

Physicochemical and phytochemical screening of *Convolvulus pluricaulis* collected from Bagalkot, Karnataka

Mallappa H. Shalavadi, V. M. Chandrashekhar, I. S. Muchchandi

Department of Pharmacology, BVVS's Hanagal Shri Kumareshwar College of Pharmacy, Bagalkot - 587101, Karnataka, India

Abstract

Aim: The present study aim was to analyze quantitatively and qualitatively the phytochemicals as well as to investigate the physicochemical and phytochemical screening of *Convolvulus pluricaulis* and its chloroform and ethanol extracts. **Materials and Methods:** In this, the physicochemical evaluation; ash values, namely total ash, acid-insoluble ash, water-soluble ash, and sulfated ash; extractive values, namely alcohol soluble extractive value, water-soluble extractive, and chloroform soluble extractive value; and loss on drying were determined. Preliminary phytochemical screening was done for the presence of carbohydrates, proteins, amino acids, glycosides, alkaloids, phytosteroids, flavonoids, saponins, tannins, and phenolic compounds, and total phenolic content was estimated by Folin-Ciocalteu method, and total flavonoid content was measured with the aluminum chloride colorimetric assay. **Results:** In results, it was found that the seed containing various phytochemicals were present in its ethanol and chloroform extracts. The total phenolic content in the ethanol and chloroform extracts was found 13.7 ± 0.4187 and 7.367 ± 0.2987 mg of gallic acid equivalent weight/g of extract, respectively, and the concentration of flavonoids in plant ethanol and chloroform extracts of *Cassia hirsuta* was found 114.6 ± 13.33 and 99.56 ± 11.6 mg of quercetin equivalent weight/g of extract, respectively. **Conclusion:** The present study concludes that the *C. pluricaulis* has the potential to act as a source of useful drugs because of the presence of various phytochemical constituents and high concentration of phenolic and flavonoid compounds.

Key words: Ash values, *Convolvulus pluricaulis*, Flavonoides, Phenols, Phytochemicals

INTRODUCTION

In India, alternative systems of health such as Ayurveda, Yoga, Unani, Siddha, Homeopathy, and Naturopathy have a very long, safe, and continuous usage of many herbal drugs. These systems have existed in India along with allopathic.^[1] Most of Indians regularly use herbal drugs as spices, health foods, home remedies as well as over-the-counter drug as self-medication or also as prescribed drugs in the non-allopathic systems. Hence, these systems are not folklore or traditional herbal practices. There are basic axioms of these systems leading to a logical and systematic structure of pathogenesis and diagnosis, which serves also as a determinant for therapy.

The herbal raw product is very prone to a lot of variation due to several factors through identity of the plants, ecotypic, seasonal, chemotypic, and genotypic variations, collection, drying,

processing, and storage conditions. Thus, it is very important for medicinal plants to the assurance of standardization in terms of safety, quality, and efficacy which has become an important issue in the present scenario. The process of standardization can be achieved by stepwise pharmacognostic studies and minimizing the inherent variation of natural product composition through quality assurance practices applied to cultivation and manufacturing processes.^[2]

Convolvulus pluricaulis Choisy also known as *Convolvulus microphyllus* belongs to family Convolvulaceae. Popularly, it is known as Shankhapushpi [Figure 1].

Address for correspondence:

Mallappa H Shalavadi, Department of Pharmacology, BVVS's Hanagal Shri Kumareshwar College of Pharmacy, Bagalkot - 587101, Karnataka, India.
E-mail: mallu.sha007@gmail.com

