Acute Toxicity, Antibacterial Activity and Chemical Investigation of *Quisqualis indica* Linn. Leaves (Dawei-Hmaing)

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Abstract

Myanmar medicinal plant; *Quisqualisindica* Linn. (Dawei-hmaing) used for the treatment of dysentery and diarrhoea, was screened for antibacterial activity by agar disc diffusion technique. Polar and non-polar solvents were employed for the extraction of leaves of *Quisqualisindica* Linn. These different crude extracts were determined for the antibacterial activity against 8 pathogenic bacteria causing common gastrointestinal infections. Petroleum ether, ethyl acetate, 95 % ethanol, 70 % ethanol and watery extracts were used to test the antibacterial activity. Among five types of extracts of *Quisqualisindic*a Linn.flavonoid extract showed more pronounced antibacterial activity against all tested bacteria.The minimum inhibitory concentration (MIC) of the active extract was evaluated by agar disc diffusion technique. The lowest MIC value of ethyl acetate extract was found to be 1.25 mg/mL. The quercetin (0.01 %) was isolated from flavonoid extract by column chromatographic method using PE: EtOAc (1:1). The isolated compound was identified by spectroscopic method. It was also confirmed by melting point determination and compared with authentic sample. Acute toxicity of watery and ethanolic extracts of *Quisqualisindica* Linn. was indicated that there was no lethality up to 20 g/kg body weight with watery extract and 16g/kg body weight with 95 % ethanolic extract.

Keywords: gastrointestinal, antibacterial, *Quisqualisindica,* quercetin

Introduction

Today, there are various types of infections. Among them, gastrointestinal infection is very common in developing countries. Gastrointestinal diseases are the most frequent causes of morbidity and mortality in developing countries. The presence of enterobacteria in foodstuffs and water is a common cause of diarrhoea and dysentery among infant population. *Escherichia coli* are a classic example of enteric bacteria capable of producing diseases (Vieira *et al*., 2001).

Quisqualis indica Linn belongs to the family combretaceae and also known as Dawei-hmaing in Myanmar. Among the herbal agents, *Quisqualis indica* Linn.is widely distributed throughout Myanmar. The leaves of *Quisqualis indica* Linn contain flavonoids. Flavonoids are secondary plant metabolites in various vegetables, fruits and herbal plants. Flavonoids show important pharmacological activities such as anti-allergic, anti-inflammatory, anti-viral, anti-carcinogenic, antioxidant and antimicrobial activities (Augustin*et al*., 1994; Basile*et al*., 2000).

In this study, *Quisqualis indica* Linn, an indigenous plant claimed to be effective against dysentery and diarrhoea, was selected. Although antibacterial activities of watery, 50 % and 95 % ethanolic extracts of *Quisqualis indica* Linn.were reported in Myanmar (Mar MarNyein, 1976; PastriciaThwin, 1979; Mar MarNyein*et al*., 1991), no paper has been published regarding the evaluation of antibacterial activity of ethyl acetate extract of *Quisqualis indic*a Linn.and the acute toxicity study of the plant extracts.

Quisqualis indica Linn was chosen as a medicinal plant in this study because it is easily available and widely used orally by our local people. These factors led to ascientific study on *Quisqualis indica* Linn. to find out antibacterial activity against infectious bacteria causing gastrointestinal infections.

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The objective of this study is to extract plant materials containing flavonoids and to find out the antibacterial activity of leaf extracts of *Quisqualis indica* Linn.by using bacterial strains isolated from clinical specimens and control strains. Then acute toxicity study of leaf extracts will be carried out on mice to determine toxic effects when given orally.

Materials and Methods

Sample

The leaves, *Quisqualis indica* Linn. (Dawei-hmaing) were collected from Dala Township, Yangon Division. The collected leaves were cleaned and dried in the shade for about two weeks and were ground to get coarse powder. The dried powder was packed in a plastic bag.

Identification of Plant Sample

Botanical identification of plant sample was confirmed by Dr Tin Sein Mar, Associate Professor and Department of Botany, Taungoo University.

Preparation of Crude Extracts

Dried powder of leaves was extracted with petroleum ether (60-80 ℃), ethanol (70 % and 95 %) and water using Soxhlet apparatus. Extraction time with each solvent was six hours. After removing each solvent by rotary evaporator, crude extract was dried and kept in desiccator.

Chemicals

Distilled water, ethanol, petroleum ether (60-80 ℃), ethyl acetate, hydrochloric acid

Apparatus

Soxhlet apparatus, water bath, rotary evaporator, conical flasks, pipettes, thermometer

(a) Determination of Antibacterial Activity of Various Crude Extracts by Agar Disc Diffusion Method

Preparation of Media

After autoclaving, 20 mL of the media was poured into 90 mm diameter petridishes and allowed to set at room temperature. It was prepared freshly before use.

