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Full Length Article

Phytochemical Analysis and Antioxidant Properties of *Teucrium stocksianum* Flower from Malakand Division, Pakistan

Gul Rahim[1], Rahmatullah Qureshi[1*], M. Arshad[1] and Muhammad Gulfraz[1]
[1]*Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Murree Road, Rawalpindi, Pakistan*
*For correspondence: rahmatullahq@yahoo.com; rahmatullahq@uaar.edu.pk

Abstract

The flower extracts of *Teucrium stocksianum* were screened for antioxidant and phytochemical constituents by using nine different solvents such as acetone, butanol, chloroform, ethyl acetate, ethanol, methanol, n-hexane, petroleum ether and water. By using these extracts, 10 phytoconstituents were screened. Saponins detected by all solvents, followed by tannin, reducing sugar (each 7), flavonoids (6), terpenoid (5), alkaloids, anthraquinone (4 each), whereas, steroids detected by 3 and phlobatannin as well as glycoside isolated by 2 solvents. Three solvents viz., chloroform, ethyl acetate and water extracted highest weight of raw material (150 g), followed by petroleum ether (130 g), n-hexane, acetone (120 g each), methanol (118 g). N-hexane yielded highest extract weight (26 g), followed by butanol (20 g), methanol (19.3 g), water (17 g), acetone (14.5 g), chloroform (13.5 g), petroleum (12.3 g) and ethanol (11.4 g). The percentage yield of extract was varied ranging from 1.73 to 29.08 with the maximum value recorded from n-hexane (21.6%). It was followed by butanol (20.3%), methanol (16.3%), acetone (12.08%), water (11.33%), petroleum ether (9.46%) and chloroform (9%). The plant possessed good antioxidant property and at the dose of 100 µg, DPPH value of flower was 412.82±0.003 that is near to the satndard (440.54±0.001). The phenolic compounds were also detected in plant that was about half of the quantity of standard (gallic acid). This study will serve as benchmark for further detailed analysis of plant extract prior to drug development and its utilization in future.

Keywords: *Teucrium stocksianum*; Traditional medicine; Phytochemical constituents; Antioxidant

Introduction

Recently it has been observed that there is steady increase in human infections, especially in tropical and subtropical developing countries (Fenner *et al*., 2005). This may be either due to arbitrary use of antibiotic drugs or increase in resistance to these synthetic drugs (Mukherjee *et al*., 2002). Medicinal plants possess certain biocompounds, which are therapeutically important as precursors for yielding important drugs (Sofowora, 2008). The phytochemicals present in such plants produce certain physiological action on the human body and relieve disease (Iqbal and Hamayun, 2004; Akinmoladun *et al.*, 2007).

Phytochemicals are naturally found in plants. These are deriving color, flavor and smell of plants. Besides, they are playing key role in natural defense mechanism in plants against diseases. They are well recognized as therapeutic potential to human suffering and disease (Okwu, 2004). The most pronouncing phytochemicals include: alkaloids, tannins, flavonoids, and phenolic compounds, which possess bacteriostatic and bactericidal effects. Most of these biocompounds other than antimicrobials can also act as effective antioxidants (Venkata *et al*., 2012).

Teucrium stocksianum Boiss. is a member of Lamiaceae family. This aromatic perennial and woody herb is mostly found in the hilly area of northern Oman as well as United Arab Emirates (UAE), (Nadaf *et al*., 2003) and Pakistan (Ahmad *et al.*, 2002) and mountainous area of Iran (Mojab *et al.*, 2003). The plant may reach up to 15–30 cm with dense branching pattern and grey-green leaves. As medicament, mostly leaves as well young branches are used to treat various ailments such as gastro-intestinal, diabetes and as well as inflammatory conditions also (Radhakrishnan *et al*., 2001). Another study reported its use to treat feet syndrome and diabetes mellitus (Barkatullah and Hussain, 2009). Experimentally, extracts of this plant were also investigated as anti-ulcerogenic and cytoprotective (Islam *et al*., 2002). Besides, extracts of this plant is reported having analgesic and anti-inflammatory activities (Radhakrishnan *et al*., 2001). Other studies discovered its use as a blood purifier, epilepsy and hypertension (Ahmad *et al.*, 2002) and throat pain (Iqbal and Hamayun, 2004). Keeping into its medicinal use for various purposes, present study therefore was carried out for screening phytochemical and antioxidant activities of *Teucrium stocksianum* flower, locally growing in Malakand division Pakistan.

