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Phytochemical Composition and antioxidant Activities of C*hlorophytum tuberosum* Leaf

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ABSTRACT

Under this study attempts have been made to determine the phytochemical and antioxidant potential of *Chlorophytum tuberosum* leaf as well as categorization of phenolic components by GC-MS and RP-HPLC using different tools which covers the experimental studies on phytochemical, nutritional and medicinal aspects. Detailed studies of *Chlorophytum tuberosum* leaf have been carried out using parameter of phytochemical analysis. Leaf powder has been evaluated for preliminary phytochemical screening. Phenolic fractions isolated from *Chlorophytum tuberosum* leaf extract have been characterized using GC-MS and RP-HPLC. Free radical scavenging effect using DPPH and total antioxidant evaluation by reducing power assay has been evaluated.

Keywords: *Chlorophytum tuberosum*, Phytochemical, Antioxidant, Free radical.

INTRODUCTION

Genus *Chlorophytum* (Family: Liliaceae) includes about 200 species and about 20 species recorded in Western ghat region of India; out of these about 11 species found in Maharashtra [1] [2]. Due to high saponin and carbohydrates content in root tuber different species of *Chlorophytum* 500-600.ton annual production of tuber is done [3]. While improved production techniques are continuously practiced by semi-arid tropic areas of India to increase commercial cultivation [4]. Various wild species of *Chlorophytum* in are traditionally considered as medicinal due to extensive use in many Ayurvedic, Allopathic, Homoeopathic and Unani medicines [5] [6] as chief ingredient which is used as rejuvenator, Immuno-modulator, remedy for diabetes etc. [7].

Chlorophytum tuberosum(Marathi name: Safed musali) is consider as economically important because root tubers and leafs are harvested as wild edible vegetable and Ayurvedic formulations[8]. *Chlorophytum tuberosum* is a leafy herb which grows about 30cm in height with spiral phylotaxy and fascicle tuber appearance tapering to basal side [9]. Leaves are fleshy about 2-4cm in width with undulated margin. Raceme type of inflorescence is present with white flower, incurved perianth, axillary placentation and capsule type of fruit with black seeds.

Wild Vegetables are used as a food resource by the Tribal people of Kalsubai-Harishchandragarh wildlife sanctuary located at Western ghat region of Ahmednagar district (MS) India. Different surveys are carried in study area and documentation has been published. About 22 plant species was reported as nutritionally rich wild edible plants [10]. Leaves, tubers, rhizomes, bulbils, fruits, seeds, flowers; of such wild edible plants is complementary diet during scarcity [11]. Documentation and data analysis of plants from northwest region of Ahmednagar may provide novel compounds for the treatment of different ailments and also new food species for coming generation which are available in only limited period.Documentation and ethnobotanical survey of wild edible plants carried out in different regions of Maharashtra has listed *Chlorophytum tuberosum* leaf as edible and medicinal [12][13][14].On the basis of above literature it is important to estimate phytochemical content and antioxidant activity of phenolic fractions to increase the market value and to urge local people to cultivate this plant in large scale.

MATERIALS AND METHODS

Collection and Identification of *Chlorophytum tuberosum*: *Chlorophytum tuberosum* leaves was procured from the local area of Sangamner and Akole tehsil of Maharashtra, India. Plant authentication was done at Department of Botany, S. N. Arts, D. J. M. Commerce and B. N. S. Science College, Sangamner, Maharashtra, India.

Sample Preparation: Collected *Chlorophytum tuberosum* leaves were washed with tap water and cut into small stripes with scissor and shade dried at room temperature for 15 days. The dried plant material was pulverized into fine powder using a grinder (mixer). Ground powder was used as plant sample for study.

Qualitative phytochemical assay of *chlorophytum tuberosum* leaf:*Chlorophytum tuberosum* leaves were subjected for qualitative phytochemical analysis of the various classes of active chemical constituents, using standard prescribed methods [15]. Observed results were subdivided as weak (+), moderate (++), strong (+++) and absent (-). Plant aqueous extract was prepared by boiling 20g of the sample powder in distilled water for 20 minutes. The solution was filtered through blotting paper. The filtrate was used for the phytochemical screening of active chemical constituents.