Preparation of Plant Extract Solution

Ethyl acetate extract of *Quisqualis indica* Linn. 0.1g was dissolved in 1 mL of ethyl acetate to form 100 mg/mL of plant extract solution. From the stock solution, 30 µL of solution were impregnated to discs resulting in (3 mg/disc). Other different types of extracts of *Quisqualis indica* Linn were done by the same procedure.

Bacterial Sample

A few colonies of the organism to be tested were picked with a wire loop from the original culture plate and introduced into a test tube containing 5 mL of nutrient broth. These tubes were then incubated at 37℃for three to four hours to produce the growth turbidity of 10^5 to 10^7 organisms per milliliter. The bacterial species used were *Escherichia coli* (ATCC), *Escherichia coli* 0157, *Salmonella typhi* (Biken, Japan), *Shigellaboydii*, *Shigelladysenteriae*, *Shigellaflexneri*, *Vibrio cholera* 01 and *Vibrio cholera* 0139.

(b) Determination of Minimum Inhibitory Concentration (MIC) of the Active Extract by Agar Disc Diffusion Method

In order to determine the least concentration of extract that inhibits the growth of microorganisms, the specific concentration of extract was prepared in serial dilution with respective solvents (e.g. ethyl acetate extract with ethyl acetate). To obtain the different concentration of extracts, firstly, 0.01 g of extract was dissolved in 1 mL of respective solvent (i.e. 10 mg/mL). From this stock solution, different concentrations of 5mg/mL, 2.5 mg/mL and 1.25 mg/mL were made with respective solvent according to serial 1 in 2 dilutions.

The discs, 6 mm in diameter, were punched from No.3 Whatman filter paper and sterilized by autoclaving followed by dry heat at 60 ℃for one hour. They were then impregnated with 30 µL of extract solution of different concentration and then allowed to dry at 37 ℃incubator. The discs were placed on prepared petridishes.

After overnight incubation, the lowest concentration of the plant extracts where organisms could not grow was taken as the minimum inhibitory concentration (MIC) of that agent. The minimum inhibitory concentration of the plant extract was recorded in terms of mg/mL.

(c) Acute Toxicity Study of both Ethanolic and Watery Extracts of *Quisqualis indica* Linn.on Albino Mice

Materials

Albino mice of both sexes weigh 25-30 grams and different concentration of ethanol and watery extract of *Qusiqualis indica* Linn.

Method

Eighty albino mice of both sexes weighing 25-30 g were used in this study. Food was withheld for the period of 12 hours. Mice were separated into nine groups so that each group contained 10 mice (5 male mice and 5 female mice). Four groups of mice were used for the administration of ethanolic extracts and the other four groups for watery extract. The preliminary experiment was conducted by giving distilled water only on albino mice according to body weight of mice. Four different doses of ethanolic extract of *Quisqualis indica* Linn. (i.e., 4 g/kg, 6 g/kg, 10 g/kg and 16 g/kg) were administered to each group of mice orally. The other four groups were given watery extract of *Quisqualis indica* Linn. orally in doses of 5 g/kg, 10g/kg, 15 g/kg and 20 g/kg body weight. After giving the extracts orally, each group of mice was kept in each cage with free access to water. They were observed carefully for 24 hours. Any mortality within the groups was recorded within 24 hours. To detect the delayed toxicity, the survivors were observed daily for two weeks (Litchfield and Wilcoxon, 1949).

(d) Isolation of Compound

Twenty-five grams of dried powder was extracted with 70 % ethanol and the ethanol soluble extract was evaporated to 1/5 volume in rotary evaporator. The extract was dissolved in 2 M hydrochloric acid solution and boiled for 45 minutes on a water bath (Harborne, 1984).The mixture was cooled and filtered. The filtrate was partitioned with ethyl acetate. The ethyl acetate layer was evaporated under reduced pressure by means of rotary evaporator. Thus ethyl acetate extract (flavonoid-rich fraction) was obtained. The ethyl acetate extract (0.5 g) was chromatographed on a silica gel column using PE: EtOAc (1:1) solvent mixture. Finally, quercetin was obtained as needle shape crystals.

Quercetin: Yellow crystalline compound, m.p. 315 ℃, UV λ_{max}^{MeOH} nm: 257, 375, FT IR υ_{max}^{kBr} cm^{-1}: 3421($_{O-H}$), 1647($_{C=0}$)

Results and Discussion

Antibacterial Activity

Screening of antibacterial activity of crude extracts has been done by filter paper disc diffusion method. In the present work, it was tested on bacteria causing gastrointestinal infections. Out of the different types of crude extracts, ethyl acetate extract showed remarkable zones of inhibition on all tested microorganisms(14 mm-30 mm) (Table 1 & Figure 1).

