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Total flavonoids, alkaloids, antioxidant assay and preliminary screening of secondary metabolites in root extract of some shrubs

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Abstract

Biochemically active constituents of plants are crucial and contribute to increase the plant`s economic and medicinal values. Different secondary metabolites such as alkaloids, flavonoids, phenols and quinones protect the plants and also consumed as traditional health remedies. Furthermore, plant extracted secondary metabolites are being utilized in modern drugs manufacturing. The current study was carried to find out the preliminary screening of secondary metabolites, total flavonoids, total alkaloids content and antioxidant potential of roots extract. The result showed that more flavonoids content was found *Capparis deciduas* (22.02mg QE/g DW) followed by *Withania somnifera* (17.21mg QE/g DW), *Salsola imbricata* (12.36mg QE/g DW) and least content was detected in *Carotalaria burhia* (07.46mg QE/g DW) root extract. Total alkaloids content was found more in *Capparis deciduas* (28.78mg/100g) following by *Withania somnifera* (23.03mg/100g) *Carotalaria burhia* (19.67mg/100g) and *Salsola imbricata* (18.98 mg/100g) respectively. Root extract of selected plant species revealed substantial antioxidant potential in following order: *Capparis deciduas* (27.08%)> *Withania somnifera* (24.42%)> *Salsola imbricata* (17.06%)> *Carotalaria burhia* (13.46%).

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Introduction

Plants are significant source of naturally found active phytochemical constituents such as phenols, flavonoids, alkaloids and saponins (Krishnaraju *et al*., 2005). These phytochemicals now-a -days termed as secondary metabolites of plants (Roopashree *et al*., 2008). Plants are the first and enormous source of secondary metabolites that are utilized for different daily uses (Molyneux *et al*., 2007). Some plants are rich source of nutrients and suggested for their therapeutic activity while some plants are considered as an important source of antioxidants (Shekhawat *et al*., 2010; Pattanaik *et al*., 2006). For instance, flavonoids, phenols, tannins and alkaloids are all the time more captivating people as they are accepted ailment preventing, health stimulating and eradicating aging process (Savita *et al*., 2010; Barile E. *et al*., 2007; Okwu *et al*., 2004).

Antioxidants compounds contribute a significant role in lowering risk of oxidative detriments caused by reactive oxygen species (ROS) and free radicals in human beings. Antioxidants such as phenolic constituents give appearance, peculiar fragrance and flavor to the plants. Different plants parts i.e. stem, leaves, roots and fruits contain versatile nature of secondary metabolites (Elmas Özeker, 1999; Breslin *et al*., 2017). Research depicted that diverse secondary phytochemicals i.e. alkaloids, terponoids and phenolic constituents of plants had high potential to combat and cure a numbers of diseases such as inflammation, diabetes , cancer and skin infections are a few of them. Hence, plants are considered as primary source of antioxidants and their derivatives comprising of multipurpose medicinal uses (Krishnaraju *et al*., 2005). Terpenoids and saponins have considerable hypocholesterolemic, antimicrobial and antidiabetic potential and are widely used for their unique characteristics (Shah *et al*., 2009).

The plant derived secondary metabolites are the prime source of modern as well as man-made or traditional drugs (A. V. *et al*., 2005; Makky *et al*., 2012). Antioxidants are frequently added to food stuffs for evading free radical reactions of oxidation. They work by restraining the initiation and production step of oxidation reactions resultant in inhibition of reaction and retard the oxidation reaction. Synthetic antioxidants are more toxic and carcinogenic so they are restricted. Therefore, plant derived antioxidants are considered healthier and effective with no adverse effect.

Root part of plants contains a diverse group of active compounds. The present research was carried out for two reasons. First, there was found little research and literature regarding to preliminary screening and quantitative estimation of secondary metabolites naturally occurring in plant roots. Second, the potent therapeutic properties of under study plants became a motive to explore and evaluate the antioxidant potential of composites present in plant's roots.

