

# Nutritional, Antioxidant And GCMS Screening of Antidesma MONTANUM bl. Leaves

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## ABSTRACTS

*Antidesma montanum* Bl. is the member of family Euphorbiaceae. Leaves of *A. montanum* were used as a wild vegetable, while traditional medicinal practitioners use the different parts of plant in case of ulcer, lumbar pain, eye disease, hematochezia, stomachache etc. Nutritional evaluation of *A. montanum* leaves shows high amount of carbohydrate, sodium, potassium, iron, polyphenols and ascorbic acid. Methanolic extract of leaves shows higher antioxidant power as compare to ascorbic acid. GCMS analysis shows presence of 9-Eicosyne, hexadecanoic acid, gamma-sitosterol respectively.

**Keywords:** *Antidesma montanum*, leaves, nutritional and antioxidant analysis, GCMS

## I. INTRODUCTION

The wild plants are rich sources of various vitamins, minerals and fibers required by human body. There is now increasing interest in antioxidant activity of phytochemical present in the diet. Antioxidants are play a very significant role in the body defense system against reactive oxygen species (ROS), which are the harmful byproducts generated during normal cell aerobic respiration (1). Increasing intake of dietary high nutritious and antioxidants may help to maintain an adequate nutrition status and normal physiological function of a living system (2). The antioxidant capacity of plant is totally depends on the nutritional and secondary metabolite composition.

Plants commonly synthesize number of secondary metabolites for the protection against attack of herbivores, insects, diseases and to survive in adverse condition. Therefore, the plants serve as a very good source of secondary metabolites with potential biological activities. Phytochemical components are the main source for the formation of a number of pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs (3). Hence for proper standardization of plants, modern method is useful to identification and quantification of chemical substances present in plant.

Wild edible plant plays significant role in the diet supplement and ayurvedic medicine preparation in rural areas. Most of them do not have any previously documented physiochemical studies, *Antidesma montanum* Bl. is one of them. The *Antidesma montanum* Bl. is wild edible plant having medicinal properties. The rural people of Kolhapur district and adjoining area used mature fruits to prepare pickle and ripened fruits were eaten. The leaves and roots are used in different ailment of diseases. Due to the significant uses of plant present investigation was undertaken.

## II. MATERIALS AND METHODS

### Collection of plant material

Leaves of *Antidesma montanum* was collected from Borbit of Kolhapur district in flowering season. Thoroughly washed it and dried under shade condition. These leaves grind with the help of electric grinder and it was used for further study.

### PROXIMATE ANALYSIS

Dry matter and moisture content of the plant material was determined with the help of moisture balance (Shimadzu MOC 63u). Ash value was determined by the method of AOAC (4). Two gram dried powder was kept in preweighed silica crucible and placed in a muffle furnace at 600°C for 6h. Then it was transferred in the desiccator for cooled to room temperature and percentage of ash was calculated. The method of Sadasivam and Manikam was used to denote crude fibre and crude fat (5). The total nitrogen content of the sample was multiplied with conversion factor 6.25 to calculate the crude protein content (4).

### NUTRITIONAL ANALYSIS

#### Carbohydrates

0.5g of plant material extracted with 50 ml 80% ethanol. These extract was filtered through Buchner's funnel using Whatman No.1 filter paper. Residue was used to determine starch content and filtrate for reducing and total sugar estimation (6). Residue with 50 ml distilled water and 5 ml conc. HCl was hydrolyzed at 15 lbs pressure for 30 minutes, cooled and neutralize with anhydrous

sodium carbonate. Filtered through Buckner's funnel, filtrate (a) was used to analyze starch content. Filtrates were condensed and add 2g lead acetate and potassium oxalate (1:1) for decolourization. In that 40 ml distilled water was added and filtered through Buckner's funnel. This volume of filtrate (b) was measured and it served as an extract for determination of reducing sugars. 20 ml of extract (b) with 1- 2 ml Conc. HCl was hydrolyzed at 15 lbs pressure for 30 minutes, cooled to room temperature and neutralize with anhydrous sodium carbonate. Mixture was filtered through Buckner's funnel and volume of filtrate (c) was measured and used to analyze total sugar.

For estimation of reducing sugars, total sugar and starch 2ml filtrate were taken in separate sets of test tubes. Add 1 ml Somogyi alkaline copper tartarate reagent and incubate in boiling water bath for 10 minutes, cooled to room temperature and carefully add 1 ml of Nelsons arsenomolybdate reagent. The reaction mixture diluted up to 10 ml with distilled water and absorbance was recorded at 620 nm. The amount of reducing sugar, total sugar and starch was estimated with help of calibration curve of standard glucose ( $0.1\text{mg}\cdot\text{ml}^{-1}$ ) and values were expressed as g/100g dry sample.

The Atwater system was used to determine the energy values. The conversion factors were multiplied by 4.186 in order to obtain energy values in kilojoules (kJ) (7).

#### **Mineral Analysis**

The acid digestion method (8) was used to analysis of inorganic constituents. 0.5 g plant material with 10 ml acid mixture (75ml  $\text{HNO}_3$  + 15ml  $\text{H}_2\text{SO}_4$  + 30ml  $\text{HClO}_4$  kept for an hour till the primary reactions subsided. Then, it was heated on hot plate till clear solution. It was allowed to cool, then diluted up to 100 ml with double distilled water and kept overnight. This was filtered through Whatman No. 42 (Ashless) filter paper and filtrate used to analyze inorganic constituents. The level of Calcium, Magnesium, Iron, Manganese, Zinc, and Copper were estimated by using Atomic Absorption Spectrophotometer.

