

Phytochemical status of some selected medicinal plants (*Eclipta alba*, *Cathranthus roseus* and *Swertia chirata*)

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ABSTRACT

In the present study three medicinal plants i.e. Ecliptic alba, Cathranthusroseus and Swertiachirata were selected and screened for different bioactive compounds of medicinal importance. For this purpose methanolic extracts of the leaves and stems of these medicinal plants were prepared and tested for the presence of different phytochemical compounds. These plants were found to contain alkaloids, carbohydrates, phenols, tannins, flavonoids, proteins, saponins and glycosides. Presence of alkaloids was higher in the leaves and stems of C. roseus as compared to E. alba and S. chirata. However, carbohydrates and proteins were similar in the methanolic extract of leaves and stems of all the three plants. Qualitative analysis showed that the total flavonoids contents were 15.5, 16.2 and 18.3 mg/g in leaves and 8.5, 9.4 and 9.2 mg/g in stems of Ecliptaalba, Cathranthusroseus and Swertiachirata, respectively. The total phenolic contents were 7.4, 8.3 and 5.5 mg/g in leaves and 4.9, 5.3 and 6.2 mg/g in stems of E.alba, C.roseus and S.chirata, respectively. Presence of different bioactive compounds in the methanolic extracts of leaves and stems of these plants suggested that they may be effectively used in the traditional medicine system for the treatment of different disease conditions.

Keywords: Phytochemicals, medicinal plants

INTRODUCTION

Popularity of herbal drugs is increasing all over day by day in the world because of their lesser side effects as compared to synthetic drugs [1]. The medicinal plants contain different organic compounds, which provide definite physiological action on the human body and these bioactive substances include carbohydrates, proteins, alkaloids, phenols, tannins, flavonoids, saponins and steroids [2]. These compounds are synthesized by primary or secondary metabolism and are present in leaves, stems, roots and bark of these plants [3]. These secondary metabolites are highly varied in structure; many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols[4,5].

Eclipta alba(Bhringraj), *Cathranthus roseus*(Sadabahar), and *Swertia chirata*(Chirata) are traditionally used as medicinal plants. Their different preparations are used for the treatment of remittent fever, oxidative stress and as a cooling laxative to children and as a refresher remedy for liver and heart. These are also reported in treatment of jaundice, ophthalmic diseases, skin diseases, ulcers, rheumatism, bronchitis and degenerative diseases etc[6].The medicinal properties of these plants have been attributed to different organic compounds like flavonoids and phenols, which acts as natural antioxidant. Concentration of these compounds has been reported to vary in different parts of these plants as flavonoids are reported to be present in larger amounts in leaves as compared to stems. In view of these facts, present studywas undertaken to assess the status of different bioactive compounds in the methanolic extracts of leaves and stems of these plants.

MATERIALS AND METHODS

Collection and preparation of samples

Three medicinal plants i. e. *E. alba*, *C. roseus* and *S. chirata* were selected and collected from the Department of Horticulture, SHIATS, Allahabad and NBRI, Lucknow. Leaves and stems of these plants were separated and dried at room temperature for 3 weeks and powdered. The powdered samples were stored in air tight plastic container for further use.

Solvent extraction of sample

About 20 g powder sample was extracted with methanol for 16 hours in Soxhlet Unit. Extract was then dried on hot plate at 30°–40°C till the solvent got evaporated and dried powder was stored in refrigerator at 4°C for further analysis.

Qualitative photochemical analysis

The dried powder of methanolic extract was used for carrying out various qualitative tests for alkaloids, carbohydrates, phenol and tannins, flavonoids, protein, saponins, glycosides and steroids as well quantification of total phenolics and flavonoids as described below.

Alkaloids

The crude extract (1 g) was mixed in 2 mL of slightly warm 1% HCl in test tubes and then added 2 mL of Mayer's, Hanger's and Wagner's reagents respectively in tubes. The formation of turbidity (creamy, yellow, dark red color) confirmed the presence of alkaloid [6].

Carbohydrates

Fehling's test

Equal volumes of Fehling A and Fehling B solutions were mixed and then 2 mL from it was mixed with 1 gm of crude extract and then heated. Formation of brick red precipitate, confirmed the presence of carbohydrates [5].

Benedict's test

Crude extract (1 g) was mixed with 2 mL of Benedict's reagent and boiled. Formation of reddish brown precipitate confirmed the presence of carbohydrates [6].

