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Evaluation of phytochemical screening and antioxidant activity of crude extracts of *Abutilon fruiticosum* Gill. & Perr.

Suraya Mushtaq*, Zaheer-Ud-Din Khan

Department of Botany, GC University, Lahore, Pakistan

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Abstract

The present study was an attempt to determine the phytochemical screening and antioxidant potentialof crude extracts of *Abutilon fruiticosum* Gill. & Perr. in *n*-hexane, chloroform, ethanol and distilled water, using Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH radical scavenging activity, ABTS assay and Metal Chelating activity.All the activities revealed the best results, the Total Flavonoid Content assay showed the best results among all the antioxidant activities. The qualitative analysis of the phytochemical screening of all the extracts directed towards the presence of alkaloids, flavonoids, cardiac glycosides, tannins, phenols and terpenoids.

* **Corresponding Author:** Suraya Mushtaq ✉ Suraya.mushtaq@ymail.com

Introduction

The free radical mediated destructions can cause many diseases, particularly coronary heart disease, cancer and diabetes (Selvam *et al.,* 2012). It is well known that cell damage and consequent tissue injury cancaused by the free radicals through mechanism of covalent bonding and lipid peroxidation. Antioxidant possessions of some flavonoids from the plant source have been recognized previously (Devasagayam *et al.,* 2004; Matlawska and Sikorska, 2002).

The use of traditional medicine is widespread in Africa and medicinal plants are still a large source of natural antioxidants that might serve as leads for the development of novel drug against free radical induced diseases. Medicinal plants are commonly used in treating and preventing specific ailments and diseases and are considered to play a beneficial role in health care (Karandikar *et al.,* 1997; Abdul Rahuman *et al.,* 2008).

In recent years, there is an increasing interest in finding antioxidant phytochemicals, because they can inhibit the propagation of free radical reactions, protect the human body from diseases and retard lipid oxidative rancidity in foods. The most effective ones seem to be flavonoids and other phenolic compounds of many plant raw materials, particularly in herbs, seeds, and fruits (Chung *et al*., 2006).

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) 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).

Now a days, there has been a greater concentration to categorize antioxidant constituents that are pharmacologically effective and having less or no any side effects to use in anticipatory medications. In food, antioxidant components play an essential role in health protection and also neutralizes the free radicals. The preventive medical research demonstrated that efficient nutrition plays a vital role in reduction of the risk factors of chronic disorders (Hodzic *et al*., 2009). 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). 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). Thus the present investigation was aimed at evaluating the antioxidant activity of *Abutilon fruiticosum* Gill. & Perr. leaves, stem, roots, flower and fruit. *Abutilon fruiticosum* belongs to family Malvaceae and is distributed in Pakistan, Ethiopia & Somaliland (Abedin, 1979). There is not any study carried out for the antioxidant activity on this plant previously but various plants of *Abutilon* species are traditionally claimed for their varied pharmacological and medicinal activities. Furthermore, different plant parts contain specific phytoconstituent responsible for their biological activity (Sikorska and Matlawska, 2008).

Materials and methods

Plant material

Fresh parts of the plant *Abutilon fruiticosum* Gill. & Perr. were collected from District Okara and authenticated with the help of flora of Pakistan. A voucher specimen has been deposited in Dr. Sultan Ahmad Herbarium. The collected plant parts (leaves, stem roots, flower and fruits) were separated, allowed shading dried andpulverized to coarse powder.

The prepared powder was used for extraction in *n*-hexane, chloroform, ethanol and distilled water, using maceration technique. The macerates were dried by rotary evaporator and subjected to use for the tests.

The concentrated crude extracts were weighed and the percentage yield was estimated

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

Photochemical analysis of plant extracts

The qualitative phytochemical screening of the plant extracts was carried out for the possible presence of bioactive components such as tannins, phenols, alkaloids, cardiac glycosides, quinones, saponins, reducing sugars and flavanoids according to Ayoola *et al.*, (2008), Raman (2006) and Patel *et al*, (2013).