#### **Estimation of Phosphorus**

Phosphorus was estimated from the same acid digest (9). 2 ml of acid digest mixed with 2 ml of 2 N  $\text{HNO}_3$  and 1 ml Molybdate-Vanadate reagent and volume was made up to 10 ml with distilled water mixed well and allowed to react for 20 minutes; the yellow colour intensity was measured at 420 nm. Standard phosphorus used for plotting standard curve and phosphorus content in plant material was expressed in  $\text{mg } 100 \text{ g}^{-1}$  on dry weight basis.

#### **Total nitrogen**

Digestion of 0.5 g powder of plant samples with pinch of microsalt (200g  $\text{K}_2\text{SO}_4$  + 5 g dehydrated  $\text{CuSO}_4$ ) and 5 ml  $\text{H}_2\text{SO}_4$  and double

distilled water (1:1) in Kjeldahl's flask was carried out up to clear solution. A few glass beads were added to accelerate the digestion and to avoid bumping of solution in flask. It was cooled to room temperature, and made total 100ml volume with double distilled water. Stored overnight at room temperature and filtered through Whatman No.1 filter paper. This filtrate was used for estimation of nitrogen. 1ml filtrate, a drop of 8%  $\text{KHSO}_4$  and 15 ml Nessler's reagent was diluted up to 50 ml with distilled water and absorbance was recorded at 520 nm on a double beam spectrophotometer. A standard curve of ammonium sulphate was prepared to calculate nitrogen content in plant material (10).

### **ANTIOXIDANT ANALYSIS**

#### **A. Non enzymatic antioxidants**

##### **a) Carotenoids**

Fresh 0.5 g plant material was homogenized in 80% chilled acetone a pinch of  $\text{MgCO}_3$  was added to neutralize the acids released during extraction. The extract was filtered through Whatman No.1 filter paper and final volume of the filtrate was adjusted to 50 ml with 80% acetone. The absorbance was recorded at 480 nm on UV- VIS double beam spectrophotometer, using 80% acetone as blank (11). The carotenoids were calculated  $\text{mg}/100\text{g}$  fresh weight.

##### **b) Total Polyphenol**

0.5 g fresh plant material was homogenized in 30 ml acetone (80%) and was filtered through Buckner funnel. The residue was extracted several times and the final volume was made 50 ml. One ml of plant extract and standard tannic acid (0.1 mg/ml) was taken in a Nessler tubes. In each tube, 10 ml  $\text{Na}_2\text{CO}_3$ (20% ) and 2 ml of Folin-Denis reagent were added Final volume of reaction mixture was made to 50 ml with distilled water and in dark allowed to stand at room temperature for 20 minutes. Absorbance was read at 660 nm and total polyphenols calculated with help of standard curve of tannic acid (0.1 mg/ml) and expressed as  $\text{mg}/100\text{g}$  fresh tissue (12).

##### **c) Ascorbic Acid (Vitamin C)**

One gram of fresh material was ground in 5 ml of 10% TCA. The extract was centrifuged at 3500 rpm for 20 minutes. The pellet was re-extracted twice and volume of supernatant was adjusted to 10 ml by TCA and used for estimation. Reaction was conducted by addition of 0.5 ml of the extract, 1 ml of DTC reagent and mixed thoroughly. The reaction mixture was incubated at  $37^\circ\text{C}$  for 3 hours and added 0.75 ml of ice cold 65%  $\text{H}_2\text{SO}_4$ . The tubes were allowed to stand at  $30^\circ\text{C}$  for 30 min. The resulting colour was read at 520 nm on spectrophotometer (13). The ascorbic acid content was determined using a standard curve prepared with ascorbic acid and the results were expressed in  $\text{mg}/100\text{g}$  fresh weight.

**d) Tocopherol ( $\alpha$ -tocopherol or Vitamin E)**

Five hundred milligram fresh plant material was homogenized with 10 ml of a mixture of petroleum ether and ethanol (2:1.6 v/v) and the extract was centrifuged at 10,000 rpm for 20 min. The upper layer of supernatant served as extract and used for estimation of  $\alpha$ -tocopherol. Reaction was conducted as 1ml of extract, 0.2 ml of 2, 2-dipyridyl (2%) in ethanol and a micro drop of 2% FeCl<sub>3</sub>, mixed thoroughly and kept in the dark for 5 min. The resulting red colour was diluted with 4 ml of distilled water. Aqueous layer of mixture was measured at 520 nm. The vitamin E content was calculated against standard graph  $\alpha$ -tocopherol and results were expressed in mg/100g fresh weight (14).

**B. Free radical scavenging and antioxidant activity of plant material**

**DPPH Radical Scavenging Activity**

Antioxidant activity of methanolic extract of leaves was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (15). The different concentrations methanolic extract were allowed to react with 3 ml of DPPH solution. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. Then the absorbance of mixture was measured using a spectrophotometer at 517 nm. The results were expressed as percent radical scavenging activity. The results were expressed as mg ascorbic acid equivalent antioxidant capacity in 1 g of sample (mg AEAC g<sup>-1</sup>).

**Ferric reducing antioxidant power (FRAP)**

Various concentrations of plant extract were allowed to react with 3 ml of the FRAP reagent. The final volume of the reaction mixture was made up to 4 ml with distilled water and incubates at 37°C for 15 minutes. Absorbance was measured at 593 nm and results were expressed as ascorbic acid equivalent antioxidant capacity (16).