Phenols

Crude extract (1 g) was mixed in 2 mL of 2% FeCl₃ solution. Formation of blue – green/ black color confirmed the presence of phenol and tannins [6].

Flavonoids

Mixed (1g) of crude extract in 2 mL of conc. H₂SO₄. Formation of orange color confirmed the presence of flavonoids [6].

Proteins

Biuret Test

Mixed 2 g extract in 4 mL of 40% NaOH. To it 2 – 10 drops of 1% copper sulphate solution was added to it. Formation of violet color indicated for the presence of proteins [5].

Xanthoprotic test

Crude extract (1 g) was mixed with 1 mL of conc. HNO₃, then boiled and cooled. After that 2 mL of 20% NaOH was added. Formation of orange color confirmed the presence of protein [5].

Saponins

Mixed 2 g extract with 4 mL of H₂O. The mixture was shaken vigorously. Formation of foam at 2 cm indicated for the presence of saponins [6].

Glycosides

Keller Killiani Test

Dissolved 1 g crude extract in 2 mL of acetic acid and transferred in to another test tube which contained 2 mL sulphuric acid. Formation of a reddish brown color at the junction, which gradually becomes blue, confirmed the presence of glycosides [5].

Steroids

Dissolved 1 g crude extract in 2 mL of chloroform, 2 mL of conc. H₂SO₄ was added followed by addition of 2 mL of acetic acid. Formation of greenish color indicated the presence of steroids[6].

Quantitative analysis of different phytochemicals**Total phenol content**

Total phenol content was estimated by Folin-Ciocalteu reagent method with slight modification [7; 8]. 5% Folin-Ciocalteu reagent (2.5 mL) and 3 mL of 2% Na₂CO₃ solution were added to 1 mL of plant extract. The mixture was incubated for 15 min at room temperature. The sample was read at 765 nm. Gallic acid (1 mg/ml) was used as standard. The result obtained from the standard curve was expressed as gallic acid equivalent (mg/g of extract).

Total flavonoids contents

The amount of flavonoids in the methanolic extract was estimated by aluminum chloride colorimetric method with some modifications [7;8]. 1g plant extract was dissolved in 4 mL methanol, 1 mL of 5% aluminum chloride, 1 mL of 1M potassium acetate and 6 mL of distilled water were added and kept for 30 min after that absorbance was taken at 420 nm. Quercetin was used as a standard (1 mg/ml). The results obtained from the standard curve were expressed as quercetin equivalent (mg/g of extract).

RESULTS AND DISCUSSION

The status of different phytochemicals present in the methanolic extract of leaves and stems of the three medicinal plants is presented in Table 1. Qualitative analysis of methanolic extract of leaves and stems of these medicinal plants revealed the presence of different phytochemicals such as alkaloids, carbohydrates, phenols, tannins, flavonoids, proteins, saponins glycosides, and steroids. And also total phenol and total flavonoids. In a report, the presence of phenols, flavonoids, tannins, alkaloids, terpenoids and steroids has been revealed in the methanolic and aqueous extracts of *E. alba*[9]. It was also reported that steroids and alkaloids were absent in the methanolic extract and tannins in the aqueous extract[9]. Similar to our observations, presence of carbohydrates, proteins, steroids, flavonoids, alkaloids, saponins and tannins has been reported in the roots of *E. alba* and it was suggested that the plant has potent therapeutic activity [4]. In a similar report, presence of alkaloids, flavonoids, glycosides, saponins, steroids and tannins has been reported in the methanolic extract of whole dried *E. alba* plant[10]. Similarly screening was done for the bioactive compounds in methanol and aqueous extracts of *Catharanthus roseus* leaves and flowers and presence of phytosterols, phenolic compounds, tannins, flavonoids, coumarin glycosides and terpenoids was reported in them [11]. In another study active principles of *Catharanthus roseus* were tested in the ethanolic extract of dried powdered, its leaves which was found to possess carbohydrates, flavonoids, saponins, and alkaloids [12]. Analysis of *S. chirata* showed the presence of tannins, alkaloids, glycosides and flavonoids in its methanol extract and only tannins and glycosides in the aqueous extract[13].

