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Nutraceutical and antioxidant evaluation of *Hygrophila auriculata* Schumach (Acanthaceae)

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Abstract
Present study was carried out to assess nutraceutical values of wild edible herb *H. auriculata* with its antioxidant potentials. Proximate analysis of the moisture, ash and crude fat content reveals 65±1.45%, 0.29±0.17% and 3±1% respectively. *H. auriculata* ranked highest in crude fibre (4.53±1.22%) and carbohydrate (64.83±1.38%) content. Protein content was observed to be low (8±0.74%). The mineral analysis revealed high amount of calcium (161.28±9.25mg/kg) and potassium (158.83±5.31mg/kg). Phytochemistry of *H. auriculata* also indicated the presence of alkaloids, flavonoids, phenols, steroids, saponins and cardiac glycosides. The plant contains a very good amount of vitamin C (48.1±2.19 mg/100gm) along with water soluble B vitamins ranged between 0.0133±0.02 to 6.9±0.40 mg/100gm. The plant also showed a good dose dependent antioxidant activity against both DPPH and H_2O_2 molecules. The outcome of the study indicates that proximate composition, mineral contents, vitamin contents and antioxidant properties of this plant could be used for the nutritional purpose.

Keywords: antioxidant, *H. auriculata*, minerals, nutritive value, vitamins

1. Introduction
From decades, different medicinal plants have been used in routine life to treat different diseases around the globe. They are used as a source of medicine. Many of the plants are described in ancient texts like the Vedas and the Bible as herbal remedies and different healthcare preparations, has been mark out to the occurrence of natural products with pharmacological importance. Actually, plants have a variety of bioactive molecules, making them a tremendous source of different kinds of medicines [1].
Since ancient times, as sources of medicinal compounds, higher plants have continued to play a dominant role in the maintenance of human health [2]. In the pharmaceutical industry, natural products play an important role in drug development and over 50% of all modern clinical drugs are of natural origin [3]. There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom.
Plants are the basic source of knowledge of modern medicine. For the synthesis of drugs, the basic molecular and active structures are provided by rich natural resources. As a source of modern medicine, plants are the basic source. This growing worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care [4].
Today the exploration and exploitation of the disease fighting properties of a multitude of photochemical found in both food and non food plants have created a renaissance in human health and nutrition research. At the same time, many opportunities for the development of novel dietary products have been created. With all new fields of study come new term knew as "Nutraceuticals" [5]. A term combining the words “nutrition” (a nourishing food or food component) and “pharmaceutical” (a medical drug), is a food or food product that provides health and medical benefits, including the prevention and treatment of disease.
Such products may range from isolated nutrients, dietary supplements and specific diets to genetically engineered foods, herbal products and processed foods such as cereals, soups and beverages which provides medical or health benefits including the prevention and/or treatment of a disease [6].
Hygrophila auriculata is an erect, stout, branched or unbranched, annual herb. Occasionally the basal part of the stem is creeping and rooting. The plant is harvested from the wild for local use as a food and a medicine. It is cultivated in water beds in west Africa both as a vegetable and for the vegetable salt it can yield, and is sold as a vegetable in the local markets of Sri Lanka and India. The plant is used as a vegetable. The young leaves are chopped and cooked alone, or are combined with other vegetables such as peas or amaranth. Coconut milk or groundnut paste is then added and the dish is served with a staple such as rice [7, 8].
The present work was aimed at examining the phytochemicals, proximate, minerals, vitamins and antioxidant potentials of *H. auriculata*, which is a prominent wild edible plant grown in India, with a view to provide useful information towards the effective usage of this plant as a good source of nutraceuticals.

2. Materials and Methods
2.1 Collection and identification of plant
Whole plant of *H. auriculata* Schumach. was collected from the South Gujarat region of Gujarat state and the identification was authenticated by Dr. Minoo Parabia. The voucher specimens were deposited at Bapalal Vaidya Botanical Research Centre, Surat, Gujarat, India. Collected plant material was sun-dried, and grounded into fine powder followed by storage in airtight containers.

