	0.94
4.	Sulphur	0.548

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The composition of carbon is 58.55 % and of hydrogen is 7.968 % in seed extracts (Table 3) which is higher as compared to the necessary elemental composition which should not be less than 45 % and 6 % respectively (40). The higher percent of carbon and hydrogen means the higher amount of carbohydrates, which provide energy to the consumers. So according to results, the seed extracts is a good source of carbohydrates. The values of nitrogen and sulphur are the 0.93 % and 0.542 % respectively, which are within limits as compare to the necessary elemental composition which should be 1% for nitrogen and <1 % for sulphur. The nitrogen is a structural component of proteins and sulphur is also present in proteins and vitamins.

The high carbon to nitrogen ratio of an organic material shows it is carbonaceous and has less nitrogen content. If the content of nitrogen is higher in plants then carbon to nitrogen ratio becomes low. This low ratio enhances the vegetative growth. The high carbon to nitrogen ratio also increases the strength of the cell wall (41). The carbon to hydrogen ratio of an organic material shows that it is a source of hydrocarbons. Hydrocarbons are oily in nature (42). The carbon to hydrogen ratio of seed pulp sample shows that it contains hydrocarbons.

CONCLUSIONS

In present study the *Amorphophallus sylvaticus* (Roxb) (Araceae) seed extracts showed the higher antioxidant activity. It is a potential source of natural antioxidants. Along with antioxidant potential and phenolic content. The study also reveals that *Amorphophallus sylvaticus* has showed necessary composition of macro elements, which are carbon, hydrogen, nitrogen, and sulphur. The antioxidant potential and other chemical constituents can enhance its ecological and pharmaceutical importance along with its other uses like wood and grazing purposes. The antioxidants can also be used in cosmetics industry after refining as they are antiaging.

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CONFLICTS OF INTEREST

"The authors declare no conflict of interest".