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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY DETERMINATION OF SOME MEDICINALLY IMPORTANT PLANTS OF BALOCHISTAN

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Abstract

The present study was conducted to investigate the existence of phytochemical constituents and antioxidant activity of medicinally important plants such as *Eremostachys thyrsiflora*, *Cousinia stocksii* and *Gaillonia macrantha* found in the north of Quetta, Balochistan. Phytochemical analysis of selected plants showed the presence of phenolics, alkaloids, steroids, flavonoids, saponins, terpenoids and tannins. The total alkaloids, phenolics, flavonoids and saponins found in the crude methanolic extracts of *Eremostachys thyrsiflora* were 8%, 3.2%, 5.9% and 1.5%, while in *Cousinia stocksii* the proportions were 7%, 2.2%, 6.8%, 1% and in *Gaillonia macrantha* these phytochemicals were found to be 8%, 2.8%, 0% and 0.5% respectively. *In-Vitro* antioxidant activities were studied spectrophotometrically by using total antioxidant activity, free radical scavenging power (DPPH) assay, ferric reducing antioxidant power assay and reducing power assays. The total antioxidant capacity of the *E. thyrsiflora, C. stocksii* and *G. macrantha* were determined and found to be 2.31, 5.44 and 9.15 mg ascorbic acid equivalent/ gram of plant extract respectively. The crude methanolic extracts of all these plants revealed antibacterial activity against *S. typhi, E. coli, K. pneumonia* and *S. aureus* and antifungal activity against *S. cerevisiae*. These results indicated that the crude methanolic extracts of the selected plants contained medicinally bioactive constituents which could be used for the treatment of different diseases and could also be used to reduce the oxidative stress.

Key words: Phytochemicals, Qualitative analysis, Quantitative analysis, Antioxidant activity, Spectrophotometer

Introduction

Plants have been used as the richest and valuable sources for traditional as well as modern medicines in Asia and Africa. The medicinal plants are good sources of drugs in traditional system for the cure of diseases. These medicinal plants contain some chemically active constituents, which can improve different physiological actions of the human body. The analysis of many chemical compounds found in plants are very important because the drugs, which were used as treatment of different diseases, were synthesized later after the study of constituents and their structure (Karunyadevi *et al*., 2009; Yusuf *et al.*, 2014).

In addition, large quantities of medicinal plants are available whose significance in the world of science is still to be investigated. In the past era, people used plants and their extracts in the discovery of new drugs which were beneficial for the treatment of numerous diseases. This resulted in the conversion most of the world's population i.e., 80-82% to use plants and their extracts as medicines for their health care. Plants can produce a large variation of natural products with various activities (Yadav & Agrawala, 2011). There are thousands of medicinal plant species which are used worldwide for the cure of different infections. These plant materials are utilized for the antimicrobial activity and numerous analyses have been done by scientists to explore their medicinal importance (Pradeep *et al.*, 2014).

Considering various biological activities, many medicinal plants have antioxidant activities which attract the attention of many researchers to study their part in the cure of different diseases such as diabetes, hypertension, atherosclerosis, cardiovascular and cancer (Vaghasiya *et al.*, 2011; Rebaya *et al.*, 2015). Recently, the analysis of medicinal plants with active antioxidant activity has gained attention due to increasing concerned for harmless and non-hazardous alternative antioxidants (Aliyu *et al*., 2013).

This study helps in enhancing the knowledge of people about the plants of Balochistan. In the present work, phytochemical analysis (qualitative and quantitative) and antioxidant properties were carried out in three plants like *Eremostachys thyrsiflora*, *Cousinia stocksii* and *Gaillonia macrantha* of Balochistan. The antioxidant properties of the extract of these three plants were measured by four *In vitro* methods such as total antioxidant activity (TAA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, ferric reducing antioxidant potential (FRAP) and Reducing power (RP).