Determination of Total phenolic content and Total flavonoid content from *Chlorophytum tuberosum* leaf:
Study was carried out for total phenol content (TPC), total flavonoid content (TFC) as phytochemicals present in *Chlorophytum tuberosum* leaf.

Determination of total phenol content (TPC): Extraction and estimation of total phenol content present in *Chlorophytum tuberosum* leaf was analyzed according to the folin-ciocalteu method suggested by Cliffe*et al*. (1994) [16]. Absorbance was measured using visible spectrophotometer at 765nm wavelength. Series of Gallic acid as standard was prepared separately. Standard curve was drawn by plotting the absorbance against concentration of Gallic acid. Total phenol content was expressed in % of gallic acid equivalents (GAE) per gram.

Determination of total Flavonoid content (TFC): Total flavonoid content of *Chlorophytum tuberosum* leaf was determined by aluminium chloride colorimetric method [17][18] following the principle that "aluminium chloride forms complexes with the ortho-dihydroxyl groups in the A-ring or B-ring of flavonoids; and measured by recording the absorbance at 510nm". Standard curve was plotted with the help of rutin (200µg/ml) as standard flavonoid and expressed in milligrams of rutin equivalents per gram.

Extraction of Phenolic compounds:*Chlorophytum tuberosum* leaf powder was subjected for phenolic compound extraction[19]. Hundred grams of finely powdered plant part was soxhlet extracted with hot 80% methanol (500 ml) and filtered. Filtrate was re-extracted successively with petroleum ether (fraction I), ethyl ether fraction (DEE fraction) and ethyl acetate fraction (EA fraction) using separating funnel. Petroleum ether fraction was discarded due to being rich in fatty substances, whereas ethyl ether and ethyl acetate fractions were analyzed for free and bound flavonoids, respectively. Ethyl acetate fraction was hydrolyzed by refluxing with 7% H_2SO_4 for 2 h (for removal of bound sugars from the flavonoids). Resulting mixture was filtered. Filtrate was acetate extract thus obtained, washed with distilled water till neutrality. Ethyl ether (free flavonoids) and ethyl acetate fraction (bound flavonoids) were dried and weighed.

Gas chromatography and Mass spectrometry analysis of isolated phenolic fractions: Gas chromatography analysis was carried out using prescribed method [20][21]. The high attainable separation power in combination with wide range of the detectors employing various detection principles to which it can be coupled makes GC an important, often irreplaceable tool in the analysis at trace level of plant phytochemical compounds. The GC-MS analysis of phenolic fraction with in absolute alcohol, was performed using a Clarus 500 Perkin Elmer gas chromatography equipped with Elite-5 capillary column (5% phenyl 95% dimethyl polysiloxane) (30nm X 0.25mm ID X 0.25µmdf) and mass detector turbomass gold of the company which was operated in EI mode. Helium was the carriers gas at a flow rate of 1ml/min and the injector was operated at 290ºC and the oven temperature was programmed as follows; 50ºC at 8ºC/min to 200ºC (5min) at 7ºC/min to 290ºC (10min).Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomas 5.2.

Quantification of phenolic components by RP-HPLC from isolated phenolic fraction of *Chlorophytum tuberosum* leaf:Mobile Phase solvent was used as acetonitrile/solvent (80:20). The gradient profile is 8% at 10 min. The column (reverse phase RP-C18, 150 mm × 4.6 mm, 5 m) was equilibrated for 15 min before injection. The flow rate was 1 ml/min and phenolic compounds were detected at 280 nm and at 320 nm. 20 µl sample of Diethyl ether and Ethyl acetate fraction of *Chlorophytum tuberosum* leaf were injected for analysis. Phenolic standards used in this study were Rutin, Benzoic acid, Salicyclic acid, Cinnamic acid, Caffeic acid, Naringin, Syringic acid, Hesperidin and Quercetin.