Received: 14-08-2018

Revised: 10-09-2018

Accepted: 18-09-2018



Figure 1: *Convolvulus pluricaulis* choisy

The plant grows all over India, distributed in Gujarat, Karnataka, Bihar, Konkan, Sindh, Himalayas, etc. Plant is procumbent or suberect, not twining and fulvous-villous. Stems suffruticose at the base, 6–20 inch long, often floriferous from near the base. Leaves $\frac{1}{2}$ –1 inch long, linear-oblong, upper elliptic, obtuse, mucronate, subsessile, villous on both sides, base tapering. Flowers axillary or on short lateral branches, solitary or 2–4 together, sessile or shortly pedunculate; bracts beneath the calyx $\frac{1}{8}$ inch long, linear-lanceolate, hairy. Sepals $\frac{1}{4}$ inch long, ovate-lanceolate, acute, hairy on both sides, the 3 outer slightly broader than the 2 inner. Corolla rose yellow $\frac{1}{3}$ – $\frac{1}{2}$ inch long, infundibuliform, with hairy bands outside; limb shallowly 5-lobed, the lobes deltoid, acute, with a tuft of hair at the apex of each. Stamens unequal. Ovary glabrous, seated on a cup-shaped disk; stigmas $\frac{1}{6}$ inch long, filiform. Capsules ellipsoid or subglobose, $\frac{1}{8}$ inch long, smooth. Seeds $\frac{1}{10}$ inch long, glabrous, scarcely papillose.^[3]

The plant contains carbohydrate-D-glucose, maltose, rhamnose, sucrose, and starch. It contains proteins, amino acids, and the alkaloid shankpushpi. The most notable constituents are tropane alkaloids and convolamine. The fresh plant contains volatile oils, fatty acids, fatty alcohols, and hydrocarbons, i.e. myristic acid (30.9%), palmitic acid (66.8%), linoleic acid (2.3%), and straight chain hydrocarbon hexatriacontane. Deshpande and Srivastava (1969) carried out a chemical examination of the whole plant of *C. pluricaulis* and reported the presence of scopoletin, β -sitosterol, and cetyl alcohol. The chloroform fraction of this contains 20-oxodotriacontanol, tetratriacontanoic acid, and 29-oxodotriacontanol. The flavonoid kaempferol and steroids phytosterol and β -sitosterol will also be found in major amounts. The leaves of Shankpushpi will be used to treat chronic bronchitis and asthma. The root will be used for childhood fever, and the oil stimulates the growth of hair. The whole herb will use medicinally in the form of a decoction with cumin and milk in fever, nervous debility, and loss of memory, syphilis, and scrofula. Shankpushpi is an astringent, hot aphrodisiac, and a nervine tonic. It improves strength, digestive power, complexion and voice and cures intestinal worms, animal poisoning, skin disease, cough, dyspnea, diabetes, dysuria,

and uterine disorder. It is helpful in epilepsy, insomnia, heart disease, and hematemesis. The whole plant is used in various formulae as a nervine tonic for the improvement of memory and intellect. In Ayurveda, it had action like majjadhathu rasayana (Rejuvenates the nervous tissue).^[4,5]

Preliminary phytochemical screening of plants is also necessary for the discovery and development of novel therapeutic agents with improved efficacy.^[6,7] The present study aim was to analyze physicochemical and phytochemicals properties from *C. pluricaulis* and its chloroform and ethanol extracts.

MATERIALS AND METHODS

Chemicals

Folin–Ciocalteu phenol reagent, gallic acid, quercetin, anhydrous sodium carbonate, methanol, deionized water, chloroform, benzene, petroleum ether, ethanol, sodium nitrite, aluminum trichloride, sodium hydroxide, and all other chemical of laboratory grade were used.

Plant Material

The plant was collected from agriculture land of Bagalkot, Karnataka. It was identified and authenticated and by Prof. S A Kapali, Department of Botany, BVVS Science College, Bagalkot-587101, Karnataka.

Preparation of Extract

The whole plant was cleaned and air-dried, then subjected to coarse powdering, and passed through a sieve #44 to get uniform powder size. The collected powder was used for physicochemical analysis and successively extracted with petroleum ether to defat and then by chloroform followed by ethanol for 24 h using Soxhlet apparatus. After the extraction, solvents were distilled off to get concentrated residue and completely dried by lyophilization and stored in airtight container under refrigeration.

Physicochemical Properties of Powder of *C. pluricaulis*

In the physicochemical evaluation, ash values, namely total ash, acid-insoluble ash, water-soluble ash, and sulfated ash, extractive values, namely alcohol-soluble extractive value, water-soluble extractive, and chloroform-soluble extractive value, and loss on drying (LOD) were determined.^[8,9] The ash values represent the inorganic salts present in the drug. Extracts obtained by exhausting crude drugs are indicative of the approximate measures of certain chemical compounds they contain the diversity in chemical nature and properties of contents of drug.

