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Evaluation of Nutritive Minerals and Antioxidants Values of *Euphorbia thymifolia* Linn.

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ABSTRACT

The chemical composition of the volatile oil from areal parts of *Euphorbia thymifolia* Linn. growing at Naini Patal, Distt-Pithoragarh, Uttarakh and was analyzed by GC-MS. The steam distillation of *E. thymifolia* areal parts ware carried out using a Clevenger apparatus in order to obtain the volatile oil (0.25%). It indicated presence of 30 compounds. The major constituents of oil were n-hexadecanoic acid (33.03%), phytol (10.36%) and tetradecanoic acid (6.58%). The oxygenated monoterpenes and sesquiterpene hydrocarbons were found in the oil as minor components. The yields of essential oil obtained from aerial parts were 0.05% (v/w). The nutrients, antioxidant and minerals composition in aerial parts of *Euphorbia thymifolia* Linn. were determined. Macro minerals viz., Na, K, Ca and Li were estimated by AIMIL, Flame Photometer while micro elements viz. Fe, Cu, Mn, Zn and Co were estimated by Atomic Absorption Spectrophotometer, model 4129, Electronic Corporation of India Ltd. The plants contained antioxidants such as β-carotene, Vitamin C and phenolics (307.40, 88.48 and 336.73 m/100 g), respectively. The plants have been reported rich in nutrients such as crude protein, carbohydrate, crude lipids, starch and crude fiber (13.42, 11.99, 4.63, 22.31 and 24.34 g/100 g), respectively. The plants have also been reported rich in minerals such as Na, K, Ca, S, P, Fe, Mn, Cu and Zn (75.40, 4786.48, 242.46, 325.24, 226.81, 121.04, 9.28, 3.45 and 6.47 mg/100 g), respectively. The study established the chemical composition of the essential oil of the plants. The plants have been found as a good source of nutrients, antioxidants and minerals. Results suggest aerial parts of plants could be used as raw materials in drug formulation.

Key words: GC-MS, essential oil, isolation, kovat indices, nutritive, antioxidant, mineral composition, *Euphorbia thymifolia*

INTRODUCTION

There is great need to evaluate the local herbs for mineral and nutrient composition, so as to determine the potential of indigenous source of medicines (Rahila *et al*., 1994). *Euphorbia thymifolia* Linn. (Euphorbiaceae) is traditionally used as blood purifier, cough, antiviral in brachial asthma and paronychia (Manickam and Rajappan, 1998). Water extract of this plants have antiviral activity (Anonymous, 1952; Lin *et al*., 2002). *E. thymifolia* Linn. are numerous reservoirs of many minerals, antioxidants and nutritive properties. Their mineral antioxidant and nutritional compounds are not determined despite their impotence in traditional medicine (Prabha and Singh, 2005). Over the years, man has acquired extensive knowledge regarding the utilization of plants

around him as food and for maintenance of his health (Okwu and Orji, 2007). Indigenous edible wild fruits are enjoyed by people especially those in the rural areas (Boateng *et al.*, 2007).

Antioxidants are integral part of the nutraceutical market. Last few years of research has confirmed that many of the common diseases and ailments of the 21st century (cardiovascular diseases, diabetes, cataracts, high blood pressure, infertility, respiratory infections, rheumatoid arthritis, Alzheimer's disease, several types of cancer, mental illness, including tumor promotion and AIDS) are associated with tissue deficiency and low dietary level of compound called "antioxidant" (Wattenberg, 1992). Vitamin C is known to be a potential antioxidant and it is essential for functioning of the central nervous system and help in fighting infectious diseases (Naik, 2003). Vitamin A and carotenoids can both accept and donate electrons and carotenoids can also quench singlet Oxygen (Devi and Fazlur-Rehaman, 2002). Flavonoids are the major class of phenolics and have been recognized for having a potential role in the prevention of several forms of cancer and cardiovascular diseases (Shetgiri and D'Mello, 2003). The green leafy vegetables are one of the source of nutrients for growth in man and animal (Dairo and Adanlawo, 2007). In developing countries, there is need for a constant search of new food resources to alleviate hunger which arises from increasing population and shortage of fertile land (Idu *et al.*, 2008). The plant has been traditionally noted for its medicinal and food values (Njoku and Akumefula, 2007).