Material and method

Plants selection and samples collection

Plants under current study were preferred for their various therapeutic applications (Table 1). Fresh root samples of four plants *Withania somnifera, Carotalaria burhia, Salsola imbricatea* and *Capparis decidua* were collected from the Cholistan desert situated in Bahawalpur, Pakistan and brought to the Life Science laboratory of The Islamia University of Bahawalpur for further processing. The fresh collected root sample were washed cautiously with distilled water and left for two hours to air- dry. Equally weighed collected root samples were dried for two weeks at room temperature. The dried root samples were pulverized into fine powdered and put in storage in air free sterile bottles for qualitative and quantitative analysis of biochemical composites.

Table 1. Description of plants used in this study and their therapeutic values.

Botanical Name	*Withania somnifera*
Family	Solanaceae
Description	Plant is small shrub, 35-75cm tall. Gloomy green leaves. Bell shaped green flower and fruit is orange to red.
Distribution	Throughout Asia

Botanical Name	*Withania somnifera*
Parts used	Root, leaves, stem, flower.
Uses	Anti-anxiety, Anti- stress, Tonic (Mishra *et al.*, 2000)
Botanical Name	*Crotalaria burhia*
Family	Fabaceae
Description	Shrub, 30-55 cm tall, numerous branches with few leaves.
Distribution	Pakistan, India, Afghanistan, Bangladesh
Parts used	Root, stem, leaves
Uses	Antioxidants, antibacterial and ant-inflammation agent and antimicrobial (Kataria *et al.*, 2010)
Botanical Name	*Salsola imbricata*
Family	Amaranthaceae
Description	Shrub, leaves are alternate, flower is bisexual.
Distribution	Pakistan, India, Sri lanka, Egypt.
Parts used	Root, stem, leaves, fruit
Uses	Diuretic, indigestion, dysentery, cold and asthma (Chopra *et al.*, 2006)
Botanical Name	*Capparis decidua*
Family	Capparaceae
Description	Plant is 4-5 m tall, grey color stem on maturity, small leaves.
Distribution	Found in subtropics and arid regions of Asia.
Parts used	Root, stem, fruit, seeds
Uses	Antimicrobial, purgative, antidiabetic, analgesic, and aphrodisiac (Dalziel, 1948)

Preparation of aqueous extract

1g of grinded powder was added to 50ml of distilled water, crushed in mortar and pestle and boiled at 60°C for half an hour on water bath. On cooling, the aqueous impure extract was centrifuged at 25rmp for 20 minutes. Then it was filtered by using Whatman filter paper and the filtrate was stored in sterile bottles for qualitative screening biochemicals (Harbone, 1973).

Preparation of methanolic extract

Fine powered material weighed 1gm of each collected root sample dissolved in 50ml of methanol as decoction agent was placed shaker for an hour. Later, the solvent extract was vaporized to yield 15ml of stock solution for qualitative analysis of secondary composites (Khalid and Siddiqui, 2011).

Phytochemical screening of prepared root extracts

The qualitative analysis was accomplished to investigate the secondary metabolites present in root extracts. Roots secondary bio-constituents were analyzed through modern techniques (Harborne *et al.*, 1998).

Total alkaloids

To determine total alkaloid content present in root samples, spectrophotometric method was used (Ajanal *et al.*, 2012).

The 100gm of each root sample was grinded and extracted using methanol in Soxhlet apparatus. The extract was evaporated on a rotary evaporator to evaporate methanol at a temperature of 38°C for quantitative screening of alkaloids.

Total flavonoids

Total flavonoids content of methanolic root extract was determined by Aluminium chloride method (Chang *et al.*, 2002). Prepared extract was added with 1.5ml methanol, 10% Aluminum chloride solution and 1.0M Potassium ethanoate followed by 2.8ml of distill water. Then sample absorbance was recorded by 405 nm and total content was measured comparing with 0.0-8 lg/mL as standard.

