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Investigation of Phytochemistry and antioxidant activity of the crude extracts of *Verbena tenuisecta* Briq

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Abstract

In vitro phytochemical analysis and antioxidant activity of various extracts of Verbena tenuisecta Briq. Were investigated through Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH radical scavenging activity, ABTS assay and Metal Chelating activity. The results revealed that leaf extracts showed remarkable antioxidant potential in all assays, whereas other extracts also showed significant results, i.e. flower extract exhibited the best %age bound iron capacity and TEAC (mM of Trolox) value was found to be maximum in stem extract. Qualitative phytochemical screening indicated the presence of alkaloids, flavonoids, saponins, tannins, phenols, cardiac glycosides and terpenoids.

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Introduction

Nature has not only served as a potential source of medicinal agents for thousands of years but a remarkable account of modern drugs is still being isolated from natural resources (Peter *et al.*, 2005). Plants have an effective quantity of secondary metabolites i.e. alkaloids, flavonoids, tannins, essential oils and phenolic compounds are some of the most essential bioactive molecules which can induce certain physiological actions on human body and thus, can be used in the treatment of chronic diseases (Diallo *et al.*, 1999; Edeoga *et al.*, 2005). Plants (fruits, vegetables, medicinal herbs) contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites, that are rich in antioxidant activity (Cotelle *et al.,* 1996; Velioglu *et al.,* 1998).

Antioxidants are a class of secondary metabolites of plants. The plant kingdom offers many polyphenolic compounds. Several isolated plant constituents as well as extracts have been recognized to possess antioxidant effects against free radicals in biological systems (Mervat *et al.,* 2009). Phenols are one of the main secondary metabolites present in the plant kingdom. They are commonly found in both edible and non-edible plants and have been reported to have multiple biological effects, including antioxidant activity (Kähkönen *et al.,* 1999). Flavonoids and other plant phenolics are especially common in leaves, flowering tissues and woody parts such as stems and bark.

Oxidation is a natural metabolic process in cell. Free radicals of different forms are normally generated at a low level in cells to help in the modulation of several physiological functions and are quenched by an integrated antioxidant system in the body. However, if free radicals are produced in excess amount they can be destructive. Super oxide, hydrogen peroxide, hydroxyl and nitric oxide radicals are such examples (Halliwell and Gutteridge, 1989).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are species with odd electrons called free radicals.

They are very reactive groups of atoms and when they are produced excessively they can damage the components of living cells (Valko *et al.*, 2007).

Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and the affiliated lipid peroxidation, protein damage and DNA strand breakage (de Sousa *et al.,* 2007). Natural antioxidants have a wide range of biochemical activities including inhibition of ROS generation, direct or indirect scavenging of free radicals and alteration of intracellular redox potential (Abdollahi *et al.,* 2005).

An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health promoting effects in the prevention of degenerative diseases (Shahidi, 1997). In addition, it has been reported that there is an inverse relationship between dietary intake of antioxidant rich food and the incidence of human diseases (Rich-Evan, 1997).

Thus, this study was mainly focused to investigate the leaves, stem, roots and flower of *Verbena tenuisecta* Briq. (Syn: *Verbena bipinnatifida* auct.) for the antioxidant potential and preliminary phytochemical screening. *Verbena tenuisecta* Briq. has been placed in the family Verbenaceae which is native to South America. It is a locally growing and ethnobotanically important plant *Verbena tenuisecta* Briq. (Jafri and Ghafoor, 1974). This plant is not studied for any activity till at that time but literature showed that the other plants of this family exhibited the antibacterial, polyphenolic, toxicity and antioxidant activity. Furthermore, different plant parts of this family have phytochemicals responsible for their biological activities.

The objective of this research is to identify good number of chemical compounds in various organic crude extracts of *V. tenuisecta* Briq. might have some medicinal benefits and to evaluate the antioxidant potential of the plant so that generated data may help the pharmacologists in the production of new drugs.

