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Antioxidant Potential of Selected Medicinal Plants of Trans- Himalayan Region

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Abstract: ***Considering the growing interest in possessing the antioxidant capacity of medicinal plants; an effort has been carried to recognize the effect of antioxidants in different plants of Trans-Himalayan region by using DPPH (1, 1–diphenyl-2-picrylhydrazyl) radical assay, ABTS (2, 2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt assay, FRAP (ferric reducing antioxidant power), total phenolic content (TPC), total flavonol, total proanthoanthocyanidin and total flavonoid content. Extraction of antioxidant compound from plants part was done with 80% methanol. The inhibitory concentration (IC_{50}) in DPPH ranged from 2.306 to 8.604µg/mg. (IC_{50}) in ABTS ranged from 1.38 to 7µg/mg and FRAP ranged from 102.66 to 385.76 $FeSO_4.7H_2O$. Highest antioxidant activity was observed in Saussaria lappa. TPC were expressed as gallic acid equivalent (GAE) in µg per mg weight. Total flavonol, total proanthoanthocyanidin and total flavonoid content were expressed as µg of quercetin equivalent per milligram of dry weight. Highest polyphenol and flavonoid content was found in Arnebia euchroma (244.58µgGAE/mg) and (17.77µgQE/mg) respectively. Highest flavonol content was found in Inula racemose (70µgQE/mg) and proanthocyanidin content was highest in R.webbanium (108.33µgQE/mg).***

Keywords: Antioxidant potential, Polyphenol, Medicinal plant, Radical scavenger, Trans- Himalayan

1. Introduction

Recently there has been an emerging interest globally for identification of antioxidant compounds which are of pharmaceutical and nutraceuticals importance which can be used in preventive medicine and the food industry. Antioxidant activity exhibited by plants might be due to their phenol compounds [1]. Antioxidant plays a vital role by preventing from oxidative damage due to free radical present in the body. A free radical is an atom, molecule or anion that contains one or more unpaired electrons and which is more reactive than their parent species. Free radicals which are formed in the body can realize oxygenation or reduction reactions that damage many biological molecule and cause food rancid [2]. Plants produce significant amount of antioxidants compounds to prevent against the oxidative stress caused by free radicals and nascent oxygen and thus they represent a potential source of new compounds with antioxidant activity. The use of various artificial antioxidants such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), gallic acid tertiary and butylated hydroquinon have been reported to cause several negative health effects [3], [4]. Therefore, strong restrictions have been made on their application and there is an erg to substitute them with naturally occurring antioxidants. The increasing demand quest for natural antioxidants for pharmaceuticals, nutraceuticals and cosmetic uses has become one of the major industrial and scientific research challenges. These create an environmental issue of the extinction of many of the medicinal plant species due to indiscriminate harvesting in large quantity from the wild to meet the increasing demand for the crude drugs. As a result of this and lack of cultivation many of the medicinal plant species has been reported threatened species. Efforts to gain knowledge regarding the antioxidants power from plants and to know their potential are therefore on the increase. The traditional herbal medicine in India and across the world has its own importance in human health care and prevention of many diseases. One of the oldest medical systems in the world that provides potential leads is ayurveda that find potent and therapeutically useful compounds from plants. The Trans-Himalayan region is the land of several medicinal plants. Some of the medicinal plants like *Saussaria lappa, Rheum webbanium, Arnebia euchroma, Inula racemosa* are used as healing sources, which thereby helps in the emerging area of antioxidant research of medicinal plants**.** Their rhizomes, stems, leaves, bark, are important parts which have medicinal properties. The root powder of *Inula racemosa* is used for hypoglycemic and hypocholesterolemic in human subjects and also considered specific for cough, dyspnea, asthma, pleurisy, tuberculosis and chest pain especially pre cordial pain. *Rheum webbanium* roots are rich sources of drugs rhein, emodin and rutin which are used as laxative, tonic and purgative. The powder is sprinkled on ulcers and wounds for quick healing and also is used for cleaning teeth [5]. Another medicinal herb *Saussurea lappa* its principal constituents, costunolide dehydrocostus used as anti-ulcer, anti-carcinogenesis. Another potent plant is *Arnebia ecrhoma*; it has have been reported to have anti-inflammatory, antimicrobial and anti-tumor activities and thus considered as one of the important compound for potentially medicinal use [6]. To the best of the knowledge, there is no report on these antioxidant properties in methanolic extracts of the mentioned species in the literature. Thus, the present investigation focuses (i) *in vitro* antioxidant activity profiles of these plant extracts by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) (ii) total phenolic compounds content of the plant extracts as gallic acid equivalents(GAE) and total proanthocyanidin, total flavonol and total flavanoid compound as quercetin equivalent(QE).