Materials and Methods

Plant Materials Collection and Identification

Teucrium stocksianum plant samples were gathered at

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flowering stage from the growing areas (Talash), Dir lower, Malakand Division, Khyber Pakhtun Khwa (KPK), Pakistan during May and June, 2011. Voucher specimen was also prepared from the fresh collection and identified through employing the Flora of Pakistan (Hedge, 1990). The voucher was maintained in the department of Botany, PMAS- Arid Agriculture University Rawalpindi for reference.

Preparation of the Extracts

Flowers were separated from the plant collection and washed 2 to 3 times through tap water and subsequently once distilled water was used to remove impurity and then shade air-dried. The dried material was blended to fine powder (80 mesh) with the help of a laboratory grinding machine and stored in refrigerator. The obtained powder material was then saturated in acetone, butanol, chloroform, ethyl acetate, ethanol, methanol, n-hexane, petroleum ether and water by shaking for 24 h at 37°C. The resultant macerate were then sieved using Whatman filter paper No. l. all these solvent extracts were evaporated and concentrated under reduced pressure at 40°C with the help of rotary evaporator. These extracts were then employed for the screening of phytochemicals.

Evaluation of Phytochemicals Screening Test

The chemical tests were carried out from nine different solvents based extracts of the *Teucrium stocksianum* flower using standard procedures for the identification of plant constituents by following the works of Egwaikhide and Gimba (2007).

Test for alkaloids: Extract in 0.2 mg was heated with 2% sulphuric acid for two minutes. Few drops of Dragendorff's reagent were added with the filtrate. An emergence of orange red precipitate was indicative of alkaloids (Egwaikhide and Gimba, 2007).

Test for tannins: A portion of plant extract was dissolved in aqua and warmed by using water bath. The test sample was then filtered and ferric chloride was added to it. Presence of dark green color indicates sign mark for tannins (Egwaikhide and Gimba, 2007).

Test for anthraquinones: An amount of 0.5 g of the test sample was heated with 10% hydrochloric acid for some time in water bath and was filtrated, cooled and the same volume of chloroform was mixed. Few drops of 10% Ammonia (NH_3) was mixed and heated. An emergence of rose-pink color was marked as presence of anthraquinone (Egwaikhide and Gimba, 2007).

Test for glycosides: Plant extract was mixed with HCl and then NaOH solution. Subsequently some drops of the Fehling's solution A and B were supplemented. An appearance of reddish precipitate revealed the incidence of glycosides (Egwaikhide and Gimba, 2007).

Test for reducing sugars: Plant extract was agitating in distilled water, filtered and simmered with some drops of the Fehling's solution A and B for some time. Presences of an orange as well as red type precipitate indicate the incidence of reducing sugars (Egwaikhide and Gimba, 2007).

Test for saponins: The plant extract in 0.2 g was dynamically shaken with 5 mL of distilled water and boiled. Appearance of bubble is evident of saponins (Egwaikhide and Gimba, 2007).

Test for flavonoids: Plant sample extract in 0.2 g quantity was mixed in diluted sodium hydroxide and after which HCl was pouring into it. Presence of yellow solution and then turning into colorless within few minutes showed the occurrence of flavonoids (Egwaikhide and Gimba, 2007).

Test for phlobatanins: An amount of 0.5 g extract was liquefied in some distilled water and then filtered. Subsequently it was heated with 2% hydrochloric acid solution. The emergence of red precipitation exposed the occurrence of phlobatanins (Egwaikhide and Gimba, 2007).

Test for steroids: About 0.5 g plant extract was added with 2 mL acetic anhydride along with 2 mL of H_2SO_4. The transformation of color from violet to green or blue showed the occurrence of steroids (Egwaikhide and Gimba, 2007).