Antioxidant Activity of *Chlorophytum tuberosum* leaf fractions:
Determination of antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method: Determination of DPPH scavenging activity of *Chlorophytum tuberosum* leaf phenolic fraction was carried using prescribed method [22]. Fractions were aliquot into different concentrations (12.5µg -200µg) for determining its ability to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals. 50µl of samples (12.5µg -200µg) were mixed with 1250µl of 0.06mM DPPH in 85% methanol. Tubes were incubated in dark for 30 minutes at RT and optimum density was measured at 517nm using visible spectrophotometer. Control was prepared by mixing 50µl of methanol with 1.25ml of DPPH

reagent. Ascorbic with dose of 1-10 µg per 50µl was served as standard to compare with fraction isolated from *Chlorophytum tuberosum* leaf.

Determination of Antioxidant Activity by reducing power assay: Determination of antioxidant activity of *Chlorophytum tuberosum* leaf was carried by reducing power assay (Khyade et al, 2017). 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of potassium ferricyanide are added to 1ml of sample dissolved in distilled water. The resulting mixture is incubated at 50°C for 20 minutes, followed by the addition of 2.5ml of Trichloro acetic acid (10% w/v). The mixture is centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5ml), mixed with distilled water (2.5ml) and 0.5ml of $FeCl_3$ (0.1%, w/v). The absorbance is then measured at 700 nm against blank sample.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of *Chlorophytum tuberosum leaf*: Preliminary phytochemical analysis of *Chlorophytum tuberosum leaf* was carried out and results were denoted as High (+++), moderate (++), low (+) and absent (-).*Chlorophytum tuberosum leaf* contain high amount of steroid; moderate amount of alkaloid, tannin, anthraquinones, flavonoids and phenol; while saponins, anthocyanosides, cardiac glycosides, protein and reducing sugar in low amount (Table 1).

Table 1: Preliminary phytochemical analysis of *Chlorophytum tuberosum leaf*

Phytochemical test	Results
Alkaloids	++
Tannins	++
Saponins	+
Anthraquinones	++
Anthocyanosides	+
Cardiac glycosides (Cardenolides)	+
Steroids	+++
Flavonoids	++
Protein	+
Reducing sugar	+
Phenol	++

Note: +++ as high, ++ as Moderate, + as low, - as absent

Total Phenolic content and Total flavonoid content of *Chlorophytum tuberosum leaf*: Total phenolic content of *Chlorophytum tuberosum* leaf was observed to be 0.15% while 0.11% of total flavonoids were observed. When compared to the preliminary phytochemical test it shows that moderate amount of TPC and TFC were recorded; which indicate that sufficient amount of active phenolic compounds are present in *Chlorophytum tuberosum* leaf and should be useful against various degenerative diseases (Table 2).

Table 2. Total Phenolic content and Total flavonoid content of *Chlorophytum tuberosum leaf*

Parameter	Concentration
Total phenol Content	0.15%
Total Flavonoid content	0.11%

Separation and Identification of compounds from fraction isolated from *Chlorophytum tuberosum* leaf by Gas Chromatography and mass spectroscopy (GC-MS):*Chlorophytum tuberosum* leaf were subjected for extraction of phenolic compounds and isolated fractions (Diethyl ether and ethyl acetate) were transferred to Central Instrumentation

laboratory, Punjab University, Chandigarh for separation of compounds by gas chromatography and identified with the help of mass spectrophotometer using NIST library. Fig. 1 shows the spectra of both the fraction. Diethyl ether fraction of *Chlorophytum tuberosum* leaf shows the presence of Ethyl iso-allocholate, Perfluorotributylamine, Dodecanoic acid, 4-[N-methylureido]-1-[4-methylaminocarbonyloxymethyl, Pyrimidin-2-one, Cyclohexasiloxane, Hexasiloxane, Oleic acid, Octasiloxane, Digitoxin and 9-Octadecenoic acid (Table 3). While Ethyl acetate fraction shows major peaks of Perfluoro(dibutylmethylamine), 4-[N-methylureido]-1-[4-methylaminocarbonyloxymethyl, Perfluorotributylamine, Cyclohexasiloxane, Cycloheptasiloxane, Benzoic acid, Tetracosamethyl-cyclododecasiloxane and Phthalic acid (Table 4).