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2. Materials and Methods

Chemicals

2,4,6-tripyridyl-s-triazine (TPTZ), sodium carbonate (Na_2CO_3), ferrous sulphate ($FeSO_4.7H_2O$), aluminium chloride ($AlCl_3$), Folin-Ciocalteu's phenol reagent, gallic acid anhydrous, 1-diphenyl-2-picrylhydrazyl radical (DPPH), naphthyethylenediamine dihydrochloride, sodium acetate ($C_2H_3NaO_2$), potassium persulfate ($K_2S_2O_8$), glacial acetic acid, butylated hydroxytoluene (BHT), ascorbic acid, 2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), quercetin, vanillin, hydrochloric acid, n-hexane, chloroform, ethanol, potassium chloride (KCl), ferric chloride ($FeCl_3 \cdot 6H_2O$), egg yolk emulsion, sulfanilic acid ($C_6H_7NO_3S$), sulphuric acid, methanol and sodium carbonate. All other chemicals used including solvents were of analytical grade.

Plant materials

The plants were collected from medicinal garden, DIHAR, Leh.

Preparation of extract

Fresh roots from the plants were shade dried and milled to fine powder using a mechanical grinder. Dried powder plant material (75g) root was taken in thimble and was extracted with 600ml, 80% methanol separately for 24 hrs by soxhlet apparatus. The extract were concentrated by rotary evaporator under reduce pressure at 40°C. Further the concentrated extract was oven dried to obtain dry power form of the crude extract and these were stored in -20°C for future analysis.

3. Procedure for determination of antioxidant

Determination of 1, 1- Diphenyl-2-picrylhydrazyl (DPPH) Assay

The effect of different extract on DPPH radical was determined using method described previously [7]. A solution of 0.135mM DPPH in methanol was prepared and 100µl of the working solution was mixed with 100µl of the different extract. The concentration of plant extracts was ranged from 5 to 500µg/ml. The reaction mixture was vortexed vigoursly and rested in the dark for 30 minute at room temperature. The absorbance of the mixture was measured spectrophotometrically at 517nm. Ascorbic acid (AA) was used as standards. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging capacity (%) = $[(Abs_{control} - Abs_{sample})] / (Abs_{control})] \times 100$

Where, $Abs_{control}$ is the absorbance of DPPH radical and methanol;

Abs_{sample} is the absorbance of DPPH radical with sample extract or standard. The (IC_{50}), half maximal inhibitory concentration for scavengers (radical scavenging $concentration_{50}$ or RSa_{50}), define as the amount of antioxidant required to decrease the initial DPPH concentration by 50%, termed as efficiency concentration (EC_{50}). The effectiveness of antioxidant and radical scavenging capacity demonstrated as antiradical power (ARP) were calculated [8], [9], [10], [11]. The RSa_{50} value was determined by plotting the scavenging capacity against the logarithm of sample concentration. The EC_{50} was calculated from the following formula: $EC_{50} = IC_{50}/$ [DPPH] in µg/ml:

The ARP was also determined as follows: ARP = $1/ (EC_{50} X 100)$

The results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC) [12] using the following equation: AEAC = $(IC_{50(AA)} / IC_{50(sample)}) X 10^5$

