



Antimicrobial activity and toxicity of *Quisqualis indica*

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SUMMARY

The plant *Quisqualis indica* (Compositae) has ethnopharmacological reputation of being used as a healing agent in Bangladesh. In this study, preliminary screenings were conducted to look at the antimicrobial susceptibility and cytotoxicity of the plant extract. The extractives of the plant were subjected to screening for inhibition of microbial growth by the disc diffusion method. The zones of inhibition demonstrated by the *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates of the methanolic extract ranged from 8 - 15 mm, 8 - 18 mm, 12 - 20 mm and 10 - 16 mm, respectively at a concentration of 400 µg/disc. All the extractives were also subjected to brine shrimp lethality bioassay for primary cytotoxicity evaluation. Here, the carbon tetrachloride soluble materials demonstrated the highest cytotoxicity with LC₅₀ of 0.826 µg/ml, while *n*-hexane, chloroform and aqueous soluble partitionates of the methanolic extract revealed the LC₅₀ of 1.254, 3.866 and 5.366 µg/ml, respectively. This is the first report of the antimicrobial activity and cytotoxicity *Q. indica*.

Key words: *Quisqualis indica*; Combretaceae; Antimicrobial; Cytotoxicity; Disc diffusion; Brine shrimp lethality

INTRODUCTION

Medicinal plants have long been the subjects of human curiosity and need. It is estimated that there are about 2,500,000 species of higher plants and the majority of these have not been examined in detail for their pharmacological activities (Ram *et al.*, 2004). The antimicrobial properties of certain Bangladeshi medicinal plants were reported based on folkloric information and a few attempts were made to study the inhibitory activity against certain pathogenic bacteria and fungi (Rashid *et al.*, 1997;

Rahman *et al.*, 2001). In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercially available antimicrobial drugs used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from medicinal plants, which are the good sources of novel antimicrobial and chemotherapeutic agents (Karaman *et al.*, 2003). One of the aims of the present study was to investigate the antimicrobial susceptibility of different extractives of *Quisqualis indica* against a wide range of pathogenic bacteria and fungi, which have not been evaluated earlier. Brine shrimp (*Artemia salina*) larvae have been used for many years in toxicological studies. Many researchers are now using brine shrimp as a pre-screen for plant extracts as it provide a quick,

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inexpensive, and desirable alternative to test on larger animals. It is known that a positive correlation exists between brine shrimp lethality and 9 KB (human nasopharyngeal carcinoma) cytotoxicity; brine shrimp are therefore used in many pre-screens for potential anti-tumor activity (Meyer *et al.*, 1982). In addition, brine shrimp bioassay effectively predicts pesticidal activities and respond to a broad range of chemically and pharmacologically diverse compounds (McLaughlin, 1998). So, the second objective of our study was to correlate the cytotoxic potency of the extractives of *Q. indica* through brine shrimp mortality.

Quisqualis indica (Family - Combretaceae, Bengali name - Modhumaloti) is an evergreen creeping shrub, which can be as much as 70 feet long in tropical climates (Welsh, 1998). It is widely distributed in all districts of Bangladesh (Ghani, 2003). The leaves of *Q. indica* are opposite and 7 - 15 cm long. Its flowers have a faint sweet aroma and they bloom in the spring, early summer or mid fall (Welsh, 1998). Fruits of this plant are used to treat ascariasis and oxyuriasis (Beers, 1999), while the decoction of the fruit is useful in toothache and nephritis. The roasted ripe seeds are beneficial in diarrhoea, fever and rickets. Furthermore, seeds macerated in oil can also be applied in parasitic skin troubles (De Padua, 1999). Decoction of leaves is useful in abdominal pain and the leaf juice is a remedy for boils and ulcers. The leaves are also used to treat various kinds of infantile disorders and skin diseases, where as the roots are used in rheumatism and diarrhoea. Besides, the plant extract has been found to be anticoccidial for veterinary purposes (Youn and Noh, 2001). Previous phytochemical studies with the *Q. indica* led to the isolation of quisquagenin, quisqualic acid, quisqualin A and quisqualin B (Dictionary of natural products, 2002). In this paper, we report the preliminary antimicrobial activity and cytotoxicity of *Q. indica* extractives for the first time.

MATERIALS AND METHODS

Plant materials

Stem bark of *Q. indica* was collected from Dhaka in August 2006 and was identified at the Department of Botany, University of Dhaka. The clean bark was cut into small pieces and air-dried for several days. The pieces were then oven dried for 24 h at 40°C and ground into a coarse powder.

Extraction

The powdered bark (750 g) of *Q. indica* was soaked in 1.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator. A portion (5 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning method (Vanwagenen *et al.*, 1993) into *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions. Subsequent evaporation of solvents afforded *n*-hexane (1.50 g), carbon tetrachloride (1.25 g), chloroform (1.00 g) and aqueous soluble (1.10 g) materials.