Proteins are the important compounds which are main organic constituent of body such as muscle, skin, hair and nail. They carry on all vital life processes in organisms. Carbohydrates are 50% of our diet and are needed to use fat efficiently in the body. Fats are the reserve energy producing substance which also provides carbon skeleton for amino synthesis. Several macro and micro minerals of plant are considered as most important part of food. Minerals are important for the growth of animal, production of milk and in the regulation of several metabolic processes in body along with vitamins. The nutritive value of plants depends on the protein content, essential amino acid, sugar, fat and essential minerals. The present study deals with the estimation of antioxidants, crude fat, crude protein, carbohydrate, crude fiber and minerals content in the aerials parts of medicinally important plants *Euphorbia thymifolia* Linn. of Uttaranchal and consequently to asses their potential usefulness as pharmaceutical raw material in the formulation of drugs.

MATERIALS AND METHODS

Plants material: The aerial parts of the plant (5.0 kg) were collected in May 2006 from Nainipatal (Disst. Pithoragarh, Uttarakhand) District identified by Prof. Y. P. S. Pangtey, Department of Botany, Kumaun University, Nainital and also from Dr. H. C. Pandey, Botanical Survey India, Dehradun. The voucher specimen was deposited in the Herbarium section at Phytochemistry Laboratory, DSB Campus Nainital. The leaves were collected from 50 plants. The aerial parts and leaves of plant were dried in shade after collection.

Oil isolation: The fresh material was steam distilled for 6 h in a Clavenger apparatus. The distillate was saturated with NaCl and the oil was extracted with hexane and dichloromethane. The organic phase was dried over anhydrous Na_2SO_4 and solvent distilled in the thin film rotary vacuum evaporator at 35°C. The yield of oil was calculated based on dried weight of plant material.

GC/MS: The GC-MS of oil was recorded on 17A-Shimatdzu interfaced with QP5050A ion mass spectrometer using Rtx.® – WAX column (30 m × 0.25 mm i.d., 0.25 μm film coating) the oven

temperature was programmed from 40°C at rise 3°C, finally at 230°C. Helium was used as carrier gas. The gas chromatogram were recorded in Nucon 5765 model, Rtx-5 columns (30 m x 0.32 mm i.d., 0.25 μm film) under temperature programme 60°C to 210°C at 3°C min^{-1} rise, N_2 was used as carrier gas and FID as detector. Comparing the mass spectra with Willey Spectral Library identified the components. The identification of the chemical constituents was assigned on the basis of comparison of their retention indices and mass spectra with those given in the study of Adams (1955), Adams (2001) and Joulain and Koenig (1988).

Biochemical analysis: The moisture content was estimated by dried in electrical oven at 80°C for 24 h and expressed on a percentage basis. The dried leafs were powdered separately in electric mill to 60 mesh size. The fine leaves powders so obtained were used for further biochemical and mineral analysis (three replication of each parameter). The carotenoids in plant sample were extracted, as described by Ranganna (1976) and Witham *et al.* (1971) methods. The chlorophyll content in dry leaves powder was estimated by method of Singleton *et al.* (1999). Total phenolics content was estimated by method Singleton *et al.* (1999) with modification. Dry leaves powder (0.5 g) was extracted with 10 time volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatant was collected. The residue was extracted three times with 80% ethanol, centrifuged and supernatant was collected. The supernatant was evaporated to dryness. The residue was dissolved in 5 mL double distilled water and 1.0 mL aliquots were added to 0.5 mL Folin-Ciocalteau reagent, followed by addition of 2.0 mL of 20% sodium carbonate solution and the absorbance measured at 650 nm. Tannins content was estimated as described by method of Schanderl (1970). Ascorbic acid content was estimated by method of Ranganna (1976) with modification. Dry leaves powder (2.0 g) was extracted with 4% oxalic acid and made up to 100 mL and centrifuged at 10,000 rpm for a 10 min. Five milliliter supernatant liquid was transferred in a conical flask, followed by addition of 10 mL 4% oxalic acid and titrated against standard dye solution (2.6 dichlorophenol-indophenol) to a pink end point. The procedure was repeated with a blank solution omitting the sample. Total carbohydrate content in plant leaves was estimated by Dubois *et al.* (1956), Starch by Hodge and Hofreiter (1962). Total nitrogen was estimated by Micro-Kjeldahl method, according to AOAC (1985). Crude protein was calculated as Kjeldahl N × 6.25 (based on assumption that nitrogen constitutes 16.0% of a protein). The content of crude fat was estimated by AOAC (1970). Amylose content in plant leave was estimated, as described method of McCready *et al.* (1950) and Juliano (1971). Cellulose content was estimated as described by method Updegraff (1969). Crude fiber content was estimated as described by methods of Maynard (1970).