Determination of ABTS Radical Scavenging Assay

The ABTS assay was performed as described by Pellegrini and proteggente [13]. The stock solutions containing 2.4mM potassium persulfate(PPS) solution and 7mM ABTS solution were prepared. The working solution was prepared by mixing equal quantities of the two stock solutions and rested them for 12 hours at room temperature in dark for its reaction. Thereafter the solution was diluted by the addition of 1 ml $ABTS^+$ solution with 60ml of methanol to obtain an absorbance of 0.706 ± 0.001 units at 734nm using spectrophotometer. The concentration of plant extracts was ranged from 5 to 500µg/ml. Plant extracts (100µl) and 100µl of the $ABTS^+$ solution was allowed to react and the absorbance was taken at 734nm after 7 min incubation at 25°C in 96 well plate. The $ABTS^+$ scavenging capacity of the extracts was compared with that of BHT. The radical scavenging percentage of different extract was calculated as follows:

ABTS radical scavenging capacity (%) = $[(Abs_{control} - Abs_{sample})] / (Abs_{control})] \times 100$

Where, $Abs_{control}$ is the absorbance of ABTS radical + methanol; Abs_{sample} is the absorbance of ABTS radical with standard or sample extract. The RSa_{50}, EC_{50}, ARP and AEAC values were also calculated as described in the previous section.

Determination of Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power of ferric ions by different plant extract was determined using FRAP assay [14]. An antioxidant capable of donating a single electron to the ferric-TPTZ (Fe^{+3}-TPTZ) complex would cause the reduction of this complex into the blue ferrous-TPTZ (Fe^{+2} -TPTZ) complex which absorbs strongly at 593nm. The antioxidant potential of the different extract was determined based on a calibration curve plotted using $FeSO_4$ concentration ranging from (20-100µg/ml).

Determination of Antioxidant Compound Concentration.

Determination of Total Phenol Content

Total polyphenolic in different extract content was determined using Folin- Ciocalteu colorimetric method as described by Ohlander and Jeppsson[15]. The phytococktail extracts (10µl) was mixed with 20µl of Folin- Ciocalteu reagent and 200µl of H_2O, and incubated at room temperature for 3 min. Thereby following the addition of 100µl of 20% sodium carbonate to the mixture. Total polyphenol was determined after 1 hours of incubation at room temperature. The absorbance of the resulting blue colour was measured at 765nm. Quantification was done with respect to the standard curve of gallic acid. The total

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polyphenol content was expressed as gallic acid equivalent (GAE).

Determination of Total Flavonoid Content

Estimation of total flavonoid in the methanol extracts was carried out using the previous method [16]. 100µl of sample and 100µl of 2% $AlCl_3$ ethanol solution were mixed together. The prepared test solution was incubated for 1 hour at room temperature. The absorbance of the test solution was measured at 420 nm. Total flavonoid content was calculated as quercetin equivalent (QE) using the derived equation based on the calibration curve: y=0.002x+0.0998, r^2=0.9704, where 'y' was absorbance and 'x' was QE (mg of extract) at a final concentration of 100µg/ml.

Determination of Total Flavonol Content

Total flavonol in the extracts was determined by the method described previously [17]. 100µl of test extract, 150µl (50g/l) sodium acetate solutions and 100µl of 2% $AlCl_3$ ethanol were added. The test mixture was mixed properly. The test mixture was rested for 2.5 hours at 20°C and absorbance at 440nm was taken. Total flavonol content was estimated as quercetin equivalent (QE) using the calibration curve: y=0.0007x+0.077, r^2=0.9819, where 'y' was absorbance and 'x' was QE (mg of extract) at a final concentration of 100µg/ml.