Material and methods

Collection and preparation of samples

Verbena tenuisecta Briq. (Verbenaceae) was collected from District Okara. The plant sample was identified by Dr. Zaheer-ud-din Khan and deposited in Dr. Sultan Ahmed Herbarium GC, University Lahore after posting voucher number. The plant parts were subjected to desiccation in shady conditions at room temperature. The prevention of any phytochemical denaturation was persuaded by drying the parts in shade. Dried parts of the plant were ground separately into the fine powder. The plant powder of each part was treated with polar and non-polar solvents (*n*-hexane, chloroform, ethanol and distilled water) using maceration technique. Ultimately, the extracts were concentrated using rotary evaporator. The concentrated extracts were dried and stored at 20°c.

Estimation of percentage yield

Percentage yield was calculated by following formula:

$$\text{Percentage extraction yield} = \frac{\text{wt.of the crude extract}}{\text{wt.of the plant part}} \times 100$$

Phytochemical screening

Qualitative analysis

The qualitative analysis of primary as well as the secondary metabolites was carried out by using the crude extracts of *Verbena tenuisecta* Briq. For the detection of different phytoconstituents present in *V. tenuisecta* Briq., standard protocols were used.

Tests for Alkaloids

For the detection of alkaloids Wagner's test (Wagner, 1993), Mayer's test (Siddique and Ali, 1997) and Dragendorff's test (Waldi, 1965) were performed.

Tests for Flavonoids

Flavonoid contents were assessed through Ferric Chloride test (Patel *et al.*, 2013), Lead Acetate test (Tiwari *et al.*, 2011) and Alkaline test (Trease and Evans, 2002).

Tests for Reducing Sugars

Fehling's test (Harborne, 1973) was performed for the detection of reducing sugars.

Tests for Tannins and Phenols

Phenolic and tannin comppunds were detected by Ferric Chloride test (Iyengar, 1995), Lead Acetate test (Kokate, 2005) Gelatin test (Bhandary *et al.*, 2012) and Ellagic Acid test (Patel *et al.*, 2012).

Tests for Terpenoids

For the detection of terpenoids, Salkowski test (Harborne, 1973) was performed.

Tests for Cardiac Glycosides

Keller Killiani test (Onwukaeme *et al.*, 2007) and Concentrated Sulphuric acid test (Patel *et al.*, 2013) were performed for the detection of cardiac glycosides.

Test for saponins

Foam test (Roopshare *et al.*, 2008) and Olive Oil test (Harborne, 1973) were applied for the detection of saponins.

Determination of Antioxidant Adequacy

The determination of antioxidant potential of *Verbena tenuisecta* Briq.was carried out in the light of following techniques.

Estimation of Total Phenolic Content (TPC)

Total Phenolic Content of extracts was estimated by method proposed by Makkar *et al.* (1993). By using a standard curve, the total Phenolic Contents were determined and expressed as mg of Gallic acid equivalents per gram of fresh weight (mg/g).

Quantification of Total Flavonoid Content

The protocol formulated by Dewanto *et al.* (2002) was used for the determination of the Total Flavonoid Content. The Total Flavonoid Content (TFC) was estimated from the standard curve of Catchecin and values were expressed as mg of Catchecin equivalents in per Gram of sample.

$ABTS^+$ assay

ABTS assay was carried out according to the protocol framed by Re *et al.* (1999).

Percentage inhabitation was determined by using the following formula:

% age Inhibition = $(1-A_f/A_o) \times 100$

Whereas A_f is the absorbance of sample and A_o is the absorbance of ABTS radical cation.

Metal Chelating Activity

The protocol followed for the determination of the chelation of ferrous ions and standards was developed by Dinis *et al.* (1994).

From the absorption value, percentage bound iron was quantified by applying the following equation:

Percentage bound iron= $(A_{control} - A_{sample} / A_{control}) \times 100$

The values of percentage bound iron, for each sample were represented in the form of a bar graph.

DPPH Radical Scavenging Activity

The protocol of Shimada *et al.* (1992) was used for the estimation of DPPH radical scavenging activity.

Following formula was used for the calculation of the % of DPPH remaining:

%age DPPH $_{remaining}$= $(A_f / A_o) \times 100$

A kinetic curve showing decrease in absorbance of DPPH with time was plotted and calculation of EC50 value was performed for each sample.