Materials and Methods

Plant collection: Fresh whole plant materials of *Eremostachys thyrsiflora*, Voucher NO. (ET.FB.04) *Cousinia stocksii*, Voucher NO. (CS.FB.05) and *Gaillonia macrantha*, Voucher NO. (GM.FB.06), were collected from Quetta district. These plants were taxonomically identified by Professor Dr. Rasool Bashk Tareen, Department of Botany University of Balochistan, Pakistan and deposited at Herbarium Botany Department, University of Balochistan, Quetta. After shade drying the plants, samples were crushed into fine powder and transferred into airtight containers for further analysis.

Preparation of extract: For preparing the extract 500 g of the three plant materials were macerated with methanol and allowed to stand for a week. Then these were filtered to remove the solid substance and filtrates were evaporated to gain crude extract, under reduced pressure by using rotary evaporator at 35°C. These extracts were dried by using freez-drier and for further analysis were stored in refrigerator at 5°C.

Phytochemical screening: The phytochemical test for the presence and identification of biologically active components in the whole plant samples under analysis were carried out in powder samples and in methanolic extracts as well by using the standard procedures as reported in literature (Khan *et al.,* 2011; Vaghasiya *et al*., 2011; Yusuf *et al.,* 2014).

Qualitative analysis

Alkaloids: 1 g of plant extract was treated with 10 mL dilute HCl, gently warmed and filtered. Filtrate was divided into three parts and allowed to react separately with reagents (Mayer's, Dragendroff's and Wagner's), presence of alkaloids were indicated by the appearance of precipitates and turbidity.

Flavonoids: 1 g of plant extracts was made fat free by treating it with petroleum ether. Then this defatted extract was treated with 30 mL ethanol (80%) and filtered to remove any un-dissolved material. The filtrate was used as: (a) 4 mL of filtrate was treated with equal volume of potassium hydroxide (1%) and yellow colour was observed for the presence of flavonoids. (b) 5 mL of filtrate was treated with 6 mL of dilute NH_4OH solution and 1 mL Conc. H_2SO_4 was added dropwise. Presence of flavonoids was confirmed by the formation of yellow colouration.

Phenols: 1 g of plant extract was dissolved in boiling distilled water and treated with 5 mL of lead acetate (10%). Phenols were confirmed by the formation of bulky precipitates.

Saponins: 1 g of the plant extract was dissolved in boiling distilled water and allow to cool. The solution was shaken. Saponins presence were indicated by the froth formation.

Steroids: a) 4 mL of plant extract was added to equal volume of chloroform and 1 mL of Conc. H_2SO_4 was added dropwise. Brown ring confirmed the presence of phytosteroids. (b) 1 g of methanolic extract was treated with 3 mL of acetic anhydride and then 2 mL Conc. H_2SO_4 was added dropwise. Brown ring confirmed the presence of steroids.

Terpenoids: 1 g of plant extract was dissolved in warm distilled water further treated with 2 mL of chloroform and then 2 mL of Conc. H_2SO_4. An interface with a reddish-brown coloration indicated the terpenoids presence.

Tannins: 1 g of plant extract was dissolved in boiling distilled water and filtrated. Few drops of ferric chloride solution (0.1%) was added to the filtrate. Tannins presence was confirmed by the formation of intense bluish-green colour.

Coumarins: 2 mL of methanolic extract was added to equal quantity of 10% sodium hydroxide. Coumarins presence was confirmed by the formation of yellow colour in solution.

Glycosides and carbohydrates: 1 g of plant extract was dissolved in boiling distilled water and filtered. The filtrate was divided into two equal parts for further analysis:

a) Molisch's test: The first part of filtrate was treated with 4 mL of alpha naphthol (10 % alcoholic) solution, further addition of 4 mL Conc. H_2SO_4 led to the formation of bluish violet zone confirming the presence of carbohydrates and glycosides.

b) Fehling's test: The second part of filtrate was treated with 3 mL each of Fehling's solution (Solution A and B). The mixture was heated, and reddish-brown precipitates indicated the presence of reducing sugars.

c) Cardiac glycosides: 3 mL of plant extract was reacted with equal volume of glacial acetic acid and 5-6 drops of 5% ferric chloride solution, and then 3 mL of Conc. H_2SO_4 was added drop wise. Cardiac glycosides presence was confirmed by brown ring formation at the interface.

