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Biochemical Composition and Nutritional Analysis of Leaves of Portulaca Pilosa L

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ABSTRACT

The Preliminary Phytochemical analysis shows the presence of Phenols, Flavones, Alkaloids, Carbohydrate, Glycosides, Tannin and Saponin. Flavones and saponins are observed in all three solvent systems namely Methanol, Acetone and Alcohol. The proximate composition of leaves of Portulaca pilosa L. has Total ash (10%), Crude Fat (20%), Crude fibre (13.5%), Crude Protein (14.81%), Dry matter (32%) and Moisture (68%). Mineral analysis showed highest amount of Potassium (42.3mg/g) followed by Nitrogen(23.7mg/g), Calcium(15.3 mg/g) and other trace elements. The Screening of Methanolic extract of leaves of Portulaca pilosa L. by Gas chromatography and Mass Spectrometry revealed the presence of fifteen bioactive compounds showing a wide spectrum of biological properties including antibacterial, anti-inflammatory, antioxidant, hypocholesterolemic etc. FTIR analysis shows the presence of different functional groups like Aromatic ether, Hydroxyl, Phosphate, Ester, Phenol, Nitrate, Saturated aliphatic alkanes etc., The results from present study offer a platform of using Portulaca pilosa L. leaves in pharmaceutical industries as well as for traditional practitioners for herbal drug formulations.

Key words: Bioactive compounds, P. pilosa, GC-MS, Nutritional analysis, Methanolic extract.

I. INTRODUCTION

The genus *Portulaca* belongs to family Portulacaceae which is commonly known as Purslane family. In India this genus *Portulaca* is represented by 9 species [7, 18]. *Portulaca pilosa* L. is perennial, robust, succulent herb, stem is densely pilose having pink showy flowers and called as hairy pigweed in English. Plant is cosmopolitan in distribution but it is native to Asia (Japan, China, Singapore) and spread in North and South America. Leaves of the plant are eaten as potherb, added in soups and salads in many Mediterranean and tropical Asian countries. [18]. Plants serve as source of secondary metabolites with interesting Biological activities. These compounds include mainly Phenolics, Flavonoids, Alkaloids, Fatty acids, Amino acids etc. [13].

The essential compounds like Fats, Carbohydrate, Proteins and phenols are synthesised as a result of Primary plant metabolism. Carbohydrates, Proteins and Fats are referred as Proximate Principles which form major portion of the diet whereas minerals are seen to play an important role in regulating the metabolic activities in the body [11]. The Gas Chromatography and Mass Spectrometry technique is used to identify important bioactive compounds. . This is a valuable technique for the analysis of non-polar compounds, volatile essential

oils, fatty acids, lipids and alkaloids [26]. Fourier transform infrared (FTIR) is used to identify the characteristic functional groups in the plant extract. It also provides the information about the structure of molecule which is frequently obtained from its absorption spectrum [15].

II. MATERIALS AND METHODS

i) Collection of Plant Material:

The plant material was collected from Badami plateau, Bagalkot District of Karnataka (15.9186° N, 75.6761° E) in early month of July and identified. The leaves of plant were washed repeatedly with distilled water and blotted gently and then shade dried. After drying the leaves were grinded into fine powder and stored in air tight containers at room temperature for further analysis.

ii) Phytochemical analysis

Phytochemical analysis was done using the methods of Evans W. C. (1977) and Brindha *et al.* (1981) [5,10]. 2g of Dried powder powder was used for soxhlet extraction. For that extraction Methanol, Alcohol (60-700C) and Acetone (40-500C) were used as solvents. After evaporation of the extract, solid residue was again reconstituted to particular solvent and was used for phytochemical analysis.

iii) Proximate analysis

For Proximate analysis standard methods such as AOAC (1990) and Sadashivam and Manikam were used.[2,24]. Total ash content, Crude fat, Crude fibre, Crude Protein, Dry Matter and moisture was calculated.

iv) Mineral analysis

Nitrogen was estimated by Hawk *et al.* (1948) method [12]. The acid digestion for analysis of inorganic constituents was done by method given by Toth *et al.* (1948) [28]. Phosphorus was estimated from acid digestion and following the method described by Sekine *et al.* (1965) [25]. Remaining inorganic constituents were estimated by using Atomic absorption spectrophotometer.

v) GC-MS and FTIR

The dried powder (2g) was extracted successively with 200 ml of methanol in Soxhlet apparatus. The obtained extract was then evaporated to dryness at 40° C and residual extract was reconstituted in methanol and stored at 4°C in refrigerator. This extract was then further used for GC-MS analysis. Fine powder was used for FTIR analysis.

