

Antioxidant activity of an ethnobotanically important plant *Quisqualis indica* Linn.

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Abstract: The antioxidant potential of leaf, stem, root and flower extracts of *Quisqualis indica* Linn. was assessed to verify its ethnopharmacological importance. Both polar and non-polar solvents like n-hexane, chloroform, ethanol and distilled water were used to obtain crude extracts. The chloroform extract of leaves showed the maximum %age yield, i.e. 27.3% while the n-hexane extract of stem showed the minimum yield, i.e. 0.2%. Five activities including DPPH free radical scavenging activity, ABTS+ assay, Total flavonoid components (TFC), Total phenolic components (TPC) and Metal chelating Assay (MC) were employed to evaluate the antioxidant activity of the plant. The ethanol extract of inflorescence of the plant displayed most elevated DPPH potential, i.e. 452.11%. Aqueous extract of root had highest value of TEAC i.e., 7.4515 mmol. The aqueous extract of flower displayed the highest level of phenolic contents with the value of 35 in terms of GAE mg/mL. On the other hand, the chloroform extract had the highest % bound iron value of 128 and the aqueous extract of flower showed a high concentration of Flavonoids having the value 347.65mg/l of Quercetin. It has been inferred that all parts of *Quisqualis indica* L. possess good antioxidant potential. Different parts showed different antioxidant potentials hence they can be used as curative agents against human and animal ailments.

Keywords: *Quisqualis indica* Linn., antioxidant activity, Percentage yield, ABTS radical cation assay, DPPH assay, Total Phenolic contents (TPC), Total Flavonoid contents (TFC), Metal Chelating assay (MC).

INTRODUCTION

Medicinal plants efficiently produce wide number of chemical products or constituents with precious selectivity (Uddin *et al.*, 2012). According to the report published by the World Health Organization, the respective conditions prevail in more or less 80% of the world's community where the synthetic drugs are either inaccessible or too exorbitant to be afforded by the local man (WHO, 1993). The oldest form of health care known to mankind is herbal medicine. Many drugs which are commonly used today are of herbal origin. There are many basic systems of herbal medicine which are part of traditional oriental medicine. These systems include Ayurveda, Homeopathy, Siddha, Unani, traditional herbalism and Chinese herbalism (Ravikumar and Rathinam, 2009).

Man of previous ages used the herbs that were easy to procure as therapeutics and used for the treatment of various diseases. Nature has blessed all living creatures with abundant plant wealth which have great medicinal value. Large number of plants are not yet been explored despite of the fact that the importance of some plants has long been published. So, there is a need to explore the uses of such plants to know the pharmacological importance and therapeutic properties of these plants (Mushtaq *et al.*, 2009). There is a wide range of plant resources in Pakistan that have therapeutic properties.

Marginal communities use more than 1,000 species having medicinal properties to cure various diseases (Latif *et al.*, 2004). Plants are one of those resources of medicines that are not harmful and have no side effects as well. Hence they serve as one of the most efficient source for a healthy life because they contain a large number of important nutrients (Ajaib *et al.*, 2011). Medicinal plants contain many components that have great therapeutic potential. Many plants contain such components that have been found to possess good antimicrobial and antioxidant potential. Plants having good antimicrobial and antioxidant potential mostly contain isocatechins, catechins, flavones, anthocyanin, flavonoids, isoflavones, lignans and coumarins (Aqil *et al.*, 2006). Diseases like atherosclerosis, diabetes, stroke, Alzheimer's disease and cancer are being treated and prevented by the drugs with great antioxidant activity (Devasagayam *et al.*, 2004). In traditional medicine, the plants which were used to treat various ailments have been found to contain physiologically active components (Adebanjo *et al.*, 1983).

There has been a great interest in treating the tissue injury induced due to free radicals using the plant components having antioxidant potential. Such antioxidants have potential to reduce such free radicals and lessen the chance of damage (Pourmorad *et al.*, 2006). Various teas, wines, fruits, vegetables spices, rosemary and sage which were used traditionally as natural antioxidants are now commercially exploited to use them as nutritional products and antioxidant additives (Schuler, 1990).