Determination of antioxidant activity

The following parameters were used to conclude the antioxidant adequacy of different the parts of *Abutilon fruiticosum* Gill & Perr. and *Ipomoea cairica* (L.) Sweet:

DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging activity of the plant extracts was inspected after Lee *et al.* (1998). The variation in absorbance was measured and the % age of DPPH radical remaining was configured using the formula:

% age DPPH $_{\text{remaining}}$ = As/Ao×100

The kinetic curve was platted displaying decline in absorbance of DPPH with time and also calculated the EC_{50} value for each sample.

Determination of Total Phenolic Content

Total phenolic ingradients of each extract of the plants were predicted by using the technique of Makkar *et al.* (1993). Total phenolic contents were measured from the standard curve and explicit as mg/g equivalents of Gallic acid (GAE).

Total Flavonoids Content Determination

The TFC content of plant fractions was determined by following method developed by Dewanto *et al.*, (2002). The total flavonoid content (TFC) was estimated using the standard curve of Catechin values, as mg of Catechin equivalents in per gram of plant sample.

Metal Chelating Activity

The ability of plant extracts to chelate Iron (II) was determined by the method devised by Dinis *et al.* (1994). The values expressed as the % age of bound iron, which can be calculated from the formula shown below or in terms of EDTA standard.

% age bound iron= [(Acontrol – Asample)/Acontrol] or Ao-As/Ao ABTS+ Assay

ABTS assay was performed according to protocol formulated by Re *et al.* (1999). The percentage inhibition for each plant extract was calculated by using this formula;

% age inhibition = (1-As/Ao) X 100

Statistical analysis of data

All the parameters were carried out in triplicates and data was presented as mean value ± S.E. (standard error). The results obtained were analyzed statistically by applying analysis of variance (ANOVA) and Duncan's Multiple Range Test at 5% significant value of analysis, after Steel *et al.* (1997).

Results and discussions

Phytochemical analysis

The phytochemical screening of leaves, stem, root, flower and fruit of *Abutilon fruiticosum* Gill & Perr. was performed and revealed the existence of secondary metabolites; tannins, flavanoids, terpenoids, alkaloids, phenols, reducing sugars, cardiac glycosides and saponnins (table 1).

Antioxidant activity

DPPH Free Radical Scavenging Activity

Stem, leaf, root, flower and fruit of *Abutilon fruiticosum* Gill & Perr. were used to calculate their DPPH free radical scavenging activity.

All the extracts of *Abutilon fruiticosum* Gill & Perr. showed appropriate antioxidant abilities and EC_{50} value was calculated. Minimum absorbance of fruit in ethanol i.e, 0.077±0.00 showed minimum percentage yield and higher DPPH free radical scavenging activity (Fig. 1-5).

Table 1. Phytochemical screening of crude extracts of *Abutilon fruiticosum* Gill. & Perr.

Constituents	Phytochemical tests	Plant Macerates																			
		Leaf				Root				Stem				Flower				Fruit			
		H	C	E	W	H	C	E	W	H	C	E	W	H	C	E	W	H	C	E	W
Alkaloids	Mayer's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	Wagner's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Drangendorff's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins and phenols	Ferric chloride test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
	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	-	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	+

+ = presence of phytoconstituents, - = absence of phytoconstituents.
H= *n*-hexane, C= Chloroform, E= Ethanol, W= Distilled Water.

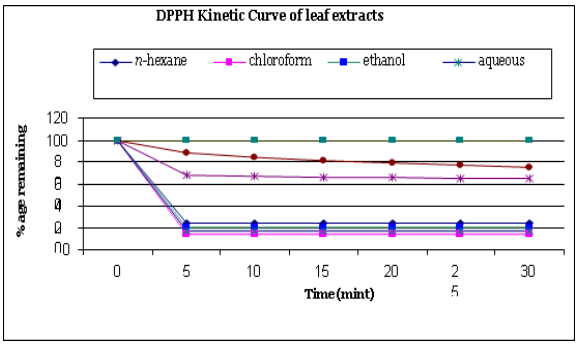

Fig. 1. Graphical representation of DPPH Assay kinetic curve of *Abutilon fruiticosum* Gill & Perr. leaf.