Quinones: 4 mL of plant extracts were treated with 3 mL of Conc. H_2SO_4. Red to blue colour formation indicated the presence of quinones.

Anthraquinones: 2 mL plant extracts were treated with 1mL of 2% HCl. Anthraquinones gave red colour precipitates.

Phlobatannins: 2 mL of plant extracts were treated with 0.5 mL of 10 % ammonia solution. Phlobatannins gave pink color precipitates.

Fats: Saponification test was performed to detect fats. 5 mL of 0.5N potassium hydroxide in methanol and 10 mL of plant extract were combined and few drops of phenolphthalein were added to the mixture. The mixture was heated on water bath for 2hrs. The soap formation indicated the presence of fats.

Xanthoprotein: 2 mL of plant extract was added to 0.5 mL of Conc. HNO_3 and 0.5 mL ammonia solution. Reddish orange precipitates formation indicated the presence of xanthprotein.

Resins: 2 mL of plant extracts were treated with 5-6 drops of acetic anhydride solution and then 1 ml Conc. H_2SO_4 was added. Resins gave colouration ranging from orange to red.

Carboxylic acid: 2 mL of plant extract was treated with equal volume of sodium bicarbonate solution. Effervesces (due to CO_2) indicated the presence of carboxylic acid.

Anthocyannins: 3 mL plant extracts were treated with 3 mL of 2N HCl and few drops of NH_3 solution was added. Anthocyannins gave pinkish red colour.

Emodins: 3 mL of plant extract was treated with 3mL NH_4OH followed by addition of 3 mL of benzene. Red coloration indicated the presence of emodins.

Volatile oil: 4 mL of plant extract was treated with 2mL dilute NaOH solution and then 3 mL of dilute HCl was added. White precipitates formation showed the presence of volatile oil.

Quantitative analysis

Determination of total alkaloids: Total alkaloids content was determined by the method of Harborne (1973) with few modifications. Powder plant samples (5 gram) were treated separately with 250 mL of ethanolic solution of acetic acid (10%). The mixtures were left for about 5 h at room temperature and filtered to gain the extracts. Then these extracts were concentrated about one-fourth by using a water bath. For the formation of precipitates Conc. NH_4OH was added drop-wise. The whole solution was allowed to stand for half hour for the complete formation of precipitates. The solution was filtered to collect the precipitates.

Determination of total phenolics: Total phenolics content was determined by the method of Hagerman *et al.,* 2000 with few modifications. Powder plant samples (5 gram) were treated separately with 250 mL of n-Hexane twice for 3 hours each. The filtrate was removed from the residue for the preparation of fat free sample. Then residue was heated on water bath for 20 mints with 100 mL diethyl ether twice and cooled at room temperature. The mixture was filtered, and filtrate was transferred into a separated funnel. In the funne l 50 mL of NaOH (10%) solution was added twice to the filtrate and was shaken well each time. Then organic layer was separated and washed twice with deionized water. HCl (10%) solution was added to the aqueous layer to make it acidic (pH 4) and 100 mL dichloromethane. Subsequently the organic layer was collected and dried over water bath and then weighed.

Determination of total flavonoids: Total flavonoids content was determined by using the method of Bohm & Kocipai-Abyazan (1994). Powder plant samples **(**5 gram) were extracted at room temperature with 200 mL of methanol (80%) twice. The mixture was then filtered to remove the solid substance and the filtrate was evaporated to gain a constant weight on dryness.

Determination of saponins: Total saponin content was determined by the method of Obadoni & Ochuko (2002) with few modifications. Powder plant samples (20 gram) were separately treated with 250 mL of ethanol (20%). The solution was heated on a water bath for about 3 h at 50ºC with constant stirring. The mixture was then filtered, and same process was done with the residue. The extracts were combined and concentrated at about 90ºC on water bath. The concentrated extracts were treated with 40 mL of diethyl ether in a separating funnel and aqueous layer was collected. About 100 mL of n-butanol was added twice. The organic layer was collected and washed with 20 mL of NaCl (5%) solution. The organic layer was evaporated on a water bath. The dry material was then weighted.