2.2 Processing of plant material
H. auriculata powder (20g) was soaked in 85% ethanol for 24 h at 37°C with vigorous shaking. Whatman no. 1 filter paper was then used for the filtration of ethanol extract. Concentrated dried extract was then obtained using a rotary evaporator. Phytochemistry and antioxidant activity was then performed using a stock solution of crude ethanol extract.

2.3 Qualitative screening of phytochemicals
Phytochemical analysis was carried out by using aqueous extract of *H. auriculata* according to the procedures described by [9-11].

2.3.1 Test for alkaloids
Plant extract was added to 1% aqueous HCL over water bath and filtered. The filtrate was treated with few drops of Wagner's reagent. Formation of a reddish brown precipitate indicates the presence of alkaloids.

2.3.2 Test for flavonoids
Plant extract was added to 5ml of Ammonium solution with a few drops of concentrated H_2SO_4. Yellow coloration indicates the presence of flavonoids.

2.3.3 Test for phenols
Equal volumes of plant extract and ferric chloride solution are added together. A deep bluish green precipitate indicates the presence of phenol.

2.3.4 Test for steroids and sterols
Five mg of plant extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound, in the extract.

2.3.5 Test for saponins
0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. The formation of foam to a length of 1cm indicated the presence of saponins and steroids.

2.3.6 Test for cardiac glycosides
0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

2.4 Proximate Analysis
The proximate analyses were carried out in triplicate using a standard procedure described by AOAC (2000) [12].

2.4.1 Estimation of ash content
Five gm of plant sample was weighed in a silica crucible and heated in the muffle furnace for about 5-6 h at 500 °C. It was cooled in a desiccator and weighed. It was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently, until the weight became constant (ash became white or grayish white). Weight of ash gave the ash content.

$$\text{Ash content (\%)} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}}$$

2.4.2 Estimation of moisture content
Two gm of fresh plant sample was taken in a flat-bottom dish and kept overnight in an air oven at 100–110°C and weighed. The loss in weight was regarded as a measure of moisture content in the sample.

$$\text{Moisture (\%)} = \frac{(\text{Weight of original sample} - \text{Weight of dried sample})}{\text{Weight of original sample}} \times 100$$

2.4.3 Estimation of crude fat content
Two gm moisture free plant powder was extracted with petroleum ether (40-60°C) in a Soxhlet apparatus for about 6-8 h. After boiling with petrol, the residual petrol was filtered using Whatman no. 40 filter paper and the filtrate was evaporated in a pre-weighed beaker. Increase in weight of a beaker gave crude fat. Percentage of fat content was calculated using the following formula.

$$\text{Crude fat (\%)} = \frac{\text{Weight of fat in sample} \times 100}{\text{Weight of dry sample}}$$

2.4.4 Estimation of crude fibre content
The crude fiber was determined by using the method described by Sadasivam and Manickam, (2008). Extract 2 gm of plant powder with ether or petrolium ether for removal of fat. After extraction with ether, add 200 ml of sulphuric acid with 2 gm of dried material for 30 minutes. Filter through muslin cloth and give washes with boiling water until washings are no longer acidic. Boil with 200 ml of sodium hydroxide solution for 30 minutes. Filter through muslin cloth again and wash with 25 ml of boiling 1.25% of sulphuric acid, 50 ml of water and alcohol. Remove the residue and transfer it to pre weighed ashing dish W1 and dry for 2 hours at 130 ± 2° C. Cool the dish in a desiccator and weigh W2. Ignition for 30 minutes at 600 ± 15°C and cool in a desiccator and reweigh W3.

$$\text{\% crude fiber in ground sample} = \frac{loss\ in\ weight\ on\ ignition\ (W2-W1)-(W3-W1)}{Weight\ of\ the\ sample} \times 100$$