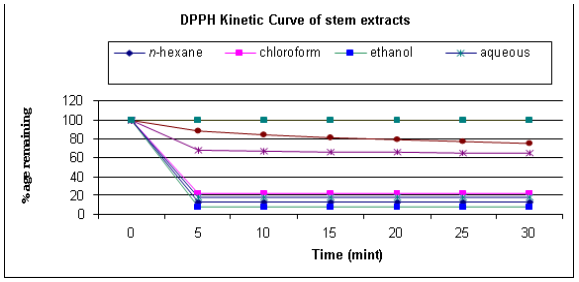

Fig. 2. Graphical representation of DPPH Assay kinetic curve of *Abutilon fruiticosum* Gill & Perr stem.

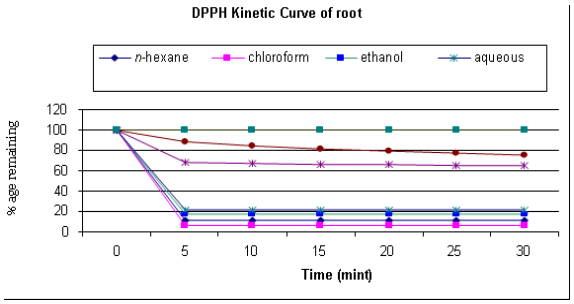

Fig. 3. Graphical representation of DPPH Assay kinetic curve of *Abutilon fruiticosum* Gill & Perr root.

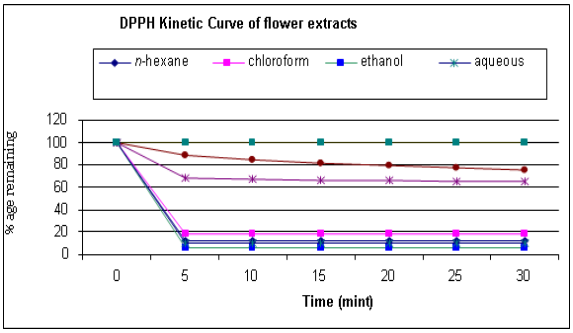

Fig. 4. Graphical representation of DPPH Assay kinetic curve of *Abutilon fruiticosum* Gill & Perr. flower.

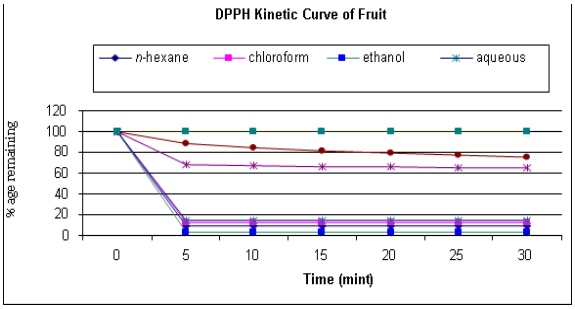

Fig. 5. Graphical representation of DPPH Assay kinetic curve of *Abutilon fruiticosum* Gill & Perr. fruit.

Total Phenolic Activity

The flower in chloroform, leaf and stem aqueous extracts of *Abutilon fruiticosum* Gill & Perr showed maximum GAE values, i.e. 114.12±0.60 µg/ml, 131.37±0.11 µg/ml and 147.25±0.12µg/ml, respectively whereas the ethanol root extract exhibited the lower GAE value, i.e. 4.00±0.05 µg/ml. The fruit and other extracts exposed significantly higher to moderate values of TPC (Fig. 6).

