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RESEARCH ARTICLE

TOTAL POLYPHENOLIC, FLAVAONOIDS AND DPPH ACTIVITY OF SOME PLANTS OF LILIACEAE FAMILY IN CHITRAKOOT REGION

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ABSTRACT

Plants play an important role on medicines since thousands of years to treat various diseases. Oxidative stress may precede and speed up to development of diabetes. In diabetic complications antioxidant action may be an important property of medicinal plant linked with diabetes. The Lily family, *Liliaceae*, consists of fifteen genera about 600 species of flowering plants with in the order. Onion (*Allium cepa*) belongs to the Lilly family, it shows only a particular vertical shoot above the ground and is used for energy storage and as a spice. It could be in a range of shapes and sizes. Garlic (*Allium Sativum*) also of the lilly family has a strong characteristic odour and taste and the bulb is used a flavoring agent. Both plants of medicinal values excluding common cold, heat disease, osteoporosis and other diseases, which may be associated with its high bioactive compounds including flavonoids, polyphenolic and antioxidant properties. In the present study antioxidant activity of Liliaceae family was assayed by DPPH scavenging activity, flavonoid and total polyphenolic content.

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INTRODUCTION

Diabetes Mellitus is a chronic disease characterized by elevated blood glucose levels, disturbances in the carbohydrate, fat and protein metabolism. Over several years diabetes mellitus has become a major health problem worldwide; reaching epidemic proportion (Gomathi *et al.,* 2012). The world prevalence of diabetes among adults (aged 20-80 years) was 6.7% affecting 295 million adults (2014), and will increase to 7.9% and 443 million adults by 2030. In India, the number of adults with diabetes was 60.8 million (2014) and will be expected to reach 90 million (2030), with a mean annual increment of 2.8 million. Type 2 diabetes mellitus is a heterogeneous metabolic disorder, characterized by defects in insulin secretion and insulin sensitivity (Bachhawat *et al.,* 2011). Mans life are survival would be impossible without 'symbosis' with and extensive use of plants and plants products (Chukwuma *et al.,* 2015). Medicinal plants are reservoirs of natural products with anti-diabetic potentials with respect of effective therapeutic approaches to treatment (Narmadha *et al.,* 2012). These plants based traditional medicine systems continue to play an essential role in health care (Mohan *et al.,* 2016). Medicinal plants have curative properties due to the presence of various complex chemical substance of different composition (Patil *et al.,* 2016).

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Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, based on their use in traditional medicine. Higher plants as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health (Kumar *et al.,* 2012). Medicinal plants have been used in traditional medicine for the treatment of several diseases. In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases and physiological abnormalities under practicals such as Ayurveda. Plants antioxidants are composed of a broad variety of different substance like ascorbic acid and tocopherols, polypenolic compounds or terpenoids. They perform several important functions in plants and humans. Herbs for diabetes treatment are not new. Plants and plants extracts are being used to combat diabetes since old times. About 150 plant species are used commercially for formulation of various drugs (Hemant *et al.,* 2011). Medicinal plants are sources of diverse molecules, which display antioxidant properties which protect human body from both pathogens and cellular oxidation (Rahman *et al.,* 2015). The lily family, Liliaceae, consists of fifteen genera and approximatrly 600 species of flowering plants within the order Liliales. Plants in this family have evolved with a fair amount of morphological diversity despite genetic similarity. Common characteristics include large flowers with parts arranged in three: with six colored or patterned petaloid tepals (undifferentiated petals and sepals) arranged in two whorls, six stamens and a superior ovary. Most

species are grown from bulbs, although some have rhizomes. First described in 1789, the lily family became a paraphyletic "catch-all" group of petaloid monocots that did not fit into other families and included a great number of genera now included in other families and in some cases in other orders. Consequently, many sources and descriptions labeled "Liliaceae" deal with the broader sense of the family. Liliaceae are widely distributed, mainly in temperate regions of the Northern Hemisphere and the flowers are insect pollinated. Many Liliaceae are important ornamental plants, widely grown for their attractive flowers and involved in a major floriculture of cut flowers and dry bulbs. Some species are poisonous if eaten and can have adverse health effects in humans and household pets. A number of Liliaceae genera are popular cultivated plants in private and public spaces. Lilies and tulips in particular have had considerable symbolic and decorative value, and appear frequently in paintings and the decorative arts. They are also an economically important product (Wikipedia).