Antioxidant activity

Total antioxidant activity (TAC): The determination of TAC was carried out by using the phosphomolybdenum method (Sadhu *et al.,* 2003). Plant extract (0.5 mL) was mixed with a mixture of 5 mL of reagent solution (0.6 M sulfuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate). The test tubes having the reaction mixture were incubated for 90 min at 95°C. Then the sample mixtures were cooled at room temperature and absorbance was measured at 695 nm using UV/VIS spectrophotometer (UV-VIS 1700, Shimadzu). Blank was prepared by the same method using methanol in place of extract. TAC is represented as the mg of equivalent of ascorbic acid.

Free radical scavenging power by using DPPH method: The determination of free radical scavenging activity was done by using McCune & Johns (2002) method. In this assay, 1 mL of plant extract (various concentration) was added with 2 mL of compound 1,1-diphenyl-2-picrylhydrazyl (DPPH) (0.004%) in methanol. This mixture was incubated in dark at room temperature for 1 hour. Then the absorbance was measured at 517 nm using UV/VIS spectrophotometer (UV-VIS 1700, Shimadzu). Methanol was used as blank in this method. An equal volume of DPPH and methanol except sample was used as control. In this method, ascorbic acid served as positive control. Following formula was used for the calculation of percentage of inhibition:

$$\% \text{ Inhibition} = (A_c - A_e / A_c) \times 100$$

where; A_c and A_e is the absorbance of control and plant extracts respectively

Ferric reducing antioxidant power (FRAP): The determination of FRAP was carried out by using the method of Maizura *et al*., 2011 with few modifications. Preparation of FRAP reagent was done by using acetate buffer (1.6 g sodium acetate and 8 mL acetic acid made up to 500 mL) (pH 3.6), 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM ferric chloride solution in ratio of 10:1:1 (volume/volume) respectively. The freshly prepared FRAP reagent was warmed to 38°C. A total of 0.5 mL plants extract and 4 mL of the FRAP reagent were added. The absorbance was measured by using UV/VIS spectrophotometer (UV-VIS 1700, Shimadzu) at 593 nm. Standard curve of ascorbic acid was made utilizing the same method. The results are shown as mg ascorbic acid /g of extract sample.

Determination of reducing power (RP): The RP of plant extract was measured by using the method of Jayanthi & Lalitha, 2011. Plant extracts (various concentrations) in methanol were treated with 2.5 mL phosphate buffer and 2.5 mL potassium ferricyanide. This solution was incubated at 50°C for half hour. After attending room temperature. Then 10 % trichloro acetic acid (2.5 mL) was mixed with the solution. The mixture was centrifuged at 3000 rpm for 5-10 minutes. A volume of 3 mL of upper layer of the solution was diluted with 3 mL of distilled water and 1 mL ferric chloride (0.1%) freshly prepared solution was added to the solution mixture. The absorbance was measured at 700 nm using UV/VIS spectrophotometer (UV-VIS 1700, Shimadzu). Various concentration of ascorbic acid was used as standard. Control was made in similar manner excluding extract. Higher reducing power of reaction mixture is indicated by the high absorbance.

Antimicrobial activity

Antibacterial activity: Agar well method was used for the determination of antibacterial activity of selected plant samples (Obeidat *et al*., 2012) using four strains of bacteria: *S. typhi, E. coli, K. pneumoniae* and *S. aureus.* Pure dimethyl sulfoxide (DMSO) and amoxicillin were used as control and standard drug respectively. The zone of inhibition was measured in millimeters (mm) after 24 hours.