**Ferrous ion chelating ability**

Different concentrations of methanolic extract was mixed with 2 ml of distilled water and 50 $\mu$ l of 2 mM FeCl<sub>2</sub> and add 100 $\mu$ l of 5 mM ferrozine solutions and allowed to react for 10 minutes at room temperature. The absorbance was measured at 562 nm on Spectrophotometer. The FeCl<sub>2</sub> and ferrozine used as a control. Distilled water instead of ferrozine solution was used as a blank. The percentage inhibition of the ferrous ion was calculated by comparing the results of test with L-ascorbic acid (0.1mg/ml) (17).

**Reducing power assay**

Various concentrations of the leaves extract mixed with 1ml of phosphate buffer (0.2M, pH 6.6) and 1ml 1% potassium ferrocyanide. The mixture was incubated at 50°C for 20 minutes, add 1ml of TCA (10%) and centrifuged at 3000 rpm for 10

minutes. The upper layer of the solution was mixed with 1.5ml distilled water and 0.1ml 0.1% FeCl<sub>3</sub>. The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated the higher reducing power (18). The results were expressed as mean value  $\pm$  standard deviations and ascorbic acid was used as standard.

**Total antioxidant capacity**

An aliquot of 0.1- 0.5ml of sample solution (2mg/ml) combined with methanol for appropriate volume and was mixed with 5 ml phosphomolybdate reagent solution. The tubes were capped and incubated at 95°C for 90 min. After the samples had cooled to room temperature and absorbance was measured at 695 nm against blank by using spectrophotometer (19). For samples total antioxidant capacity is expressed as equivalents of ascorbic acid.

**Enzymatic antioxidants**

**a) Catalase**

0.5 g fresh plant material was homogenized in 10 ml (1/15M) phosphate buffer (pH 6.8) and filtered through muslin cloth. The filtrate was centrifuged at 10,000 rpm for 20 minutes at 4°C and supernatant was used as an enzyme source. The reaction mixture contained 3 ml of 10% H<sub>2</sub>O<sub>2</sub> and 0.2 ml enzyme extract was added and immediately  $\Delta$ OD was recorded at 240 nm up to reaction went on (5). The enzyme activity was expressed as unit  $\Delta$ OD/min/mg protein (20).

**b) Peroxidase**

0.5 g of plant material homogenized in 10 ml ice-cold (0.1M) phosphate buffer (pH 7) and filtered through muslin cloth. The filtrate was centrifuged at 10,000 rpm for 20 minutes and supernatant was used as source of enzyme. The reaction mixture contained 2 ml 0.1M phosphate buffer (pH 7), 1 ml 20 mM guaiacol and 1 ml enzyme extract. The reaction was initiated by the addition of 0.05 ml H<sub>2</sub>O<sub>2</sub> (20 mM). Changes in optical density due to oxidation of guaiacol was recorded immediately up to reaction goes on at 470 nm (21). The enzyme activity was expressed as  $\Delta$ OD/min/mg protein.

**c) Superoxide dismutase**

Enzyme was extracted by homogenizing 0.5 g fresh plant material in 15 ml, 150 mM cold potassium phosphate buffer (pH-7.8) containing 1% Polyvinylpyrrolidone (PVP), to protect enzyme from the action of polyphenols. Then it was filtered through four layered muslin cloths. The filtrate was centrifuged at 10,000 rpm for 20 min at 0 to 4°C. The supernatant was used as an enzyme source. Assay contained 2 ml potassium phosphate buffer (pH 7.8), 0.2 ml methionine (13mM), 0.1 ml Nitrobluetetrazolium (75  $\mu$ M), 0.5ml EDTA (0.1mM), and 0.5 ml enzyme. Then 0.1 ml riboflavin (2 $\mu$ M) was added and immediately absorbance was measured at 560nm on spectrophotometer (22). Then

the assay mixture was exposed to full sunlight for 30 min and again absorbance was read at 560 nm. The enzyme activity was expressed as  $\Delta\text{OD}/\text{min}/\text{mg}$  protein. Enzyme activity (catalase, peroxidase and superoxide dismutase) was calculated with the help of standard curve of protein (23).

### GCMS ANALYSIS

GC-MS analysis of leaves was carried out by using the methanol extract. Analysis was carried out using Shimadzu Make QP-2010 with non-polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at  $40^\circ\text{C}$  and held for 3 min and the final temperature of the oven was  $480^\circ\text{C}$  with rate at  $10^\circ\text{C} [\text{min. sup.}-1]$ . The chemical components from the methanolic extracts were identified by comparing the retention times of chromatographic peaks using Quadra pole detector with National Institute Standard and Technology (NIST) Library having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

### III. RESULTS

The local people of Kolhapur district and adjoining area used the young leaves of *A. montanum* as vegetable and to prepare tea as tonic whereas different parts of plant for the medicinal purposes. Leaves paste of *A. montanum* is used in chest pain, lumbar pain, headache, ulcer and malaria. Whereas decoction of leaves taken by women after child birth as tonic. Stem bark paste applied on injury and root paste in case of stomachache. Due to dual significance of the plant was selected for the present study to aware the nutritional as well as antioxidant value and chemical constituent of the plant leaves.