Biological screenings

Antimicrobial assay

The disc diffusion method (Bauer *et al.*, 1966) was used to test antimicrobial activity of the extractives against twelve bacteria and three fungi (Table 1). Solutions of known concentration ($\mu\text{g/ml}$) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette and the residual solvents were completely evaporated. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of kanamycin (30 μg /disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively.

Table 1. Antimicrobial activity *Q. indica* extractives at 400 µg/disc

Test microorganisms	Diameter of zone of inhibition (mm)				
	HX	CT	CF	AQ	Kan
Gram positive bacteria					
<i>Bacillus cereus</i>	10.23 ± 0.33	15.16 ± 1.14	15.35 ± 0.68	15.41 ± 0.33	25.13 ± 0.84
<i>Bacillus megaterium</i>	15.19 ± 0.24	8.24 ± 1.17	20.22 ± 0.94	13.36 ± 0.75	30.21 ± 0.61
<i>Bacillus subtilis</i>	10.33 ± 0.70	14.26 ± 0.28	12.33 ± 1.30	16.28 ± 0.73	23.16 ± 1.10
<i>Staphylococcus aureus</i>	12.45 ± 0.56	15.48 ± 0.33	16.48 ± 0.68	12.26 ± 0.67	26.33 ± 0.67
<i>Sarcina lutea</i>	11.21 ± 1.10	15.26 ± 0.15	12.37 ± 0.57	14.30 ± 0.77	24.15 ± 0.25
Gram negative bacteria					
<i>Escherichia coli</i>	-	12.37 ± 0.28	15.65 ± 0.91	12.20 ± 1.12	22.31 ± 0.34
<i>Pseudomonas aeruginosa</i>	10.33 ± 0.84	11.45 ± 0.31	14.23 ± 1.30	13.33 ± 1.10	20.16 ± 1.33
<i>Salmonella paratyphi</i>	8.37 ± 0.54	10.13 ± 1.24	13.47 ± 0.82	12.32 ± 2.12	25.20 ± 1.14
<i>Salmonella typhi</i>	11.27 ± 0.33	17.41 ± 0.98	15.35 ± 1.35	14.26 ± 0.62	25.24 ± 0.42
<i>Shigella dysenteriae</i>	8.35 ± 0.87	10.33 ± 1.32	10.33 ± 1.12	11.18 ± 0.69	25.18 ± 0.84
<i>Vibrio mimicus</i>	15.11 ± 1.11	10.98 ± 1.50	20.21 ± 1.24	15.27 ± 0.57	28.17 ± 0.70
<i>Vibrio parahemolyticus</i>	12.34 ± 0.24	16.40 ± 0.89	16.41 ± 1.67	11.11 ± 0.37	25.26 ± 0.61
Fungi					
<i>Candida albicans</i>	11.16 ± 0.31	14.15 ± 0.56	15.33 ± 0.84	13.33 ± 1.39	25.33 ± 1.13
<i>Aspergillus niger</i>	14.25 ± 0.33	18.23 ± 0.29	13.22 ± 1.61	10.12 ± 1.14	25.21 ± 0.54
<i>Sacharomyces cerevaceae</i>	08.24 ± 0.35	14.17 ± 0.68	12.11 ± 1.33	10.17 ± 1.22	20.27 ± 0.57

The diameters of zones of inhibition are expressed as mean ± S.D. (n = 3); a diameter less than 8 mm was considered inactive; HX: *n*-hexane soluble fraction of the methanolic extract; CT: carbon tetrachloride soluble fraction of the methanolic extract; CF: chloroform soluble fraction of the methanolic extract; AQ: Aqueous fraction of the methanolic extract; Kan: standard kanamycin (30 µg/disc).

These plates were then kept at low temperature (4°C) for 24 h to allow maximum diffusion. There was a gradual change of test materials concentration in the media surrounding the discs. The plates were then incubated at 37°C for 24 h to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment was carried out in triplicate.

Cytotoxicity study

Brine shrimp lethality bioassay (Persoone, 1980; Meyer *et al.*, 1982; McLaughlin *et al.*, 1998) technique was applied for the determination of cytotoxic property of the plant extractives.

Preparation of positive control group

Vincristine sulphate was used as the positive control. Measured amount of vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 µg/ml from which serial dilutions were made using DMSO to get 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.078125 µg/ml, 0.0390 µg/ml. Then the solutions were added to the premarked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water.

Preparation of negative control group

100 µl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii. If the brine shrimps in these vials show a rapid mortality, then the test is considered as invalid as the nauplii died due to

some reasons other than the cytotoxicity of the compounds.

Preparation of test groups

4 mg of each of the partitionates obtained by Kupchan fractionation was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563 and 0.781 µg/ml were obtained by serial dilution technique. Then the solutions were added to the premarked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water.

Counting of nauplii

After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC₅₀) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

Statistical analysis

For each of the extracts, three samples were prepared for each of the bioassays. The zone of inhibition and LC₅₀ were calculated as mean ± S.D. (n=3) for the antimicrobial screening and brine shrimp lethality bioassay, respectively.