GC-MS analysis of the extract was performed using instrument model GC-MS TQ8050 Shimadzu, Japan. It is well equipped with SH-Rxi-5 sil MS fused silica capillary column of 3m in length, 0.25mm inner diameter and .25mm thickness. Carrier gas used was helium (99.9%) at constant flow rate of 1mL/min with 2µl injection volume at temperature of 250°C.The spectrum data was interpreted by using the database of National institute of standard and technology (NIST-08 LIB) and WILEY-08 LIB. The FTIR spectrum was used for identification of the functional group of the active components based on the peak value in the region of infrared radiation.

III. RESULT AND DISCUSSION

Phytochemical studies of Leaves of *Portulca pilosa* L. showed presence of Flavones and saponins in all three solvents namely Methanol, Acetone and Alcohol. Apart from saponins and Flavones the Methanolic extract of leaves also showed presence of Phytochemicals like Alkaloids, Phenols, Carbohydrates and Glycosides. In case of Acetone, Tannins and carbohydrates were also present and in alcoholic extract Alkaloids and Glycosides

were observed along with Flavones and saponins. [Table no. 1] Investigation of phytochemicals of *Portulaca quadrifida* L. showed good proportion of secondary metabolities in Ethanolic and aqueous extract as compared to Petroleum ether and chloroform [19].

Proximate analysis revealed total ash (10%), Crude fat (20%), Crude Fibre (13.5%), Crude Protein (14.81%), Moisture (68%) and dry matter (32%). Crude Protein and Crude fibre are present in good quantity whereas plant has high moisture content. Quantification of minerals showed highest amount of potassium followed by Nitrogen, Calcium and Magnesium [Fig no.3]. These mineral elements are very important in human nutrition. Calcium, potassium and magnesium are required for repair of worn out cells, strong bones and teeth in humans, building of red blood cells and for body mechanisms [30]. Trace elements like Iron, Manganese, copper, zinc and sodium are also present in small quantities which are essential for enzymes metabolism. *Protulaca oleracea* L. has great nutritive value. Proximate and mineral analysis of *Portulaca oleracea* L. showed Proteins, Fats, Fibre, Calcium, Magnesium, Phosphorus and Potassium in relatively high amount [6].

Total fifteen compounds were detected through the GC-MS analysis on the basis of molecular formula, molecular weight, retention time and peak area. The major compounds present in the leaves were n-Hexadecanoic acid (26.66%), Benzoic acid (18.15%), Hexadecanoic acid, methyl ester- (10.37%), 2-Pentadecanone 6, 10, 14-trimethyl (6.83%), Heptadecanoic acid, 16-methyl-, methyl ester (5.46%) etc., with some other minor and major compounds. These compounds could contribute to the medicinal quality of the plant. The mass spectrum of the different compounds is shown above (Fig no. 4 and Table 4). n-Hexadecanoic acid has the highest peak area percentage as per results of GC-MS. Earlier GC-MS analysis of Ethanolic extract of dried whole plant of *Portulaca oleraceae* L. revealed the presence of esters of cyclopropanepentanoic acid, hexanedoic acid, octadecanoic acid besides n-nonadecanol, phosphoric acid, dibutyl 3-trifluoromethyl-3-pently ester, 9,12,15 octadecatrienal. These compounds have been tested for various antibacterial, antiviral and other pharmaceutical applications [9]. Results of GC-MS analysis of *P. oleracea* vary depending on the growth conditions and time of harvest also plays important role in determining the Phytochemicals and their amount [21].

The powder of *Portulaca pilosa* L. was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. FTIR analysis confirmed the presence of Aromatic ether, Hydroxyl, Phosphate, Phenol, Ester, Nitrate, Saturated aliphatic alkane and alkyl functional groups which shows peaks at 1243.24, 3339.03, 1016.91, 1458.02, 1730.93, 822.57, 2916.72 and 2849.76, (Fig no.5 Table no. 5) (John Coates 2000). The absorption spectral lines of *P.pilosa* L. and *P. quadrifida* L. were very similar. The absorption peak at 3470 indicates the presence of phenolic and flavonoids; it also suggests that there are also amines, acids (oxalic acid, succinic acid, citric acid, propionic acid, lactic acid and butyric acid and amino acids. Rear absorption at 2156 cm^{-1} is related to unsaturated compounds and amino acids. Peaks 1300 to 1400 cm^{-1} indicate poly sulphur and polyphenolic compounds. Very weak absorption signals observed at 3525-3515 cm^{-1} indicates the presence of stretching [27].