Mineral analysis: Ash content was estimated by AOAC (1985) and ash insoluble content was estimated by method of Peach and Tracy (1955) and Mishra (1968). Mineral content in plant was estimated by wet digestion method. A 1.0 g plant material was first digested with conc. HNO_3 (5 mL each), followed by application of 15 mL of tri-acid mixture (HNO_3, $HClO_4$ and H_2SO_4, 10:4:1, v/v) heated at 200°C and reduces to 1 mL. The residue after digestion was dissolved in double distilled water, filtered and diluted to 100 mL. This solution was used for the estimation of minerals. Macro minerals viz., Na, K, Ca and Li were estimated by AIMIL, Flame Photometer while micro elements viz. Fe, Cu, Mn, Zn and Co were estimated by Atomic Absorption Spectrophotometer, model 4129, Electronic Corporation of India Ltd. Phosphorous and sulphur content was estimated by method of Allen (1977).

RESULTS AND DISCUSSION

The gas chromatogram shows the presence of 30 compounds and 79.89% of essential oil has been identified (Table 1). The major constituents of oil were n-hexadecanoic acid (33.03%), phytol (10.36%) and tetradecanoic acid (6.58%) of total oil. 8.28% of the oil are monoterpene as the minor components of the oil. 2.86% of the oil are sesquiterpenes and 8.28% diterpenes. 12.06% of oil are aliphatic/ aromatic hydrocarbon and oxygenated compounds. 32.45% of the oils are fatty acid as a major component of oil.

Antioxidant content in aerial parts of *Euphorbia* plants is presented in Table 2. β-carotene in aerial parts of plants was found 307.40±0.38 mg/100 g on a dry weight basis. The range of β-carotene was found 307.04–307.93 mg/100 g. The content of Vitamin C in aerial parts of plants was found 88.48±0.95 mg/100 g on dry weight basis. The range of Vitamin C was 87.37–89.68 mg/100 g.

The content of chlorophyll-a and chlorophyll-b in aerial parts of plants were found 115.37±1.05 and 72.98±0.51 mg/100 g on dry weight basis. The content of phenolic and tannins in plant was

Table 1: Composition of essential oil from aerial parts of *E. thymifolia*

Compound	Area%	Chemical formula	RI	Mode of identification
Cymol	0.20	$C_{10}H_{14}$	1161	a, b
(-) isopinocampheol	0.99	$C_{10}H_{18}O$	1535	a, b
Limonene	1.20	$C_{10}H_{16}$	1528	a, b
2,6,6-Trimethyl-1-cyclohexene-1-carboxaldehyde	1.84	$C_{10}H_{16}O$	1574	a, b
Safranal	1.04	$C_{10}H_{14}O$	1595	a, b
2-(4-Methyl-3-cyclohexene-1-yl)-2-propanol	1.35	$C_{10}H_{18}O$	1637	a, b
Piperitone	2.44	$C_{10}H_{16}O$	1648	a, b
(E,E) 2,4-Decadienal	1.17	$C_{10}H_{16}O$	1698	a, b
A-Caryophyllene	0.72	$C_{15}H_{24}$	1615	a, b
Caryophyllene oxide	2.86	$C_{15}H_{24}O$	1774	a, b
Phytol	10.36	$C_{20}H_{40}O$	1959	a, b
2-N-Ppentylfuran	0.50	$C_9H_{14}O$	1106	a, b
1-Pentanol	0.20	$C_5H_{12}O$	1141	a, b
Nonanal	1.87	$C_9H_{18}O$	1350	a, b
2,4-Heptadienal	1.57	$C_7H_{10}O$	1451	a, b
Benzaldehyde	0.81	C_7H_6O	1480	a, b
2,3-Heptanedione	0.53	$C_7H_{12}O_2$	1489	a, b
2-N-Ppentylfuran	0.50	$C_9H_{14}O$	1106	a, b
1-Pentanol	0.20	$C_5H_{12}O$	1141	a, b
Nonanal	1.87	$C_9H_{18}O$	1350	a, b
2,4-Heptadienal	1.57	$C_7H_{10}O$	1451	a, b
Benzaldehyde	0.81	C_7H_6O	1480	a, b
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1-Pentanol	0.20	$C_5H_{12}O$	1141	a, b
Nonanal	1.87	$C_9H_{18}O$	1350	a, b
2,4-Heptadienal	1.57	$C_7H_{10}O$	1451	a, b
Tetradecanoic acid	6.58	$C_{14}H_{28}O_2$	1974	a, b
Pentadecanoic acid	1.01	$C_{15}H_{30}O_2$	1999	a, b
n-Hexadecanoic acid	33.03	$C_{16}H_{32}O_2$	2034	a, b