Test for terpenoids (salkowski test): The plant extract taken in 0.2 g was mixed with 2 mL chloroform and then 3 mL concentrated sulphuric acid (H_2SO_4). An appearance of reddish brown color was indicated the occurrence of terpenoids. The percentage of crude extracts yield (%) was determined by the formula given below (Dellavalle *et al.*, 2011):

$$\text{Yield} = \frac{\text{Weight of lyophilized extract}}{\text{Weight of dried flower}} \times 100$$

Antioxidant Bioassay (DPPH Radical-scavenging Activity)

The evaluation of DPPH (Sigma-Aldrich, Germany: MW. 394.32) quenching activity was carried out according to the protocols defined by Yıldırım *et al.* (2003) with a slight amendment. For this purpose, in methanol 1mM DPPH radical solution was prepared. One mg/mL of plant extract was prepared in methanol. Besides, 6×10^{-5} mol/L DPPH in methanol was also prepared. One mL quantity was taken from the said solution that was amalgamate with 3 mL test solutions in methanol having different dilutions in the range of (20, 40, 60, 80,100 µg) and positive control (Ascorbic Acid: Merck: M.W. 176.13). All the test sample dilution and positive control were kept in a dark room After 30 min. at ordinary room temperature. The test samples and standard absorbance was recorded at 517 nm spectrophotometrically in triplicate and converted into the percentage antioxidant activity using the following percent radical scavenging equation:

$$\%\ \text{DPPH scavenging capacity} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100$$

Statistical Analysis

The statistical analysis was done for means and Standard Error of the Mean (SEM) for antioxidant and phenolic activities by using the following formula:

$$SEM = \frac{s}{\sqrt{n}}$$

Where, s = sample standard deviation and n= size (number of samples).

Assessment of Total Phenolic Content

The concentrations of phenolic compounds were measured following the protocol developed by Li *et al.* (2007). The diluted sample in 0.50 mL was mixed to 2.5 mL of 1:10 diluted form of (FCR) reagent. After 10 min, 2 mL of sodium carbonate saturated solution (about 75 g/L) was loaded to it. After 2 hours of interval at normal temperature, the mixture spectrophotometric absorbance was recorded at 760 nm. For comparison with plant extract Gallic acid a reference standard was and all the obtained results were shown as mg gallic acid equivalent (mg GAE)/g) with dry weight of plant tested material.

Results

Physical Properties

All solvent based extracts possessed brown color with some extent of variations ranging from Blackish to light brown (Table 1). Most of these extracts were semisolid and non-stick, however resinous to sticky and powdery characters were also recorded from petroleum ether, ethyl acetate and butanol, respectively. The highest weight of raw material (150 g) used for chloroform, water and ethyl acetate, followed by petroleum ether (130 g), acetone, n-hexane, (120 g each), methanol (118 g), whereas for ethanol and butanol low raw material (10 g each) was used. With reference to extract's weight, n-hexane yielded highest weight (21 g), followed by butanol (20 g), methanol (19.3 g), water (17 g), acetone (14.5 g), chloroform (13.5 g), petroleum (12.3 g) and ethanol (11.4 g), while rest of solvents extracted least yield. The percentage yield of extract was varied ranging from 1.73 to 29.08 with the maximum value recorded from n-hexane (21.6%). It was followed by butanol (20.3%), methanol (16.3%), acetone (12.08%), water (11.33%), petroleum ether (9.46%) and chloroform (9%). Interestingly the least values were detected from ethyl acetate (1.73%).

Phytochemical Constituents

Results of phytochemical screening of flowers of *T. stocksianum* are provided in Table 2. Maximum plant constituents were detected by methanol, followed by butanol (9), ethanol (7), acetone, ethyl acetate (5 each), petroleum ether, chloroform (4 each), water (3) and n-hexane (2). Saponins detected by all solvents, followed by tannin, reducing sugar (each 7), flavonoids (6), terpenoid (5), alkaloids, anthraquinone (4 each), whereas, steroids detected by 3 and phlobatannin as well as glycoside isolated by 2 solvents (Fig. 1).

Antioxidant Activity

Methanol solvent detected all selected plant constiteunts and therefore taken for antioxidant properties. Various concentrations/dilutions were made from the flower extract and used to assess antioxidant property. DPPH was used for

Table 1: Color, consistency and percentage yield of extracts from various solvents

Solvent used for extraction	Color of the extracts	Consistency	Weight of raw material (g)	Weight of the extract (g)	% Yield
Chloroform	Blackish Brown	Semisolid and non-sticky	150	13.5	9.0
Methanol	Brownish Black	Semisolid and sticky	118	19.3	16.3
n-hexane	Blackish	Semisolid and sticky	120	26	21.6
ethanol	Brownish Black	Semisolid and non-sticky	100	11.4	11.4
Petroleum ether	Blackish Green	Resinous and Sticky	130	12.3	9.46
Ethyl Acetate	Light brown	Gummy	150	2.6	1.73
Butanol	Dark Brown	Powder	100	20.3	20.3
Acetone	Dark Brown	Semisolid and non-sticky	120	14.5	12.08
Water	Brown	Semisolid and non-sticky	150	17.0	11.33