Table 1: Status of different phytochemical constituents in the methanolic extracts of *Eclipta alba*, *Catharanthus roseus* and *Swertia chirata*

Phytochemical constituents	Test done for identification	Leaf			Stem		
		<i>E. alba</i>	<i>C. roseus</i>	<i>S. chirata</i>	<i>E. alba</i>	<i>C. roseus</i>	<i>S. chirata</i>
Alkaloids	Mayers	++	++++	++	++	++++	++
	Hangers	++	++++	++	++	++++	++
	Wagners	++	++++	++	++	++++	++
Carbohydrates	Fehlings	++	++++	++	++++	++++	++++
	Benedicts	++++	++++	++	++++	++++	++++
Phenol & Tannins		++++	++++	++++	++++	++++	++++
Flavonoid		++++	++++	++++	++++	++++	++++
Protein	Biuret	++++	++++	++++	+++	++++	++++
	Xanthoprotic	++++	++++	++++	++++	++++	++++
Saponins		++++	++	++++	++	++++	++++
Glycosides		++++	++++	++++	++++	++++	++++
Steroids		++++	++++	++++	++++	++++	++++

Observation: ++++ Present, ++ Less present

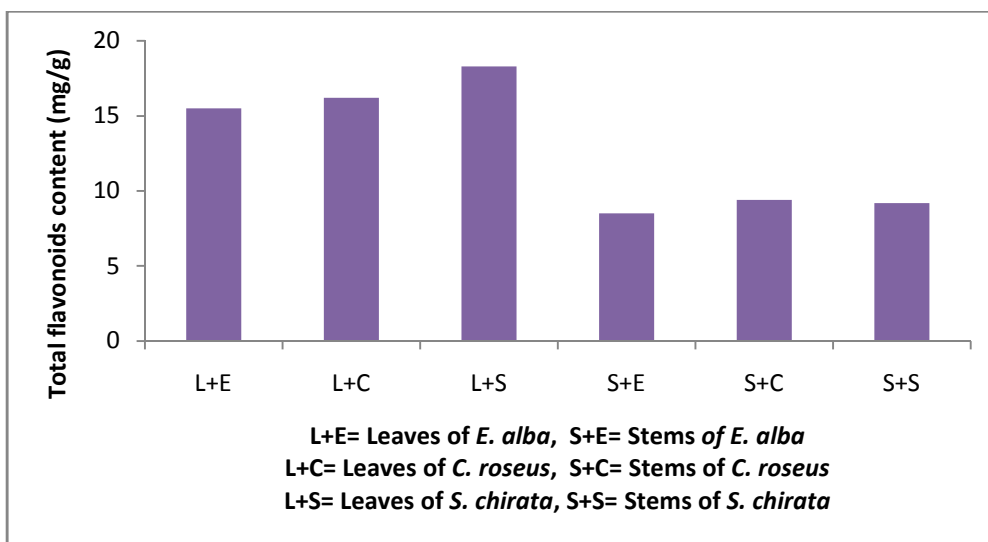


Fig1. Total flavonoids in the methanolic extract of leaves and stems of selected medicinal plants