2.4.5 Estimation of carbohydrate content
Total carbohydrate was estimated by Anthrone method. 100 mg of the sample took into a boiling tube to hydrolyze by keeping it in a boiling water bath for three hours with 5 ml of 2.5N HCl and cool to room temperature. Solid sodium carbonate used to neutralize it until the effervescence ceased. Made up the volume to 100 ml and centrifuge. The supernatant was collected and took 0.5 and 1ml aliquots for analysis. The standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standards. '0' served as blank. Made up the volume 1 ml in all the tubes including the sample tubes by adding distilled water. Then 4 ml of anthrone reagent added. Heated for eight minutes in a boiling water bath. Cooled rapidly and read the green to dark green colour at 630nm Drew a standard graph by plotting concentration of the standard on the x-axis versus absorbance on the y-axis. From the graph calculated the amount of carbohydrate present in the sample.

$$\text{Amount of carbohydrate in 100mg of the sample} = \frac{\text{mg of glucose}}{\text{volume of the sample}} \times 100$$

2.4.6 Estimation of protein content

Protein estimation was carried out by the method of Lowery *et al*, (1951) [13]. Powder (dried) 0.1g was crushed, homogenized and extracted in 10 ml of 80% ethanol. The resulting slurry was centrifuged and residue obtained, to the residue, 10 ml 5% perchloric acid was added and incubated at 80ºC for 20 min. It was brought to room temperature and centrifuged. The supernatant was discarded and precipitates were washed with 10 ml distilled water. Centrifuged and discarded the supernatant. Then to the residue, 10 ml of reagent A was added and incubated at 30ºC for 1 hour. The protein was extracted in supernatant by centrifugation and residues were discarded. This supernatant was used as the sample for protein estimation and the total volume of sample was recorded. To 1 ml or 0.1 ml sample solution 5 ml of reagent C was added and incubated for 10 mins. To this add 1 ml of reagent D and mixed thoroughly. The optical density was measured at 660nm before 30 mins. Bovine serum albumin was used as a standard protein (1mg/ml). Double distilled water added to the aliquots of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml of the stock solution to adjust final volume 1 ml. Then to this 5 ml of reagent C was added and incubated for 10 mins. 1 ml of reagent D was added and optical density was measured at 660nm. Blank was prepared using distilled water. The standard graph was obtained by plotting optical density on y-axis and concentration on x-axis.

2.4.7 Estimation of energy content

The three components of foods which provide energy are protein, carbohydrate and fat. One gram carbohydrate and protein each yield four kcal energy whereas one gram fat yields nine kcal energy. Therefore, the energy (kcal/100gm) contents of each plant sample were determined by multiplying the values obtained for protein, fat and available carbohydrate by 4.00, 9.00 and 4.00, respectively and adding up the values.

2.5 Estimation of minerals

About 0.25 g of plant material was weight accurately in a test tube and placed in an aluminium block or sand bath containing a thermometer (0 – 400 °C). Add 5 mL of a mixed nitric perchloric digesting acid (1 mL 70% $HClO_4$ and 4 mL 70% HNO_3). Heat the block for 2 hours at 120 °C, then slowly increase the temperature to 180 °C over a three hour period to drive off the nitric acid. White fumes from the perchloric acid will indicate the end of the digestion procedure. It is important not to allow the digestate to dry out. Carry out the digestion under strict supervision in a protected fume hood. On completion of the digestion the contents of the test tube are rinsed into a 25 mL volumetric flask and made up to the mark with distilled deionized water. Determination of mineral elements were carried out through atomic absorption spectroscopy (AAS) (AA 800, Perkin-Elmer Germany). Standard solution of each element was prepared and calibration curves were drawn for each element using AAS. All assays were carried out in triplicates.

2.6 Analysis of vitamins

The amount of vitamin A, E, C, B_1, B_2, B_5, B_6, B_7 and B_9 were determined using the method described by Bureau of Indian Standards (1992) [14].