Proximate analysis results were represented in the Table 1; it showed the comparative value of proximate composition in leaves. It shows high amount of moisture content ( $65.09 \pm 0.03\%$ ) which is followed by dry matter ( $34.91 \pm 0.03\%$ ), crude fibre ( $16.741 \pm 0.001\%$ ), total ash ( $8 \pm 0.03\%$ ), crude fat ( $3.87 \pm 0.06\%$ ) and crude protein ( $3.5 \pm 0.02\%$ ) respectively.

Carbohydrate and mineral analysis results were represented in Table 2. Leaves of *A. montanum* contain considerable amount of total carbohydrate ( $614 \pm 0.43 \text{ mg}/100\text{g DW}$ ), reducing sugar ( $49 \pm 0.01 \text{ mg}/100\text{g DW}$ ), total sugar ( $214 \pm 0.6 \text{ mg}/100\text{g DW}$ ) and starch ( $437 \pm 0.44 \text{ mg}/100\text{g DW}$ ). In macronutrients the leaves shows very high amount of nitrogen ( $1323 \pm 0.04 \text{ mg}/100\text{g}$ ) is much higher than potassium ( $840 \pm 0.002 \text{ mg}/100\text{g}$ ), phosphorus ( $530 \pm 0.002 \text{ mg}/100\text{g}$ ), calcium ( $100 \pm 0.01 \text{ mg}/100\text{g}$ ), sodium ( $80 \pm 0.01 \text{ mg}/100\text{g}$ ) and magnesium ( $8.82 \pm 0.01 \text{ mg}/100\text{g}$ ). Whereas in micronutrients higher concentration of iron ( $42 \pm 0.001 \text{ mg}/100\text{g}$ ) then manganese ( $7 \pm 0.01 \text{ mg}/100\text{g}$ ),

zinc ( $5 \pm 0.05 \text{ mg}/100\text{g}$ ) and copper ( $4 \pm 0.06 \text{ mg}/100\text{g}$ ) found in leaves. The higher amount of nitrogen is may be due to the wild plant and the soil is rich in nitrogen content.

In the biological systems four general sources of antioxidants that is enzymatic, non-enzymatic, hormones and vitamins. On the other hand, there are number of free radical and oxidants that act as antioxidants having different physical and chemical properties. Single antioxidants act in multiple mechanisms in a single system or for single mechanisms different types of antioxidants are required. Additionally, antioxidants may respond in a different manner to different radical sources.

The non-enzymatic antioxidants of *A. montanum* leaves are shown in graph 1. In observation we found that  $6.09 \pm 0.02 \text{ mg}/100\text{g}$  carotenoids,  $401 \pm 1.09 \text{ mg}/100\text{g}$  total polyphenols,  $482 \pm 0.65 \text{ mg}/100\text{g}$  ascorbic acid and  $16.34 \pm 0.46 \text{ mg}/100\text{g}$   $\alpha$ -tocopherol.

Radical scavenging activity of methanolic extract of leaves was undertaken with different types of assays; DPPH radical scavenging activity of leaves shown in graph 2. Where radical scavenging activity increased remarkably with increasing of extract and inhibitory capacity of the leaves extracts was comparatively similar to the ascorbic acid. At  $40 \mu\text{g}/\text{ml}$  concentrations of leaves extract of *A. montanum* shows  $98.89 \pm 0.01\%$  inhibition of DPPH.

The ferric reducing antioxidant power (FRAP) of extract of leaves was represented in the graph 3. Extract showed higher ability to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  ion with increase in concentration of extract. *A. montanum* leaves having  $0.418 \pm 0.051 \text{ mg AAE}$  FRAP activity at  $0.5 \text{ mg}/\text{ml}$  of extract.

The ion chelating ability of the leaves extracts increases with increasing concentration (graph 4). At  $1 \text{ mg}/\text{ml}$  of leaves extract, percent-scavenging activity is found in *A. montanum*  $36.51 \pm 0.05\%$ . Reducing capacity of sample served as an indicator of potential antioxidant power (graph 5). Leaves showed absorbance at  $0.5 \text{ mg}/\text{ml}$  of extract of *A. montanum*  $0.798 \pm 0.001$ . Total antioxidant capacity by Phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. Graph 6 represents the total antioxidant capacity of leaves. Total antioxidant capacity increase with the increasing concentration of extract.

Enzymatic antioxidants in leaves are studied by using parameters like catalase, peroxidase and superoxide dismutase and results represented in graph 7. *A. montanum* leaves shows  $0.341 \pm 0.002 \Delta\text{OD}/\text{min}/\text{mg}$  protein catalase,  $0.042 \pm 0.00 \Delta\text{OD}/\text{min}/\text{mg}$  protein peroxidase and  $0.004 \pm 0.001 \Delta\text{OD}/\text{min}/\text{mg}$  protein superoxide dismutase.

GCMS analysis of *A. montanum* leaves are represented in graph 8 and table 3. Graph represents



methanolic extract of leaves having 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, 9-eicosyne, hexadecanoic acid, Tridecanoic acid and gamma-sitosterol.

#### IV. DISCUSSION

The proximate analysis *Securine gavirosa* Roxb. Ex Willd. contain lower amount of moistur, crude fibre and crude protein and higher amount of crude fat and ash than the leaves of *A. montanum*. While *S. virosa* leaves contain lower amount of Na, K, Ca, Fe, Mn, Zn, P and higher amount of Mg (24). *Euphorbia hirta* L. and *Croton zambesicus* Muell. Arg. having lower content of ash fat, moisture and crude fibre whereas maximum amount of crude protein and carbohydrate than the leaves of *Antidesma* (25).

