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Qualitative and quantitative phytochemical studies of *helianthus tuberosus* L.

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Abstract

Helianthus tuberosus L. is a biotechnologically and medicinally important plant. Five accessions of *H. tuberosus* were analyzed for phytoconstituents to identify the accession with the highest content of medicinal compounds. The aqueous and ethanolic extracts of leaves and shoots were qualitatively tested for secondary metabolites. The total phenolic contents were estimated in leaves and tubers of five accessions of *H. tuberosus* to select the accessions with highest total phenolic content. In the present study qualitative analysis confirmed that the presence of various phytoconstituents like saponins, alkaloids, phenolics, tannins, steroids, glycosides. In leaves, the total phenolic content ranged from 87.83 ± 1.05 to 127.23 ± 1.34 mg GAE/g. In tubers, the total phenolic content ranged from 7.06 ± 0.27 to 20.43 ± 0.69 mg GAE/g. The accessions TUB-1715 identified showed highest total phenolic content in both leaves and tubers. The plant extracts have many applications like antioxidant, anticancer and antifungal activities.

Keywords: *Helianthus tuberosus* L., total phenolic content

Introduction

In ancient times, Ayurvedic (herbal) medicines were majorly used to treat diseases because plants contain natural antioxidant products like polyphenols and flavonoids and also pharmacological and active biological ingredients. Besides being used as medicine, herbs are increasingly used for various purposes like food, flavor, dyes, health drinks, energy drinks, fragrance, etc. Industries like pharma, cosmetics and food use herbal acidic compounds [1]. Ayurvedic (herbal) medicines were majorly used to treat diseases because plants contain natural antioxidant products like polyphenols and flavonoids and also pharmacological and active biological ingredients. Besides being used as medicine, herbs are extensively used for various other purposes like food, clothing, dyes, flavours etc. [2].

Helianthus tuberosus L. has been cultivated in various countries for fodder, vegetable and also in the industry [3, 4]. *H. tuberosus* is also used as livestock feed due to its high nutritional value of essential amino acids [5]. Inulin present in tubers is not digestible in human intestine and has less caloric value compared to the other carbohydrates.

Bioactive compounds found in *H. tuberosus* exhibit three properties namely, antioxidant properties, anticancer properties and antifungal properties. Phytochemical analysis of *H. tuberosus* showed that it is rich in total phenols and also showed the strongest free radical scavenging activities. In this ethyl acetate fraction total, six phenolic compounds strongly showed *in vitro* free radical scavenging activity. Leaves of *H. tuberosus* contain 11 sesquiterpene lactone and flavones [6]. The wild flowers of *H. tuberosus* are known to have antioxidant properties. A huge correlation between the DPPH and FRAP value and the overall phenolic content was reported in flowers extracts, which can help in prevention and treatment of diseases as a result of oxidative stress [7].

Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutical and industrial importance [8].

The present study deals with the phytochemical analysis of different plant parts of *Helianthus tuberosus* L. for the presence of Alkaloids, Tannins, Glycosides, Resins, Steroids, Saponins, Flavonoids and Phenols. Quantitative analysis of leaf and tuber extract for total phenolic content was also carried out.

Material and methods

Preparation of plant extracts for qualitative analysis

Phytochemical analysis (qualitative and quantitative) of the leaves and shoots of *Helianthus tuberosus* L. was carried out for phytoconstituents. The plant materials of *H. tuberosus* (leaves/shoots) were washed with running tap water and sun dried until the moisture content

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was reduced to a level of 10%. The leaves/shoots were then powdered in a mortar, and further ground into a fine powder. The ground leaves/shoots were sieved and 50 grams of powder was put into different conical flasks and 200ml of absolute ethanol and cold aqueous was added to each of the conical flasks and placed on mechanical shaker for 48 hours at 190 rev. per. min. After 48 hrs, the extract was decanted and filtered through a muslin cloth and Whatman No.1 filter paper. Filtrate was evaporated to dryness at 45 °C, and part of the residue reconstituted in 95% ethanol as 250mg/ml ethanolic extract. The rest of the filtrate was reconstituted in distilled water as 250mg/ml aqueous extract.