It was also observed that the petroleum ether extracts of *Quisqualisindica*Linn.yielding non-polar or lipophilic substance did not show any antibacterial activity on the tested bacteria. The watery and 95 % ethanol extracts of *Quisqualis indica* Linn.inhibited on *Vibrio cholera* 01 and *Vibrio cholera* 0139.

Minimum Inhibitory Concentration (MIC) of the Active Extracts by Agar Disc Diffusion Method

The minimum inhibitory concentrations (MIC) of the antibacterial active extract of *Quisqualis indica* Linn. were determined by agar disc diffusion method. It was found that MIC of *Quisqualis indica* Linn. on tested bacteria ranged from 1.25 mg/mL to 10 mg/mL concentration (Table 2& Figure 2).

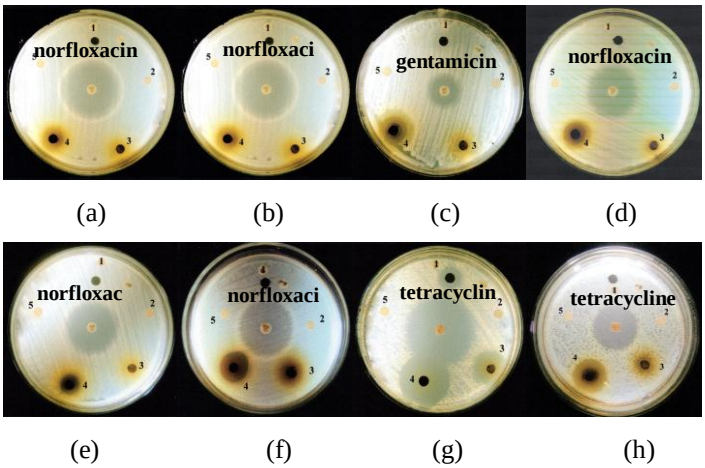
The MIC of ethyl acetate extract of *Quisqualis indica* Linn. was in the range of 1.25 to 10 mg/mL. The MICs of ethyl acetate extract of *Quisqualis indica* Linn. On *Shigella flexneri* and *Vibrio cholera* 0139 were 1.25 mg/mL on *Vibrio cholera* 01 was 2.5 mg/mL, on *E. coli* 0157 and *Shigella boydii* were 5 mg/mL and *on E. coli* ATCC, *Salmonella typhi* and *Shigella dysenteriae* were 10 mg/mL.

Acute Toxicity Test

The acute toxicity showed that no lethality of the mice was observed up to 14 days, even with the practically administrable maximal dose of 16g/kg for ethanolic extract and 20g/kg for watery extract. At above these doses, the extracts were unable to dissolve in the volume of water that can be administered to mice. Therefore, it was observed that the toxic doses will be more than 16 g/kg for ethanolic extract and 20 g/kg for watery extract or the extracts were free from acute toxic or harmful effects. The preliminary experiment done by giving distilled water only on albino mice showed no lethality. The results of acute toxicity study of both extracts of *Quisqualis indica* Linn on albino mice were shown in Table 3 and 4.

Identification of Isolated Compound

The active flavonoid extract of *Quisqualis indica* Linn was separated on silica gel using PE:EtOAc (1:1). Quercetin (0.01%) was isolated as crystal. The purity of compound was checked on TLC. It was identified by spectroscopic method namely UV and FT IR. It was also confirmed with melting point determination and compared with authentic quercetin. The melting point of isolated quercetin was found to be 315-316 ℃. It was agreed with the literature value, 316 ℃ (Merck index, 2001).In this study, ethyl acetate extract of *Q.indica* L. inhibited all tested bacteria with the zone diameter (14 mm to 30 mm). This was due to the aglycone portion (quercetin) of flavonoids in ethyl acetate extract of *Q.indica* L.



(a) (b) (c) (d)

(e) (f) (g) (h)

(a) *Escherichia coli* ATCC
(b) *Escherichia coli* 0157
(c) *Salmonella typhi*
(d) *Shigella boydii*
(e) *Shigella dysenteriae*
(f) *Shigella flexneri*
(g) *Vibrio cholera* 01
(h) *Vibrio cholera* 0139
(1) PE extract
(2) control (PE)
(3) watery extract
(4) EtOAc extract
(5) control (EtOAc)

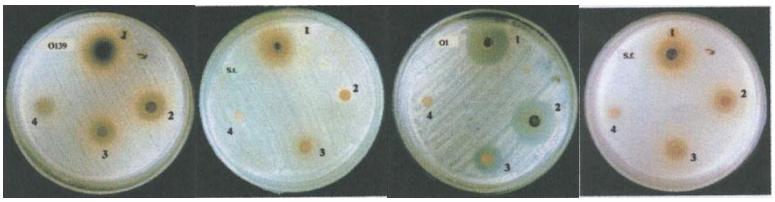
Figure 1. Antibacterial activity of crude extracts of *Quisqulisindica* Linn.