The leaves of *Manihot esculenta* Cranz. and *Ricinus communis* L. shows lower amount of carbohydrate than the present studied leaves of *Antidesma* (26 and 27). *Euphorbia granulate* Forssk. leaves shows potassium and sodium contents are near to same in *A. montanum* (28). Ascorbic acid found in *Croton zambesicus* Mull. Arg. and *Euphorbia hirta* L. was lower (25) whereas in *Antidesma acidus* Retz. (29), *Hevea brasiliensis* Mull. Arg. and *Phyllanthus maderaspatensis* L. higher total carotenoids (30).

Decoction of *Euphorbia regis-jubae* (webb and berth) shows higher reducing power activity (31). Leaves of *Euphorbia hirta* L., *Acalypha indica* L., *Phyllanthus niruri* L. and *Euphorbia prostrate* Aiton. shows lower DPPH radical scavenging activity (32,33,34 and 35).

The phytocontents like palmitic acid (hexadecanoic acid), 10-octadecanoate, 9-hexadecenal, glycerol 1, 3-dipalmitate, 2, 13-octedecadiene – 1 –ol, dioctyl ester and heptanoic acid (9-dece-1-yl ester) have antimicrobial property, whereas methyl 14-methylpentadecanoate antifungal property. 3, 5-di-t-butyl phenol shows anti-inflammatory, antimicrobial and antioxidant property (36). Sixteen compounds were identified in ethanolic extracts of leaves of *Cleidion nitidum* (Muell. – Arg.) Thw. Ex Kurz. among them phytol and vitamin E are

the common compounds found in *Antidesma* (37). Whereas phytol and hexadecanoic acid in leaves oil of *Euphorbia hirta* Linn. (38). In leaves of *Acalypha ciliate* Forssk. 9-eicosyne and hexadecanoic acid was found (39). Hexadecanoic acid and n-octadecadienoic acid was found in *Cnidocolus aconitifolius* (40), *Phyllanthus amarus* L. (41), *Cleistanthus collinus* Roxb. (42 and 43), *Jatropha maheswarii* Subram and Nayar. (44) *Euphorbia hirta* Linn. (45).

In GCMS analysis of fruits of *Embllica officinalis* Gaertn., 1, 2, 3-benzenetriol, 2-Furancarboxaldehyde, 5-(hydroxymethyl), 2-Acetyl-5-methylfuran, Benzoic acid, 3, 4, 5-trihydroxy compounds was identified (46). While, 2, 5-furandione, 3-methyl, hexadecane and pentadecanoic acid in the fruits of *Antidesma ghaesembilla* (47). This work is showed the species of *A. montanum* have bioactive potential for the management of certain health conditions.

We believe that these plants could be used the rural people for nutritional purposes due to their demonstrated good nutritional qualities and can help to overcome the nutritional deficiency especially in rural areas. Nutritional values of wild plant foods are of considerable significance, as they help to pinpoint traditional food resources of Kolhapur district. Due to lack of awareness and negative approach towards the wild food plants, it is important to create community awareness to accept wild food plants as useful as the cultivated ones.

#### V. CONCLUSION

*A. montanum* is a wild edible plant having high amount of nutrients, antioxidants, vitamins. The GCMS analysis of leaves shows presence of valuable secondary metabolites. The data generated from these studies can be added in to the food composition, database; which can then be used as a tool for nutrition education and encourage people to consume greater quantities of wild edible plants. In a parallel, the conservation of these indigenous plants should be a major food security concern.

**Table No. 1- Proximate analysis of *A. montanum* leaves**

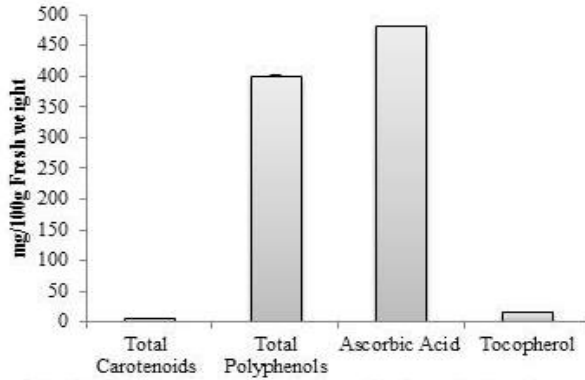
Sr. no.	Compound	Percentage (%)
1	Dry matter	34.91 ± 0.03
2	Moisture	65.09 ± 0.03
3	Total ash	8 ± 0.03
4	Crude fibre	16.741 ± 0.001
5	Crude fat	3.87 ± 0.06
6	Crude protein	3.5 ± 0.02

**Table No. 1- Carbohydrate and mineral analysis of *A. montanum* leaves**

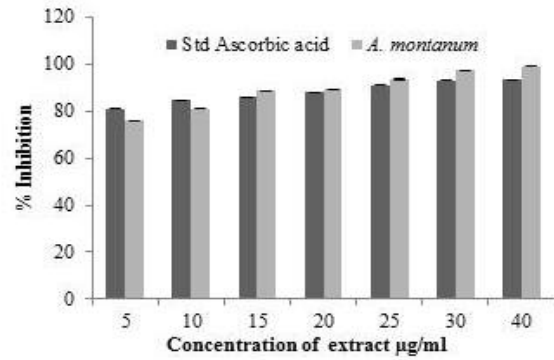
Sr. no.	Compound	Value mg/100g
1	Reducing Sugar	49 ± 0.01
2	Total Sugar	214 ± 0.6
3	Starch	437 ± 0.44
4	Total Carbohydrates	6140 ± 0.43
5	Nitrogen	1323 ± 0.04
6	Phosphorus	530 ± 0.002
7	Potassium	840 ± 0.002
8	Calcium	100 ± 0.01
9	Magnesium	8.82 ± 0.01
10	Sodium	80 ± 0.01
11	Iron	42 ± 0.001
12	Manganese	7 ± 0.01
13	Zinc	5 ± 0.05
14	Copper	4 ± 0.06

