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Antimicrobial potential of the crude extracts of an ethno botanically important plant *Quisqualis indica* Linn.

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Abstract

The organic and inorganic crude extracts of different plant parts of *Quisqualis indica* Linn; were obtained by maceration technique in n-hexane, chloroform, ethanol and water. The phytochemical screening of the crude extracts disclosed the presence of a variety of secondary metabolites, such as alkaloids, tannins, saponins, terpenoids, flavonoids, cardiac glucosides and reducing sugars. The crude extracts were also tested for their antibacterial and antifungal activities by using agar well diffusion method against gram-positive bacteria, i.e. *Escherichia coli* & *Pseudomonas aeruginosa*, gram-negative bacteria, i.e. *Bacillus subtilis* & *Staphylococcus aureus* and fungal strains *Aspergillus niger* & *Aspergillus oryzae*. The broad spectrum of potential in the form of zone of inhibition was exhibited against bacterial and fungal strains ranging from 9±0.3mm to 53±0.1mm and ±0.3mm to 51±0.4mm respectively. The maximum zone of inhibition, i.e. 53±0.1mm was developed by the ethanol root extract against *B. subtilis,* while the second and third largest zones of inhibition, i.e. 51±0.4 and 46±0.5 were shown by chloroform and ethanol root extracts respectively against fungal strains.

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Introduction

Finding healing powers in plants is an ancient idea. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory. The plants are still widely used in ethno medicine around the world. Historically, therapeutic results have been mixed; quite often cures or symptom relief resulted (Cowan, 1999).

Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. It is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. It is reported that, on average, two or three antibiotics derived from microorganisms are launched each year (Cowan, 1999). In the recent era, the use of plants is again increasing as scientists realize that the effective life span of any antibiotic is limited. Moreover, public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. A multitude of plant compounds (often of unreliable purity) is readily available over-the-counter from herbal suppliers and natural-food stores, and self-medication with these substances is common place. The aim of this study is to check the effectiveness of selected plant extracts and to study that how this plant could be used as an alternative form of medical treatment.

Herbal medicine is the oldest form of health care known to mankind. Many drugs commonly used today are of herbal origin. Herbal medicine can be broadly classified into various basic systems like Ayurveda, Homeopathy, Siddha and Unani, Traditional and Chinese herbalism, and is part of traditional oriental medicine (Ravikumar *et al*., 2009).

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted.

For example, the use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tee tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents (Heinrich *et al.,* 2004).

Ethnomedical plant-use data in many forms has been heavily utilized in the development of formularies and pharmacopoeias, providing a major focus in global health care, as well as contributing substantially to the drug development process (Graham *et al.,* 2000). Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments (Adebanjo *et al.,* 1983) as they contain anti-microbial properties (Sokmen *et al.,* 1999). These medicinal herbs constitute indispensable components of the traditional medicine practiced worldwide due to the low cost, easy access and ancestral experience (Martin-Bettolo, 1983). It is believed that plants which are rich in a wide variety of secondary metabolites, belonging to chemical classes such as tannins, terpenoids, alkaloids and polyphenols are generally superior in their anti-microbial activities (Cowan, 1999). This suggests that the strength of biological activities of a natural product is dependent on the diversity and quantity of such constituents. Therefore, simultaneous determination of the compounds that is possibly responsible for any biological activity would, interalia, facilitate decision-making process as in the selection of the plants for in-depth future investigations.

In developing countries, microorganisms are frequently the cause of prevailing diseases, presenting a serious public health issue in the significant segment of the population uncovered by either private or official health care systems.

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing.

This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immune suppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Graybill, 1988; Ng, 1994; Dean & Burchard, 1996; Gonzalez *et al.,* 1996). In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections (Sieradzki *et al.,* 1999).

According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Elloff, 1999).

Pakistan has been bestowed with ample wealth of plant resources that have therapeutic properties. More than 1,000 species have been reported that have medicinal values and are used by marginal communities to cure various diseases (Latif *et al,* 2004).

The plant of interest "*Quisqalis indica* Linn." belongs to the family Combretaceae Members of the Combretaceae family occur widely in tropical and subtropical areas. *Quisqualis indica* (Rangoon Creeper) is an extremely spectacular vine that blooms throughout the year. Plant is an evergreen, freely branching perennial climber and *attains the height of* 10' - 40' or more and the rate of growth is quick. Plant has the ability of medium salt tolerance and grows in a rich, moist and well-drained soil.