Antifungal activity: Agar well method was used for the determination of antifungal activity of selected species (Saha & Paul, 2012) using yeast (*S. cerevisiae)* as fungal strain. Pure dimethyl sulfoxide (DMSO) and amoxicillin were used as control and standard drug respectively. The inhibition zones were measured in mm after 48 hours.

Results and Discussion

The qualitative phytochemical screening of three selected plant extracts are shown in 'Table 1'. Phenols, flavonoids, alkaloids, steroids, saponins, terpenoids, tannins, coumarins, glycosides, carbohydrates, fats, xanthoproteins, carboxylic acids and volatile oils were present in *E. thyrsiflora* but quinones, anthraquinones, phalobatannins, resins, anthocyanins and emodins were absent. In *G. macrantha*, all phytochemical constituents were presents except flavonoids, coumarins, anthraquinones, phalobatannins, resins, anthocyanins and emodins. In *C. stocksii*, all the phytochemical constituents were present except terpenoids, anthraquinones, phalobatannins, xanthoproteins, resins and anthocyanins. These results showed that all the three species were rich in phytochemicals. This study also encompasses some quantitative tests for the determination of concentrations of alkaloids, phenols, flavonoids and saponins (Table 2). The greater number of alkaloids were found in both *E. thyrsiflora* and *G. macrantha*, but lower amount was found in *C. stocksii.* The phenolics were observed in high quantity in *E. thyrsiflora* followed by *G. macrantha* while low quantities were found in *C. stocksii.* High number of flavonoids were found in *C. stocksii* and low in *E. thyrsiflora* but none were detected in *G. macrantha.* Saponin was recorded to be present in high amount in *E. thyrsiflora* followed by *C. stocksii* but low amount was found in *G. macrantha.* Qualitative and quantitative studies revealed that all the three plant extracts were rich in phytochemical constituents and could play very important medicinal and physiological role (Khan *et al.*, 2011; Yadav & Agarwala, 2011). The phenolics are one of the largest chemical components of plants secondary metabolites. These are found to be involved in the biological activities such as anti-inflamation, cardiovascular protection, antiapoptosis and anticarcinogen (Han *et al*., 2007). Many researchers revealed that the antioxidant activities of the medicinal plants depend on the amount of phenolic compounds present in them (Angeloni *et al*., 2015). Flavonoids are hydroxylated phenolic compounds and are reported to show antimicrobial activity (Yadav & Agarwala, 2011), cardiovascular disease, antiallergic, anticancer and antithrombotic activities (Samiullah *et al.*, 2015). They are also found to show effective antioxidant property (Yadav & Agarwala, 2011). The alkaloids are reported to be used as medicines for more than 4000 years ago and play an important role in the formation of modern drug industries. Several researchers have reported that alkaloids show antibacterial, antihypertensive, antispasmodic and gastrointestinal activities (Amirkia & Heinrich, 2014). Steroids have been reported to show antibacterial activity. Saponins are reported to exhibit immune stimulating and anti-inflammatory activities (Okwu, 2005).

The TAC in the methanolic extracts of three selected plant samples were calculated from linear regression equation (y = 0.9277 x-0.008 with R^2=0.9993, where y was the absorbance and x was the concentration of ascorbic acid in mg/mL) of the calibration curve (Fig. 1a). The total antioxidant capacity of the *E. thyrsiflora, C. stocksii* and *G. macrantha* was 6.14, 8.00 and 13.27 mg ascorbic acid equivalent/ gram of plant extract respectively (Table 3). The antioxidant activity of the selected plant samples was also determined by using ferric reducing antioxidant power. In this method the antioxidant activity of plant extracts was measured by their power to reduce ferric ions to ferrous ions (Aliyu *et al.*, 2013).