2.7 Antioxidant assays

2.7.1 Determination of free radical scavenging effects of antioxidants using DPPH method

Antioxidant activity of *H. auriculata* crude extract was measured against DPPH free radicals in relation to radical scavenging capability [15]. Four tubes containing 2 ml of 6×10^{-5} M of DPPH solution in DMSO were mixed with the crude extract of varied concentrations (25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml), followed by incubation in dark for 1 hour. After the period of incubation, decrease in absorbance was measured at 517 nm. Ascorbic acid was used as a standard, DMSO was used as a blank and control was DPPH solution without crude extract. Later, following equation was used for calculating the scavenging of DPPH radicals:

$$\text{DPPH scavenging activity (\%)} = (A_0 - A_1)/A_0 \times 100$$

Where,

A_0 = absorbance of the control

A_1 = absorbance of the sample.

2.7.2 Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was measured as per the method used by Adnan *et al*., (2018) [16]. H_2O_2 (1 ml, 2mM) solution, prepared in phosphate buffer (0.1M, pH 7.4), was mixed with 1 ml of crude extract (25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml). The tubes were then incubated for 10 minutes at room temperature, followed by absorbance measurement at 230nm. Absorbance was determined against a blank solution (phosphate buffer without hydrogen peroxide), while, ascorbic acid was used as positive control. Following formula was then used to calculate the percentage of hydrogen peroxide scavenged:

$$\text{\% inhibition} = (A_0 - A_1) / A_0) \times 100$$

Where

A_0= absorbance of the control

A_1= absorbance of the extract/standard

3 Results

3.1 Phytochemicals present in *H. auriculata*

H. auriculata plant extract was analyzed for the presence of various phytochemical constituents. Results of qualitative estimation of phytochemicals are presented in table -1. It is revealing that alkaloid, flavonoid, phenols, steroids & sterols, saponins and cardiac glycosides are present.

3.2 Proximate analyses

The proximate composition of these plants has been presented in table 2. The proximate analysis of *H. auriculata* revealed ash content 0.29±0.38 gm per 100gm dry sample and moisture content 65 ± 1.45 gm per 100 gm dry plant. The plant was found to contain protein, crude fat, crude fibre and carbohydrate 8 ± 0.74%, 3 ±1%, 4.53 ± 1.22% and 64.83 ± 1.38% respectively. The energy content of the plant was found to be 319.46±1.070/100gm (Fig. 1).

3.3 Minerals content

Table 3 shows the minerals content in mg per kg of the dry plant. Sodium content of the fruit was found to be 0.35±0.12 mg/kg. The plant was found to be rich in minerals like potassium (158.83±5.31 mg/kg), calcium (161.28±9.25 mg/kg), magnesium (73.29±5.40 mg/kg), phosphorus

(42.06±6.12 mg/kg) and iron (3.26±0.71 mg/kg) respectively. An appreciable amount of copper, zinc and manganese were also detected in *H. auriculata* (Fig. 2).

3.3 Vitamins profile

The quantity of all detected vitamins of in *H. auriculata* is expressed as mg/100gm dry plant material and data presented in table 4. Retinol (A), ascorbic acid (C), thiamine (B1), riboflavin (B2), pantothenic acid (B5), pyridoxine (B6) and biotin (B7) were present in *H. auriculata* (Fig. 3).

3.4 Antioxidant activity

H. auriculata crude extract was analysed for its antioxidant potential against DPPH and H_2O_2 molecules in comparison to ascorbic acid. It exhibited notable free radical scavenging activity against both DPPH and H_2O_2 molecules. Antioxidant activity was higher against DPPH free radicals than H_2O_2 molecules. Antioxidant potential by *H. auriculata* crude extract was found to be dose dependent. Increase in concentration (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml), escalates the antioxidant potentiality (Fig. 4).