Fig no.1 Habit

Fig no. 2 Flowering twig

Table no- 1. Preliminary Phytochemical Analysis of Leaves of Portulaca pilosa L.

Sr. No.	Test	Methanol	Acetone	Alcohol
1	Alkaloids	+	_	+
2	Phenols	+	+	_
3.	Tannin	+	+	_
4.	Saponin	+	+	+
5.	Flavones	+	+	+
6.	Anthraquinone	_	_	_
7.	Carbohydrate	+	+	_
8.	Xanthoprotein	_	_	_
9.	Coumarine	_	_	_
10.	Glycosides	+	_	+

+ → Presence of phytochemical - → Absence of phytochemical

Proximate analysis

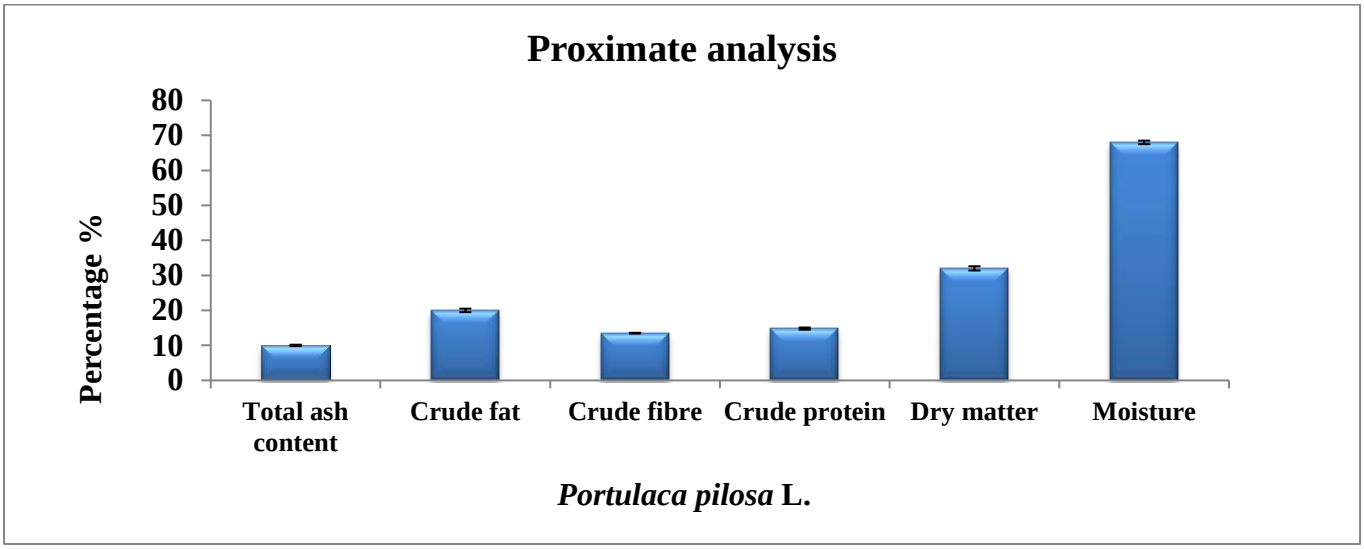

Fig no.3 Graphical representation of results obtained by proximate analysis

Mineral analysis

Table No.2

Sr.no	Macronutrients	Quantity (%)
1.	N	2.37±0.07
2.	P	0.24±0.06
3.	K	4.23±0.10
4.	Ca	1.57±0.07
5.	Mg	0.58±0.02
6.	S	0.06±0.04

Table no. 3

Sr no.	Micronutrients	Quantity (ppm)
1	Fe	530.59±0.08
2	Mn	541.05±0.1
3	Zn	34.33 ±0.08
4	Cu	9.73±0.01
5	Na	0.17 ±0.02