The ferric reducing antioxidant power in methanolic extract was calculated using regression line equation (y = 3.0941x+0.5138 with R^2 =0.9195, where y was the absorbance and x was the concentration of ascorbic acid in mg/mL) of the calibration curve (Fig. 1b). The antioxidant activity of the *E. thyrsiflora, C. stocksii* and *G. macrantha* was 2.18, 2.48 and 3.02 mg ascorbic acid/ gram of plant extract respectively (Table 3). The scavenging power of the selected three plant samples were related with the ascorbic acid and used as standard. The result of plant extracts and ascorbic acid reported depend on their concentrations (Fig. 2). The reducing power of the selected three plant samples were found to be linked with increasing absorbance related with standard ascorbic acid (Fig. 3). Various studies have shown that for determination of antioxidant activity of plant extract quantitatively and accurately, different methods should be used (Rebaya *et al.*, 2015). The measurement of TAC is based on the formation of phosphomolybdenum complex. In this method plant extract reduce the Mo (VI) to a green colour Mo (V) complex (Uddin *et al.*, 2013; Do *et al.*, 2014). It evaluates both water and fat-soluble antioxidants (Aliyu *et al.*, 2013).

Table 1. Qualitative analysis of methanolic extracts of *E. thyrsiflora, C. stocksii,* and *G. macrantha*.

Phytochemical constitutes	*E. thyrsiflora*	*C. stocksii*	*G. macrantha*
Alkaloid	+	+	+
Phenolic	+	+	+
Flavonoid	+	+	-
Saponin	+	+	+
Steriod	+	+	+
Terpenoid	+	-	+
Tannin	+	+	+
Coumarin	+	+	-
Glycoside and carbohydrate	+	+	+
Quinones	-	+	+
Anthraquinones	-	-	-
Phlobatannins	-	-	-
Fats	+	+	+
Xanthoprotein	+	-	+
Resins	-	-	-
Carboxylic acid	+	+	+
Anthocyanins	-	-	-
Emodins	-	+	-
Volatile oil	+	+	+

* Present + and Absent -

Table 2. Quantitative analysis of methanolic extracts of *E. thyrsiflora, C. stocksii,* and *G. macrantha*

Phytochemical constitutes (Content w/w %)	*E. thyrsiflora*	*C. stocksii*	*G. macrantha*
Alkaloid	8	7	8
Phenolic	3.2	2.2	2.8
Flavonoid	5.9	6.8	N. D
Saponin	1.5	1	0.5

*N. D= not detected

Table 3. Determination of total antioxidant activity.

Parameter	Regression line	Quantitaive value (mg ascorbic acid equavalent/g palnt extract)		
		E. thyrsiflora	*C. stocksii*	*G. macrantha*
Total antioxidant capacity	y = 0.9227x-0.008	2.31	5.44	9.152
Ferric reducing antioxidant power	y = 3.0941x+0.5138	2.18	2.48	3.02

Table 4. The DPPH antioxidant activity of the plant extracts of *E. thyrsiflora, C. stocksii,* and *G. macrantha*.

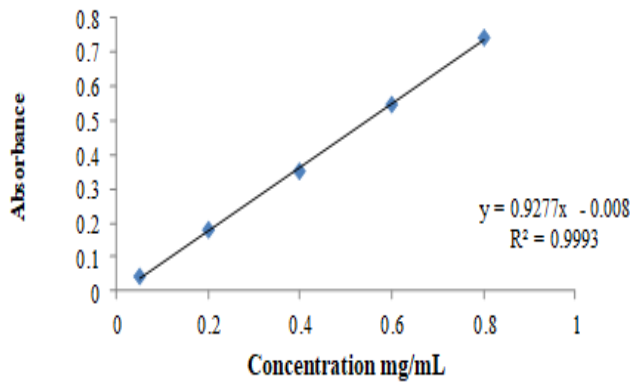
Concentration (mg/mL)	*E. thyrsiflora*	*C. stocksii*	*G. macrantha*	Standard
0.05	4.51 ± 0.16	9.22 ± 0.49	12.66 ± 0.31	98.50
0.2	27.25 ± 0.27	32.83 ± 0.33	53.43 ± 0.43	98.50
0.4	59.01 ± 0.30	70.17 ± 0.27	82.83 ± 0.25	98.93
0.6	88.63 ± 0.53	86.91 ± 0.36	88.41 ± 0.34	98.36
0.8	96.57 ± 0.26	92.70 ± 0.13	91.41 ± 0.15	99.36