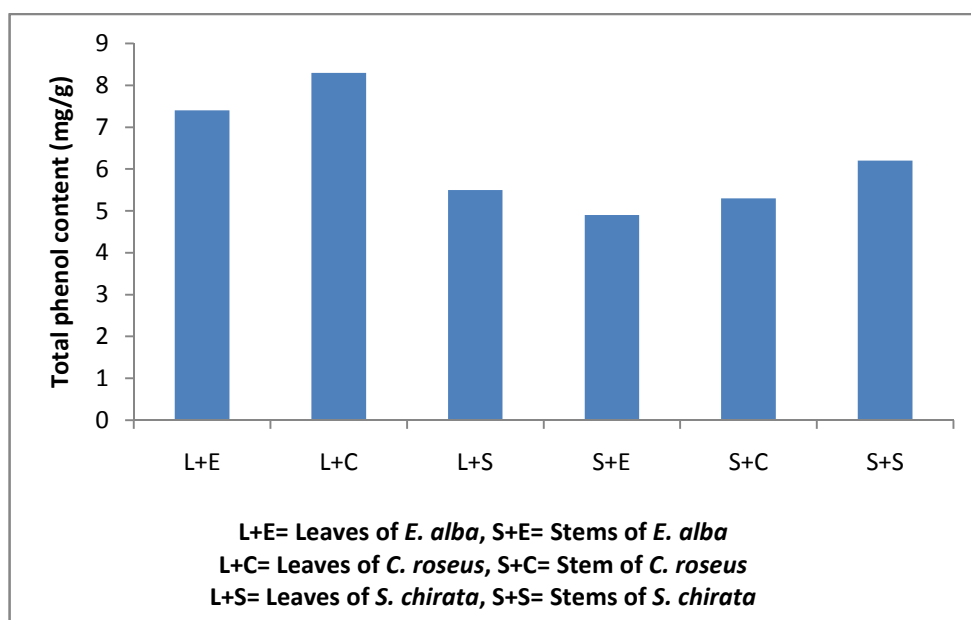
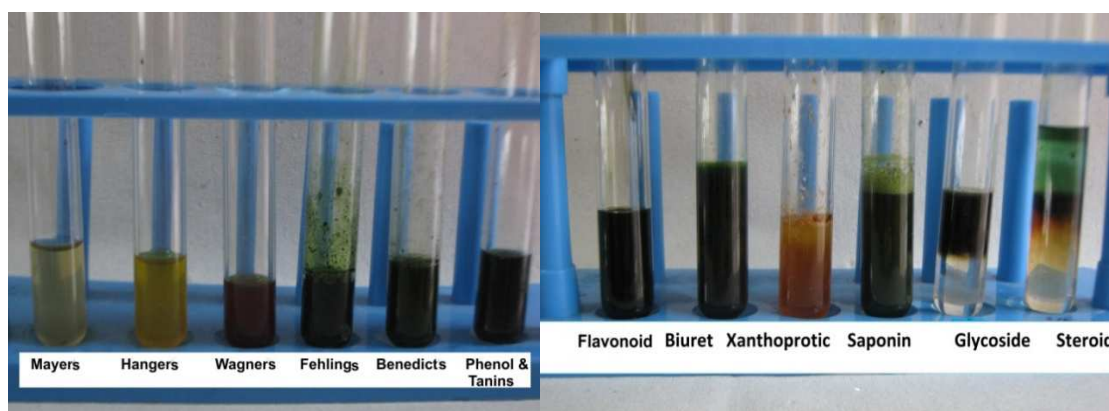
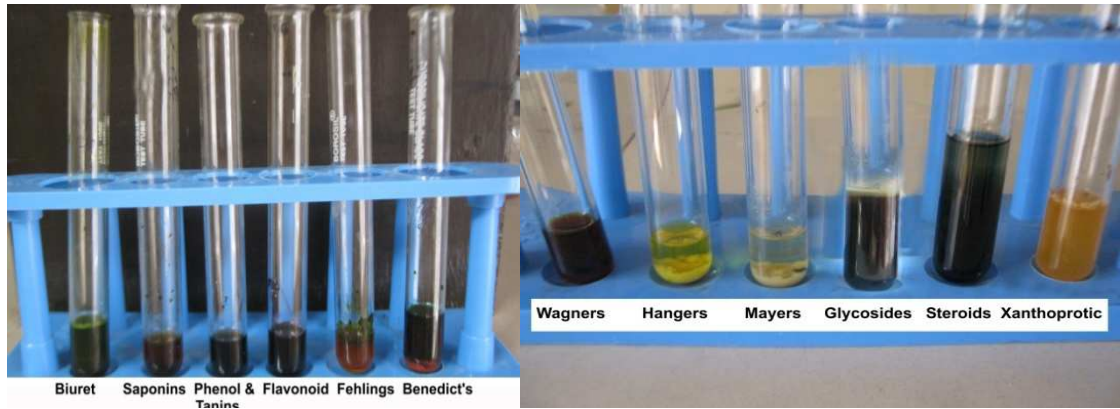


Fig 2.Total phenolic contents in methanolic extract of leaves and stems of selected medicinal plants



(a) (b)