Table 2: Phytochemical screening of *T. stocksianum* flower using different solvents

Plant constituents	Solvent								
	Chl.	Meth.	n-hex.	Eth.	P. ether	E. Acet.	But.	Ace.	Water
Alkaloids	P	P	A	A	A	P	P	A	A
Tannin	A	P	A	P	P	P	P	P	P
Saponins	P	P	P	P	P	P	P	P	P
Anthraquinone	A	P	A	P	A	A	P	P	A
Steroid	A	P	A	P	A	A	P	A	A
Phlobatannin	A	P	A	A	A	A	P	A	A
Terpenoid	P	P	A	P	A	P	P	A	A
Flavonoids	A	P	P	P	P	A	P	A	P
Glycoside	A	P	A	A	A	A	A	P	A
Reducing sugar	P	P	A	P	P	P	P	P	A

P= Present and A= Absent

the evaluation of antioxidant activity. Result of the assay is expressed in Table 3 and Fig. 2. The flower extract of *Teucrium stocksianum* were diluted in the range of 20 µg to 100 µg to seek the optimum level of activity. The plant extract exhibited activity in the range of 222.58±0.001 to 412.82±0.003 at 20 µg to 100 µg concentration respectively, while control had 233.33±0.001 to 440.54±0.001 at the same concentrations. Comparing with control (Ascorbic acid), flower extract parallely expressed good activity (Fig. 2). At the dose of 100 µg, the DPPH value of flower was 412.82±0.003 that is near to the satndard (440.54±0.001).

The result of antioxidant properties of *Teucrium* flower was due to the presence of phenolic compound in the plant. Table 4 revealed that phenolic compounds were present in plants sample about half of the quantity of standard (gallic acid).

Discussion

Nine solvents were used for the evaluation of yield of extracts, detection of phytochmicals and antioxidant activity of the *T. stocksianum* flower. The highest weight of extract was obtained by n-hexane (21 g), followed by butanol (20 g), methanol (19.3 g), water (17 g), acetone (14.5 g), chloroform (13.5 g), petroleum (12.3 g) and ethanol (11.4 g). On the other hand, high percentage yield of extract also recorded from n-hexane (21.6%), followed by butanol (20.3%), methanol (16.3%), acetone (12.08%), water (11.33%), petroleum ether (9.46%) and chloroform (9%). The ethyl acetate yielded the least percentage yield (1.73%).

This study discovered various important phytochemicals in *T. stocksianum* flower like alkaloids, tannin, saponins, anthraquinone, steroid, phlobatannin, terpenoid, flavonoids, glycoside and reducing sugar (Table 1). These chemicals were detected variously by the selected solvents. The presence of alkaloids flower extracts of *Teucrium stocksianum* makes them recommendable for patient as alkaloids posses a significant pharmacological property. This study reported presence of alkaloid in the plant and endorse the findings of Awoyinka *et al.* (2007) who detected alkaloid in *Cnidoscolus aconitifolius.*

With reference to efficacy of individual solvents for the detection of phytochmicals, methanol was the most efficient one that detected all phytochemicals (Table 2). It was followed by butanol (9), ethanol (7), acetone, ethyl acetate (5 each), petroleum ether, chloroform, (4 each), water (3) and n-hexane (2). With reference to chemicals, saponins detected by all solvents, followed by tannin, reducing sugar (each 7), flavonoids (6), terpenoid (5), alkaloids, anthraquinone (4 each), whereas, steroids detected by 3 and phlobatannin as well as glycoside isolated by 2 solvents (Fig. 1).

For analyzing optimum antioxidant activity, the flower extract was diluted in various concentation.