*Each value is expressed as mean± standard deviation, n=3

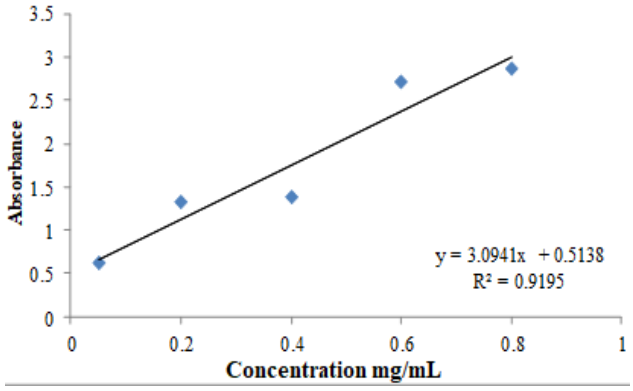
Table 5. The Reduction Power of plant extracts of *E. thyrsiflora, C. stocksii,* and *G. macrantha*.

Concentration (mg/mL)	*E. thyrsiflora*	*C. stocksii*	*G. macrantha*	Standard
0.05	0.053 ± 0.008	0.011 ± 0.003	0.096 ± 0.01	0.587
0.2	0.25 ± 0.035	0.40 ± 0.017	0.703 ± 0.017	0.958
0.4	0.71 ± 0.016	0.72 ± 0.043	0.962 ± 0.04	1.294
0.6	0.97 ± 0.056	1.02 ± 0.013	1.416 ± 0.006	2.135
0.8	1.33 ± 0.294	1.46 ± 0.017	2.045 ± 0.029	2.894

*Each value is expressed as mean± standard deviation, n=3



a). Total antioxidant capacity



b). Ferric reducing antioxidant power

Fig. 1. Standard calibration curve for the determination of (a) total antioxidant activity and (b) ferric reducing antioxidant power of *E. thyrsiflora*, *C. stocksii*, and *G. macrantha*.

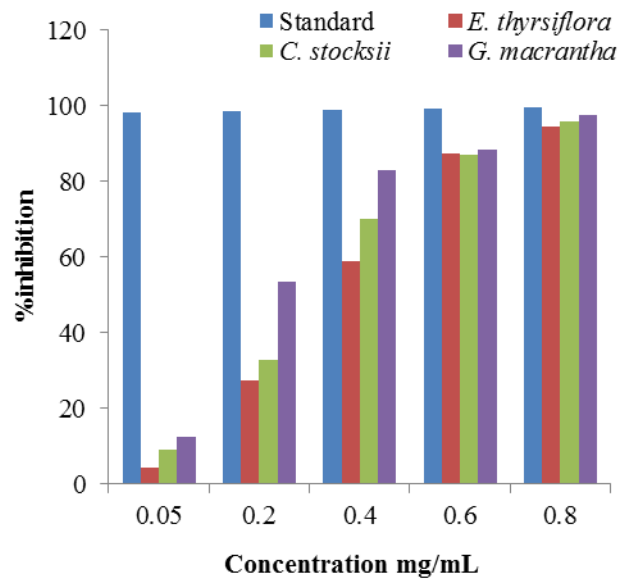


Fig. 2. The DPPH antioxidant activity of the plant extracts of *E. thyrsiflora*, *C. stocksii*, and *G. macrantha*.