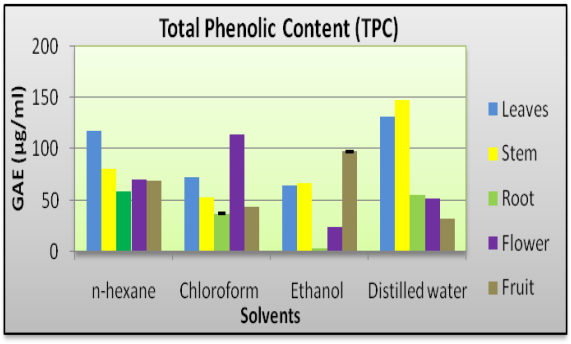

Fig. 6. Graphical representation Total Phenolic Content of leaf, stem, root, flower and fruit of *Abutilon fruiticosum* Gill & Perr.

Total Flavonoid Content Assay

The TFC of the *Abutilon fruiticosum* Gill & Perr. showed overall best results for all the extracts. The root extracts had highest TFC values, i.e. 1206.18±2.88 mg/g (*n*-Hexane), 1749.18±5.19 mg/g (chloroform) and 1838.90±5.13 mg/g (ethanol) than others. All the other extracts had the TFC values ranging from 103.45±0.84 mg/g to 1562.54±1.46 mg/g (Fig. 7).

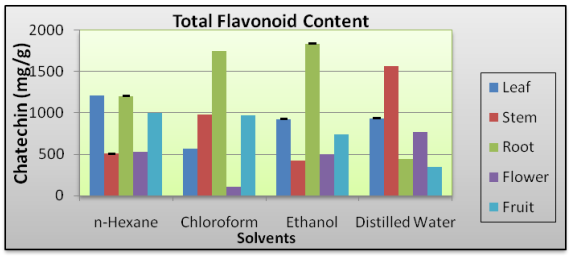

Fig. 7. Graphical representation of Total Flavonoid Content of *Abutilon fruiticosum* Gill & Perr. leaf, stem, root, flower and fruit.

ABTS Assay of Abutilon fruiticosum Gill & Perr.

7.96±0.19, 7.60±0.03 and 7.45±0.21 mM of Trolox was the maximum TEAC values reported in the aqueous, *n*-hexane and ethanol extract of stem, respectively, while the minimum value was of the chloroform fruit extract 0.19±0.00mM and *n*-hexane and chloroform root extracts 0.42^{n}±0.05, 0.31^{no}±0.00mM of Trolox (Fig. 8).

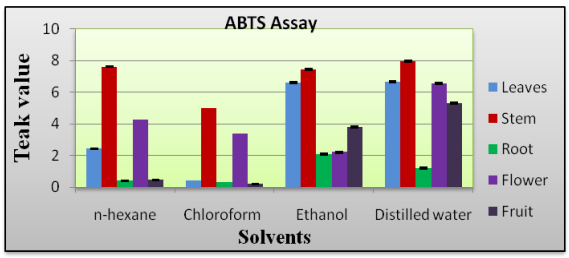

Fig. 8. Graphical representation ABTS Assay of leaf, stem, root, flower and fruit of *Abutilon fruiticosum* Gill & Perr.

Metal chelating assay of Abutilon fruiticosum Gill & Perr extracts

Flower extract in chloroform and fruit extract in *n*-Hexane showed maximum and minimum percentage inhibition in *Abutilon fruiticosum* Gill & Perr. i.e. 70.8% and 1.4%, appropriately (Fig. 9).

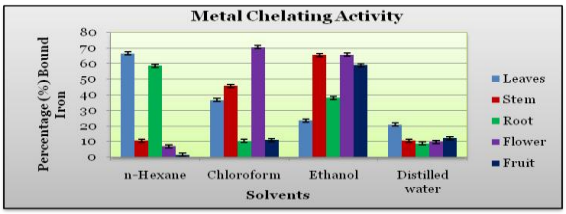

Fig. 9. Graphical representation Metal Chelating Activity of leaf, stem, root, flower and fruit of *Abutilon fruiticosum* Gill & Perr.