Determination of Total Proanthocyanidin Content

Total proanthocyanidin test was determined by vanillin- HCl assay with minor modification [18]. (1%) Vanillin reagent was prepared by mixing methanol and incubated at 30°C before use. (8%) HCl solution in methanol was prepared. The working reagent was prepared by mixing equal part of 8% HCl solution and 1% vanillin solution. The reaction mixture contained and plant extracts (20µl) and working vanillin reagent (100µl). The absorbance at 500nm was taken after 20 min at 30°C. Total proanthocyanidin content was expressed as quercetin equivalent QE using calibration curve: y=0.0003x+0.0435, r^2=0.9847, where 'y' was absorbance and 'x' was QE (mg of extract) at a final concentration of 100µg/ml.

4. Statistical Analysis

All the measurement was done in triplicate. The results are expressed as the mean value ± S.E.M. Statistical analysis was performed using ANOVA, Duncan's multiple range tests was performed. Statistical significance was set at $p<$ 0.05.

5. Result & Discussion

To determine antioxidant activities of *Inula racemosa, Rheium webbanium, Arnebia echroma* and *Saussaria lappa* root were dried and sequentially extracted with 80% methanol in a soxhlet apparatus. Table 1 shows the extraction yields for the plant extracts. The Percentage yield of Methanolic extracts varied from (1.77% to 51.65%). *S.lappa* produced the highest yield (51.65%) followed by *Rheium webannium* (31.76%) and *Inula racemosa* (6.4%) and lowest produced in *A.euchroma* (1.77%). These entire fractions were used to evaluate their *in vitro* antioxidant potential.

Table 1: Percentage yield of Methanol extracts of medicinal herb

Sources of extract	Weight of the plant powder (g)	Weight of the crude extract obtained (g)	Percentage of yield (%)
Inula racemosa	75	4.8	6.4
Saussaria lappa	75	38.74	51.65
Rheium webannium	75	23.82	31.76
Arnebia euchroma	75	1.33	1.77

Determination of Antioxidant Capacity

The antioxidant activity of the wild herbs was analysed using *in vitro* methods such as DPPH radical scavenging, ABTS radical cation scavenging activity and ferric reducing antioxidant power (FRAP) activity.

DPPH scavenging activity

Antioxidants compound in extract was react with DPPH, which is a stable free radical and is reduced to the DPPH-H and as result of which the absorbance's starts decreasing from the DPPH radical to the DPPH-H form. The degree of discoloration marks the scavenging potential activity of the antioxidant compounds or extracts in terms of hydrogen donating ability [19]. Radical scavenging activity of the crude extracts from selected four high altitude medicinal plants was quantitatively determined using a DPPH assay as presented in table 2. The dosage of extract is expressed in µg of dry weight of the extract (compound) per ml of the assay mixture. The free radical scavenging capacity of the phytococktail methanol extract of different medicinal herb was compared with ascorbic acid with their ability to scavenge DPPH radical. The free average radical-scavenging activity ranges from (39.56%) to (68.94%) (Table 2). The methanolic root extract of *Saussaria lappa* (68.94%) has maximum radical scavenging activity followed by *Arnebia euchroma* (67.17%), *Rheum webbanium* (46.26%), and least radical scavenging activity was found in *Inula racemosa* (39.56%). The antioxidant activity of the plants varied considerably in terms of IC_{50} value (Table 2). The IC_{50} value for each fraction, defined as the concentration at which 50 percent inhibition of free radicals has been occurred. The IC_{50} has been determined and tabulated (Table 2). Since IC_{50} is a measurement of inhibitory concentration, a lower value; reflect greater antioxidant activity of the fraction. The antioxidant potential of an extract is inversely proportional to IC_{50} value, which was calculated from the linear regression of the percentage antioxidant activity versus, extracts concentrations. The free radical-scavenging activity in terms of IC_{50} values ranging from (2.306 µg/mg) to (8.604 µg/mg) among the medicinal herb. It was also observed that the DPPH activity of wild herb extract increases in a dose-dependent manner. The various concentrations of the fractions which showed radical scavenging activity greater than 60% were found to be significant ($p < 0.05$).