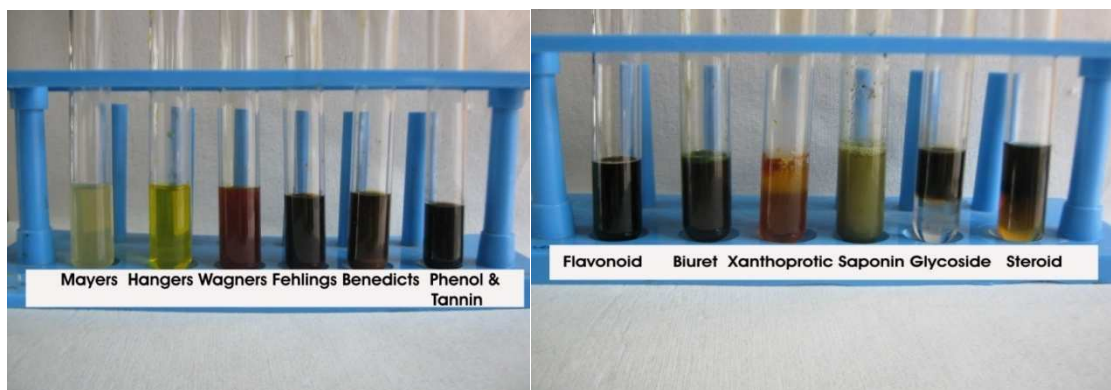
Fig 1.1: (a,b): Screening of phytochemicals for leaves of *E. alba*



(c)

(d)

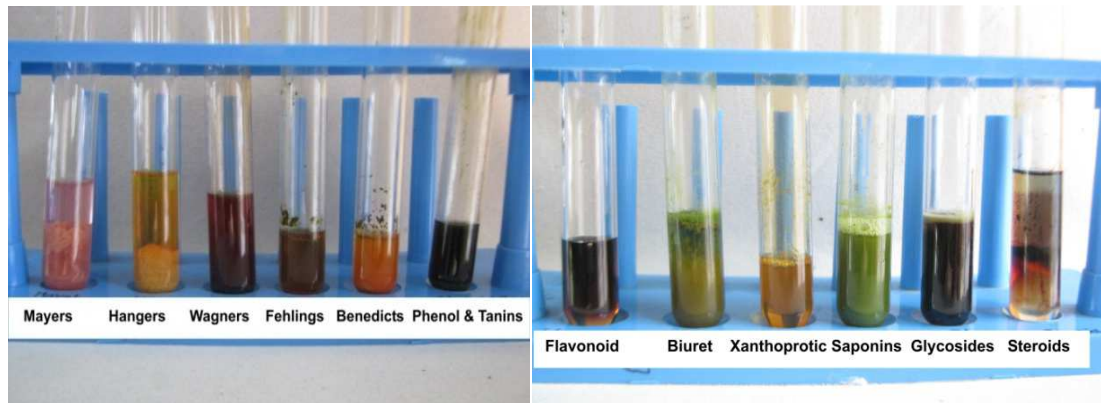
Fig 1.2: (c,d): Screening of phytochemicals for leaves of *C.roseus*



(e)

(f)

Fig 1.3: (e,f): Screening of phytochemicals for leaves of *S.chirata*



(g)

(h)