Qualitative tests of the leaf and shoot extracts (ethanolic and aqueous) was carried out for secondary metabolites.

1. Alkaloids

To 3ml of extract, 5ml of 1% HCl was added by keeping it on a steam bath for twenty 20 minutes. Solution was cooled and filtered and a few drops of Mayer's reagent/picric acid was added. Cream color. Indicates presence of Alkaloids.

2. Tannins

1ml of extract and 1ml of freshly prepared 10% KOH were mixed. Cream color precipitate indicates presence of Tannins.

3. Glycosides

To 3ml of extract, 2ml of chloroform was added and sulphuric acid was added slowly to form a lower layer. Appearance of reddish brown color at the junction of the layers and bluish green upper layer indicates glycosides.

4. Steroids

To 1ml of extract, acetic anhydride (2ml) was added and 2ml sulphuric acid was added slowly. Colour change to blue green indicates presence of Steroids.

5. Test for saponins

1ml of extract and 5ml of distilled water was added and shaken thoroughly until it froths. 3 drops of olive oil was added and shaken for the formation of emulsion which indicates presence of Saponins.

6. Test for flavonoids

In 3ml of extract and 1ml of 10% sodium hydroxide was mixed for yellow colour development which indicates Flavonoids.

7. Test for phenols

To 5ml of extract, 5% ferric chloride was added to form a green precipitate which indicates Phenols.

Quantitative estimation of phenols in leaves and tubers of *Helianthus tuberosus* L.

a) Preparation of leaf/tuber extract

Five accessions (TUB-1715, TUB-107, TUB-115, TUB-1705 and TUB-07) of *H. tuberosus* were presently studied. Collected leaves/tubers were air dried at room temperature and powdered. Ten grams of leaf powder was dissolved in 70% ethanol and incubated for three hours. The extract was filtered, dried and dissolved in 25ml methanol was subjected to spectrophotometric analysis^[9].

Estimation of total phenols

Folin-Ciocalteu method was used to estimate total phenols in leaf/tuber extracts with standard (gallic acid)^[10]. To 0.5ml extract, 0.5ml of freshly prepared Ciocalteu reagent, 2ml of

10% w/v sodium carbonate solution and 3.0ml distilled water were added and incubated for 2 h at room temperature in the dark and the total phenols estimated spectrophotometrically at 760 nm. Total phenol was expressed in terms of gallic acid equivalents (mg/g of extract).

Results

The leaves and shoots (aqueous and ethanolic extracts) were tested qualitatively as to their secondary metabolite content.

The aqueous leaf extract of *H. tuberosus* contained saponins, alkaloids, phenolics, tannins, steroids, glycosides except flavonoids. All the secondary metabolites were present in ethanolic extract of *H. tuberosus*, but, the saponins and steroids were not detectable (Table-1).

In aqueous shoot extract, saponins, alkaloids, phenols, tannins and steroids were detected except flavonoids and glycosides. In the ethanolic extract, saponins, alkaloids, phenolics, tannins, flavonoids and glycosides were detected except steroids (Table-2).

These phytoconstituents may possess many activities like antioxidant activities, antimicrobial activities and wound healing activities. The observed results (given in a later page) further support the view that some traditionally used Indian medicinal plants are promising important sources of potential antioxidant compounds and medicinally important compounds.

Quantitative estimation of phenols in leaves and tubers of *Helianthus tuberosus* L.

Quantitative phytochemical analysis was carried out in five accessions of *H. tuberosus* for the estimation of total phenolic content in leaf and tuber extracts.

In leaves, the total phenolic content ranged from 87.83 ±1.05 to 127.23±1.34mg GAE/g. Highest total phenolic content was recorded in TUB-1715 accession (127.23±1.34mg GAE/g), and the lowest was recorded in TUB-07 accession (87.83 ±1.05mg GAE/g) (Table-3; Fig- 1).