The climber bears slender-tubed, fragrant flowers, 1.5 - 3" long with 5 spreading lobes, in pendent, terminal racemes, 4" long, initially white, change to pink, purplish red, then bright red over a 3 day period. Fruit is a small dry drupe-like seed with five angles and five wings. *Quisqualis indica* is used for traditional medicine.

Leaves are used to relieve pain caused by fever while the roots are used treat rheumatis (Qaiser & Quaiser, 1978).

Fig. 1. *Quisqualis indica* Linn. (Rangoon creeper).

Materials and methods

Plant Material

Quisaualis indica Linn. was collected from GC University Lahore. The plant specimen was identified, authenticated and deposited in Dr. Sultan Ahmad Herbarium GC University Lahore.

Extraction and Fractionation

The shade-dried ground plant parts were extracted successively with non- polar and polar solvents, like n-hexane, chloroform, ethanol and water by maceration, in each of the solvents respectively. The extracts were then dried and used to prepare fractions of various concentrations in µg/ml to evaluate their antimicrobial potential.

Physical Screening

The physical analysis including its color, texture, order and % yield was conducted before subjecting to the further evaluations.

Phytochemical Screening

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

Antimicrobial activity

All the crude extracts were studied for their antibacterial activity using agar well diffusion method according to Ortega and Julian (1996) and Ferreira *et al.* (1996), Minimum Inhibitory Concentration (MIC) of only the ethanolic extract in mg/ml was carried out according to Murray *et al.* (1999) using modified Broth dilution assay with the help of Spectrophotometer at 595nm. The antimicrobial activity was checked against Gram-positive bacteria (*Escherichia coli* & *Pseudomonas aeruginosa*), Gram-negative bacteria (*Bascillus subtilis* and *Staphylococcus aureus*) and two fungal strains (*Aspergillus niger* and *Aspergillus oryzae*).

The antimicrobial screening of the plant extracts prepared through maceration procedure was carried out by taking into consideration the zone of inhibition assay in addition to the evaluation of the Minimum Inhibitory Concentration (MIC). The standard discs were used to determine the susceptibility of the bacterial and fungal strains. The negative control was established by evaluating the solvents in which the extracts were prepared against the bacterial and fungal strains used as test organisms (Table 2 and 3).

Statistical Analysis

All the parameters executed to exploit the potential of different parts of *Quisqualis indica* Linn were carried out in triplicates and the facts were organized in accordance to the mean of the values accomplished along with the standard error. The statistical arrangement was carried out utilizing Microsoft Excel 2010.

Results

Percentage Yield

The chloroform extract of the leaves had maximum yield (27.3%) in comparison to all the extracts, while the minimal yield was obtained from the n-hexane extract of stem, i.e. 0.2% (Table 3). As far the plant parts were individually concerned, the aqueous extract of leaves (18.5%), the hexane extract of roots (5.16%), n-hexane and ethanol extracts of flowers had provided the maximal yield (Fig. 2)

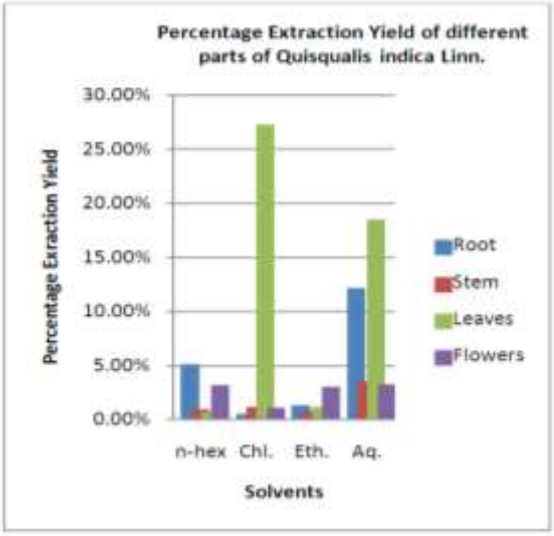

Fig. 2. Graphical representation of the % extraction yield of the different parts of *Quisqualis indica* Linn.

Phytochemical Analysis

Phytochemical screening of crude extracts of stem, leaf, root and flower of *Quisqualis indica* Linn, performed using standard procedures following Ayoola *et al.*, (2008), showed the presence of secondary metabolites such as alkaloids, tannins, saponins, terpenoids, flavonoids, cardiac glycosides and reducing sugars (Table 1).