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Table 2: DPPH radical scavenging activity of methanol extract of medicinal plant

	Radical Scavenging Capacity (%)			
Conc. (µg/ml)	*I.racemosa*	*S. lappa*	*R. webbanium*	*A. euchroma*
5	21.74± 1.13a	33.33 ± 1.53a	6.91 ± 1.4a	27.43 ± 1.83a
10	24.26± 1.9ab	39.67 ± 0.58b	11.45± 1.22ab	34.70 ± 2.38b
20	24.26± 0.73ab	48.81 ± 0.87c	15.69± 0.71bc	55.79 ± 3.93c
25	26.21± 0.38b	62.63 ± 0.75d	18 ±0.76 c	71.06 ± 1.5d
40	27.28 ± 0.19b	69.33 ± 0.25e	23.25 ± 3.64d	71.70 ± 1.26de
50	30.60 ± 1.44c	79.99 ± 1.88f	32.97 ± 1.32e	72.21 ± 1.68de
100	33.48 ± 1.43cd	81.93 ± 1.93f	53.99 ± 3.31f	74.94 ± 0.99def
125	34.34 ± 0.62d	82.65 ± 0.69f	71.78 ± 2.01g	78.69 ± 0.29efg
200	44.06 ± 0.82e	82.72 ± 0.87f	78.11 ± 1.78	78.47 ± 0.61efg
250	55.87 ± 0.26f	82.86± 1.12f	79.7± 0.25h	79.41 ± 3.42fg
400	69.18 ± 1.46g	81.14 ± 0.8f	81.14 ± 0.85	79.34 ± 3.5fg
500	83.51 ± 0.8h	82.22 ± 0.9f	82.22 ± 0.19h	82.29 ± 0.57g
MEAN±S.E.M	39.56 ±3.22	68.94 ±3	46.26 ±5.03	67.17 ±3
IC 50	8.604	2.306	6.95	2.51
EC 50	161.724	43.35	130.642	47.208
ARP	0.006	0.023	0.008	0.021
AEAC	117500.81	438411.54	145464.32	402779.68

The value means of three replicates ± standard error mean. The values followed by different alphabets differ significantly when subjected to Duncan's multiple range test at 0.5 subset

ABTS Radical Scavenging Activity

The ABTS radical scavenging capacity (%) of methanol extracts of the different medicinal herb has been compared in (Table 3). The extracts scavenged the ABTS radical in a dose dependent manner at concentration of 5-500µg/ml. Average ABTS radical scavenging capacity ranged from (47.54 to 66%). Highest radical scavenging activity showed by *Saussaria lappa* (66%) followed by *Arnebia euchroma* (64.05%), *Rheum webbanium (*55.47%) and least scavenging activity was shown by *Inula racemosa* (47.54%). The medicinal plant having lesser IC_{50} value has higher antioxidant activity; *A. euchroma* and *S. lappa* have the lower value thus exhibiting higher antioxidant activity. The concentrations of the various fractions which showed scavenging activity greater than 60% were found to be significant (p<0.5).

Table 3: ABTS Radical Scavenging Activity of methanol extra extract of medicinal herb