4. Discussion

From decades, natural products have been well known for their therapeutic applications. In recent era, as a replacement of modern medicine, there is a growing interest in nutraceuticals which provide health benefits. Nutraceutical rich leafy vegetables are an important part of healthy diet. In the time of Fast food, people often have a negative attitude towards these vegetables and fail to appreciate their taste. Promoting the nutritional value of traditional leafy vegetables will have a good impact on encouraging their use. Nutraceutical potential of the wild leafy vegetables here is discussed in light of the role of nutrient components and bioactive molecules they contain, and the medicinal uses mentioned in literature.

Proximate analysis data reval that, *H. auriculata* contain a good amount of protein, crude fat, crude fibre, carbohydrate, ash and moisture contents and also energy content. The moisture content determines the actual quality of the food before consumption which involves the amount of water present in the food. It affects the chemical and physical aspects of food which transmit the freshness and stability for the storage of the food for a long period of time [17]. The moisture content was found 64.83±1.38%. Dietary fibre though cannot be considered as nutrient, plays an important role in disease prevention such as heart diseases, hypertension, overweight, constipation, serum cholesterol, colon and breast cancer and health promotion [18]. Crude fibre estimated here constitutes insoluble dietary fibre which adsorbs bile salts, it also facilitates intestinal movements. As per the WHO recommendation, an intake of 22-23 kg of fibre for every 1000 k. cal. of diet is important for digestion and elimination of wastes. *H. auriculata* possessed a good amount of crude fibre (4.53±1.22%) which is higher to commercial fruits and vegetables like apple (3.2 %), broad beans (8.9 %), cabbage (2.8 %), potato (1.7 %) and spinach (2.5 %). Therefore, this wild leafy plant can be used in the diet to fulfil WHO recommendation. Evaluation of ash content, a part of proximate analysis for nutritional evaluation of food, is very much important for few reasons. It represents the total mineral content in foods. The leafy vegetables used in the present study contained good amounts of ash indicating that these plants are rich in minerals and could provide a considerable amount of mineral elements in our diet. However, *H. auriculata* possessed a lower amount of ash content (0.29±0.17%). Values of fat & oils are quite satisfactory. Fat & oils were also found lowest in *H. auriculata* (3±1%). Fats provides few important fatty acids, important for controlling inflammation, brain development and blood clotting which are not made by body and must be obtained from food. It also acts as the storage substance for the body's extra calories. It is also important for the absorptivity of fat soluble vitamins like Vitamin A [19]. Carbohydrate is the major nutrient of any food material and a class of energy yielding substances like starch, glucose, cane sugar, milk sugar etc. Carbohydrate content in the *H. auriculata* 64.83±1.38%. These content is considerably higher than the known values when compared to some wild edible vegetables like beans (29.1 %), potato (20.9%), jack fruit seeds (25.8%) etc [19]. Proteins are one of the vital nutrient required by the body and needed in adequate amounts in the diet. They required performing for various body functions like for growth and repair of body tissues, for the proper functioning of antibodies, for the regulation of enzymes and hormones etc [19]. Protein content was found 8±0.74% in *H. auriculata*. The energy value of foods is often more easily calculated from the analysis of foods for proteins, fat and carbohydrates and multiplication of the content of these components with appropriate factors. The results obtained from this study revealed systematic that calorific value of this plant is (319.46±1.070/100gm), higher than potato (97 kcal/100gm), beans (158 kcal/100gm), jack fruit (133 kcal/100gm) etc [19].

Alkaloids are naturally occurring heterogeneous group of compounds which is found in different part of the plant body. According to Ayoola *et al*., (2008) [20] alkaloids found in different spcies of *Hygrophila* fruits which is act as pain relievers or tranquilizers, stimulate nervous system, cause paralysis, and lower blood pressure. In this phytochemical study shown that *H. auriculata* contain alkaloids, flavonoids, phenols, steroid & sterols, saponins and cardiac glycosides. Flavonoids have been reported for various biological activities, such as anti allergic, anti inflammatory, antiproliferative, anti-carcinogenic and also effects on mammalian metabolism [21, 22]. Saponins, was known for their hormonal as well as antimicrobial properties. Tannins act as natural defense mechanism against microbial infections. Phenols known for their insecticides properties as well as medicinal drugs such as aspirin [23]. Saponin, tannins and phenols was detected in *H. thebaica* and *B. aethiopum* [23] and also detected in *H. auriculata* in this study. However, important constitute which has high therapeutic value such as cardiac glycoside was present in *H. auriculata* but absent in other species like *H. thebaica* and *B. aethiopum.*