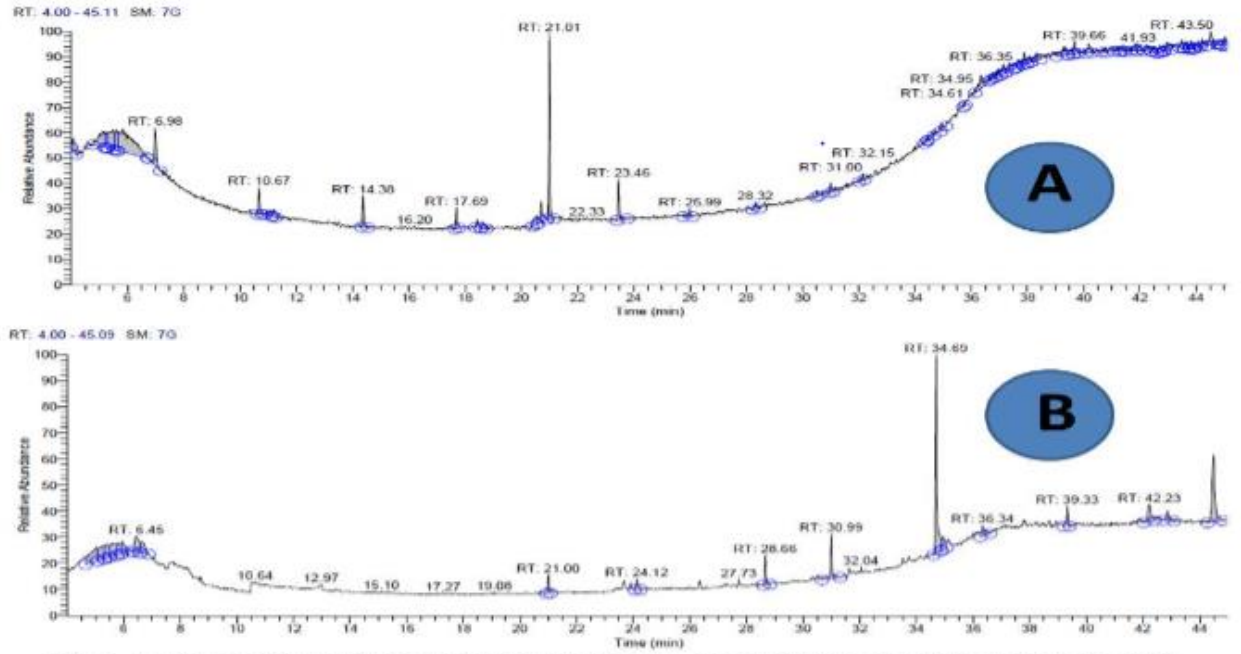

Fig. 1. A. Mass spectra of Diethyl ether Fraction B. Mass spectra of ethyl acetate Fraction

Table 3: Major compounds identified by gas chromatography and mass spectroscopy from Diethyl ether fraction *Chlorophytum tuberosum* leaf

Retention time (Minute)	Name of compound	Molecular formula	% peak area
4.07	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	1.26
5.13	Perfluorotributylamine	$C_{12}F_{27}N$	4.60
5.28	Dodecanoic acid	$C_{12}HF_{23}O_2$	1.51
5.50	4-[N-methylureido]-1-[4-methylaminocarbonyloxymethyl	$C_{13}H_{19}N_5O_5$	4.65
5.61	Pyrimidin-2-one,	$C_{13}H_{19}N_5O_5$	2.43
6.98	Cyclohexasiloxane	$C_{12}H_{36}O_6Si_6$	3.88
10.67	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	$C_{18}H_{52}O_7Si_7$	1.97

14.38	Hexasiloxane	$C_{14}H_{42}O_5Si_6$	2.09
17.69	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	1.33
20.55	Oleic acid	$C_{38}H_{74}O_2$	1.33
20.70	Octasiloxane	$C_{16}H_{50}O_7Si_8$	2.77
21.01	Dibutyl phthalate	$C_{16}H_{22}O_4$	11.03
23.46	Digitoxin	$C_{41}H_{64}O_{13}$	3.06
36.35	9-Octadecenoic acid	$C_{36}H_{70}O2$	2.58
37.14	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	1.18
37.34	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethy	$C_{16}H_{50}O_7Si_8$	1.54
37.66	Glycine	$C_{26}H_{43}NO_5$	1.05
37.88	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol	$C_{26}H_{36}O_8$	1.13
39.30	Milbemycin B	$C_{33}H_{47}ClO_7$	2.17
39.66	Octadecane	$C_{39}H_{80}O_2$	1.23
40.18	9-Desoxo-9x-hydroxy-7-ketoingol3,8,9,12-tetraacetate	$C_{28}H_{38}O_{10}$	1.26