Discussions

The present ethnopharmacological study was executed in order to find out the antioxidant potential of medicinally important plant, *Abutilon fruiticosum* Gill & Perr. through Phytochemical screening, and antioxidant activities. Various parts of the plants were separated, shade dried, ground to fine powder and macerated in polar and non-polar solvents. The percentage yield of all crude extracts of *I. cairica* (L.) Sweet and *A. fruticosum* Gill & Perr. was computed and the highest yield was documented by aqueous flower extract of *A. fruiticosum* Gill & Perr. i.e. 17.34%.

The antioxidant activity was evaluated by using five assays, i.e. DPPH free radical scavenging activity, metal chelating activity, ABTS assay, TPC, and TFC assay. The leaf, stem, root, flower and fruit extract of *A. fruiticosum* Gill & Perr. were subjected to DPPH free radical scavenging activity to assess their maximum antioxidant potentials. All of the extracts showed appropriate DPPH scavenging ability. The ethanolic extract of the fruit of *A. fruiticosum* Gill & Perr. showed maximum DPPH value as the percentage remaining i.e. 2.56%. All the ethanol extracts of the *A. fruiticosum* Gill & perr. except the root extract showed the maximum DPPH free scavenging activity (Fig. 1-5).

The metal chelating therapy is common practice of neutralizing iron overload in the body. The results of metal chelating ability of extracts of different parts of the *A. fruiticosum* Gill & Perr. were recorded and the maximum ability was shown. The % bound iron of ethanol extracts of fruit and stem, i.e. 59.2%, 65.6%, *n*-Hexane extract of leaf and root, i.e. 66.7%, 58.8% and chloroform extract of flower, i.e. 70.8% showed the maximum metal Chelating potential. Therefore, chelating of metal ions by natural phytochemicals from this plant can prove to be of therapeutic importance (Fig. 9).

The results of ABTS assay were determined on the percentage inhibition shown by the different extracts of Trolox, which was used as a standard in this assay. Depending upon the %age inhibition shown by various extracts and comparison with the percentage inhibition shown by the Trolox Equivalent Antioxidant Concentration (TEAC) of the extracts determined. The highest TEAC value was exposed by the ethanol and aqueous extracts of *A. fruiticosum* Gill & Perr. while all the stem extract exhibited higher TEAC values (Fig. 8).

Phenolic compounds comprised a part of unsaponifiable matter called minor constituents of oils. These components are determinent for some characheristics of oils as flavour, shelf life and resistance against oxidation. The concentration of total phenols in the methanolic extracts was estimated with Folin-Ciocalteau's reagent. The total phenolic content (TPC) of the extracts of the *A. fruiticosum* Gill & Perr. was determined and calculated using the standard curve of Gallic acid and expressed as Gallic Acid Equivalent (GAE). All the parts showed maximum values of the TPC. The aqueous extract of *A. fruiticosum* Gill & Perr. showed the maximum value 147.25±0.12µg/ml (Fig. 6).

The components such as flavonoids, hold hydroxyl group are responsible for the radical scavenging ability in the plants. The TFC capacity of the *A. fruiticosum* Gill & Perr. was calculated with the comparison of Catech in standard curve in mg/g. The plant showed highest TFC values for all the extracts, as the extracts the best results from 103.45±0.84 mg/g to 1838.90±5.13 mg/g. Overall, the root of the A. *fruiticosum* Gill & Perr. showed the remarkable results (Fig. 7).

Conclusions

The present study concluded that *Abutilon fruiticosum* Gill & Perr. had some quantity of the active phytochemicals and antioxidant compounds which can be used as an alternative of the synthetic medicine for the treatment of different human diseases. Overall, *Abutilon fruiticosum* Gill & Perr. had significant antioxidant activity, thus supporting its traditional use in medicinal practices.

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