**Table No. 3. GCMS analysis of *A. montanum* leaves**

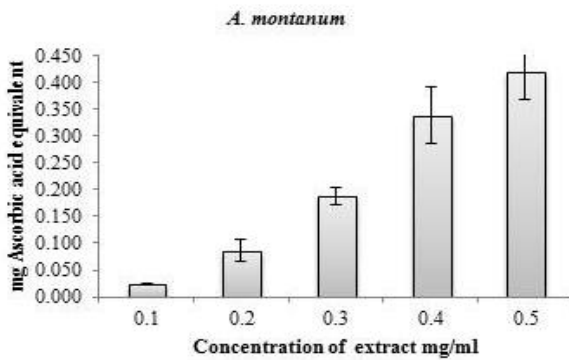
Compound Analyzed	Retention Time (min.)	Area of Peak (%)	Mol. Wt.	Functional Group
3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	15.645	6.42	296	Terpane alcohol
9-Eicosyne	15.643	2.06	278	Alkyne
Hexadecanoic acid	16.388	2.16	270	Acid
Tridecanoic acid	16.740	7.53	214	Fatty acid
gamma-Sitosterol	24.504	58.80	414	Steroid



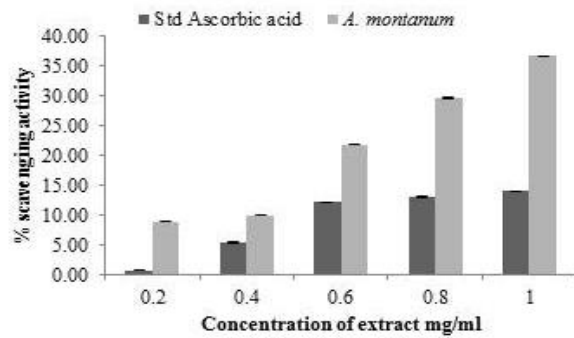
Graph 1. Nonenzymatic antioxidant analysis of *A. montanum* leaves



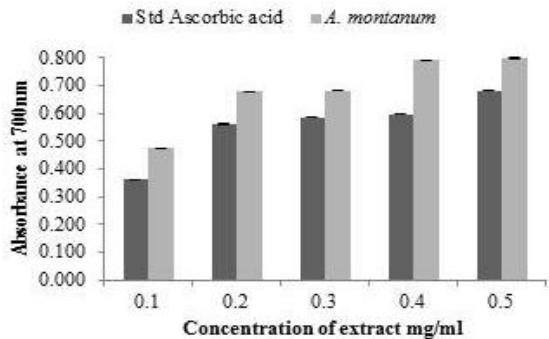
Graph 2. DPPH radical scavenging activity of *A. montanum* leaves



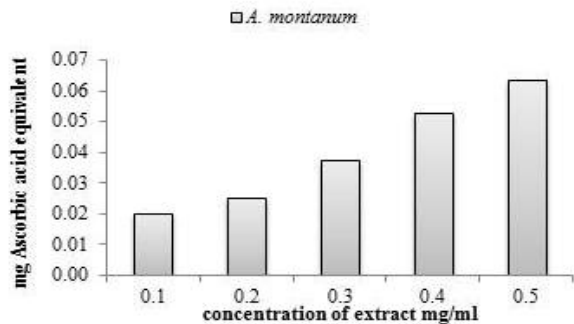
Graph 3. Ferric reducing antioxidant power (FRAP) of *A. montanum* leaves



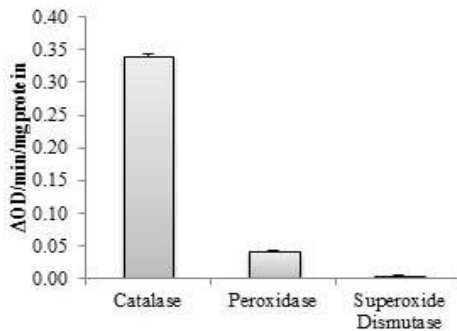
Graph 4. Ferrous ion chelating ability of *A. montanum* leaves



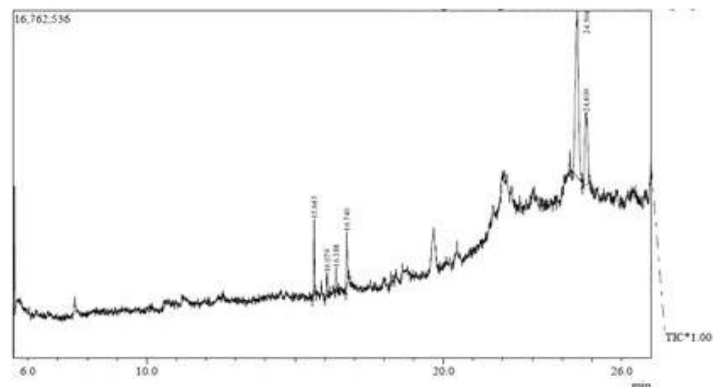
Graph 5. Reducing power assay of *A. montanum* leaves