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Phenolic components are found to be the reason of antioxidant potential (Duh *et al.*, 1999). Polyphenolic components in plants like flavonoids possess properties of free radical scavenging and anti-inflammatory action (Frankel, 1995). Medicinal plants are found to be an efficient and safer source of antioxidant components as they do not need any strict quality control measurements. Thus, phytotherapeutics prove to be a cheaper and time saving source than any other drugs (Niero, 2010).

The plant of interest, *Quisqualis indica* Linn. belongs to the family Combretaceae (Indian Almond/White Mangrove family/Rangoon Creeper family). Plants of the Combretaceae family occur mostly in tropical and subtropical areas. They can be trees, shrubs or climbers and are easily recognized by the appendages of their fruits which are wing shaped and are most widely used for medicinal purposes (Rogers and Verotta, 1996).



Fig. 1: *Quisqualis indica* Linn. (Rangoon Creeper)

This study contains the antioxidant activity of the extracts from different parts of the plant *Quisqualis indica* Linn. The antioxidant potential of the roots, stem, leaves and flowers of the selected plant was evaluated using various antioxidant tests including total phenolic contents (TPC), total flavonoids contents (TFC), free radical scavenging (DPPH) assay, (ABTS) radical cation assay and metal chelating activities.

MATERIALS AND METHODS

Plant material

Quisqualis indica Linn. was collected from the botanic garden of GC University, Lahore. The plant specimen was recognized, authenticated and submitted in Dr. Sultan Ahmad Herbarium, GC University Lahore, as voucher specimen.

Extraction and fractionation of antioxidants

Plant parts were dried in shade, grounded and extracted in polar and non-polar solvents like n-hexane, chloroform,

ethanol and water using the maceration method. The extracts were macerated for 10 days in each solvent respectively. These were then dried and used to prepare plant fractions of various concentrations to evaluate their antioxidant potential.

Physical screening

The plant extracts of *Q. indica* were subjected to physical analysis including its color, texture and % yield before using them for further evaluations.

Phytochemical screening

The presence of secondary metabolites was investigated by performing phytochemical analysis, using the standard procedure after Ayoola *et al.*, (2008). Phytochemical screening included Test for the presence of Reducing sugars (Fehling's test), Test for the evaluation of Terpenoids (Salkowski test), Test for Flavonoids, Test for Saponins, Test for Tannins, Test for Alkaloids and Test for Cardiac glycosides (Keller-Killiani test).

Antioxidant assays

Antioxidant activity of all the extracts was assessed using the following standard procedures.

DPPH radical scavenging activity

The DPPH radical scavenging activity of all extracts of plant was examined. Then the activity of all extracts was compared with a standard antioxidant, butylated hydroxytoluene (BHT) after the method of (Lee *et al.*, 2001).

Total phenolic contents

Phenolic content of the extracts of plant was assessed by using the method of Makkar *et al.*, (1993).

ABTS assay protocol

ABTS•+ Assay was determined using the method of Re *et al.*, (1999). All the samples were run three times and their absorbance was noted at 734nm. Then the mean value of absorbance was measured. Absorbance of Trolox was calculated at 734nm and its dose response curve was plotted by using the absorbance value. Standard formula was used to calculate percentage inhibition of every sample.

Total flavonoid contents determination

The Total Flavonoid Content of plant extracts was determined using the method of Dewanto *et al.* (2002).

Metal chelating activity

Method by Dinis *et al.* (1994) was used to test the ability of extracts to chelate Iron (II).

STATISTICAL ANALYSIS

All the parameters used to assess the antioxidant potential of different plant extracts of *Quisqualis indica* Linn. were

carried out three times. The values were then organized by taking the mean values along with the standard errors using Microsoft Excel 2010.