The minerals are very important for the different biochemical process occurring in the human cells and its necessary for structural stability and functional activity and act as a electrolytes [24]. Sodium and potassium help to balance osmotic pressor, calcium surved as synthesis of bones, iron helped in blood formation and fouctioning of central nervous system (CNS), wheras copper, manganese and zinc play an important role in different methabolic pathways regulation. The mineral analysis profiling revealed that, *H. auriculata* contain higher amount of calcium and

potassium content that is 161.28±9.25 mg/kg and 158.83±5.31mg/kg respectively. Also, moderate amount of magnesium (73.29±5.40 mg/kg) and phosphorus (42.06±6.12 mg/kg), and trace amount of iron (3.26±0.71 mg/kg), manganese (0.54±0.01 mg/kg) and zinc (0.11±0.06 mg/kg).

Vitamins are the organic molecules, and human body need small quantity for the proper regulation of metabolism. Vitamins profile shown that *H. auriculata* contain high amount of vitamin C, which has function as antioxidant [25]. Also, various amount of vitamin B complex present which act as cofactor and precourses of many enzymes. However, Vitamin A is important for the cell growth and differentiation.

The antioxidant is the substance which present at low concentrations, and helps to inhibits oxidation of oxidizable substrate and prevent damage to a target molecule [26]. Its believed that antioxidants play an important role in a body defence system against Reactive Oxygen Species **(**ROS) [27]. Antioxidant constituents of the plant material act as radical scavengers, which helps in converting the radicals to less reactive species [28]. The result of DPPH and H_2O_2 scavenging activity shown that, plant *H. auriculata* had antioxidant activity. At 500µg/ml maximum inhibition was shown in both DPPH and H_2O_2 scavenging methods.

5. Tables

Table 1: Phytochemical screening of powdered extract of *H. auriculata*

Phytochemicals	Qualitative screening
alkaloid	+
flavonoids	+
phenols	+
steroid & sterols	+
saponins	+
cardiac glycosides	+

Table 2: Proximate composition of *H. auriculata*

Sr. No.	Nutrient	Amount (%)
1	Total carbohydrates	64.83±1.38
2	Protein	8±0.74
3	Crude fibre	4.53±1.22
4	Oil & Fat	3±1
5	Total sugar	4.10±0.12
6	Energy K cal.	319.46±1.070
7	Moisture content	65±1.45
8	Ash content	0.29±0.17

Table 3: Mineral Profile of *H. auriculata*

Sr. No.	Mineral	Amount (mg/kg)
1	Sodium	0.35±0.12
2	Potassium	158.83±5.31
3	Calcium	161.28±9.25
4	Iron	3.26±0.71
5	Phosphorus	42.06±6.12
6	Magnesium	73.29±5.40
7	Manganese	0.54±0.01
8	Copper	0.005±0.02
9	Zinc	0.11±0.06

Table 4: Vitamins profile of *H. auriculata*

Sr. No.	Vitamin Name	Inference	Amount (mg/100 gm)
1	Vitamin A	+	0.0266±0.05
2	Vitamin C	+	48.1±2.19
3	Vitamin E	-	-
4	Vitamin B_1	+	0.0293±0.06
5	Vitamin B_2	+	0.0133±0.02
6	Vitamin B_5	+	1.9±0.36
7	Vitamin B_6	+	0.0301±0.04
8	Vitamin B_7	+	6.9±0.40
9	Vitamin B_9	-	-