Table 4: Major compounds identified by gas chromatography and mass spectroscopy from ethyl acetate fraction *Chlorophytum tuberosum* leaf

Retention time (Minute)	Name of compound	Molecular formula	% peak area
6.07	Perfluoro(dibutylmethylamine)	$C_9F_{21}N$	12.60
6.41	4-[N-methylureido]-1-[4-methylaminocarbonyloxymethyl	$C_{13}H_{19}N_5O_5$	8.44
6.72	Perfluorotributylamine	$C_{12}F_{27}N$	7.89
6.99	Cyclohexasiloxane	$C_{12}H_{36}O_6Si_6$	21.91

10.68	Cycloheptasiloxane	$C_{14}H_{42}O_7Si_7$	4.49
14.38	Benzoic acid	$C_{16}H_{30}O_4Si_3$	4.07
17.70	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	2.56
21.01	Phthalic acid	$C_{19}H_{28}O_4$	33.32

Quantification of phenolic components from diethyl ether and ethyl acetate fraction of *Chlorophytum tuberosum* leaf by Reverse phase high performance liquid chromatography (RP-HPLC):Nine phenolic standards were subjected along with Diethyl ether and ethyl acetate fraction for RP-HPLC. Phenolic component from fractions were identified using retention time of compound (Fig.2) and quantification was done by calibration with standard peak area measurement; whereas validation for linearity was checked according to ICH guideline. Linear plots for standard phenolic components were calculated using windows Excel 2010. Diethyl ether fraction of *Chlorophytum tuberosum* leaf shows the match with four Standard Phenolic Components. Table 5 indicates that Syringic acid, Rutin, Cinnamic acid and Quercitin in 83.5µg/mg, 69µg/mg, 60.5µg/mg and 8.35µg/mg, respectively. Whereas, ethyl acetate fraction contains high amount of rutin (330µg/mg) and Syringic acid (293.85µg/mg). Equal amount of Cinnamic acid and Salicyclic acid (49µg/mg) was evaluated (Table 6). Results indicate that fractionation of extracts using polarity of solvent increases the amount of purified component.

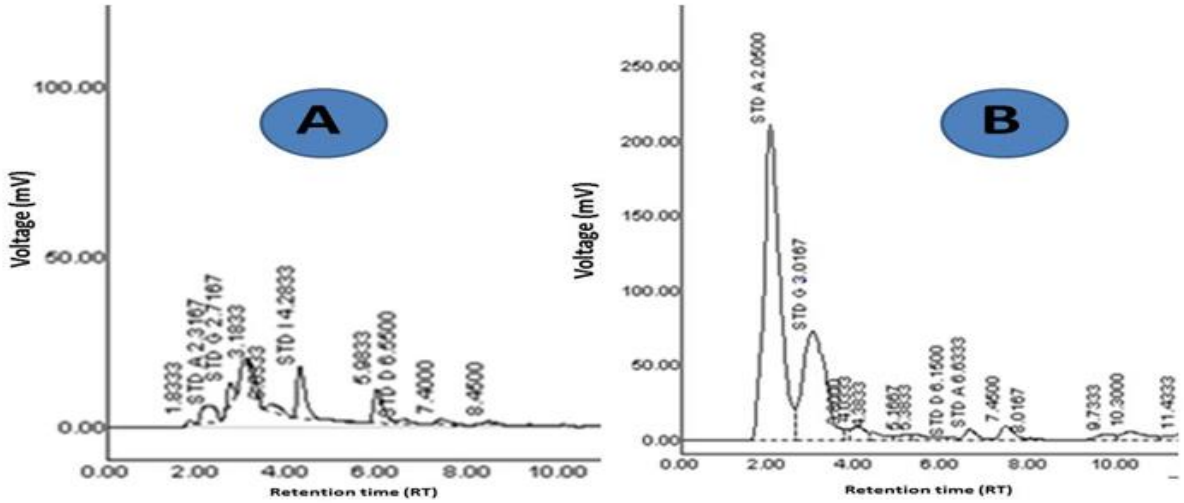

Fig. 2. A. HPLC Spectra of Diethyl ether fraction. B. HPLC Spectra of ethyl acetate fraction.