RESULTS

Percentage yield

The chloroform extract of the leaves showed maximum yield of 27.3% as compared to all the extracts whereas the minimum yield of 0.2% was observed in n-hexane extract of stem. As far the plant parts were individually concerned, the maximal yield of 18.5%, 5.16%, 3.13% & 3.0% was expressed by the aqueous extract of leaves, the n-hexane extract of roots and the n-hexane and ethanol extracts of flowers respectively (fig. 2).

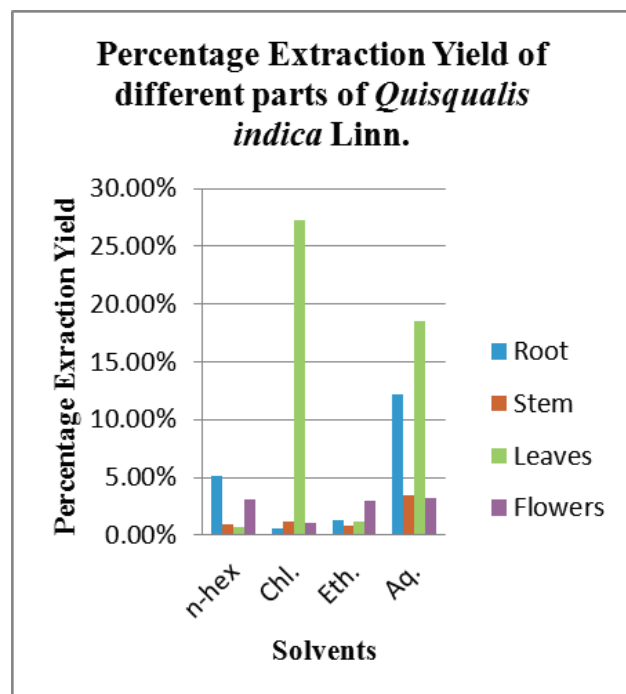


Fig. 2: Graphical representation of the % extraction yield of the different parts of *Quisqualis indica* Linn.

Phytochemical analysis

Phytochemical screening of crude extracts of stem, leaf, root and flower extracts was performed using standard procedures of Ayoola *et al.* (2008). Analysis showed that extracts had variety of secondary metabolites such as alkaloids, tannins, saponins, terpenoids, flavonoids, cardiac glycosides and reducing sugars (table 1).

Antioxidant activity

DPPH radical scavenging activity: % DPPH free radical quenching potential of n-hexane, chloroform, ethanol and aqueous extracts of different parts of *Quisqualis indica* Linn. was evaluated (table 2). It was observed that increasing the concentration of fractions in assay increased the scavenging activity as well. The results obtained were compared with a standard known as BHT. The ethanol extract of inflorescence of the plant

displayed the highest DPPH radical scavenging activity as compared to other fractions. It showed $452.11 \pm 0.44\%$ of DPPH radical inhibition. The DPPH potential of the plant extracts was found to be lying within the range of 452.11 to 28.17%.

ABTS assay protocol

Although the scavenging activity was comparatively low, almost all the extracts scavenged the ABTS radical cation which shows that all of them had some antioxidant potential. All the results of assay were expressed in terms of Trolox equivalent antioxidant capacity, which is the measure of antioxidant activity. All extracts showed small antioxidant potential because of their lower TEAC value. The ethanol extract of roots had the least antioxidant activity having the TEAC value of 0.6842 mmol of Trolox equivalence. Aqueous extract of root had highest value of TEAC i.e., 7.4515 mmol (table 2).

Total phenolic contents

The total phenolic contents present within the root, stem, leaf and inflorescence of the Rangoon Creeper were obtained in relation to Gallic acid. All the values were estimated by comparing the values with the standard curve of Gallic acid. Results were expressed in terms of GAE mg/ml (table 2). The aqueous extract of flower displayed highest level of phenolic content while the leaf extract macerated in distilled water showed the least concentration (fig. 3). Moreover, leaf, stem and root showed mixed trend thus establishing that the phenols were unevenly distributed in plants.