Table 3: Antioxidant property of *Teucrium* flower extract and ascorbic acid

Concentration (µg)	Ascorbic Acid	Flower
20	233.33±0.001	222.58±0.001
40	263.63±0.000	250.87±0.001
60	316.66±0.001	292.15±0.001
80	412.82±0.001	344.44±0.000
100	440.54±0.001	412.82±0.003

Table 4: Total phenolic compound obtained from plant as compared with the standard

	A	B	C	Mean
Teucrium stocksianum flower	1.142	0.850	1.362	1.118±0.14
Gallic Acid	3.00	2.74	2.90	2.88±0.07

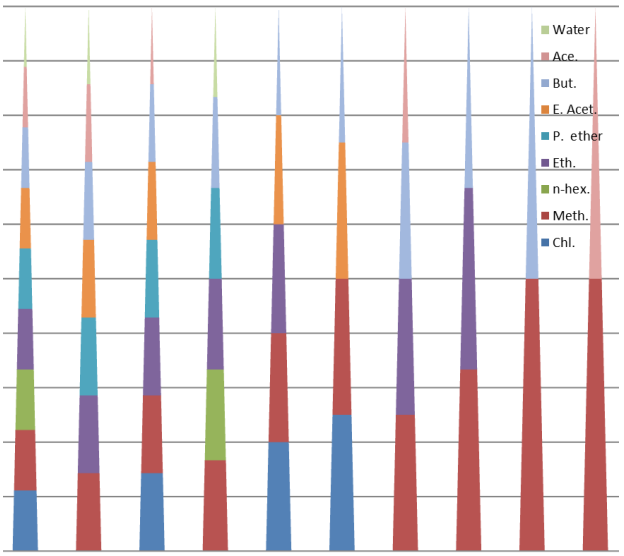

Fig. 1: Detection of selected phyto-constiteunts by nine solvents from *Teucrium* flower

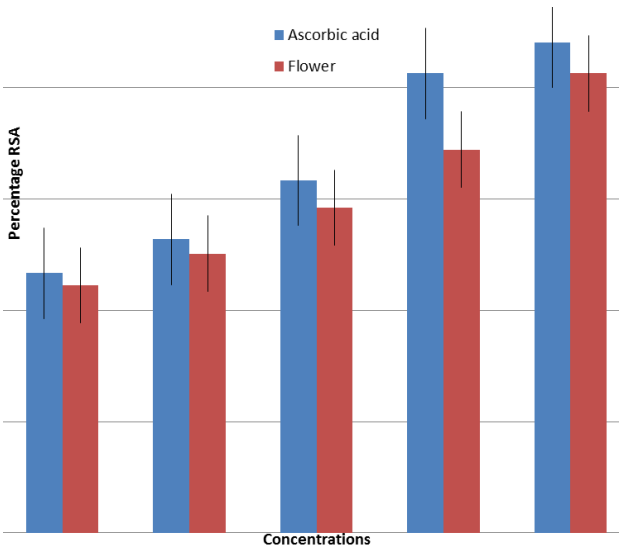

Fig. 2: Antioxidant properties of flower extract by DPPH method

The plant extrcat exhibited parallely good activity with the standard (Fig. 2). At 100 µg, the DPPH value of flower was 412.82 that is near to the control (440.54). Results revealed that flower extracts of *Teucrium stocksianum* contained flavonoid. These compounds are water soluble antioxidants as well as free radical scavengers responsible for preventing oxidative cell damage and possess anticancer properties and reduce the process of carcinogenesis (Okwu, 2004). The present study exhibited that steroids are frequently present in the flower extracts of *Teucrium stocksianum*. These compounds enhance sex and are of great importance in Pharmacy (Okwu, 2001). Our results are in the line of results by Iniaghe *et al*. (2009) who screened *A. hispida* and *A. racemosa* and report the presence of phlobatannin in these plants.

The microbial infections can cause extensive damage to cells and tissues (Kouam *et al.*, 2006). The detected compounds (Phenol) from plant extract exhibited marked radical scavenging activity against the stable DPPH free radical (Table 4). The activity of the phenolic compound was almost half of the Gallic acid. Although the crude extract of this plant showed a relatively low activity compared to that of reference material however, this may either be due to low concentration of this compound used in the extract or to an opposed effect with other phytochemicals of the extract.

It can be concluded that different solvents extracts of *Teucrium stocksianum* flower possess sufficient amount of phytochemicals such as flavonoids, saponins and phenolic compounds. These phyto-constituents demonstrate free radical scavenging and high antioxidant activities. The *in vitro* assay revealed that flower extracts can be used as a natural antioxidant that may help in preventing oxidative stress. This is preliminary study that requires a detailed study on fractionation components responsible for free radical scavenging and antioxidant activity. Thus, more study is required to segregate and to know about the antioxidant compounds normally present in the plant extract. On the other hand, *in vivo* antioxidant activity should also be evaluated prior to its clinical use. These results also endorsed the ethnobotanical use of this plant from the collected territory due to presence of various chemicals.

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