IV. GAS CHROMATOGRAPHY MASS SPECTROMETRY

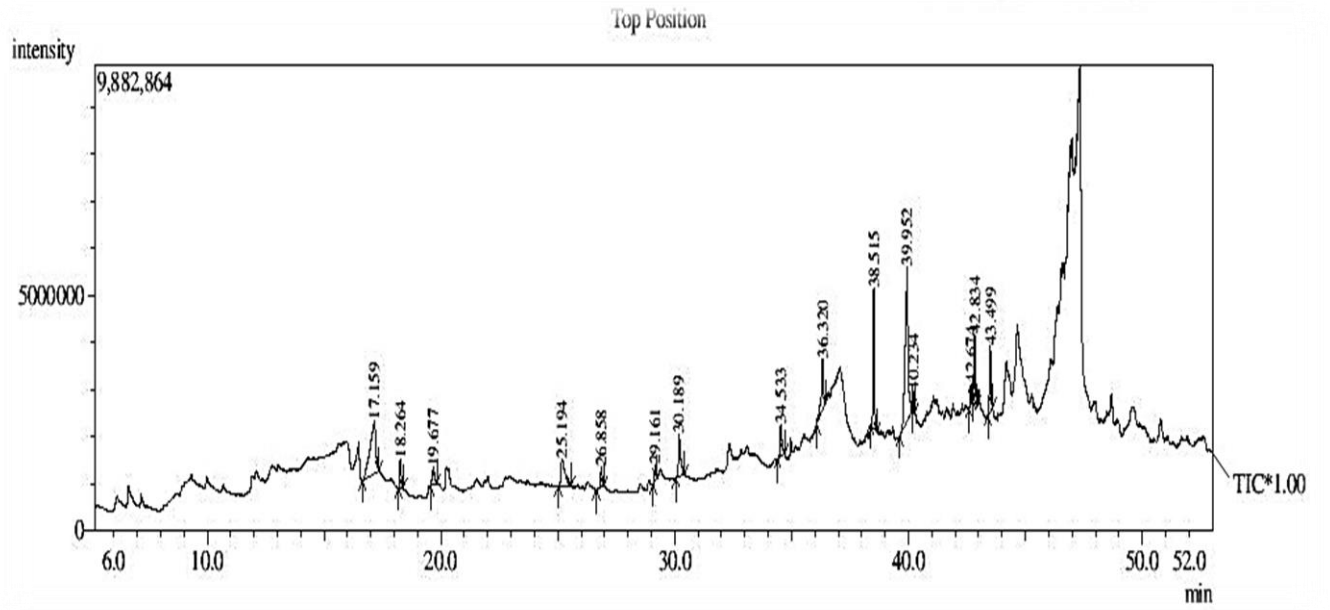

Fig no. 4: GC-MS chromatograph of methanolic extract of leaves of *Portulaca pilosa* L.