	Radical Scavenging Capacity (%)			
Conc. (µg/ml)	*I.racemosa*	*S. lappa*	*R. webbanium*	*A. euchroma*
5	12.37 ± 2.74a	39.83±0.21a	20.54± 0.2a	42.77 ± 1.45a
10	23.27 ± 3.32b	45.49±0.55ab	22.64 ± 0.36a	53.88 ± 2.36b
20	25.58 ± 0.21bc	51.14±2.41bc	33.54 ± 0.91b	61.01 ± 0.72c
25	30.4 ± 1.99c	55.97±0.95c	37.94± 1.37c	63.10 ± 0.75cd
40	44.65 ± 1.31d	63.52±0.36d	49.48 ± 0.55d	63.73 ± 0.55cd
50	46.33 ± 0.21d	65.82±1.1de	56.39 ± 0.55e	66.67 ± 0.36cd
100	57.02 ± 0.21e	68.67±4.95dc	67.51 ± 1.27f	68.34 ± 0.42cd
125	58.28 ± 1.37e	70.23±3.45ef	74.00 ± 0.55g	68.97 ± 4.01cd
200	64.15 ± 0.63f	75.26±2.57fg	74.63 ± 0.21g	69.18± 3.8d
250	68.97 ± 0.55g	80.08±1.04gh	75.24± 0.22g	70.65 ± 3.9d
400	69.39 ± 0.21g	85.74±0.75hi	75.45± 0.02g	70.86 ± 2.72d
500	70.02 ± 1.11g	90.14±0.55j	78.2 ± 0.91h	69.39 ± 2.36
MEAN±S.E.M	47.54 ± 3.31	66±2.59	55.47± 3.55	64.05 ± 1.86
IC 50	7.00	2.81	5.61	1.38
EC 50	1.82	0.732	1.46	0.36
ARP	0.55	0.13	0.69	2.79
AEAC	57143.87	142351.28	71302.51	289860.22

The value means of three replicates ± standard error mean. The values followed by different alphabets differ significantly when subjected to Duncan's multiple range test at 0.5 subset

Ferric Reducing Antioxidant Power (FRAP)

The reduction of the ferric ions by various root extract was determined using the FRAP assay. Aydemir reported that a significant correlation between reducing power and antioxidant activity [20]. Reducing power of a compound is said to be the indicator of its antioxidant capacity. The results of present study of reducing power of plant part extracts are shown in table 4. A large range values was obtained for ferric reducing activity. The ferric reducing activity in methanolic extracts ranged from (182 $FeSO_4.7H_2O$µg/ml to 1022 $FeSO_4.7H_2O$µg/ml) at sample concentration 500µg/ml (Table 4). Average antioxidant activity was found highest in *Saussaria lappa* (385.76

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$FeSO_4.7H_2O$µg/ml) followed by *Arnebia euchroma* (308.52 $FeSO_4.7H_2O$µg/ml), *Rheum webbanium* (104.96 $FeSO_4.7H_2O$µg/ml), and least radical scavenging activity was observed in *Inula racemosa* (102.66 $FeSO_4.7H_2O$µg/ml) respectively. Higher ferric reducing antioxidant potential with *Saussaria lappa;* it suggests that the extract contains significantly higher total antioxidant capacity (TAC). The difference in FRAP value between the different concentration (5 to 500µg/ml) showing the lowest and highest value was 1- 10 folds in *Saussaria lappa*. All the extracts showed reducing power in a dose-dependent manner [21]. This highlights the importance of plant species for determining ferric reducing antioxidant potential of root extract from different plant species. Antioxidant activity capacity observed in the above result DPPH, ABTS, FRAP was analogous to each other.

Table 4: FRAP Radical Scavenging Activity of methanol extract of medicinal herb

Radical Scavenging Capacity (%)				
FRAP ($FeSO_4.7H_2O$µg/ml)				
Conc. (µg/ml)	*I.racemosa*	*S. lappa*	*R. webbanium*	*A euchroma*
5	76.55±1.39a	100.11±0.8a	67.82±0.46a	94.6±1.17a
10	80.57±0.79ab	108.5±6.73a	68.16±1.51a	100.8±2.6a
20	83.33±1.09b	126.9±0.72b	68.51±0.46ab	117.24±2.78b
25	84.37±2.12b	156.09±6.6c	71.26±1.09abc	134.48±2.6c
40	86.89±2.09bc	159.88±2.07c	76.78±0.5bc	163.34±2.95d
50	91.15±2.99cd	188.16±1.67d	78.74±2.59cd	227.24±2.49e
100	92.87±0.41cd	340.92±0.98e	85.75±4.51d	231.15±1.13e
125	95.52±1.05d	388.39±0.3f	105.63±3.04e	298.05±0.92f
200	102.02±1.91e	510.92±7.2g	110.12±2.46e	400.35±8.45g
250	118.39±0.11f	592.53±6.67h	139.31±0.69f	472.18±6.78h
400	137.7±3.46g	933.79±4.14i	184.48±0.71g	667.24±3.63i
500	182.53±4.08h	1022.87±4.78j	202.98±6.39h	795.52±2.63j
MEAN±S.E.M	102.66±4.97	385.76±51.99	104.96±7.61	308.52±37.51