a: Retention index of gas chromatography, b: GC-MS, RI: Retention index

Table 2: Antioxidant phytochemical composition investigated in aerial parts of *Euphorbia thymifolia* Linn.

Antioxidants	Composition (mg/100 g)	Range (mg/100 g)
β-Carotene	307.40±0.38	307.04-307.93
Vitamin C	88.48±0.95	87.37-89.68
Chlorophyll-a	115.37±1.05	114.16-116.73
Chlorophyll-b	72.98±0.51	72.32-73.57
Phenolics	336.73±0.55	336.25-337.50
Tannins	2465.74±0.72	2464.92-2466.67

All values are Mean±SD of triplicate determinations expressed on dry weight basis

Table 3: Nutrients composition investigated in aerial parts of *Euphorbia thymifolia* Linn.

Biochemical parameter	Composition (g/100 g)	Range (g/100 g)
Moisture	78.60±0.59	77.96-78.99
Crude protein (Kjeldhal N x 6.25)	13.42±0.37	12.96-13.87
Crude fat	4.63±0.44	4.01-4.98
Total carbohydrate	11.99±0.35	11.58-12.44
Starch	22.31±0.48	21.78-22.95
Amylose	1.39±0.28	1.14-1.79
Amylopectin	20.93±0.58	20.42-21.74
Cellulose	4.35±0.69	3.38-4.96
Crude fiber	24.34±0.64	23.47-24.98
Ash	10.29±0.08	10.18-10.34
Acid soluble ash	7.07±0.38	6.53- 7.37
Acid insoluble ash	3.24±2.9	3.03- 3.65
Calorific value (Kcal/100 g DM)	143.31	–

All values are Mean±SD of triplicate determinations expressed on dry weight basis

found 336.73±0.55 and 2465.74±0.72 mg/100 g. The range of phenolic was 336.25–337.50 and tannin was 2464.92–2466.67 mg/100 g.

The amounts of certain nutrients in aerial parts of plants are presented in Table 3. Crude lipid, protein and total carbohydrate content in aerial parts of plants were found 4.63±0.44, 13.42±0.37 and 11.99±0.35 g/100 g respectively on dry weight basis. The range were, 4.01-4.98, 12.96–13.87 and 11.58–12.44 mg/100 g of crude lipid, protein and total carbohydrate respectively. Starch, Amylose and Anylopeetin content in *Euphorbia* were found 22.31±0.48, 1.39±0.28 and 20.93±0.58 g/100 g, respectively.

The cellulose, crude fiber and moisture content were found 4.35±0.69, 24.34±0.64 and 78.60±0.59 g/100 g respectively. The ash content was found 10.29±0.08 g/100 g on dry weight basis. Acid insoluble ash was found 3.24±2.9 g/100 g and acid soluble ash was found 7.07±0.38 g/100 g. The energy content of plant aerial parts of plants was determined by multiplying the crude protein, crude lipid and total carbohydrate content by the factor 4, 9 and 4, respectively (Osborne and Voogt, 1978). Calorific values of the plant aerial parts of plants were found 141 k.cal/100 g.