MATERIALS AND METHODS

Chemicals

Methanol (HPLC grade), Water (HPLC grade), Tris HCl, Folin & Ciocalteu's Phenol Reagent (PCP), DMSO HPLC grade, Sodium acetate, Quercetin, So¬dium carbonate AR grade and Ascorbic acid were obtained from SRL, India, while 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) were purchased from Alfa acer, Britain. All chemicals were analytical grade.

Sample collection of Plant Materials

The plant leaves of allium cipa, allium sativum and aloevera, were collected in March 2015 from the campus of M.G.C.G.V. Chitrakoot Satna (M.P.) and identified. All plant leaves were collected, washed with fresh water and dried under shade at room temperature separately. The leaves were grinded coarsely and then powdered, filtered through sieve (30No.) stored in sterile and air tight container for further use.

Preparation of Plant extracts

100 mg powdered sample of plant leaves were extracted with 10 ml HPLC grade methanol through open air reflux pro¬cess at 40°C for 6 hours till dried than make the volume again 10 ml with methanol and reflux, this process was repeated several times. The extracts were filtered through filter paper (Watman no.1) to remove free un-extractable substances. The filtrates of plant extract were evaporated at room tem-perature at dryness, finally dissolve with 10 ml DMSO and preserved at 4-5°C for further process. The crude samples were subjected to antioxidant and total polyphenolic content.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

The assay for free radical DPPH was done by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) modified method adopted as it is described by Tripathi *et al.* In brief, a 96-well microplate, 125µl of various dilutions of methanolics extract 50 µl of tris –HCl buffer (0.1M, pH 7.4) and 125 µl of DPPH solution (0.004 in methanols) were added. The reaction mixture was shaken well. The DPPH decolorisation was recorded at 517 nm on a Bio Tek^{synergyH4} multi-mode micro plates reader, Bio Tek Instruments, Instruments, Inc Winooski, VT, USA after 30 min incubation in dark. The percentage of DPPH scavenging by plant extracts obtained in terms of ascorbic acid equivalent concentration. Quantification was performed with respect to the standard curve of ascorbic acid ($Y= 5.558X + 42.31$, $R^2= 0.983$). Results were expressed as milligrams of ascorbic acid equivalent per ml of the extract.

Determination of Total Polyphenolic content

Total polyphenolic content of plant leaves extracts was meas-ured by using Folin-Ciocalteu reagent (Tripathi *et al.,* 2013). The 25 µl of plant extract diluted with 125 µl water followed by addition of 150 µl of Folin-Ciocalteu reagent (1N) & 25 µl of Na_2CO3 (20%w/v) and incubated at 45^oC for 60 min then absorbance was measured spectrophotometrically at 765nm (Bio Tek^{synergyH4} multi-mode micro plates reader, Bio Tek Instruments, Instruments, Inc Winooski, VT, USA). Absorbance was recorded triplicates. Quantification was performed with respect to the standard curve of Catechol ($y= 0.004x+0.086$;$R^2 =0.985$). Result was expressed as milligram of Catechol equivalent per ml of extract.

Determination of Flavonoid content

Total flavonoid content in the plant extracts, in brief, 100µl of sample (100 times diluted the original sample with methanol) followed by 100µl 2% $AlCl_3.6H_2O$ in ethanol and 150 µl sodium acetate (50g/L) solution were added. The absorbance at 420 nm monitored (Bio Tek^{synergyH4} multi-mode micro plates reader, Bio Tek Instruments, Instruments, Inc Winooski, VT, USA) after 2.5h of incubation at 20^0C. Total flavonoid content was calculated with respect to the standard curve of the flavonoid quercetindihydrate. Quantification was performed with respect to the standard curve of Quercetin ($y= 0.007x+0.096$; $R^2 =0.997$). Results were expressed as micrograms of quercetin dehydrated equivalents (QE) per ml of the extract.