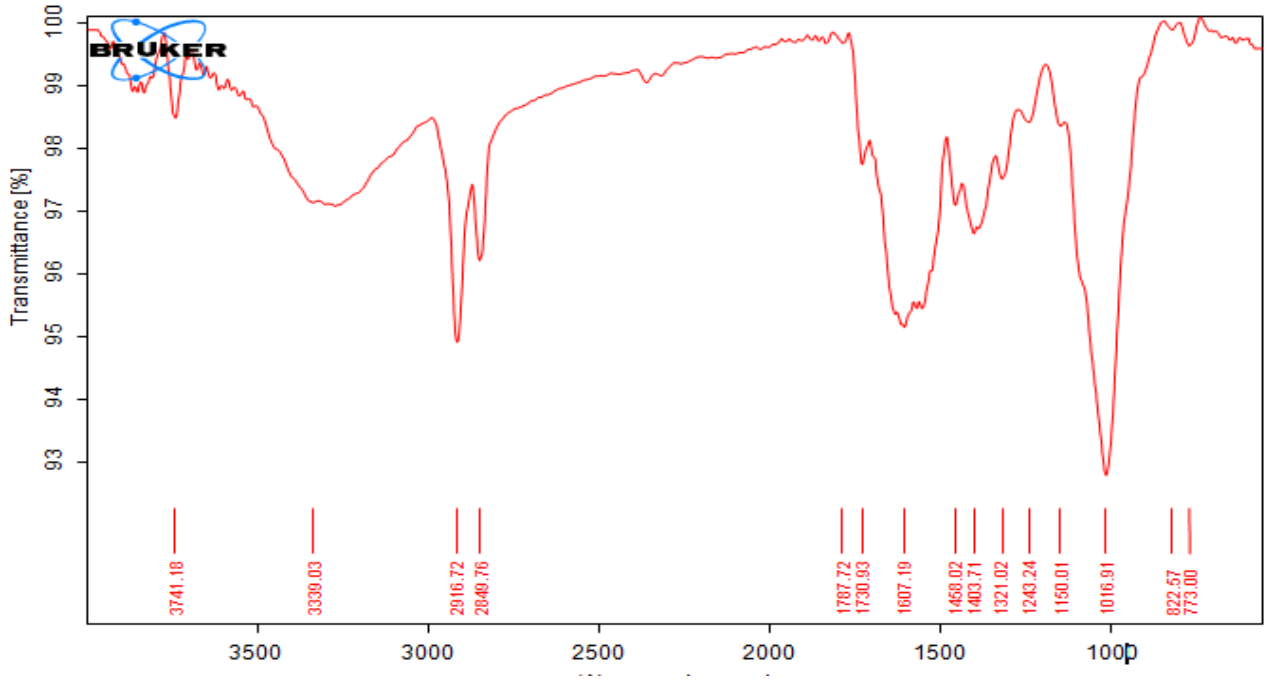

Fig no.5 FTIR spectrum of *Portulaca pilosa* L. leaves

Table no.4: Compounds identified from GC-MS analysis and their biological activity of methanolic extract of *P.pilosa* L. Leaves

Sr no	Name of the Compound	Molecular formula	Retention time	Peak area (%)	Biological activity
1	Benzoic acid	$C_7H_6O_2$	17.159	18.15	Anti-sickling, Antimicrobial [17,23]
2	2-Decenal, (E)-	$C_{10}H_{18}O$	18.264	2.74	Not reported
3	Nonanoic acid	$C_9H_{18}O_2$	19.677	2.46	Antimicrobial [1]
4	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	25.194	6.74	Not reported
5	Phenol, 2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	26.858	1.83	Antibacterial, Antioxidant [3]
6	E-15-Heptadecenal	$C_{17}H_{32}O$	29.161	1.22	Fatty acid amide hydrolase [8]
7	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	30.189	5.50	Not reported
8	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	34.533	3.84	Antioxidant, Antimicrobial [23]
9	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	36.320	6.83	Antibacterial, Allelopathic [4]
10	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	38.515	10.37	Antifungal, 5-Alpha reductase inhibitor, Pesticide [4]
11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	39.952	26.66	Antioxidant, Hypocholesterolemic Nematicide, Antiandrogenic, haemolytic.[30]
12	1-Heptacosanol	$C_{27}H_{56}O$	40.234	1.09	Anticancer, Nematicide, Antioxidant, Flavour and fragrance agent, cholesterol Lowering, Antimicrobial and Cytotoxicity [3,20]
13	n-Propyl 9,12-octadecadienoate	$C_{21}H_{38}O_2$	42.674	1.22	Not reported
14	6-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	42.834	5.90	Not reported
15	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	43.499	5.46	Used against skin cancer protein.[14]

Table no.5. Fourier- Transform Infrared Spectroscopy (FTIR) analysis for *Portulaca pilosa* L. leaf powder

Sr No.	Absorption Frequency	Functional group
1.	3741.18	Amine NH stretch
2.	3339.03	Normal polymeric OH stretch
3.	2916.72	Saturated aliphatic Methylene
4.	2849.76	Saturated aliphatic Methylene
5.	1787.72	Carbonyl compound
6.	1730.93	Ester
7.	1607.19	Quinone or conjugated ketone
8.	1458.02	Phenol
9.	1403.71	Amide
10	1321.02	Alkyl ketone
11.	1243.24	Aromatic ethers
12.	1150.01	Secondary amine CN stretch
13.	1016.91	Phosphate ion
14.	822.57	Nitrate ion
15.	773.00	Aliphatic chloro compounds C-Cl strech

V. CONCLUSION

Present investigation reveals that the leaves of *Portulaca pilosa* L. have the potential to act as source of useful drugs because of presence of various phytochemical constituents such as flavonoids, alkaloids, phenols, saponins and carbohydrates. These constituents play important role in improving the health status. The results of proximate analysis showed that plant is edible has considerable amount of crude protein, fat and fibre. The mineral content is within the permissible range for human consumption. An impressive and growing number of bioactive compounds have been identified that have potentially important health benefits. Through GC-MS five major compounds detected had Hypocholesterolemic, Anti-androgenic, Haemolytic, Antimicrobial, Antifungal, 5-Alpha reductase inhibitor and Allelopathic activity. Antioxidant activity is shown by n-Hexadecanoic acid which is found in high amount in the sample. In FTIR analysis absorption frequency of observed functional groups ranges from 773 to 3741.18 cm^{-1}.The above study about nutritional value of *Portulaca pilosa* L. reveals the knowledge about edibility, habitat, distribution, medicinal uses of *Portulaca pilosa* L.

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