Graph 6. Total antioxidant capacity of *A. montanum* leaves



Graph 7. Enzymatic antioxidants of *A. montanum* leaves



Graph 8. GCMS analysis of *A. montanum* leaves

## References

- [1] JMC Gutteridge and B Halliwell, Free radicals and antioxidants in the year 2000 – A historical look to the future. *Ann. N. Y. Acad. Sci.*, 2000; 899: 136-147.
- [2] IR Record, IE Dreosti, JK McInerney, Changes in plasma antioxidant status following consumption of diets high or low in fruit and vegetables or following dietary supplementation with an antioxidant mixture. *Brit. J. Nutri.* 2001; 85: 459-464.
- [3] N Savithramma, RM Linga and Beenaprabha, Phytochemical studies of *Dysophylla myosuroides* (Roth.) Benth. In. wall. and *Talinum cuneifolium* (Vahl.) Willd. *Res. J. Phyto.* 2011; 5 (3): 163-169.
- [4] AOAC. Official Methods of Analysis. Association of official Analytical Chemists, Washington. DC. 1990.
- [5] S Sadashivam and A Manikam, Biochemical method for agricultural sciences, Wiley, Eastern Ltd.: 105; 1992.
- [6] N Nelson, A Photochemical adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.*, 1944; 153: 375-380.
- [7] WHO/FAO/UNU, Report: Energy and protein Requirement: WHO technical report series No.724: 220(WHO Geneva) 1985.
- [8] CA. Black (ed.), Method of Soil Analysis, Part 2, Chemical and Microbiological Properties, American Society of Agronomy, Inc, Publisher, Madison, Wisconsin USA 1965.
- [9] T Sekine, T Sasakawa, S Morita, T Kimura. and K Kuratom, cf. laboratory manual for physiological studies of Rice (Eds.) Yoshida, S., Forno, D., Cook, J. B. and Gomez, K. A. Pub. International Rice Research institute, Manila, India. 1972.
- [10] PB Hawk, BL Oser and WH Summerson, Practical physiological chemistry (Publ.). The Blockiston Co. USA, 1948.
- [11] JO Kirk and RL Allen, Dependence of chloroplast pigment on actidione *Arch. Biochem. Biophys. Res. Commun.* 1965; 21: 523-530.
- [12] O Folin and W Denis, A colorimetric estimation of phenols (and phenolic derivatives) in urine. *J. Bio. Chem.* 1915; 22: 305-308.
- [13] ST Omaye JD Turnbull and HE Sauberlich, Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. *Methods in Enzymology.* 1979; 62: 3-11.
- [14] H Backer, O Frank, B De Angells and S Feingold, Plasma tocopherol in man at various times after ingesting free or ocetylaned tocopherol. *Nutrition Reports Int.* 1980; 21: 531-536.
- [15] HC Lee, JH Kim, SM Jeong, DR Kim, JU Ha and KC Nam., Effect of far infrared radiation on the antioxidant activity of rice hulls. *J. Agri. Food Chem.* 2003; 51 (15): 4400-4403.
- [16] IFF Benzie and JJ Strain, The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power: the FRAP assay. *Analytical Biochem.* 1996; 239: 70-76.
- [17] EA Decker and B Welch, Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.* 1990; 38: 674-677.
- [18] M Oyaizu, Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutri.* 1986; 44: 629-632.
- [19] P Prieto, M Pineda and M Aguilar, Spectrophotometric quantification of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem.* 1999; 269: 337-341.
- [20] H Luck, In: methods in enzymatic analysis. II (ed.) Bergmeyer. (Publ.) Academic Press, New York.: 885, 1974.
- [21] AC Maehly, Methods in biochemical analysis (Ed.) Glick D. (Publ.) Interscience publishers Inc. New York: 1954; 385-386.
- [22] CN Giannopolitis and SK Reis, Superoxide dismutase I. Occurrence in higher plants. *Plant Physio.* 1977; 59: 309-314.
- [23] OH Lowry, NJ Rosenbrough, AL Furr and RJ Randall, Protein measurements with folin phenol reagent. *J. Bio. Chem.*, 1951; 193: 262-263.
- [24] U Danlami, BM David and SA Thomas, The Phytochemical, proximate and elemental analysis of *Securinega virosa* leaf extracts. *Res. J. Eng. App. Sci.* 2012; 1 (6): 351-354.
- [25] OO Abidemi, Proximate composition and vitamin levels of seven medicinal plants. *Int. J. Eng. Sci. Inv.* 2013; 2 (5): 47-50.
- [26] T Balamurugan, and S Anbuselvi, Physicochemical characteristics of *Manihot esculenta* plant and its waste. *J. Chem. Pharma. Res.* 2013; 5 (2): 258-260.
- [27] G Dastagir, F Hussain, F Khattak, and Khanzadi, Proximate analysis of plants of family Zygophyllaceae and Euphorbiaceae during winter. *Sarhad J. Agric.* 2013; 29 (3): 395-400.
- [28] M Parvez, F Hussain, B Ahmad, and J Ali, *Euphorbia granulata* Forssk. as a source of mineral supplement. *American-Eurasian J. Agric. Environ. Sci.* 2013; 13 (8): 1108-1113.
- [29] P Rajyalakshmi, K Venkata lakshmi, TVN Padmavathi, and V Suneetha, Effect of processing on beta-carotene content in forest green leafy vegetables consumed by tribals of south India. *Plant Foods Hum. Nutri.* 2003; 58: 1-10.
- [30] P Rajyalakshmi, K Venkatalaxmi, K Venkatalakshamma, Y Jyothsna, K Balachandramani Devi, and V Suneetha, Total carotenoid and beta-carotene contents of forest green leafy vegetables consumed by tribals of south India. *Plant Foods Hum. Nutri.* 2001; 56: 225-238.
- [31] F Lahlou, F Hmidid, M Loutfi, and N Bourhim, Antioxidant activity and determination of total phenolic compounds content of *Euphorbia regis-jubae* (webb and berth) from methanol and aqueous extracts. *Int. J. Pure App. Biosci.* 2014, 2 (3): 112-117.
- [32] P Purohit, M Panda, and R Rosalin, Phenolic potency and antioxidant activities of plants of a disturbed area. *Int. J. Innov. Res. Studies.* 2014; 3 (8): 388-397.
- [33] A Rauf, M Qaisar, G Uddin, S Akhtar, N Muhammad, and M Qaisar, Preliminary phytochemical screening and antioxidant profile of *Euphorbia prostrata*. *Middle-East J. Med. Plant Res.* 2012; 1 (1): 9-13.
- [34] NK Sharma, S Dey, and R Prasad, In vitro antioxidant potential evaluation of *Euphorbia hirta* L. *Pharmacol.* 2007; 1: 91-98.
- [35] PM Satya JD Suman K Narendra, K Srinivas, BJ Srilakshmi, CM Lakshmi and SA Krishna, Phytochemical and pharmacological evaluation of Euphorbiaceae family plant leaves- *Acalypha indica* L., *Croton bonplandianum* Baill. *Mintage J. Pharma. Med. Sci.* 2015; 4 (3): 17-22.
- [36] UT Mamza, OA Sodipo, and IZ Khan, Gas chromatography-mass spectrometry (GC-MS) analysis of bioactive components of *Phyllanthus amarus* leaves. *Int. Res. J. Plant Sci.* 2012; 3 (10): 208-215.
- [37] N Mohan Das, SS Sivakama, S Karuppusamy, V Mohan and B Parthipan, GC - MS analysis of leaf and stem bark of *Cleidion nitidum* (Muell. Arg.) Thw. *Ex Kurz.* (Euphorbiaceae). *Asian J. Pharma. Clin. Res.* 2014; 7 (2): 41-47.
- [38] M Ogunlesi, W Okiei, E Ofor, and AE Osibote, Analysis of the essential oil from the dried leaves of *Euphorbia hirta* Linn. (Euphorbiaceae), a potential medication for asthma. *African J. Biotech.* 2009; 8 (24): 7042-7050.
- [39] NE Okoronkwo, KA Mbachu, AB Nnaji, OS Igboanugo, Evaluation of chemical compositions and antimicrobial activities of *Acalypha ciliata* leaves. *Int. J. Plant Sci. Eco.* 2015; 1 (3): 67-71.
- [40] AE Omotoso, K Ezealisiji, and KI Mkparu, Chemometric profiling of methanolic leaf extract of *Cnidioscolus aconitifolius* (Euphorbiaceae) using UV-VIS, FTIR and GC-MS techniques. *Peak J. Med. Plant Res.* 2014; 2 (1): 6-12.
- [41] S Pandurangan, A Mohan, B Sethuramali and S Ramalingam, GC-MS analysis of methanolic extract of *Phyllanthus amarus* leaves collected from Salem region. *Asian J. Pharma. and Toxicol.* 2015; 3 (10): 54-59.