Total flavonoid components

The aqueous extract of flower had a high concentration of Flavonoids. Other extracts also contained significant but comparatively lower quantities of Flavonoids (table 2 & fig. 4). The increasing order of Flavonoid contents of *Quisqualis indica* Linn. is shown below:

Flowers > Stem > Leaves > Root

Metal chelating activity

The maximum value of percentage of bound iron was shown by the chloroform extract (table 2). Other extracts also showed the value of percentage of bound iron but it was comparatively less than the chloroform extract of stem (fig. 5). The trend explaining the order of percentage of bound iron in different parts of *Quisqualis indica* Linn. was:

Stem > Leaves > Root > Flowers

DISCUSSION

Basically to diminish the unpaired condition of electrons, an antioxidant neutralizes the free radicals by donating or accepting an electron. It changes the free radical to neutral form and itself becomes an antioxidant free radical. Now, the radical formed is comparatively less reactive than the radical which was neutralized. Hence, the chance of

damage is reduced (Halliwell *et al.*, 2000). Antioxidants may be exogenous or endogenous. Exogenous antioxidants are the dietary supplements that include Vitamin C, Vitamin E, carotenoids, minerals, synthetic antioxidants and various phytoconstituents and phytonutrients. Such antioxidants play an important role in the control of damage caused by free radicals (Ames, 1999).

Various phytoconstituents such as phenols, terpenoids, alkaloids, flavonoides, etc present in plants have been found to contain variable antioxidant potentials. Such compounds show radical scavenging activity. Green tea, grapes, wines, soyabeans, ginko biloba are the source of antioxidant active plant sources. Such medicinal plants have been exploited commercially to get the antioxidants which are later used as dietary supplements or antioxidant additives. Some examples of the plants containing antioxidants are Rosemary & Sage and some species e.g. cloves, cinnamon, etc. (Gupta, 2010).

The qualitative identification of plant constituents was done by phytochemical screening. Such tests help us to exploit phytochemicals commercially in order to recognize the medicinal value of plant. The phytochemical screening exposed the presence of phytochemicals including reducing sugars, tannins, saponins, alkaloids, flavonoids, terpenoids and cardiac glycosides. Reducing sugars and cardiac glycosides were present in all extracts while terpenoides were absent in aqueous extract of root. In the same way saponins showed positive response for all extracts except the ethanol extract of stem. All extracts showed the presence of flavonoids except the n-hexane, chloroform and ethanol extract of root. Tannins were present in all extracts of flower and in the chloroform extract of stem while the alkaloids were present in the chloform extracts of stem and leaf only (table 1).

The root, stem, leaf and flower extracts of *Quisqualis indica* Linn. were subjected to DPPH free radical scavenging activity to assess their possible antioxidants potential. Basically DPPH is a well known free radical and is a scavenger for other radicals. All the extracts of plant showed the DPPH activity more than 50% (table 2). Almost all the extracts of *Quisqualis indica* Linn. reported to possess strong antioxidant potential as displayed by its % DPPH assay. The maximum efficiency was exhibited by the ethanol extract of root with the % DPPH assay of 452.11%. Similar results were documented by Jindal and Mohamad (2012) while estimating the antioxidant activity of *Ardisia crispa*. However, this test only helps us to check the total amount of antioxidants in particular extract and doesn't help to identify any particular phytochemical present in extract. Thus the percentage inhibition of DPPH only confirms that various phytochemicals are present in the different

extracts of *Q.indica* in variable quantity that could be used for the treatment of various diseases.