Table 5: Identification and Quantification of standard phenolic components from diethyl ether fraction of *Chlorophytum tuberosum* leaf by RP-HPLC.

Standard Phenolics	Slope line Equation	Area[mV*s]	Concentration (in µg/mg)
Rutin	y = 66.645x + 94.857	2.31	69.00
Syringic acid	y = 35.658x + 62.456	2.71	83.5
Cinnamic acid	y = 90.209x + 115.91	6.55	60.5
Quercitin	y = 65.848x + 15.311	4.28	8.35

Note: mV*s = Milivolt per second, µg/mg= Microgram per Miligram.

Table 6. Identification and quantification of standard phenolic components from ethyl acetate fraction of *Chlorophytum tuberosum* leaf by RP-HPLC.

Standard Phenolics	Slope line Equation	Area[mV*s]	Concentration (in µg/mg)
Rutin	y = 66.645x + 94.857	535.23	330.00
Syringic acid	y = 35.658x + 62.456	272.00	293.85
Cinnamic acid	y = 90.209x + 115.91	27.48	49.00
Salicyclic acid	y = 10.119x + 4.124	14.06	49.09

Note: mV*s = Milivolt per second, µg/mg= Microgram per Miligram.

Antioxidant assay of *Chlorophytum tuberosum* leaf Dietyl ether (CtuDEE) and Ethyl acetate (CtuEA) fraction:Both Diethyl ether (CtuDEE) and Ethyl acetate (CtuEA) fraction of *Chlorophytum tuberosum* leaf was subjected to DPPH assay to evaluate the Scavenging ability and reducing power assay to quantify reducing ability of both fractions. Fig.3 indicates that ethyl acetate fraction has highest ability to scavenge DPPH molecule at highest dose of 50µg and concentration of doses (10µg-50µg) shows direct proportionality with amount of inhibition(% inhibition) , which indicates that presence of different phenolic component with its concentration shows synergetic effect. Results of reducing power assay (Fig.4) prove that reducing ability of fractions and standard scavenge DPPH molecules.