Statistical analysis

All the parameters were performed three times (in triplicates) and the data thus obtained was analyzed statistically by applying ANNOVA and Duncan's Multiple Range Test (DMRT) at 5% significance level by following Steel *et al.* (1997).

Results and discussions

Phytochemical analysis

Phytochemicals are naturally occurring biochemical of plants, regarded as the secondary metabolites of plants.

Qualitative phytochemical screening of the different crude extracts of *Verbena tenuisecta* Briq. was carried out to determine the presence or absence of bioactive compounds.

The phytochemical investigation of crude extracts of stem, root, leaf and flower (Table 1.1) of *V. tenuisecta* Briq. was appraised using the standard protocols. The results showed stem, root, leaf and flower macerates had a wide range of bioactive compounds.

Reducing sugars, terpenoids, alkaloids, flavonoids, tannins, saponins, cardiac glycosides and phenols were present in almost all extracts but with different ratio.

Table 1. Phytochemical screening of stem extracts of *Verbena tenuisecta* Briq.

Constituents	Phytochemical tests	Plant Macerates															
		n-hexane				Chloroform				Ethanol				Distilled Water			
		S	L	R	F	S	L	R	F	S	L	R	F	S	L	R	F
Alkaloids	Mayer's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Wagner's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Drangendorff's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins and	Ferric chloride test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
phenols	Ellagic acid test	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
	Gelatin test	+	+	+	+	+	-	+	+	+	-	-	+	+	+	-	+
	Lead acetate test	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Reducing sugars	Fehling's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	Salkowski test	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Saponins	Olive oil test	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+
	Foam test	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+
Flavonoids	Ferric chloride test	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
	Lead acetate test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Alkaline test	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-
Cardiac glycosides	Kellarkiliani test	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Conc. Sulphuric acid test	-	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-

Key words: - = absence of constituent, + = presence of constituent

S= stem, L= leaf, R= root, F= flower

Antioxidant Activity

Total Phenolic Content

Results showed great variation in total phenolic content ranging from 21.12±0.11-100.12±0.17 (Fig. 1). Highest total phenolic content was recorded in chloroform extract of leaf of *V. tenuisecta* Briq. while lowest content was found in ethanolic extract of leaf.

Total Flavonoid Content

Among all the extracts of *V. tenuisecta* Briq. maximum flavonoid content was found in ethanol extract of leaf, i.e. 1036.18^{a}±1.99 (Fig. 2).

ABTS assay

The results were expressed in terms of TEAC value, which is "Trolox Equivalent Antioxidant Capacity". Results were found to be ranging from 11.16^{a}±0.34-1.827^{e}±0.40 (Fig. 3). Highest TEAC value was 8.261mM of Trolox, recorded in ethanol extract of stem.

Metal Chelating Activity

Metal chelating activity of *V. tenuisecta* Bri. was assessed in the terms of %age bound iron. Among all the extracts, ethanolic extract of flower owned the highest %age bound iron, i.e. 87.25% (Fig. 4).

DPPH radical scavenging activity

The results of DPPH redical scavenging activity of *V. tenuisecta* were expressed in terms of %age DPPH remaining (Fig.5, 6, 7 & 8). A lower value of %age DPPH remaining indicated a higher antioxidant potential.

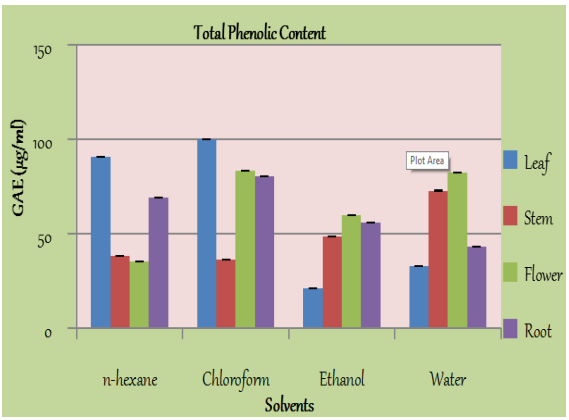

Fig. 1. Graphical representation of TPC of crude extracts of *V. tenuisecta* Briq.