RESULTS AND DISCUSSION

Analysis of Total Polyphenolic Contents

Polyphenolic compounds are commonly found in both edible and inedible plants, and reported for multiple biological effects, including antioxidant activity.

The antioxidant effect of plant phenolics has been studied in relation to the prevention of coronary diseases and cancer, as well as age-related degenerative brain disorders. It was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation (Tripathi *et al.,* 2013). Total polyphenolic content of selected medicinal plants were shown in table-1, and graphical representation of standard curve of Catechol for estimation of total polyphenolic content were shown in Graph-1.

Table 1. Total polyphenolic Content of selected medicinal plants

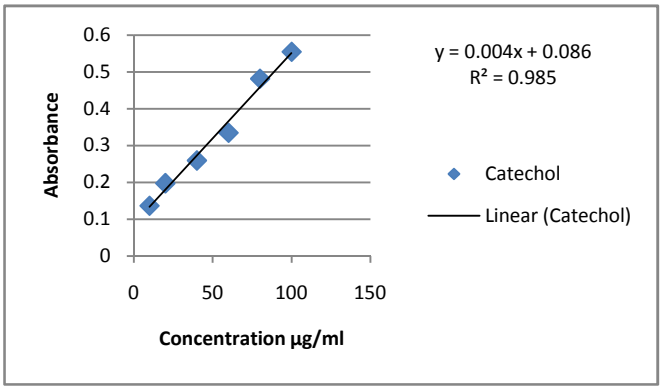
S.No.	Plant Name (Botanical Name)	Total Polyphenolic Content Equivalent to Catechol µg/ml
1.	Onion Leaf (*Allium Cipa*)	2.50
2.	Onion Bulb (*Allium Cipa*)	18.00
3.	Garlic Leaf (*Allium Sativum*)	23.25
4.	Garlic Bulb (*Allium Sativum*)	18.50
5.	Aloe Vera Leaf (*Aloe Vera (L)Burm.f*	27.75

Table 2. Total Flavonoid Content of selected medicinal plants

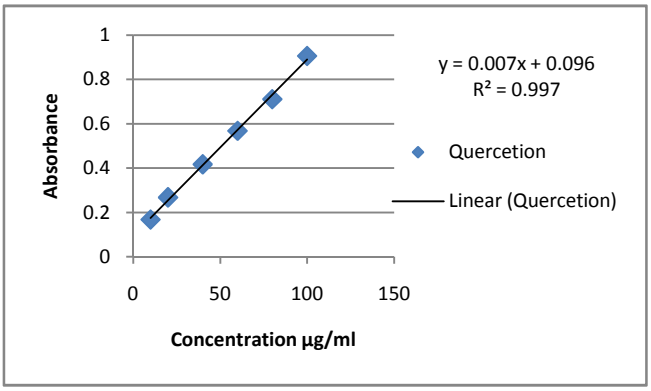
S.No.	Plant Name (Botanical Name)	Total Flavonoid Content Equivalent to Quercetin µg/ml
1.	Onion Leaf (*Allium Cipa*)	8.26
2.	Onion Bulb (*Allium Cipa)*	7.12
3.	Garlic Leaf (*Allium Sativum*)	8.50
4.	Garlic Bulb (*Allium Sativum*)	7.50
5.	Aloe Vera Leaf *(Aloe Vera (L)Burm.f*	6.75