Determination of Total Ash Value

About 3 g of powder of *C. pluricaulis* was taken in a tared silica crucible and incinerated at a temperature not exceeding 450°C until free from carbon. The resultant ash was cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

Acid-Insoluble Ash Value

The total ash obtained from 3 g of root powder was boiled for 5 min with 25 ml of dilute hydrochloric acid, and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited, and weighed. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

Water-Soluble Ash Value

The total ash obtained from 3 g of root powder was boiled for 5 min with 25 ml of water, and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited, and weighed. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Determination of Sulfated Ash Value

The total ash obtained from 3 g of root powder was moistened with 1 ml of concentrated sulfuric acid, heated gently until the white fumes were no longer evolved, ignited, and weighed. The percentage of sulfated ash was calculated with reference to the air-dried drug.

Determination of Alcohol-Soluble Extractive Value

Accurately weighed powder (5 g) of *C. pluricaulis* was taken and macerated with 100 ml of 95% alcohol for 24 h in a airtight container. The contents were frequently shaken during the first 6 h and allowed to remain for 18 h. After 24 h, the extract was filtered and the filtrate was evaporated, finally, the extract was dried at 105°C to a constant weight and extractable value was calculated as % (w/w) with reference to air-dried drug.

Determination of Water-Soluble Extractive Value

Water-soluble extractive value was determined using the procedure described for alcohol-soluble extractive, except that chloroform water was used for maceration.

Determination of Chloroform-Soluble Extractive Value

Chloroform-soluble extractive value was determined using the procedure described for alcohol-soluble extractive, except that chloroform was used for maceration.

LOD

LOD is the loss in weight in % (w/w) resulting from water and volatile matter of any kind that can be driven off under specified conditions. Weigh accurately about 1.5 g of the powdered drug in a tared porcelain dish and dried at 105°C in a hot air oven to get constant weight and then weighed. From the difference in weight, the percentage LOD with reference to the air-dried substance was calculated.

Qualitative Phytochemical Analysis of Chloroform and Ethanol Extract of *C. pluricaulis* (EECP)

About 1g of the chloroform and EECP were dissolved in 100 ml of its own mother solvents to obtain a stock of concentration 1% (w/v). The extracts thus obtained were subjected to preliminary phytochemical screening following the standard procedure.^[8,10]

Tests for Carbohydrates

Molisch's test

To 2–3 ml aqueous extract, added few drops of α -naphthol solution in alcohol and shaken and added concentrated H_2SO_4 from sides of the test tube were observed for violet ring at the junction of two liquids.

Fehling's test

Fehling's A and Fehling's B solutions, each 1ml were mixed and boiled for 1 min and 2 ml of extracts were added heated in boiling water bath for 10 min. Appearance of yellow and then brick red precipitate indicates the presence of reducing sugars.

Tests for Proteins and Amino Acids

Biuret test

To 3 ml test solution, added 4% NaOH and few drops of 1% $CuSO_4$ solution which is observed for violet or pink color.

Million's test

Mixed 3-ml test solution with 5-ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red color was observed.

Ninhydrin test

3 ml of test solution and 3 drops of 5% Ninhydrin solution were heated in boiling water bath for 10 min which is observed for purple or bluish color.

Tests for glycosides

Hydrolysis of extract

A minimum quantity of the extracts is hydrolyzed with hydrochloric acid for few minutes on water bath, and the hydrolysate is subjected to the following tests.

Legal's test

To the hydrolysate, 1 ml of the pyridine and few drops of sodium nitroprusside solution added, and then, it is made alkaline with sodium hydroxide solution. Color change shows the presence of glycosides.

Borntrager's test

Hydrolysate is treated with chloroform, and the chloroform layer is separated. To this, an equal quantity of dilute ammonia solution is added. Color changes in the ammoniacal layer show the presence of glycoside.

Baljet's test

A test solution is observed for yellow to orange color with sodium picrate.

Tests for Alkaloids**Mayer's test**

To the 1 ml of extract, add 1 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish-yellow or cream-colored precipitate indicates the presence of alkaloids.