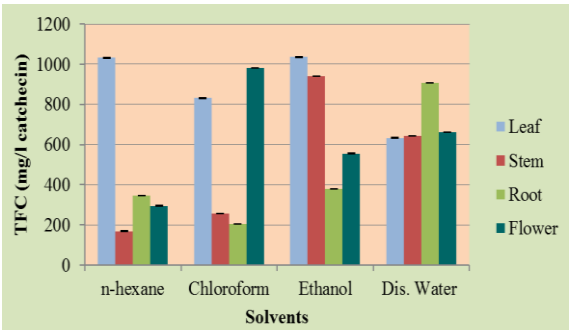

Fig. 2. Graphical representation of TFC of crude extracts of *V. tenuisecta* Briq.

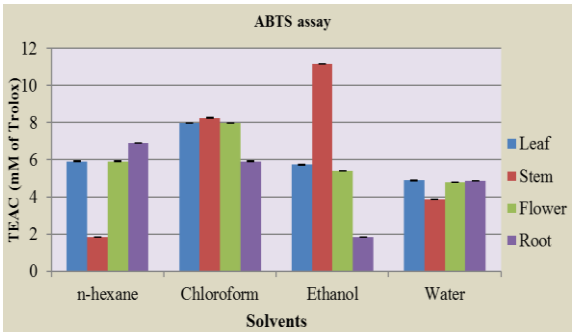

Fig. 3. Graphical representation of ABTS assay of extracts of *V. tenuisecta* Briq.

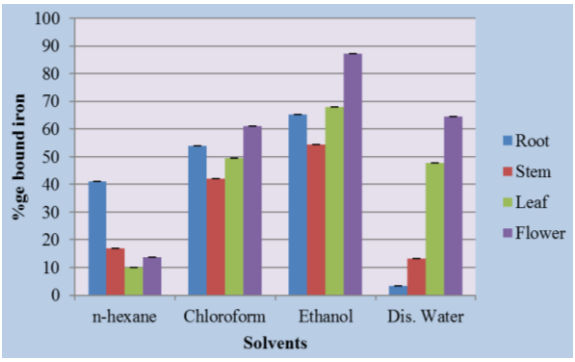

Fig. 4. Graphical representation of %age bound iron of extracts of *V. tenuisecta* Briq.

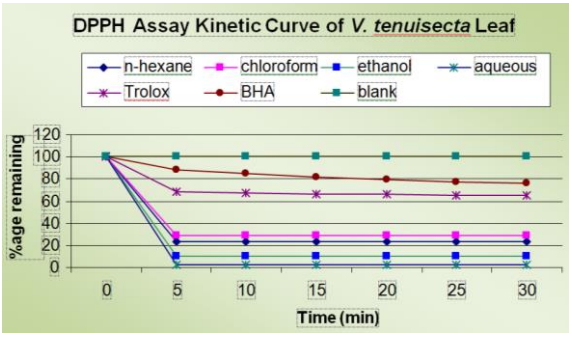

Fig. 5. DPPH kinetic curve of crude extract of leaf of *V. tenuisecta* Briq.

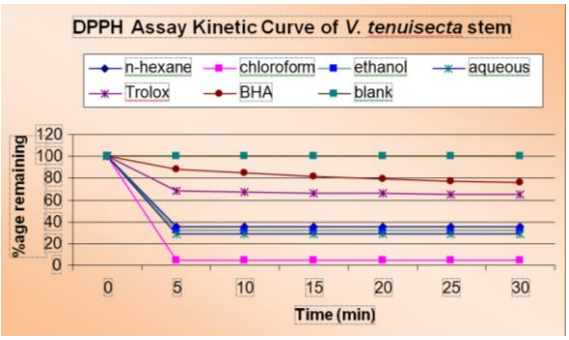

Fig . 6. DPPH Kinetic curve of crude extracts of stem of *V. tenuisecta* Briq.