In order to measure the antioxidant capacities of foods, agricultural industry and food industry frequently use the ABTS assay (Huang *et al.*, 2005). In this assay, sodium persulphate is added to convert ABTS to its radical cation. Radical cation of ABTS has blue color and it absorbs light at 734nm (Re *et al.*, 1999). Most antioxidants like phenolics, thiols and Vitamin C are able to react with the ABTS radical cation (Walker *et al.*, 2009). ABTS cation is converted from blue color to its neutral colorless form upon reaction. The reaction can be monitored by using spectrophotometer. This assay is often called as the Trolox equivalent antioxidant capacity (TEAC) assay. Trolox is the standard used to compare the reactivity of various antioxidants. Trolox is basically a water-soluble analog of vitamin E (Barclay *et al.*, 1985). All the extracts of *Quisqualis indica* Linn. had the antioxidant potential because they all scavenged the ABTS radical cation but the results showed that the scavenging activity was comparatively low. The results of ABTS assay were expressed in terms of Trolox value. Aqueous extract of root had highest value of TEAC i.e., 7.4515 mmol (table 2). The antioxidant capacity of food products is determined using ABTS assay based on special chemical properties of free radicals. Various compounds like polyphenols can quench free radicals inside human body and exist widely in various fruits. Thus, such fruits prevent oxidative damage by free radicals. ABTS assay has been used to determine the antioxidant potency of plant extract or food products. The antioxidant activity analysis of Hibiscus products is the example along with detailed method (Zhen *et al.*, 2016).

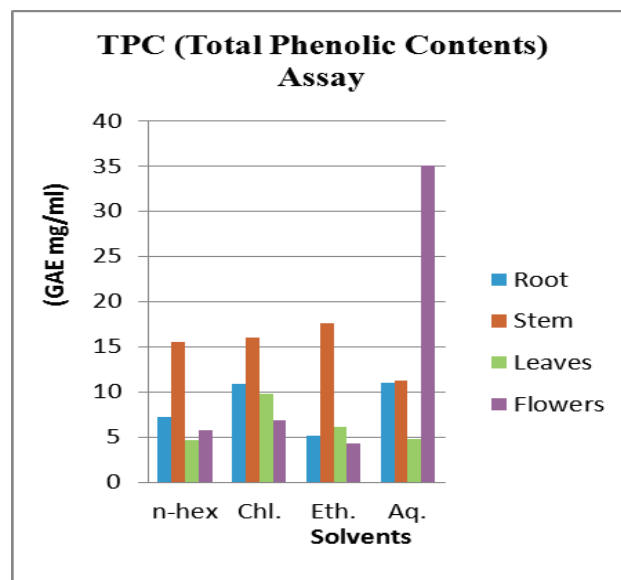


Fig. 3: Graphical Representation of the Total Phenolic Contents (TPC) assay for the extracts of different parts of *Quisqualis indica* Linn.

Vascular plants contain more than 4000 phenolic compounds (flavonoids, monophenols and poly phenols). Important plant constituents also include phenolic compounds such as quercetin, rutin, narigin, catechin, caffeic acid, gallic acid and chlorogenic acid (Qusti *et al*, 2010). Phenolic content is determined using Folin-ciocalteu. F.C reagent can be reduced by the phenolate anion which is formed by polyphenols under basic conditions. Results were articulated in terms of Gallic Acid Equivalent. The amount of reduction of F.C reagent was taken into consideration to determine the total phenols present in particular extract. Total Phenolic Contents (TPC) test was executed for all the extracts of *Quisqualis indica* Linn. (table 1 & fig. 3). The aqueous extract of flower displayed the highest level of phenolic content. Moreover, leaf, stem and root showed mixed trend thus establishing that the phenols were unevenly distributed in plants. Moreover phytochemical tests also supported the results of the total phenolic contents. Similar results were reported by Zheng and Whang, (2001) while determining antioxidant potential in some herbs. The test showed that the aqueous extract of flower had a high concentration of Flavanoids (fig. 4). Similar results were acknowledged while reporting the antioxidant potential of *Cotinus cogyria* Scop. by Riaz *et al*. (2012)

Oxyradical generation and the consequent oxidative damage can be avoided by metal ion chelating activity of an antioxidant molecule. Concentration of the catalyzing transition metal can be reduced by metal ion chelating capacity. Ferrozine assay is used to determine the chelation of iron. The results of Metal Chelating ability of extracts of different parts of *Quisqualis indica* Linn. were impeded with ferrous-ferrozine complex and it was observed that the chloroform extract had the highest % bound iron value which means it has highest metal chelating activity with the value of 120.2% (table 1 & fig. 5). Similar results were reported by Serteser *et al.*, (2009) while determining the antioxidant activity of some Turkish plants using metal chelating activity method.