Antioxidant activity (DPPH assay)

To analyze antioxidant potential of root extract, DPPH (1, 1-diphenyl-2-pictylhydrazyl radical-scavenging activity) assay method was used (Bibi *et al.*, 2012). DPPH methanol solution (180µl) mixed with 0.02ml in 100lg/mL dimethyl sulfoxide and samples were kept in dark for 20 minutes at room temperature. Then absorption was evaluated using microplate photometer at 518nm.

Results and discussion

Screening of secondary metabolites

Preliminary qualitative profile of active bio-component under study revealed that root filtrate contained secondary metabolites (Table 2).Secondary metabolites like steroids, phenols, saponins, flavonoids and carboxylic acids were detected both in aqueous and methanol root extract of all selected plant samples. Presence of steroids, phenols, flavonoids, saponins and terpenoids in both aqueous and methanol extracts show that those were the core secondary metabolites of all root samples. In present study, aqueous and methanol extracts showed positive and relatively moderate presence of steroids in *Withania somnifera* and *Carotalaria burhia*. In *Salsola imbricata,* slight detection of steroids was observed in methanol extract as compared to the *Caparis decidua* and relative moderate presence was noticed in *Caparis decidua* aqueous extract.

Uzma Munir *et al.* (2014) results showed analogous detection of biochemical composites in leaf extract of *Salsola imbricata*. Significant presence of carboxic acids and flavonoids were detected in all root samples. Both aqueous and methanol extracts showed negative results for gums in all root extract and only quinones were not found in *Capparis decidua*. Moreover, bioactive constituents like resins, alkaloids and terpinoids were screened positively in aqueous extract of all the root samples. Saidulu Ch *et al*., (2014), Balram Soni, (2014), Rathee *et al*, (2010 and Uzma Munir *et al*., 2010) findings in different parts (leaves, twigs, seeds) of *Withania somnifera, Crotalaria burhia , Capparis decidua* and *Salsola imbricata* are in support of our results. Our study confirms the presence of secondary metabolites such as steroids, flavonoids phenols, terpenoids and saponins in significant quantity.

Table 2. Qualitative screening of aqueous (Aq.) and methanol (Mat.) extract of roots.

S.No.	Secondary metabolites	Chemical test	Root Extract							
			Withania somnifera		*Salsola imbricata*		*Caparis decidua*		*Carotalaria burhia*	
			Aq.	Mat.	Aq.	Mat.	Aq.	Mat.	Aq.	Mat.
1.	Steroids	Salkowski,s test	++	++	++	+	+	++	++	++
2.	Cardiac Glycosides	Benidict's	_	++	+	+	+	++	+	++
3.	Phenols	Ferric Chloride	++	++	+	++	++	++	+	++
4.	Tannins	lead acetate test	++	–	++	+	++	+	+	+
5.	Saponins	Foam's test	++	+	+	+	++	+	++	+
6.	Gums	Potassium hydroxide	_	_	_	_	_	_	_	_
7	Quinones	Spot test	+	–	+	+	–	–	+	+
8	Carboxylic Acids	Sodium Bicarbonate	++	++	++	++	+++	++	++	+
9	Terpenoids	Salkowski test	++	++	+	+	–	++	+	++
10	Flavonoids	Sodium hydroxide	++	++	++	++	+	++	+	++
11	Alkaloids	Mayer's test	_	+	+	+	–	+	+	++
12.	Resins		+	+	+	+	+	+	–	+

Hint: + = slightly present, ++ = moderately present, +++ = highly present, - = Not present .

Total Flavonoids

Flavonoids being secondary metabolites are of great importance in view of the fact that have biological active potential to restrain enzymes, antimicrobial and anti-aging activities. The total flavonoids content of root samples is shown in Table 2.

The variation in total flavonoids content with current study of plant root samples varied considerably, ranging from 07.46 to 22.02mg QE/g DW of roots methanol extract. Total flavonoids content was calculated to be more in root filtrate of *Capparis deciduas* (0.02mg QE/g DW) followed by *Withania somnifera* (17.21mg QE/g DW). The low flavonoids content was measured in *Carotalaria burhia* (07.46mg QE/g DW).