Table 3. % Inhibition of DPPH activity of selected medicinal plants

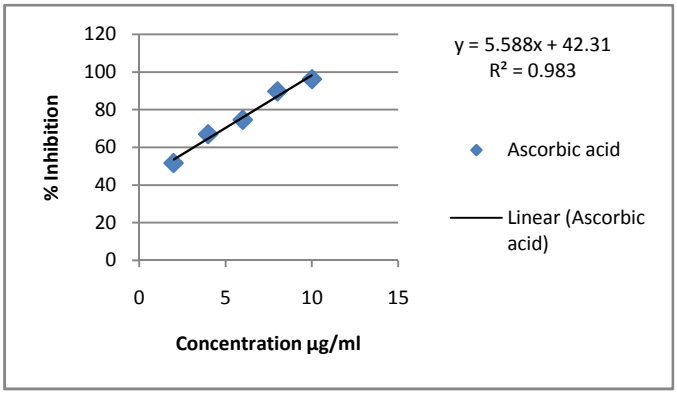
S.NO.	Concentration (µg/ml)	Ascorbic acid	Concentration (µg/ml)	A. Cepa leaf	A. Cepa bulb	A. Sativum leaf	A. Sativum bulb	Aloe vera
1.	2	51.69	10	20.16	24.69	9.87	10.18	8.02
2.	4	66.96	20	31.08	39.19	28.08	12.96	17.90
3.	6	74.63	30	41.50	65.43	31.17	17.90	24.07
4.	8	89.72	40	47.81	75	33.95	19.13	25.00
5.	10	96.19	50	50.08	77.16	41.35	20.06	27.16



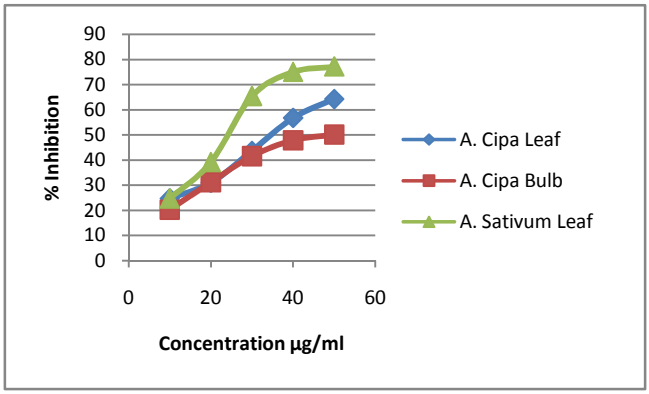
Graph 1. Standard Curve of Catechol for Estimation of Total polyphenolic Content



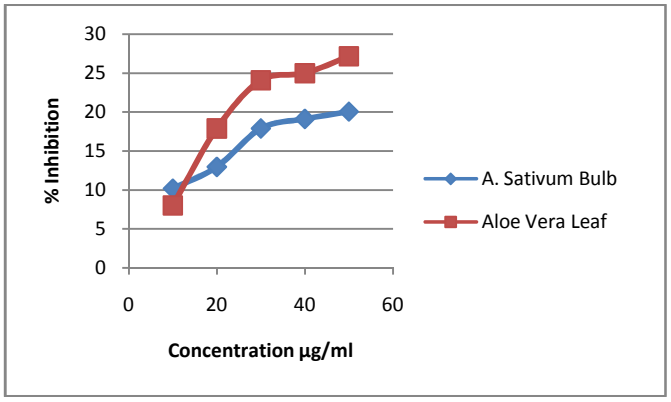
Graph 2. Standard Curve of Quercetin for Estimation of Total Flavonoid Content



Graph 3. % Inhibition of DPPH assay of ascorbic acid



Graph 4. % Inhibition of DPPH assay of selected medicinal plants



Graph 5. % Inhibition of DPPH assay of selected medicinal plants

Table 4. The IC_{50} value of selected medicinal plants and ascorbic acid

S.No.	Plant Name	IC_{50} Values µg/ml
1.	Ascorbic acid	1.38
2.	Onion Leaf	35.71
3.	Onion Bulb	45.55
4.	Garlic Leaf	25.54
5.	Garlic Bulb	161.13
6.	Aloe Vera	95.32

Estimated the highest value of total phenolic content of Aloe vera is 56.82% following by leaves extract about 33.00% and gel extract. In the present study we have estimated Onion Leaf, Onion Bulb, Garlic Leaf, Garlic Bulb and Aloe Vera it contain the 2.5 µg/ml, 18 µg/ml, 23.25 µg/ml, 18.5 µg/ml and 27.75 µg/ml polyphenolic contents respectively (Mohsin *et al.,* 2006).