The value means of three replicates ± standard error mean. The values followed by different alphabets differ significantly when subjected to Duncan's multiple range test at 0.5 subset

Table 5: Total phenolic, flavanol, flavonoid, proanthocyanides of methanol extract of medicinal herb

Sample	Total polyphenol (µgGAE/mg of sample)	Total flavanol (µgQE/mg of sample)	Total flavonoid (µgQE/mg of sample)	Total proanthocyanidin µgQE/mg of sample
Inula racemosa	167.92 ± 91a	70 ± 23d	3.1±0.28a	50.56 ± 6.76a
Sassuria lappa	202.92 ± 6b	15.71 ± 1.64b	8.77 ± 3.18a	41.67 ± 0a
Rheum webbanium	227.08 ± 2.2c	57.14 ± 2.18c	5.1 ± 1.5a	108.33 ± 1.92b
Arnebia euchroma	244.58 ± 5.07d	0.95 ± 0.47a	17.77 ± 0.6b	43.89 ± 4.84a

The value means of three replicates ± standard error mean. The values followed by different alphabets differ significantly when subjected to Duncan's multiple range test at 0.5 subset

Total Polyphenol, Flavonoid, Flavonol and Proanthocyanidin Content

The total concentration of polyphenol, flavonol, flavonoid and proanthocyanidin content in methanol extracts of different medicinal herb at a concentration (100µl/ml) is determined and shown in table 5. The total polyphenol content is expressed as µg of gallic acid equivalent (GAE) per milligram of dry weight. *Arnebia euchroma* had a total polyphenol content of 244.58µgGAE/mg followed by *Rheum webbanium* (227µgGAE/mg), *Sassuria lappa* (202.92µgGAE/mg) and least polyphenol content was *Inula racemosa* (167.92µgGAE/mg). The total flavonoid and flavonol content among the various extracts was expressed in the term of quercetin equivalent per milligram of dry weight. Flavonoid content ranged from (3.1µgQE/mg to 17.77µgQE/mg dry) with of different extract and flavonol ranged from (0.95 µgQE/mg to 70µgQE/mg). Concentration of flavonoid increased by the order *Inula racemosa* (3.1µgQE/mg) < *Rheum webbanium* (5.1µgQE/mg) < *Sassuria lappa* (8.77µgQE/mg) < *Arnebia euchroma* (17.77µgQE/mg). Similarly, flavonol content increases with the order *Arnebia euchroma* (0.95µgQE/mg) < *Sassuria lappa* (15.71µgQE/mg) < *Rheum webbanium* (57.14µgQE/mg) < *Inula racemosa* (70µgQE/mg). Total proanthocyanidin content was estimated by general procedure. The proanthocyanidin expressed as µg of quercetin equivalent per milligram of dry weight. Proanthocyanidin ranges from (41.67µgQE/mg to 108.33µgQE/mg). *Rheum webbanium* had the highest total proanthocyanidin (108µgQE/mg) followed by *Inula racemosa* (50.56µgQE/mg), *Arnebia euchroma* (43.89µgQE/mg) and the lowest total proanthocyanidin content was in *Saussaria lappa* (41.67µgQE/mg).

6. Conclusion

In the present study, all the plants showed phenol, flavonoid, flavonol and proanthocyanidin content and exhibit antioxidant activity. All these natural antioxidants from plants extract have pharmaceutical and nutraceuticals importance and they can have significant impact on human health and disease prevention. The present study also supports the scientific basis of use of these plant extracts in traditional health care system. For this reason, further work

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should be done for isolation and identification of the antioxidative components of medicinal plants.

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