Dragendorff's test

To 1 ml of the extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Hager's test

To 1 ml of the extract, add 1 ml of Hager's reagent (saturated aqueous solution of picric acid). A yellow-colored precipitate indicates the presence of alkaloids.

Wagner's test

To 1 ml of the extract, add 1 ml of Wagner's reagent (iodine in potassium iodide solution). Formation of reddish brown precipitate indicates the presence of alkaloids.

Tests for Phytosteroids

A small quantity of extract is dissolved in 5 ml of chloroform separately. The above-obtained chloroform solutions are subjected to Salkowski and Liebermann-Burchard tests.

Salkowski test

To the 1 ml of the above-prepared chloroform solution, few drops of concentrated sulfuric acid are added. Formation of brown ring indicates the presence of phytosterols.

Liebermann-Burchard test

The above-prepared chloroform solutions are treated with few drops of concentrated sulfuric acid followed by 1 ml

of acetic anhydride solution. A bluish-green color solution shows the presence of phytosterols.

Tests for Flavonoids**Shinoda test**

To dried powder or extract, added 5 ml 95% ethanol, few drops of concentrated HCl, and 0.5 g magnesium turnings. Pink color was observed.

Ferric chloride test

Test solution with few drops of ferric chloride solution shows intense green color.

Alkaline reagent test

Test solution when treated with sodium hydroxide solution shows an increase in the intensity of yellow color, which becomes colorless on the addition of few drops of dilute acid.

Lead Acetate Solution Test

Test solution with few drops of lead acetate solution (10%) gives yellow precipitates.

Tests for Saponins**Foam test**

The drug extract or dry powder shaken vigorously with water. Persistent foam was observed.

Tests for tannins and phenolic compounds

To 2–3 ml of extract, add few drops of following reagents:

5% FeCl₃ solution: Deep blue-black color.

Lead acetate solution: White precipitate.

Dilute HNO₃: Reddish to yellow color.

Quantitative Phytochemical Analysis of Chloroform and EECP**Determination of total phenol content**

Total phenolic content was estimated by Folin-Ciocalteu method. 1 ml of aliquots and standard gallic acid (12.5, 25, 50, 100, and 200 µg/ml) was positioned into the test tubes, and 5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent were mixed and shaken. After 5 min, 1.5 ml of 20% sodium carbonate was added and volume made up to 10 ml with distilled water. It was allowed to incubate for 2 h at room temperature. Intense blue color was developed. After incubation, absorbance was measured at 750 nm using UV-visible spectrophotometer. The extracts were performed in triplicates. The blank was performed using a reagent blank with solvent. Gallic acid was used as a standard. The

calibration curve was plotted using standard gallic acid. The data for total phenolic contents of extracts were expressed as mg of gallic acid equivalent (GAE) weight/1 g of extract.^[11,12]

Determination of total flavonoid content

Total flavonoid content was measured with the aluminum chloride colorimetric assay. 1 ml of aliquots and 1 ml of standard quercetin solution (50, 100, 200, and 400 µg/ml) were positioned into test tubes, and 4 ml of distilled water and 0.3 ml of 5% sodium nitrite solution were added into each. After 5 min, 0.3 ml of 10 % aluminum chloride was added. At 6th min, 2 ml of 1 M sodium hydroxide was added. Finally, volume was making up to 10 ml with distilled water and mix well. Orange yellowish color was developed. The absorbance was measured at 510 nm using UV-visible spectrophotometer. The blank was performed using distilled water. Quercetin was used as standard. The samples were performed in triplicates. The calibration curve was plotted using standard quercetin. The data of total flavonoids of extracts were expressed as mg of quercetin equivalents/1 g of extract.^[12-15]

RESULTS

Physicochemical Properties of *C. pluricaulis*

The ash values, namely total ash, acid-insoluble ash, water-soluble ash, and sulfated ash; extractive values, namely alcohol-soluble extractive value, water-soluble extractive, and chloroform-soluble extractive values; and LOD were calculated and recorded. Results are summarized in Table 1.