RESULTS

The extractives of *Q. indica* demonstrated varying degrees of inhibition to growth of microorganisms and strong cytotoxic activity against *A. salina*. The *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions of the methanolic extract were subjected to screening for inhibition of microbial growth against 12 bacteria and 3 fungal strains. Table 1 represents the summary of the antimicrobial activity of the samples with respect to each of the test organisms. The average zones of

Table 2. LC₅₀ data of test samples of *Q. indica*

Samples	LC ₅₀ (µg/ml)
VS	0.200 ± 0.11
HX	1.254 ± 1.33
CT	0.826 ± 0.26
CF	3.866 ± 1.25
AQ	5.366 ± 1.33

The values of LC₅₀ are expressed as mean ± S.D. (n = 3). VS: vincristine sulphate (Std.); HX: *n*-hexane soluble fraction of the methanolic extract; CT: carbon tetrachloride soluble fraction of the methanolic extract; CF: chloroform soluble fraction of the methanolic extract; AQ: aqueous fraction of the methanolic extract.

inhibition produced by the *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions of the methanolic extract were found to be 8 - 15 mm, 8 - 18 mm, 12 - 20 mm and 10 - 16 mm, respectively. On the other hand, the cytotoxicity of the samples was evaluated against *Artemia salina* (Table 2) where significant effects were demonstrated by the *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions of the crude methanol extract with LC₅₀ values of 1.254, 0.826, 3.866 and 5.366 µg/ml, respectively.

DISCUSSION

In the antimicrobial susceptibility test by the disc diffusion method, the chloroform soluble material demonstrated the highest inhibitory activity in the present screening at a dose 400 µg/disc. It showed the strongest activity against *V. mimicus* and *B. megaterium* having the zone size of 20.21 and 20.22 mm, respectively. The growth of *S. aureus* (16.48 mm), *V. parahemolyticus* (16.41 mm), *E. coli* (15.65 mm), *B. cereus* (15.35 mm), *S. typhi* (15.35 mm) and *P. aeruginosa* (14.23 mm) was also strongly inhibited whereas moderate activity was found against *S. paratyphi* (13.47 mm), *S. lutea* (12.37 mm) and *B. subtilis* (12.33 mm). Among the fungi, the growth of *C. albicans* was strongly inhibited with zone size 15.33 mm. The growth of *A. niger* (13.22 mm) and *S. cereviceae* (12.11 mm) was moderately inhibited.

At the same time, the carbon tetrachloride partitionate of the methanolic extract showed significant inhibitory activity against *S. typhi* having zone of inhibition 17.41 mm. The growth of *V. parahemolyticus* (16.40 mm), *S. aureus* (15.48 mm), *S. lutea* (15.26 mm), *B. cereus* (15.16 mm) and *B. subtilis* (14.26 mm) was also inhibited significantly. In addition, it showed moderate inhibition of growth of *E. coli* (12.37 mm), *V. mimicus* (10.98 mm) and *P. aeruginosa* (11.45 mm). In case of fungi, the growth of *A. niger* was strongly inhibited (18.23 mm) by this extractive while the growth of *C. albicans* and *S. cereviceae* was also inhibited with zones of inhibition of 14.15 and 14.17 mm, respectively. The aqueous soluble materials of the methanolic extract of *Q. indica* showed moderate to strong activity against the tested microorganisms. The growth of *B. subtilis* (16.28 mm) was strongly inhibited. It also showed similar activity against *B. cereus* (15.41 mm), *V. mimicus* (15.27 mm), *S. lutea* (14.30 mm) and *S. typhi* (14.26 mm). Moderate activity was found against *B. megaterium* (13.36 mm), *S. aureus* (12.26 mm), *E. coli* (12.20 mm) and *S. paratyphi* (12.32 mm). At the same time, mild to moderate inhibition (10 - 13 mm) was found against the tested fungi by the extractive.

Following the procedure of Meyer *et al.* (1982) the lethality of *n*-hexane (HX), carbon tetrachloride (CT), chloroform (CF) and aqueous soluble partitionates (AQ) of the methanolic extract to brine shrimps was determined. The degree of lethality was found to be directly proportional to the concentration of the extract ranging from the lowest concentration (0.781 µg/ml) to the highest concentration (400 µg/ml). The LC₅₀ obtained from the best-fit line slope when the mortality of shrimp was plotted against the concentration of the sample were 0.200, 1.254, 0.826, 3.866 and 5.366 µg/ml for positive control (vincristine sulphate), HX, CT, CF and AQ, respectively. In comparison with positive control, the cytotoxicity exhibited by the carbon tetrachloride and hexane soluble fraction of methanolic extract was promising.

The significant antimicrobial activity of the

chloroform, carbon tetrachloride and aqueous soluble fractions of the crude methanol extract against a wide range of pathological microbial species and strong cytotoxicity of the *n*-hexane, chloroform, carbon tetrachloride and aqueous soluble fractions of the methanolic extract against brine shrimp support the traditional uses of *Q. indica* as a remedial agent.

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