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	+	+	+	+	–	–	–

Plant Part	Solvents	Presence or Absence of Phytochemical Constituents						
		Reducing sugars	Cardiac Glycosides	Terpenoids	Flavonoids	Saponins	Tannins	Alkaloids
	Aqueous	+	+	+	+	+	–	–
Leaves	N-hexane	+	+	+	+	+	–	–
	Chloroform	+	+	+	+	+	+	+
	Ethanol	+	+	+	+	+	–	–
	Aqueous	+	+	+	+	+	–	–
Flowers	N-hexane	+	+	+	+	+	–	–
	Chloroform	+	+	+	+	+	+	+
	Ethanol	+	+	+	+	+	–	–
	Aqueous	+	+	+	+	–	–	–

Table 2. Zone of Inhibition (mm) produced by the bacterial strains and the solvents against standard antibiotic discs.

Antibacterial Disc	Concentration (µg/mL)	Zone of Inhibition (mm)			
		S.aureus	*E.coli*	*P.aeruginosa*	*B.subtilis*
Ampicillin	10	28±3.9	16±2.5	13±0.5	29±1.5
Erythromycin	15	22±1.0	18±0.7	-	21±0.6
Gentamicin	10	13±3.5	14±2.2	12±0.5	15±2.5
Final Response		Intermediate	Intermediate	Resistant	Intermediate
Solvents	Quantity (mL)	*S.aureus*	*E.coli*	*P.aeruginosa*	*B.subtilis*
N-hexane	1.5	0±0	0±0	0±0	0±0
Chloroform	1.5	0±0	0±0	0±0	0±0
Ethanol	1.5	0±0	0±0	0±0	0±0
Aqueous	1.5	0±0	0±0	0±0	0±0
Final Response		Negligible	Negligible	Negligible	Negligible

Antimicrobial Activity

The Zone of Inhibition produced by the extracts of the root, stem, leaf and flower of the *Quisqualis indica* Linn. against the bacterial strains was documented in mm (Table 2). The maximum Zone of Inhibition was reported by the ethanol extract of roots of the respective plant, i.e. 53±0.1mm against *B. subtilis* while the minimal activity was documented by the n-hexane extract of the leaves exhibiting the zone of 9±0.3mm against *S. aureus*.

The root of the Rangoon Creeper *Quisqualis indica* Linn. displayed activity within the range of 11±0.3 to 53±0.1mm and stem documented the potential within the range of 10±1.2 to 35±0.5mm. In addition, the leaf provided antibacterial potential variation from 9±0.3 to 32±1.5mm. The flower of the Rangoon creeper showed antibacterial potential variations from 11±0.5 to 44±0.3mm.

The maximum potency was yielded by the root extracts with their effectiveness ranging from 11±0.3 to 53±0.1mm.

The antimycotic potential of the crude extracts evaluated against two fungal strains, i.e. *Aspergillus niger* and *A. oryzae* had displayed varying potential against the fungal strains with the highest potency been exhibited by the root extract macerated in chloroform and ethanol, i.e. 51±0.4 and 46±0.5mm respectively (Table 2).

However, the n-hexane extract of leaves and aqueous extract of flower had shown minimal activity, i.e. 11±0.4 and 10±0.3mm respectively. The ethanolic extracts were only employed for the MIC estimation. The most resistant antibacterial MIC value of the plant under investigation was 0.1mg/mL while the most resistant antifungal MIC value was 0.4mg/mL.

Table 3. Zone of Inhibition (mm) produced by the bacterial strains and the solvents against standard antifungal discs.

Antifungal Standard Disc	Conc. (µg/mL)	Zone of Inhibition (mm)	
		A. niger	*A. oryzae*
Griseofulvin	100	23±1.5	27±0.5
Nystatin	100	24±2.0	32±1.5
Final Response		Intermediate	Intermediate
Solvents	Quantity mL	*A. niger*	*A. oryzae*
N-hexane	1.5	0±0	0±0
Chloroform	1.5	0±0	0±0
Ethanol	1.5	0±0	0±0
Aqueous	1.5	0±0	0±0
Final Response		Negligible	Negligible

Table 4. Percentage Extraction Yield, Zone of Inhibition exhibited by different parts of *Quisqualis indica* Linn. against bacterial and fungal test organisms.