Preliminary Phytochemical Studies

Preliminary phytochemical screening to detect the different chemical principles present in chloroform extracts, namely

carbohydrates, proteins, amino acids, glycosides, alkaloids, and flavonoids, was present and in ethanol extract, namely carbohydrates, proteins, amino acids, glycosides, alkaloids, steroids, flavonoids, tannins, and phenolic compounds, was present. Results are summarized in Table 2.

Total Phenolic Contents

The total phenolic contents in the examined chloroform and ethanol extracts were found 7.364±0.4133 and 21.57±0.064 mg of GAE weight/g of extract, respectively. The highest concentration of phenols was measured in ethanol extract, and chloroform extracts contain considerably smaller concentration of phenols. The total phenolic contents in plant extracts of the species *C. pluricaulis* depend on the type of extract, i.e., the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides a high concentration of these compounds in the extracts obtained using polar solvents for the extraction. Results are summarized in Table 3 and Figure 2.

Total Flavonoid Content

The concentration of flavonoids in plant chloroform and EECP was found 103.2±10.6 and 227±5.6 mg of quercetin equivalent weight/g of extract, respectively. Ethanol extracts contain the highest flavonoid concentration, and low flavonoid concentration was measured in chloroform extract. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation. Results are summarized in Table 4 and Figure 3.

DISCUSSION

Intentional misidentification of herbal material by merchants and non-intentional misidentification of medicinal herbs or their products by scientific community makes a great loss to the human health system. Thus, it is required a critical examination and identification of herbal drugs which can improve the genuine nature of herbal medicine and its therapeutic effect. The pharmacological activity of herbal extract depends on specific chemical constituents present in it, and it is necessary to find a lead chemical constituent present and its therapeutic activity. Hence, there is a need to evaluate the plant for its physicochemical and photochemical properties.

The amount of impurities present in a herbal drug can be analyzed based on ash content, and ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards. The total ash, acid-insoluble ash, water-soluble ash, and sulfated ash was found to be 12.71%, 7.23%, 5.09%, and 12.69%, respectively. This percentage clearly indicates that the *C. pluricaulis* is best for drug action and effects. High extractive value indicates the presence of bioactive compounds in

Table 1: Physicochemical properties of seeds of *C. pluricaulis*

Parameters	Values in % (w/w)
Ash value	
Total Ash value	12.71
Acid-insoluble ash value	7.23
Water-soluble ash value	5.09
Sulfated ash	12.69
Extractive value	
Alcohol-soluble extractive value	9.4
Water-soluble extractive value	28.4
Chloroform-soluble extractive value	5
LOD	6.493

LOD: Loss on drying

Table 2: Qualitative phytochemical analysis of chloroform and EECP

Phytochemical constituents	Name of the test	CECP	EECP
Carbohydrates	Molisch's test	Positive	Positive
	Fehling's test	Positive	Positive
Proteins and amino acids	Biuret test	Positive	Positive
	Million's test	Negative	Positive
	Ninhydrin test	Positive	Positive
Glycosides	Legal's test	Positive	Negative
	Baljet's test	Positive	Positive
Alkaloids	Mayer's test	Negative	Positive
	Dragendorff's test	Positive	Positive
	Hager's test	Negative	Negative
	Wagner's test	Negative	Positive
Phytosteroids	Salkowski test	Positive	Positive
	Liebermann–Burchard test	Positive	Positive
Flavonoids	Shinoda test	Negative	Positive
	Ferric chloride test	Positive	Positive
	Alkaline reagent test	Negative	Positive
	Lead acetate solution test	Negative	Positive
Saponins	Foam test	Negative	Positive
Tannins and phenolic compounds	5% FeCl ₃ solution	Negative	Positive
	Lead acetate solution	Negative	Positive
	Dilute HNO ₃	Negative	Positive

CECP: Chloroform extract of *C. pluricaulis*, EECP=Ethanol extract of *C. pluricaulis*. *C. pluricaulis*: *Convolvulus pluricaulis***Table 3:** Total phenolic content of chloroform and EECP

Extract	Phenolic content (mg of GAE weight/g of extract)
CECP	7.364±0.4133
EECP	21.57±0.064