After *Salsola imbricata* weighed against *Capparis deciduas* and *Withania somnifera*. Naik *et al.* (2013) and Monika *et al.* (1967) reported the presence of

flavonoids in overall plant, Leaves and roots of *Withania somnifera* respectively. Various other researchers also reported the presence of flavonoids in above ground parts of plants. Our results are confirmation for flavonoids occurrence in underground parts of plants in significant proportion.

Total alkaloids

The Alkaloids are active organic constituents of plants categorized as secondary metabolites .These nitrogen containing organic compounds of plants have made considerable addition to medicines due to its enormous use. In our present results, C*apparis decidua* has shown significant extractive absorbance and a alkaloid content (28.78mg/100g) followed by *Withania somnifera* (23.03mg/100g) as shown in table 2. While *Salsola imbricata* and *Carotalaria burhia* showed relative less alkaloids content as compared with other two *Capparis decidua* and *Withania somnifera* samples. Moza and Singh (1967) and Naik *et al.*, (2013) have reported alkaloids content present in above ground parts of *Withania somnifera*. Akhtar *et al.* (2015) and Cybulska *et al.* (2014) reported the presence of alkaloid in C*apparis decidua* and *Salsola imbricata* respectively**.** Hence, our results also confirm the presence of alkaloids in plant roots.

Antioxidant activity (DPPH assay)

Literature analysis confirms that a lot of plant extracts display free radical scavenging effects. Antioxidants potential of methanolic root extract from *Withania somnifera, Carotalaria burhia, Salsola imbricate* and *Capparis decidua* was assessed by DPPH assay method. The DPPH (free radical scavenging activity) was expressed as inhibition percentage and outcomes obtained are shown (Table 2). Root extracts displayed promising antioxidant potential evidenced as a result of DPPH assay. DPPH assay exhibited the considerably high antioxidant potential reducing free radicals in *Capparis deciduas* (27.08%) root extract followed *Withania somnifera* (24.42%), *Salsola imbricata* (17.06%) and least antioxidant potential was measured in *Carotalaria burhia* (13.46%). Muhammad Zia-Ul-Haq *et al.*, (2011) and Abdalrahman *et al.*, (2015) witnessed the significant antioxidant activity of *Capparis deciduas* leaves as well as fruits while Khalighi-Sigaroodi *et al.* (2012) reported the antioxidant activity of *Carotalaria burhia* stem and their results support our findings indicting *Capparis decidua* all parts possess potential to reduce free radicals.

Bhattacharya *et al.*, (2002) has also reported that leaf extract of *Withania somnifera* showed effective antioxidant activity. In general, the examined root extracts were capable to ease the stable 1, 1-diphenyl-2-pictylhydrazyl free radicals to varying extents.

Table 3. Evaluation of Total flavonoids, alkaloids content and DPPH assay of roots extract of some shrubs. The presented values are the mean ± SD of three repeated measured quantities.

S.No.	Plant species	Total flavonoids (mg QE/g DW)	Total alkaloids (mg/100 g)	DPPH (%)
1.	*Withania somnifera*	17.21 ± 1.44	23.03 ± 1.02	24.42±1.22
2.	*Salsola imbricata*	12.36 ± 1.06	18.98 ± 1.43	17.06 ± 0.76
3.	*Capparis decidua*	22.02 ± 1.32	28.78 ± 1.68	27.08 ±1.46
4.	*Carotalaria burhia*	07.4 6 ± 0.98	19.67 ± 1.12	13.46 ±0.94

Conclusion

Plants are rich in some secondary metabolites and their significance cannot be ignored. Secondary metabolites like terpenoids, tannins, flavonoids, alkaloids and steroids are present in active ingredients forms in the plant extracts. Like stem, fruits and other plant parts.

Roots also have a significant value. Contagious diseases such as pneumonia, ulcer, diabetes, malaria, typhoid and tumors can be cured by root extracts.

From the present study, it is summarized that roots are also a significant source of secondary metabolites and be as an alternative source to reduce use of synthetic pharmaceutics.

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