Plant part	Solvents	% Yield	Zone of inhibition (mm)				Zone of Inhibition (mm)	
			S. aureus	*E. coli*	*P. aeruginosa*	*B. subtilis*	*A. niger*	*A. oryzae*
Root	N-hexane	5.16%	16±0.3cdef	11±0.2b	11±0.3bc	11±0.5d	10±0.4e	12±0.4ef
	Chloroform	0.54%	44±0.5a	12±0.3b	11±0.5c	23±0.1bcd	18±0cde	51±0.4a
	Ethanol	1.34%	14±0.7def	12±0.5b	17±0.5b	53±0.1a	33±0.5a	46±0.5ab
	Aqueous	12.2%	11±0.3ef	12±0.8b	23±0.5a	11±0.5d	12±0.8e	15±0.1ef
Stem	N-hexane	0.9%	12±0.3def	12±0.5b	11±0.5bc	44±0.8ab	17±0.8de	22±0.3cdef
	Chloroform	1.18%	25±1.2bcd	14±1.1b	13±0.8bc	14±0.5d	12±0.3e	13±0.4ef
	Ethanol	0.8%	21±0.4bcdef	11±0.3b	10±1.2bc	15±0.8d	24±0.8bcd	18±0.6def
	Aqueous	3.5%	23±0.5bcde	35±0.5a	13±0.3bc	11±0.3d	25±0abc	31±0.7bc
Leaves	N-hexane	0.68%	21±0.5bcdef	11±0.6b	10±0.5c	14±0.5d	14±0.7e	11±0.4ef
	Chloroform	27.3%	32±1.5bc	11±0.3b	10±1.0bc	21±1.2cd	13±0.3e	13±0.4ef
	Ethanol	1.2%	25±0.3bcd	11±1.0b	12±0.8c	11±1.1d	14±0.1e	12±0.8f
	Aqueous	18.5%	9±0.3f	32±0.8a	12±0.5c	25±0.3bcd	37±0.5ab	23±0.3cde
Flowers	N-hexane	3.13%	20±0.3bcdef	16±0.5b	25±0.5a	44±0.3abc	14±0.1e	13±0.7ef
	Chloroform	1.08%	35±0.5ab	18±0.5b	12±1.2bc	22±0.3bcd	15±0.3de	16±0.5def
	Ethanol	3.03%	22±1.0bcdef	11±0.5b	24±1.0a	31±1.1bcd	18±0.2de	18±0.5def
	Aqueous	3.21%	25±0.8bcd	35±0.5a	13±0.5bc	11±0.6d	10±0.3e	28±0cd

Fig. 4. Zone of inhibition produced by ethanolic extract of root against *Bacillus subtilis*.

Fig. 3. Zone of inhibition produced by ethanolic extract of flowers against *S. aureus*.

Fig. 5. Zone of inhibition produced by chloroform extract of root against *Apergillus oryzae.*

Discussion

Plant oils and plant extracts have been utilized for thousands of years, serving many purposes, such as food preservatives and medical therapeutic agents (Hammer *et al.,* 1999). The compounds that are found in some spices and produced by herbs act as self-defense mechanisms to protect the plant against infectious organisms (Tiwari *et al,* 2009). The use of plants for healing dates to prehistory. As early as 60,000 years ago, the Neanderthals, in present-day Iraq, used plants including hollyhock for healing. These plants are still used globally (Cowan, 1999). The use of drugs and dietary supplements derived from plants have accelerated in recent years. Ethno pharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties (Cowan, 1999).

"An apple a day keeps the doctor away." This is a Traditional American rhyme which explains the benefits of a common fruit as it has various health benefits due to its medical properties. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds.

Phytochemical tests confirmed the presence of various secondary metabolites including terpenoids, alkaloids, tannins, reducing sugars, cardiac glycosides, flavonoids, phenols and saponins. The plant of interest *Quisqualis indica* Linn. was found to exhibit the antimicrobial potential. Flavonoids were found in almost all extracts and these might be the reason of strong antimicrobial potential exhibited by plant. For example, Flavonoids are hydroxylated phenolic substances and they are known to be synthesized by plants in response to microbial infection (Dixon *et al.*, 1983). It should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms.

Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya *et al.,* 1996). These flavonoids have been extensively researched due to their occurrence in oolong green teas. It was noticed some time ago that teas exerted antimicrobial activity and that they contain a mixture of catechin compounds (Toda *et al.,* 1989). In the same way, tannins were found in most of the extracts of plant. This group of compounds has received a great deal of attention in recent years, since it was suggested that the consumption of tannin-containing beverages, especially green teas and red wines, can cure or prevent a variety of ills (Serafini *et al.,* 1989). Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins (Haslam, 1996).

Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. Tannins in plants inhibit insect growth (Schultz, 1988) and disrupt digestive events in ruminal animals (Butler, 1988).

On the other hand the antimicrobial potential of plant was also due to presence of terpenoids. The fragrance of plants is carried in the oil fraction. These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure. In 1977, it was reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria (Chaurasia *et al.,* 1977). Oil of basil, a commercially available herbal, was found to be as effective as 125 ppm chlorine in disinfecting lettuce leaves (Wan *et al.,* 1998). Heterocyclic nitrogen compounds are called alkaloids. Phytochemical tests confirmed the presence of alkaloids in various extracts. The first medically useful example of an alkaloid was morphine, isolated in 1805 from the opium poppy *Papaver somniferum* (Fessenden *et al.,* 1982). Other alkaloids may be useful against HIV infection (Mc Mohan et al., 1995; Sethi, 1979) as well as intestinal infections associated with AIDS (McDevitt *et al.,* 1996). While alkaloids have been found to have microbiocidal effects (including against *Giardia* and *Entamoeba* species (Ghoshal *et al.,* 1999) the major antidiarrheal effect is probably due to their effects on transit time in the small intestine. As the selected plant possessed the good antimicrobial potential so it is confirmed that there are certain alkaloids present in the plant which are responsible for this activity of plant.

Another phytochemical test performed was to confirm the presence of cardiac glycosides. Therapeutic uses of cardiac glycosides primarily involve the treatment of cardiac failure. Their utility results from an increased cardiac output by increasing the force of contraction. By increasing intracellular calcium as described below, cardiac glycosides increase calcium-induced calcium release and thus contraction. From ancient times, humans have used cardiac-glycoside-containing plants and their crude extracts as arrow coatings, homicidal or suicidal aids, rat poisons, heart tonics, diuretics and emetics. In modern times, purified extracts or synthetic analogues of a few have been adapted for the treatment of congestive heart failure and cardiac arrhythmia (Singh *et al.,* 1970). So the presence of such metabolites in various extracts clearly explain the use of plant for cardiac treatment. Similarly the presence of some other metabolites like saponins also confirm the medical benfits of *Quisqualis indica* Linn. Saponins have biological role as membrane-permeabilising, immune ostimulant and hypochole sterolaemic properties and it has found to have significant affect growth and feed intake in animals. These compounds have been observed to kill protozoans, to impair the protein digestion and the uptake of vitamins and minerals in the gut and to act as hypoglycemic agent. These compounds thus affect animals in both positive and negative ways (Das *et al.,* 2012).

All the phytochemical tests as well as the antimicrobial potential of crude extracts confirmed that the plant can be used for various human ailments in future. Antimycotic and antibacterial activity of the plant was due to the presence of various secondary metabolites which have been studied by different scientists due to their effectiveness. Hence this proves that various extracts of the plant *Quisqualis indica* Linn. can be used to treat various ailments in the near future.

Scientists from various fields are investigating plants for their antimicrobial usefulness. Urge to study these useful plants increases as the pace of species extinction continues. Scientists working all over the world have found thousands of phytochemicals which have inhibitory effects on almost all types of microorganisms. More of these compounds should be subjected to animal and human studies to determine their toxicity level as well as an examination of their beneficial effects on microorganisms. It would be advantageous to standardize methods of extraction and invitro testing so that the search could be more systematic and more clear results could be obtained. Attention to these issues could usher in a badly needed new era of chemotherapeutic treatment of infection by using plant-derived principles (Cowan, 1999).

Conclusion

The root, stem, leaf and flower of an ethno botanically important plant *Quisqualis indica* Linn. were investigated for its antimicrobial potential. Present study confirmed that the plant can be used to treat various human ailments. The antimicrobial potency of *Quisqualis indica* is more effective than standard antimicrobial drugs so this plant can be used as an alternative of synthetic standard medicine. Overall the plant showed significant antimicrobial activity confirmed by phytochemical tests which supports its traditional medicinal practices.

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