In tubers, the total phenolic content ranged from 7.06±0.27 to 20.43±0.69mg GAE/g. Highest total phenolic content was recorded in TUB-1715 accession (20.43±0.69mg GAE/g) and lowest was recorded in TUB-07 accession (7.06±0.27mg GAE/g) (Table-4; Fig- 2).

Table 1: Qualitative analysis of aqueous and ethanolic leaf extracts of *H. tuberosus*.

S. No.	Phytoconstituent (secondary metabolite)	Aqueous extract	Ethanolic extract
1	Saponins	+	-
2	Alkaloids	+	+
3	Phenolics	+	+
4	Tannins	+	+
5	Steroids	+	-
6	Flavonoids	-	+
7	Glycosides	+	+

+ = Positive presence; -- = Absence

Table 2: Qualitative analysis of aqueous and ethanolic shoot extracts of *H. tuberosus*.

S. No.	Phytoconstituent (Secondary metabolite)	Aqueous extract	Ethanolic extract
1	Saponins	+	+
2	Alkaloids	+	+
3	Phenolics	+	+
4	Tannins	+	+
5	Steroids	+	-
6	Flavonoids	-	+
7	Glycosides	-	+

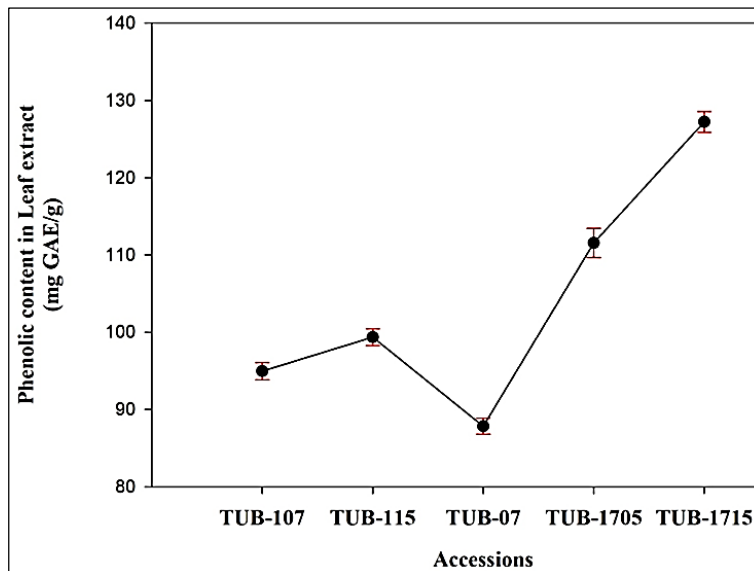
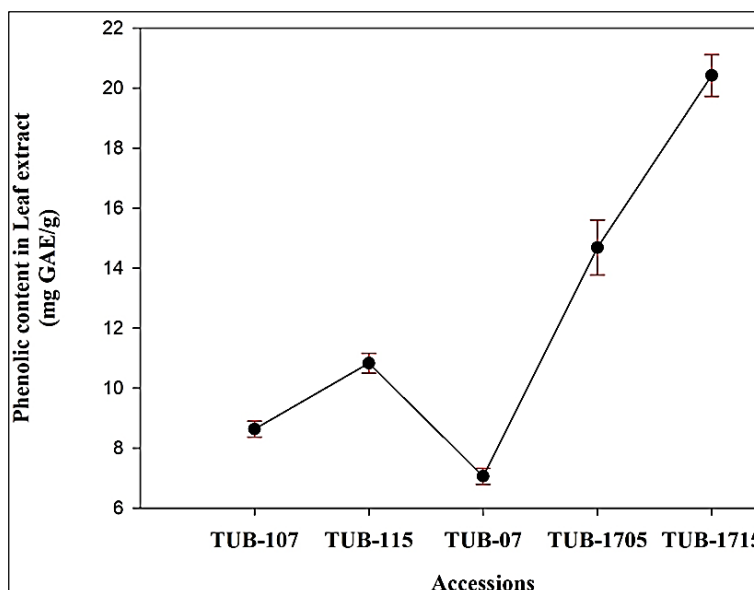
+ = Positive presence; -- = Absence