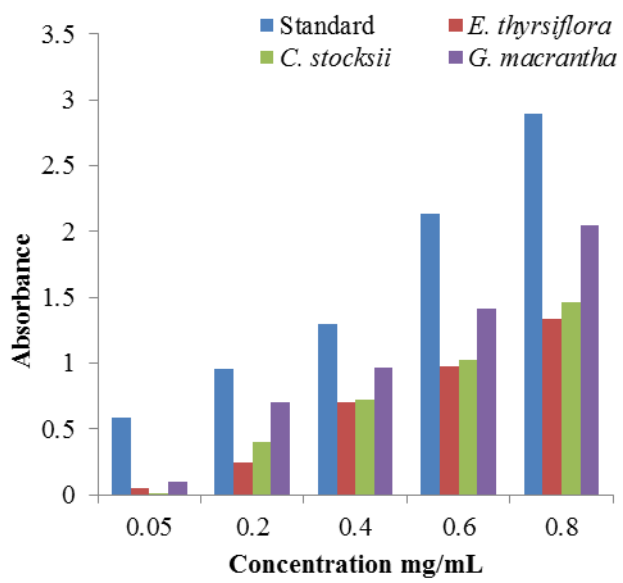


Fig. 3. The Reduction Power of plant extracts of *E. thyrsiflora*, *C. stocksii*, and *G. macrantha*.

Table 6. Results of biological activity method used was agar well method.

Samples	(Inhibition zone mm) *				
	E. coli	***S. typhi***	***K. pneumoniae***	***S. aureus***	***S. cerevisiae* (yeast)**
E. thyrsiflora	18	11	21	22	21
C. stocksii	15	14	21	19	18
G. macrantha	15	13	22	20	20
Amoxicillin	28	26	28	25	32
DMSO	0	0	0	0	0

*Well size= 6mm

The FRAP method is simple and rapid. Its reaction is consistent and associated to the molar concentration of antioxidants found in the plant extracts (Uddin *et al.*, 2013). This method measures antioxidant activity of the compounds in the reaction medium due to its reducing ability (Lahouar *et al.*, 2014). DPPH is widely used as stable organic radical used to analyze the antioxidant activities of plant samples (Onyeulo *et al.*, 2018) with an absorption maximum at 517 nm (Fig. 2). The absorbance value of DPPH decreased with the accepting of hydrogen atom resulting in the change of colour from purple to yellow. It is sensitive enough to detect active compounds at even low concentration and can also analysis many samples in short period of time (Ferreira *et al.*, 2007; Uddin *et al.*, 2013; Karafakioglu *et al.*, 2018). The DPPH of plant extracts and standard ascorbic acid were calculated (Table 4). The reducing power measures the inactivation of oxidants by describing as redox reactions.

The reducing power of chemical constitutes function as an important indicator for their potential antioxidant activity. Increased reducing power of the compound is indicated by the high absorbance (Uddin *et al.,* 2013) which can be seen in case of the three selected plant extracts (Fig. 3). Their relative reduction power and standard ascorbic acid were calculated and listed in 'Table 5'.

The antibacterial and antifungal activities of the selected plant extracts were carried out by Agar well assay and the activities were compared with the standard drug amoxicillin. These assays are already used by many researchers for the antimicrobial i.e. antibacterial and antifungal activities of medicinally active plants (Irshad *et al*., 2011; Obeidat *et al*., 2012; Saha & Paul, 2012). All the three plant extracts showed positive activity against four strains of bacteria i.e., *E. coli, S. typhi, K. pneumoniae* and *S. aureus*. Among these plants, *E. thyrsiflora* showed highest activity followed by *G. macrantha* and *C. stocksii*. Antifungal activity was highest for *E. thyrsiflora* than *C. stocksii* and least in *G. macrantha* (Table 6). Antimicrobial activity is enhanced by the antioxidant compounds due the toxic effect of antioxidant against bacterial cells (Akbar *et al*., 2014). The plants that contain high amount of antioxidant compounds show high antibacterial activities.

Conclusion

The results of selected plant samples *Eremostachys thyrsiflora, Cousinia stocksii and Gaillonia macrantha* indicated the presence of abundant phytochemical constituents which could be used in the pharmaceutical industries for the production of drugs. These drugs are used for the treatment of different diseases and also reduce the oxidative stress. High amounts of antioxidants were also investigated. Such plants can be used as rich sources of antioxidants. The biological activities of all the three plants also confirmed their significance. In future, this study will help the researchers to isolate bioactive compounds from these selected plants.

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