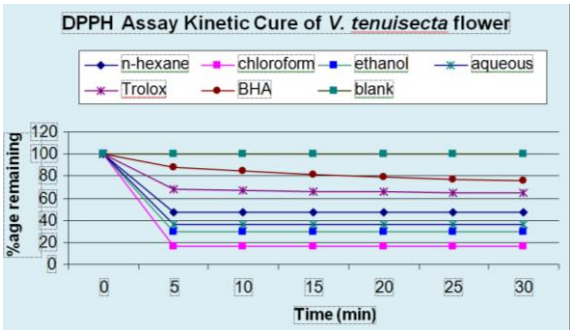

Fig. 7. DPPH kinetic curve of crude extracts of flower of *V. tenuisecta* Briq.

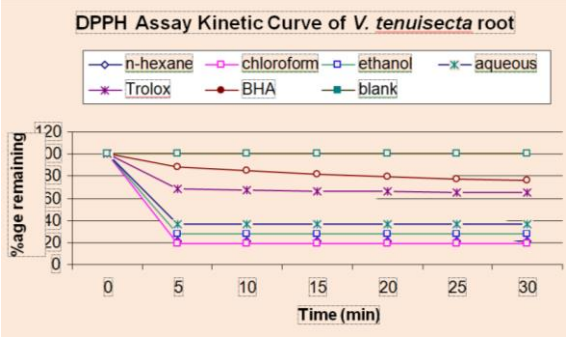

Fig. 8. DPPH kinetic curve of crude extracts of root of *V. tenuisecta* Briq.

Discussion

Pakistan has the great biodiversity and therefore great opportunities for producing advanced plant-derived products with antioxidant properties. Medicinal plants are a great source of information for an extensive variety of chemical constituents. The present investigation revealed, wide range of phytochemicals were present in the different crude extracts of *V. tenuisecta* Briq. Alkaloids, flavonoids, terpenoids, Tannins, Phenols, reducing sugars and saponins showed positive results for all extracts of plant.

These phytoconstituents were associated with antimicrobial, anti-mutagenic, anti-carcinogenic and antioxidant activities (Sen *et al.*, 2005).

So, presence of these phytocompounds in an effective manner showed a positive correlation with antioxidant capacity of *V. tenuisecta* Briq. because all the parts of the plant possessed a good range of primary as well as the secondary metabolites with the specific activities to treat various health diseases and chronic diseases as well.

The antioxidant adequacy of the crude extracts of *V. tenuisecta* Briq. was estimated through five assays. The results revealed through antioxidant activity of *V. tenuisecta* Briq. extracts for Total Phenolic Content showed that chloroform extract of leaf possessed the highest phenol concentration 100.12±0.17, whereas the ethanol extract of leaf exhibited the highest concentration of flavonoids 1036.18±3.56.

The antioxidant activity of phenols is primarily due to their redox properties, which makes them to act as reducing agents and singlet oxygen quenchers. Flavonoids are very active scavengers of most oxidizing molecules, including singlet oxygen, and several other free radicals involved in numerous diseases. Flavonoids suppress reactive oxygen development, scavenge reactive species and protect antioxidant defenses.

Among all the crude extracts of *V. tenuisecta* leaf macerate in distilled water showed the highest DPPH free radical scavenging activity i.e. 1.79. A lower value of %age DPPH remaining indicated a higher antioxidant potential. The results of ABTS assay were expressed in terms of TEAC value. The maximum TEAC value was documented by ethanol extract of stem 11.16^{a}±0.92. As surplus free irons have been associated in generation and formation of free radicals in biological systems, metal chelating activity of *V. tenuisecta* was assessed in terms of %age bound iron. Among all the crude extracts flower macerates in ethanol had displayed best %age bound iron capacity i.e. 87.25%.

Conclusion

The results of the present study had put forward the conclusion that *V. tenuisecta* have an effective quantity of antioxidant and free radical scavenging molecules including vitamins, terpenoids, phenolic acids, tannins, flavonoids, alkaloids, and other secondary metabolites that might be useful as the constituents of antimicrobial and antioxidant drugs. This study illustrated that the indication of chemical compounds in different crude extracts from *V. tenuisecta* could be used as a vital source of natural antioxidant for food and pharmaceutical industry.

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