Fig 1.4: (g,h): Screening of phytochemicals for stems of *C.roseus*

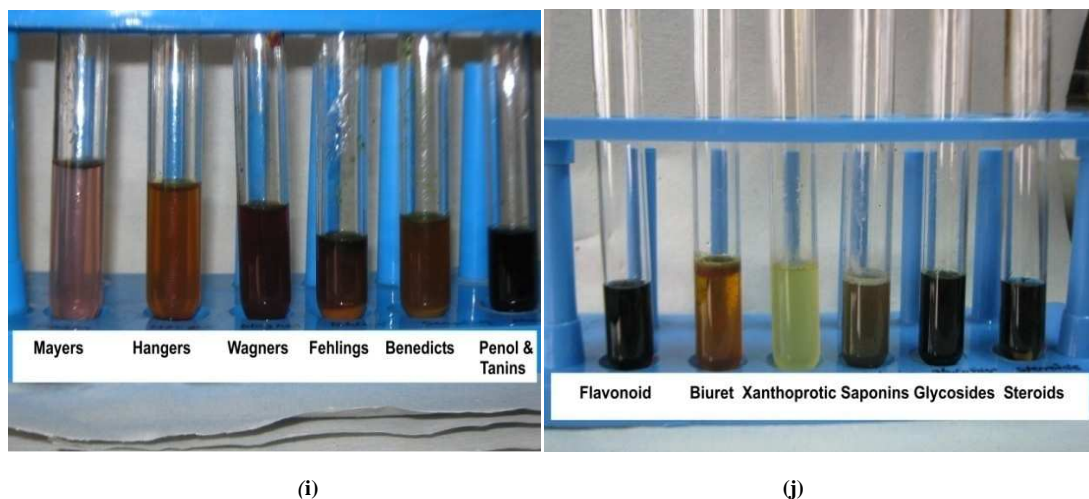


Fig 1.5: (i,j): Screening of Phytochemicals for stems of *E. alba*

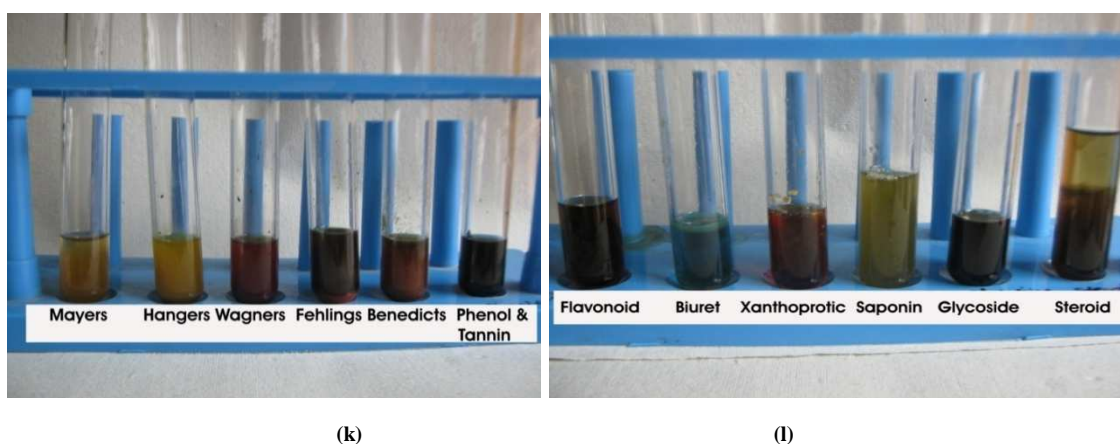


Fig 1.6: (k,l): Screening of Phytochemicals for stems of *S. chirata*

The concentrations of total flavonoids and total phenolics in the leaves and stems the in different plants have been presented in Fig: 1 and 2, respectively. The total flavonoids contents were 15.5, 16.2 and 18.3 mg/g in leaves and 8.5, 9.4 and 9.2 mg/g in stems of *E. alba*, *C. roseus* and *S. chirata* and total phenolics were 7.4, 8.3 and 5.5 mg/g in leaves and 4.9, 5.3 and 6.2 mg/g in the stems of *E. alba*, *C. roseus* and *S. chirata*, respectively. Estimation of the total phenolic content in aqueous and ethanolic extract of *S. chirayita*, *S. nervosa* and *Andrographis paniculata* plants showed that in general, ethanolic extracts had slightly higher total phenolic content than aqueous extracts. It was also found that the levels of total phenolic content were highest in *S. chirayita* (5.6 mg/g GAE –Gallic Acid Equivalent), followed by *S. nervosa* (4.7 mg/g GAE) and lowest in *Andrographis paniculata* (4.5 mg/g GAE) [14]. The total phenolic and total flavonoids content were determined in different solvent extracts of *C. roseus* shoots and it was observed that the amounts of total phenolic and total flavonoids in the extracted of *C. roseus* shoots in different solvent systems ranged from 3.2 to 8.5 (GAE) g/100g per dry matter and 1.8 to 5.4 (CE) g/100g per dry matter, respectively [15]. It was also observed that the total phenolic and total flavonoids were higher in the methanolic extract as compared to other extractions of *C. roseus* shoots [15]. In another study on *S. chirata*, it was observed that the total phenolics were higher in the methanolic extract than ethanolic extract being 273 and 189 mg gallic acid equivalents/g of dry weight of extract, respectively and same trend was observed in the concentration of total flavonoids, which was 3.38 and 3.17 mg catechin equivalents/g of dry weight of extract, respectively [16].

CONCLUSION

Presence of different bioactive compounds such as alkaloids, carbohydrates, phenols and tannins, flavonoids, proteins, saponins, glycosides, and steroids as well as total phenol and total flavonoids in the methanolic extracts of leaves and stems of *E. alba*, *C. roseus* and *S. chirata* suggested that they may be effectively used in the traditional medicine system for the treatment of different disease conditions.

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