- [42] S Parasuraman, R Raveendran, and C Madhavrao, GC-MS analysis of leaf extracts of *Cleistanthus collinus* Roxb. (euphorbiaceae). *Int. J. Ph. Sci.* 2015; 1 (2): 284-286.
- [43] T Suman, K Chakkaravarthi, and R Elangomathavan, Phytochemical profiling of *Cleistanthus collinus* leaf extracts using GC-MS analysis. *Res. J. Pharma. Tech.* 2013; 6 (11): 1173-1177.
- [44] BM Sumathi and F Uthayakumari, GC MS analysis of leaves of *Jatropha maheswarii* Subram and Nayar. *Sci. Res. Reporter.* 2014; 4 (1): 24-30.
- [45] C Suresh, S Senthilkumar, and K Vijayakumari, Phytochemical and GC-MS analysis of *Euphorbia hirta* Linn. leaf. *Int. J. Institutional Pharma. Life Sci.* 2012; 2 (3):135-139.
- [46] S Balasubramanian, D Ganesh, P Panchal, M Teimouri, and VVSS Narayana, GC-MS analysis of phytochemicals in the methanolic extract of *Emblica officinalis* Gaertn. (Indian Gooseberry). *J. Chem. Pharma. Res.* 2014; 6 (6): 843- 845.
- [47] SR Valvi, VD Jadhav, SS Gadekar, and DP Yesane, Assessment of bioactive compounds from five wild edible fruits, *Ficus racemosa*, *Elaeagnus conferta*, *Grewia tillifolia*, *Scleichera oleosa* and *Antidesma ghaesembilla*. *Acta Biologica.* 2014; 3 (1): 549- 555.