Summarizing the results of antioxidant activities, all extracts exhibited good antioxidant activities in all parameters except ABTS assay protocol. Plant has been found to have good antioxidant potential. But, before using the plant for ethnopharmacological purpose, the possible risks due to the toxicity of plant should be determined in order to avoid any possible damage. Quality of the plant material used is the parameter on which the therapeutic efficiency depends. But it is useful only under the conditions that plant contains active principles which should be clearly examined to justify the actual value of plant (Zaveri and Suvitha Jain, 2010).

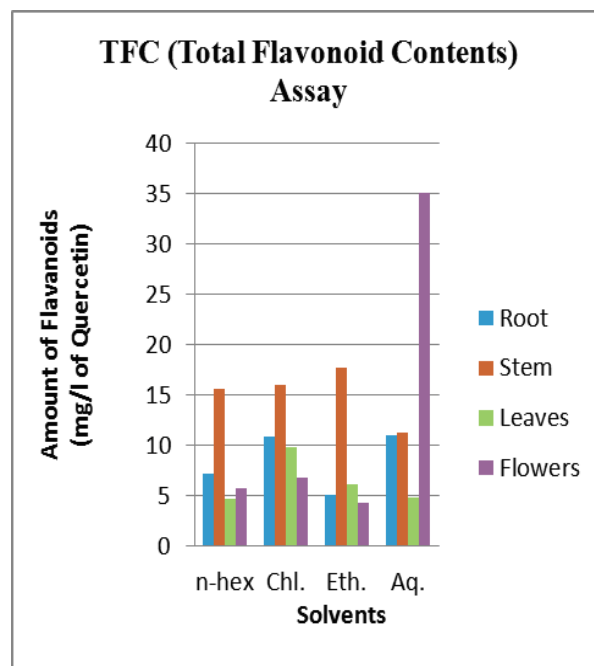


Fig. 4: Graphical Representation of the Total Phenolic Contents (TFC) assay for the extracts of different parts of *Quisqualis indica* Linn.

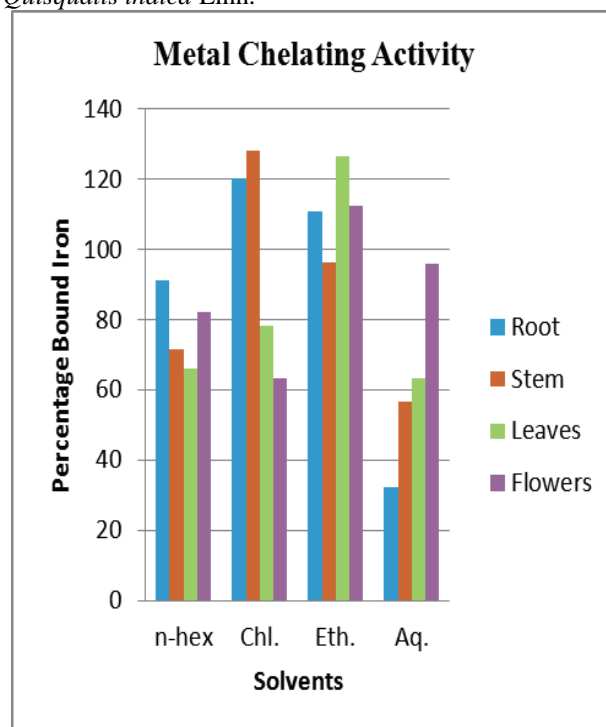


Fig. 5: Graphical Representation of the metal chelating activity for the extracts of different parts of *Quisqualis indica* Linn.