Table 1. Antibacterial Activity of Crude Extracts of *Quisqualis indica* Linn.

Inhibition zone diameter (mm)

Tested organisms	Extracts							
	PE	Water	Ethyl acetate	95% ethanol	70% ethanol	Norfloxacin 10 g	Gentamicin 10 g	Tetracycline 30 g
Escherichia coli ATCC	-	-	14	-	-	23	-	-
Escherichia coli 0157	-	-	16	-	-	10	-	-
Salmonella typhi	-	-	16	-	-	-	15	-
Shigellaboydii	-	-	14	-	-	31	-	-
Shigelladysenteriae	-	-	14	-	-	28	-	-
Shigellaflexneri	-	-	15	-	8	22	-	-
Vibrio cholera 01	13	20	30	10	14	-	-	35
Vibrio cholera 0139	-	9	20	10	12	-	-	25

disc diameter = 6 mm (-) = No activity



(a) (b) (c) (d)

(a) = *Shigellaflexneri*
(b) = *Vibrio cholera* 0139
(c) = *Vibrio cholera* 01
(d) = *Salmonella typhi*

Figure 2. Minimum inhibitory concentration of active ethyl acetate extract from *Q. indica*Linn. (Dawei-hmaing)

Table 2.Minimum Inhibitory Concentration of Active Extract of *Quisqualis indica* Linn. (Dawei-hmaing)

Minimum Inhibitory Concentration (mg/mL)

Extract	Tested organisms*							
	1	2	3	4	5	6	7	8
EtOAc	10	5	10	5	10	1.25	2.5	1.25

*1 = *Escherichia coli* ATCC
2 = *Escherichia coli* 0157
3 = *Salmonella typhi*
4 = *Shigellaboydii*
5 = *Shigelladysenteriae*
6 = *Shigellaflexneri*
7 = *Vibrio cholera* 01
8 = *Vibrio cholera* 0139

Table 3. Results of Acute Toxicity Test of Ethanolic Extract of *Quisqualis indica* Linn. on Albino Mice

Sr. No.	Dose (g/kg) body weight	Observed period	No. of dead per tested	Lethality (%)
1	16	Two weeks	0/10	0
2	10	Two weeks	0/10	0
3	6	Two weeks	0/10	0
4	4	Two weeks	0/10	0

Table 4.Results of Acute Toxicity Test of Watery Extract of *Quisqualis indica* Linn. on Albino Mice

Sr.No.	Dose (g/kg) body weight	Observed period	No. of dead per tested	Lethality (%)
1	20	Two weeks	0/10	0
2	15	Two weeks	0/10	0
3	10	Two weeks	0/10	0
4	5	Two weeks	0/10	0

Conclusion

This present research works on “Acute Toxicity, Antibacterial Activity and Chemical Investigation of *Quisqualisindica* Linn. Leaves (Dawei-Hmaing)”, the following conclusions can be drawn.

All types of crude extracts (petroleum ether, ethyl acetate, water, 95 % ethanol and 70 % ethanol) of *Quisqualisindica*Linn. (Dawei-hmaing) had antibacterial activity against *Vibrio cholera* 01.

Ethyl acetate extract (flavonoid extract) of *Quisqualisindia*Linn. (Dawei-hmaing) showed antibacterial activity against organisms causing gastrointestinal infections such as *Escherichia coli* ATCC, *Escherichia coli* 0157, *Salmonella typhi, Shigellaboydii, Shigelladysenteriae, Shigellaflexneri, Vibrio cholera* 01 and *Vibrio cholera* 0139. Thus, *Quisqualisindica*Linn.can be used for the treatment of dysentery and diarrhoea.

Watery and alcoholic extracts (polar extracts) were active against *Vibrio cholera* 01. This is useful information on a promising efficacy of aqueous decoction which is normally the dosage form in traditional medicine system.

The isolated compound (quercetin) was identified by TLC, UV and FT IR spectroscopic method. It was also confirmed by melting point determination and compared with authentic sample.

There was no acute toxic effect and lethality up to the maximum administrable dose of 16 g/kg for ethanolic extract and 20 g/kg for watery extract of *Quisqualisindica*Linn.

Therefore, it can be inferred that aqueous extract of *Quisqualisindica* Linn.may be used for the treatment of gastrointestinal infections (Dysentery and Diarrhoea).

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