Table 3: Quantitative estimation of total phenols in leaf extracts of five accessions of *H. tuberosus*

S. No.	Accessions of <i>H. tuberosus</i>	Total phenolic content (mg GAE/g) (Mean \pm S.E.)
1	TUB-107	94.96 \pm 1.12
2	TUB-115	99.36 \pm 1.10
3	TUB-07	87.83 \pm 1.05
4	TUB-1705	111.56 \pm 1.88
5	TUB-1715	127.23 \pm 1.34

Table 4: Quantitative estimation of total phenols in tuber extracts of five accessions of *H. tuberosus*

S. No.	Accessions of <i>H. tuberosus</i>	Total phenolic content (mg GAE/ g) (Mean \pm S.E.)
1	TUB-107	8.63 \pm 0.26
2	TUB-115	10.83 \pm 0.33
3	TUB-07	7.06 \pm 0.27
4	TUB-1705	14.69 \pm 0.92
5	TUB-1715	20.43 \pm 0.69

**Fig 1:** Quantitative estimation of total phenols in leaf extracts of five accessions of *H. tuberosus***Fig 2:** Quantitative estimation of total phenols in tuber extracts of five accessions of *H. tuberosus*

Discussion

Qualitative phytochemical analysis was carried out with leaf (aqueous and ethanol) extracts. The aqueous leaf extract of *H. tuberosus* contained saponins, alkaloids, phenolics, tannins, steroids, glycosides except flavonoids. All the secondary metabolites were present in ethanolic extract of *H. tuberosus*, but, the saponins and steroids were not detectable. One study revealed the presence of Saponins and Steroids, but reported the absence of terpenoids, alkaloids, glycosides, tannins and flavonoids in ethanol and aqueous extracts. In aqueous tuber extract, all the secondary metabolites i.e. saponins, alkaloids, phenolics, tannins and steroids were detected except flavonoids and glycosides^[11].

The accession TUB-1715 has shown highest total phenolic content found in both leaves (127.23 \pm 1.34 mg GAE/g) and tubers extracts (20.43 \pm 0.69 mg GAE/ g). Data of present investigation revealed that the total phenolic content varied from 87.83 \pm 1.05 GAE/g to 127.23 \pm 1.34 GAE/g in leaf extracts, whereas in tubers the total phenolic content ranged from 7.06 \pm 0.27 GAE/ g to 20.43 \pm 0.69 GAE/ g. In the leaf extract highest total phenolic content (127.23 \pm 1.34 mg GAE/g) was recorded in TUB-1715 accession, whereas lowest (87.83 \pm 1.05 GAE/g) recorded in TUB-07 accession. In the tuber extract, the highest total phenolic content (20.43 \pm 0.69 GAE/g) was recorded in TUB-1715 and the lowest (8.63 \pm 0.26 GAE/g) was recorded in TUB-107

accession. Higher total phenolic content in leaves (114.28mg GAE/g) than in tubers (22.22mg GAE/g) was also reported [12].

Conclusion

It is concluded that *Helianthus tuberosus* L. is a plant with a variety of medicinal properties. The qualitative analysis of *H. tuberosus* shows the presence of bioactive compounds such as alkaloids, flavonoids, phenols, tannins, saponins, steroids and glycosides. The accession TUB-1715 has yielded highest total phenolic content both in leaves and tubers. The values of total phenolic content are higher than earlier reports. This is a valuable information for preparation of drugs in pharmaceutical industry and stress the need for more intensive research since they play a great role in healthcare.

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