CONCLUSION

Current study concluded that *Quisqualis indica* Linn. might be utilized to cure human diseases such as colds, dermal diseases, urinary infections, abdominal pains,

Table 1: Phytochemical analysis of different parts of *Quisqualis indica* Linn.

Plant Part	Solvents	Presence or absence of phytochemical constituents						
		Reducing sugars	Cardiac Glycosides	Terpenoids	Flavonoids	Saponins	Tannins	Alkaloids
Root	N-hexane	+	+	+	—	+	—	—
	Chloroform	+	+	+	—	+	—	—
	Ethanol	+	+	+	—	+	—	—
	Aqueous	+	+	—	+	+	—	—
Stem	N-hexane	+	+	+	+	+	—	—
	Chloroform	+	+	+	+	+	+	+
	Ethanol	+	+	+	+	—	—	—
	Aqueous	+	+	+	+	+	—	—
Leaves	N-hexane	+	+	+	+	+	—	—
	Chloroform	+	+	+	+	+	+	+
	Ethanol	+	+	+	+	+	—	—
	Aqueous	+	+	+	+	+	—	—
Flowers	N-hexane	+	+	+	+	+	—	—
	Chloroform	+	+	+	+	+	+	+
	Ethanol	+	+	+	+	+	—	—
	Aqueous	+	+	+	+	—	—	—

Table 2: Percentage extraction yield, %DPPH inhibition, TEAC value (mM of Trolox equivalent), GAE(mg/ml), amount of flavonoids (mg/l of Quercetin) and percentage of bound Iron (%) value of the root, stem, leaf, and flower extracts of *Quisqualis indica* Linn.

Plant Part	Solvent	% Yield	% DPPH inhibition	TEAC value (mM of Trolox equivalent)	(GAE mg/ml)	Amount of flavanoids (mg/l of Quercetin)	Percentage of bound iron (%)
Root	n-hexane	5.16%	143.24f	6.449b	7.175d	117.65n	91.3g
	Chloroform	0.54%	130g	3.274e	10.894c	196.62h	120.2c
	Ethanol	1.34%	71.12lp	0.684g	5.140de	230.76e	110.6e
	Aqueous	12.2%	107.88j	7.451a	10.98c	151.10i	32.4n
Stem	n-hexane	0.9%	65.35m	2.101f	15.54b	145.59j	71.7j
	Chloroform	1.18%	281.83c	5.716c	15.98b	347.65a	128a
	Ethanol	0.8%	319.15b	3.567e	17.64b	210.07g	96.4f
	Aqueous	3.5%	121.69h	6.523b	11.24c	289.38c	56.7m
Leaves	n-hexane	0.68%	45.77h	1.233g	4.65e	255.24d	66.2k
	Chloroform	27.3%	270.98o	3.530e	9.82c	321.10b	78.1i
	Ethanol	1.2%	113.38d	5.619c	6.16de	221.10f	126.34b
	Aqueous	18.5%	243.52i	7.426a	4.77e	128.69m	63.2 l
Flowers	n-hexane	3.13%	56.197e	1.258g	5.80de	57o	82h
	Chloroform	1.08%	104.366n	4.776d	6.79de	132.14 l	63.1 l
	Ethanol	3.03%	452.11k	5.900c	4.31e	140.41k	112.3d
	Aqueous	3.21%	28.17a	3.444e	35a	348a	95.9f

backache, chest coughs, diarrhoea, fever, headache, gastrointestinal infection, skin and soft tissue infections, pneumonia, syphilis, toothache, general weakness and other mycotic diseases. It showed significant antioxidant power. The results were compared with standard antioxidants and it was found that plant has better antioxidant potential therefore it might be subjected to further *in vivo* and clinical studies. Overall *Quisqualis indica* Linn. showed significant antioxidant activity thus supporting its traditional medicinal practices.

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