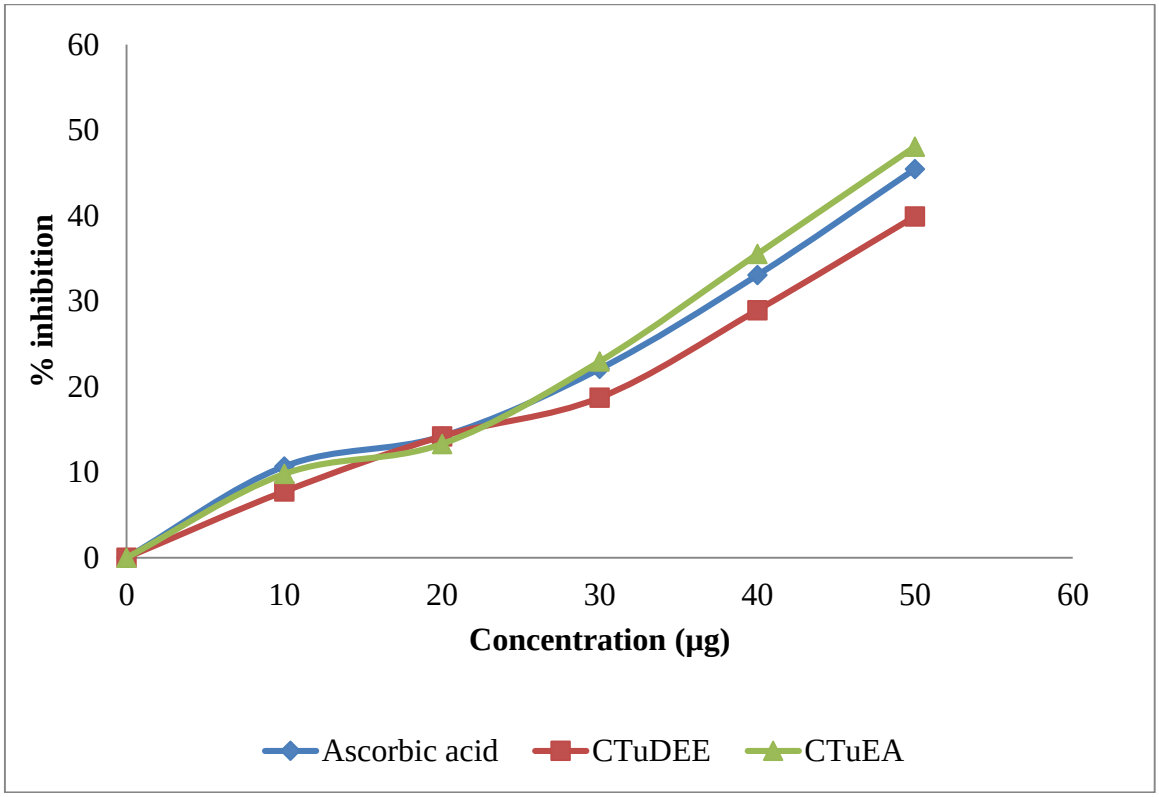

Fig. 3: DPPH antioxidant assay of *Chlorophytum tuberosum* leaf Diethyl ether (CtuDEE) and Ethyl acetate (CtuEA) fraction with Ascorbic Acid.

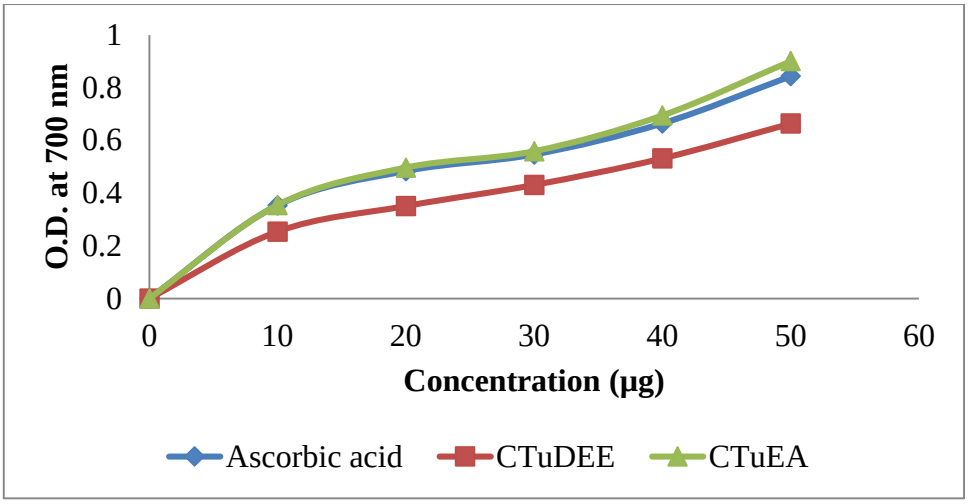

Fig.4. Reducing power assay of *Chlorophytum tuberosum* leaf Diethyl ether (CtuDEE) and Ethyl acetate (CtuEA) fraction along with Ascorbic Acid as standard.

CONCLUSION

Detailed studies of leaf for phytochemical analysis reveals that *Chlorophytum tuberosum* is an important wild vegetable consumed by tribal people.Preliminary phytochemical analysis suggests that it can be beneficial for isolation of nutraceutical components if cultivated in large scale on hilly region.Isolated phenolic components from *Chlorophytum tuberosum* leaf studied using GC-MS reveals that chromatographic separation followed by mass analysis shows different phenolic component with their mass, molecular formula and peak area gives preliminary information of chemical diversity. While quantification of some marker phenolic components via RP-HPLC shows *Chlorophytum tuberosum* leaf contains beneficial phenolic and flavonoid component.Antioxidant assays carried out using phenolic fraction reveals that it is potential for scavenging DPPH molecules while possess considerable ferric reducing ability.Present study will be important for agricultural sector to study cultivation practices for *Chlorophytum tuberosum* as well as important link in biochemical sciences.

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