Minerals are called a "spark plugs of life" because they are required to activate hundred of enzymes reactions within the body. Life is dependent upon the body's ability to maintain balance between the minerals (Watts, 1997). The mineral content of aerial parts of plants is presented in Table 4. The contents of sodium, potassium, calcium and lithium in aerial parts of plants were found 75.40±0.74, 4786.48±0.81, 242.46±0.56 and 46.64±1.36 mg/100 g, respectively on

Table 4: Mineral composition investigated in aerial parts of *Euphorbia thymifolia* Linn.

Mineral	Composition (mg/100 g)	Range (mg/100 g)
Sodium–Na	75.40±0.74	74.36-76.05
Potassium–K	4786.48±0.81	4785.34-4787.14
Calcium-Ca	242.46±0.56	241.68-242.96
Lithium–Li	46.64±1.36	45.06-48.37
Nitrogen–N	2151.85±1.35	2150.49-2153.69
Phosphorus–P	226.81±0.47	226.15-226.32
Sulphur–S	325.24±0.76	324.19-325.96
Iron–Fe	121.04±0.38	120.63-121.55
Copper-Cu	3.45±0.29	3.04-3.68
Manganese–Mn	9.28±0.30	8.96-9.69
Zinc–Zn	6.47±0.32	6.04-6.78
Cobalt-Co	0.00	-

All values are Mean±SD of triplicate determinations expressed on dry weight basis

dry weight basis. Ranges were 74.36-6.05, 4785.34-4787.14, 241.68-242.96 and 45.06-48.37 mg/100 g of Na, K, Ca and Li, respectively.

The contents of Nitrogen, phosphorus and sulphur were found 2151.85±1.35, 226.81±0.47 and 325.24±0.76 mg/100 g respectively on dry weight basis. The contents of iron, copper, manganese, zinc and cobalt in aerial parts of plants were found 121.04±0.38, 3.45±0.29, 9.28±0.30, 6.47±0.32 and 0.00 respectively on dry weight basis. The ranges were 120.63-121.55, 3.04-3.68, 8.96-9.69, 6.04-6.78 and 0.0 mg/100 g of Fe, Cu, Mn, Zn and Co, respectively.

The minerals contained in this medicinal plant may play important role in human nutrition. Magnesium, calcium and potassium in the human required for building red blood cell and for body mechanism (WHO, 1996). A deficiency of copper may cause hypertension, antibiotic sensitivity, hyperactivity, hyperglycemia, manic disorders, insomnia, allergies and osteoporosis (Watts, 1997). Calcium plays a major role in CNS function. Calcium is essential for nerve impulse conduction and activates some enzymes which generate neurotransmitters (Watts, 1997). Phosphorous is tied to calcium is bone structure and plays a significant role in CNS function. Many enzymes contain as a base phosphoproteins. Phospholipids are involved in nerve conduction. Phosphate is the primary ion in extra and intracellular fluid. It aids absorption of dietary constituents; help to maintain the blood at a slightly alkaline level regulates enzyme activity and is involved in the transmission of nerve impulses (Karade *et al.*, 2004). Potassium has many functions for protein synthesis, activation of many enzymes, stimulation of the movement of the intestinal tract etc. excess of potassium can produce neurological disturbances such as numbness of hand and feet (Watts, 1997). Zinc is extremely important for numerous body functions. Zinc deficiencies are associated with mental impairments. Zinc deficiency may be associated with mental lethargy, emotional disorder and irritability (Watts, 1997). Iron plays significant role in oxygen transport in the body. Disturbance in mental function can be caused by flows in the metabolic pathways that require iron. This is because of too little oxygen reaching the brain. Iron required for DNA synthesis. Iron is also necessary for the activation of enzymes involved in brain neurotransmitters (Watts, 1997). Thus this plant could serve as good source of minerals when consumed. This conformed to the observation of some researchers who opened that green vegetables are good source of iron, copper and zinc (Barasi and Mottram, 1987).

CONCLUSION

We conclude that the plant aerial parts of plants contain good amount of antioxidants, nutrients and minerals. The distribution of these components in common medicinal plants has important application for the health of people in addition to the basic need of developing countries. There is a great need to further research.

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