All values are expressed in Mean±SEM. CECP=Chloroform extract of *C. pluricaulis* and EECP=Ethanol extract of *C. pluricaulis*, GAE: Gallic acid equivalent. *C. pluricaulis*: *Convolvulus pluricaulis*

remarkable quantity and with fever impurity. The water-soluble extractive value plays an important role in the evaluation of crude drugs. Less extractive value indicates the addition of exhausted material, adulteration, or incorrect processing during drying or storage. The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value. The water-soluble extractive value was higher than alcohol-soluble extractive value in this study, and it was found to be 28.4%. This shows that the constituents of the drug are more extracted and soluble in water as compared to alcohol. Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always

desirable for higher stability of drugs. The moisture content of the crude drug was found below 6.493%.

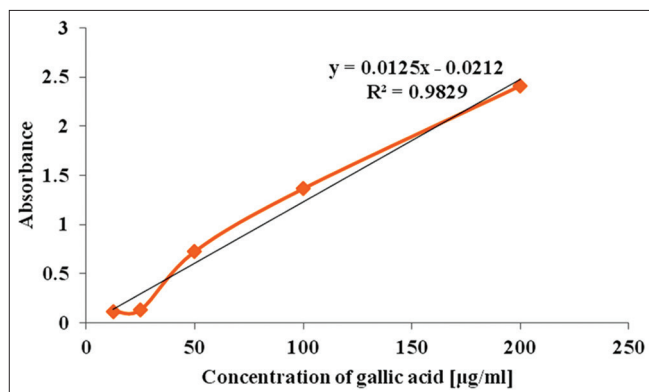
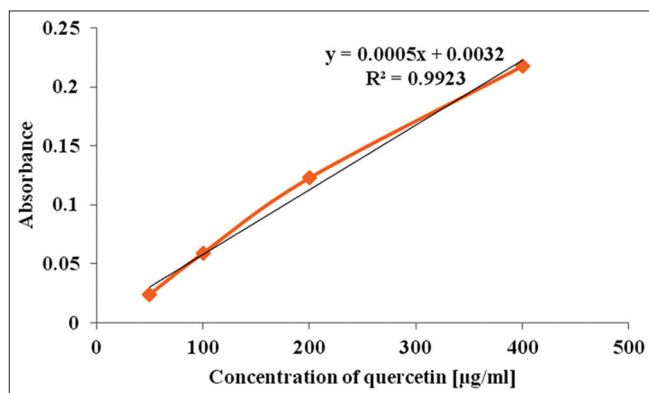
The phytochemical screening of chloroform and ethanol extract of *Convolvulus pluricaulis* showed presence of carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, flavonoids, tannins, and phenolic compounds are present in ethanol extract and apart from saponins, tannins and phenolic compounds all other constituents also present in chloroform extract. The variations in phytochemical contents of the plant are due to a number of environmental factors such as climate, altitude, and rainfall.^[16] These variations of phytochemical constituents of the plant seemed to be the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that have vital role for good health.^[17]

Phenolic compounds have redox properties, which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoids, including flavones, flavanols, and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH.

Table 4: Total flavonoid content of chloroform and EECP

Extract	Flavonoid content (mg of quercetin equivalent weight/g of extract)
CECP	103.2±10.6
EECP	227±5.6

All values are expressed in mean±SEM. CECP=Chloroform extract of *C. pluricaulis* and EECP=Ethanol extract of *C. pluricaulis*; *C. pluricaulis*: *Convolvulus pluricaulis*

**Figure 2:** Total phenolic content for standard gallic acid**Figure 3:** Total flavonoid content for standard quercetin

Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo*.^[18-20]

CONCLUSION

The present study concluded that the *C. pluricaulis* has the potential to act as a source of useful drugs due to the presence of various phytochemical constituents such as alkaloids, flavonoids, phenol, terpenoids, saponin, and carbohydrates. These phytoconstituents seemed to be the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital role for good health. With this, it was also found high contents of phenolic and flavonoid compounds, indicating that these compounds contribute to the antioxidant activity.

ACKNOWLEDGMENTS

We are thankful to Principal, H.S.K. College of Pharmacy, Bagalkot, Karnataka, India, for providing necessary facilities during the course of this study.

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Source of Support: Nil. **Conflict of Interest:** None declared.