Analysis of Flovonoid Contents

Flavonoid contents were evaluated by aluminium chloride method and Quercetin as standard. It was reported that Flavonoid contents were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation. Total Flovonoid Content of selected medicinal plants is demonstrated in Table-2. Standard Curve of Quercetin for estimation of Total Flovonoid Content is showing in Graph-2, which is used as the standard curve for the quantification of Flavonoid content equivalent to Catechol µg/ml in methanolic extracts of selected plant leaves. In the present study we have estimated Onion Leaf, Onion Bulb, Garlic Leaf, Garlic Bulb and Aloe Vera it contain the 8.26 µg/ml, 7.12 µg/ml, 8.5 µg/ml, 7.5 µg/ml and 6.75 µg/ml flovonoid contents respectively.

Free Radical Scavenging Activity (DPPH)

Natural free radical scavengers are closely related to their bio-functionalities. Free radicals are highly reactive molecules or chemical species capable of independent existence. Assay based upon the use of DPPH is the most popular spectrophotometric methods for determination of the free radical scavenging capacity of food, beverages and vegetable extracts. This chromogen radical compound can directly react with antioxidants. Additionally, DPPH scavenging method has been used to evaluate the antioxidant activity of compounds due to the simple, rapid, sensitive, and reproducible procedure. Radical scavenging activity is very important due to the deleterious role of free radicals in biological systems. Chemical assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation end-point. In this study DPPH scavenging assay of selected plants was compared with standard ascorbic acid. The graphical representation of the data is shown in graph-3 representing graph of % inhibition of DPPH assay Ascorbic acid. Graph 4 & 5, representing graph of % inhibition of DPPH assay of some medicinal plants. Table-3 shows % Inhibition of Absorbance of some plants. Table 4 shows the IC_{50} values of selected plant extracts and ascorbic acid. In the present study methanolic extracts of the plants of Garlic leaf (*Allium sativum*) show better free radical scavenging activity than other plants. IC_{50} value of methanolic extracts of Onion Leaf, Onion Bulb, Garlic Leaf, Garlic Bulb and Aloe Vera is 35.71 µg/ml, 45.55 µg/ml, 25.54 µg/ml, 161.13 µg/ml and 95.32 µg/ml respectively. Garlic leaf (*Allium sativum*) demonstrates strongest scavenging activity among these two plants. Kumar *et al*. 2013 calculated the IC_{50} value for *Allium cepa* and ascorbic acid were found to be 82.5 and 97.2 µg/ml respectively. Reena Lawrence *et al.* 2011 examined the DPPH and nitric oxide scavenging assays of *Allium sativum* and repoted 0.5µg/ml and 50µg/ml respectively. Mohsin *et al*, 2006 calculated the highest antioxidant activity of Aloe vera about 66.44% where leaves 50.68% and gel 16.19%.

Conclusion

The designed method (open reflection) for extracting the phytoconstituents from powdered leaves of plant is best extraction method because it consumes less amount of solvent. All the extracts were subjected to dose dependent studies to calculate IC_{50} values of different pharmacological activities. The overall result, obtained by the present study we observed that total polyphenolic contents were highest in Aloevera leaf and lowest in onion leaf. Highest flavonoid contents were found to be in onion leaf while it was lowest in aloevera leaf. Best antioxidant activity was found in Garlic leaf where as weakest antioxidant activity was found in Garlic bulb. Further, there is still a need of a thorough investigation to identify, isolate, purify and characterize some more biologically active constituents which will be helpful to cure chronic diseases.

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