6. Figures

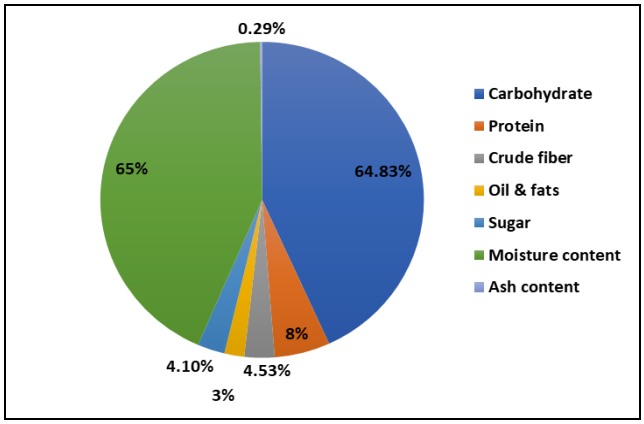

Fig 1: Nutrient content of *H. auriculata*

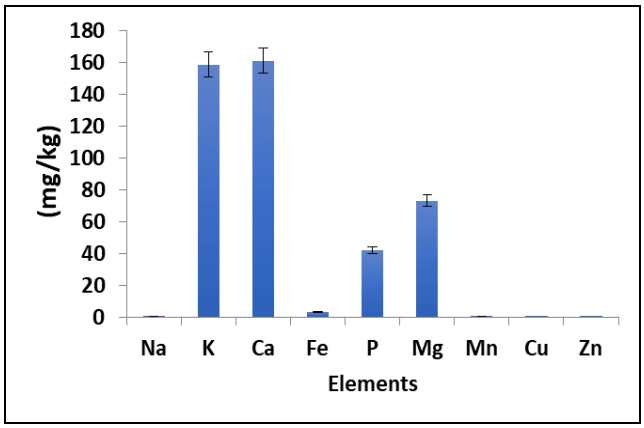

Fig 2: Mineral contents of *H. auriculate*

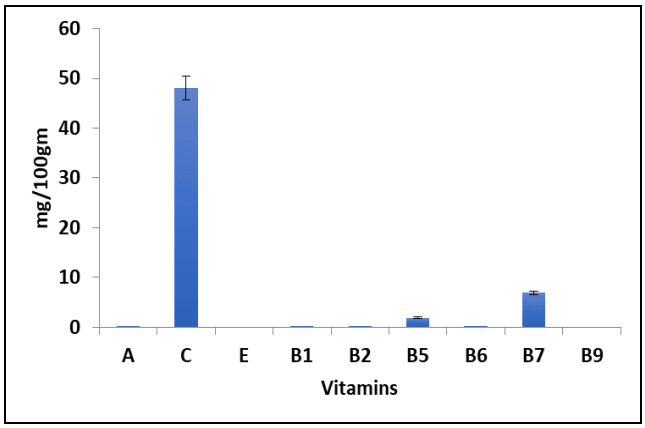

Fig 3: Vitamin contents of *H. auriculata*

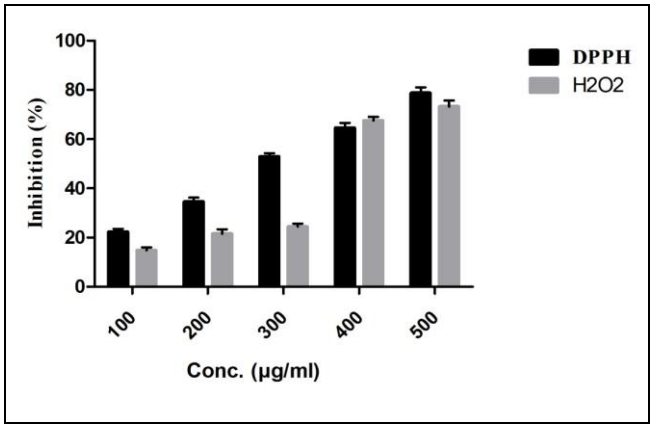

Fig 4: Antioxidant potential of *H. auriculata* against DPPH and H_2O_2 molecules

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