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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF LEAVES OF MEDICINAL PLANT *HIBISCUS TILIACEUS* L.

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Summary

The ethanol extract of the dried leaves of *Hibiscus tiliaceus* L. (Family - Malvaceae) was investigated for its possible antioxidant and antimicrobial activities. The extract of *Hibiscus tiliaceus* L. showed antioxidant property (IC_{50}=86.5µg/ml) and IC_{50} of the sample (ascorbic acid) was 15.00µg/ml. The ethanol extract of *Hibiscus tiliaceus L.* also showed activity against three strains of bacteria *Staphylococcus aureus* (gram positive), *Escherichia coli* (gram negative)and *Salmonella paratyphi* (gram negative). The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key Words: antioxidant activity, antimicrobial activity, *Hibiscus tiliaceus* L.

Introduction

Hibiscus tiliaceus L. is an evergreen, sprawling tree that typically grows to 3–10 m (10–33 ft) in height with a sprawling form. It is indigenous to many parts of the tropics and has been introduced to new regions by people. It is most at home in coastal and near-coastal environments, but it has been introduced into agricultural environments up to 800 m (2600 ft) elevation. Once established, the tree often persists and spreads, especially in moist gullies, streambeds and other wet areas[1].

Hibiscus tiliaceus L is a very common evergreen tree in Bangladesh. The plant traditionally use for treatment of diarrhea, dysentery and typhoid Upon significant literature survey it has found that very few information was found on the species and there are no significant research work has been performed on the species to judge its use in pharmaceutical industry. From the existing information it is evident that the plant may possess some important biological activities. The main objective of this study was to evaluate the antioxidant and antimicrobial activities of the ethanol extract of dried leaves of *Hibiscus tiliaceus* L.

Materials and Methods

Plant Material

Leaves of *Hibiscus tiliaceus* L.were collected from Khulna University campus, Khulna, Bangladesh in June 2011 and were authenticated by the experts at National Herbarium (Accession Number: 37531). After collection, leaves were sun dried for several days to remove moisture. After drying, the dried leaves were ground into course powder by 'Hammer' mill. About 400 gm of powdered leaves was taken in a clean, flat-bottomed glass container and soaked in 1,300 ml of 80% ethanol. The container with its contents was sealed and kept for a period of 7days accompanying occasional shaking and stirring. The whole mixture was then underwent a coarse filtration by a piece of cotton followed by a filtration through filter paper and the filtrate thus obtained was concentrated using a rotary evaporator to get the crude extract.

Drug

For the investigation of antimicrobial activity, Kanamycin (30 µg/disc) standard disc was used as the reference standard antimicrobial agent.

Antioxidant activity

Qualitative assay

Qualitative assay is very important method for determining antioxidant activity[2]. Test samples were developed with a suitable solvent system on a TLC plate and sprayed with 0.004% w/v DPPH solution in MeOH using an atomizer. The positive activity was detected by the discolored (pale yellow) spots on a reddish purple background. Ascorbic acid was used as the positive control.

Quantitative assay

The method used was adopted with suitable modifications to our particularCircumstance[3]. At first 9 test tubes were taken to make aliquots of 9 conc. (1.57, 3.13, 6.25, 12. 5, 25, 50, 100, 200 and 400) µg/ml for plant extract and 9 test tubes were taken to make aliquots of 7 conc.(1.57, 3.13, 6.25,12.5, 25, 50,100,200,400) µg/ml for ascorbic acid. Plant extract and ascorbic acid were weighed 3 times and dissolved in ethanol to make the required concentrations by dilution technique. Here ascorbic acid was taken as positive control. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogeneously magnetic stirrer was used. After making the desired concentrations, 6 ml of 0.004% DPPH solution was applied on each test tube by pipette and then 2 ml of different concentrations was mixed in each test tube. Test tubes were kept for 30 minutes in dark to complete the reactions. DPPH was also applied on the blank test tubes at the same time where only 2ml ethanol was taken as blank. After 30 minutes, absorbance of each test tube was determined by

UV spectrophotometer at 517 nm. % of inhibition was calculated and IC_{50} was determined from % inhibition vs. log conc. graph.

The formula used for % inhibition ratio is:

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%inhibition = (Blank OD-Sample OD/Blank OD) X 100
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Antimicrobial activity

Antimicrobial activities were done according to the disc diffusion method[4-5]. Disk diffusion technique is widely acceptable for the preliminary evaluation of antimicrobial activity. It is essentially a qualitative or semi-quantitative test indicating the sensitivity or resistance of microorganisms to the test materials[6]. In this method measured amount of the test samples were dissolved in definite volumes of solvent to give solutions of known concentration (µg/ml) such as 250 µg/disc and 500 µg/disc. Standard antibiotic discs (Kanamycin (30 µg/disc)) and discs on which the solvent used to dissolve the samples were used as positive and negative control, respectively. These discs were then placed in petridishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop for anti-microbial evaluation. The plates were then kept at 4°C for facilitating maximum diffusion. The test material diffused from the discs to the surrounding medium. The plates were then kept in an incubator (37 °C) for 18-24 hour to allow the growth of the microorganisms. The test material inhibited the growth of microorganism giving a clear, distinct zone called "zone of inhibition". The antibacterial activity of the test agent was determined by measuring the diameter of the zone of inhibition in term of millimeter.

Results

Antioxidant activity

Absorbance at 517 nm was determined by taking the crude extract of different concentration of *Hibiscus tiliaceus* L.

see Table 1.

see Table 2.

see Table 3.

see Fig. 1

see Fig. 2

see Table 4.

Antimicrobial activity

After proper incubation, the antibacterial activity of *Hibiscus tiliaceus L*. was determined by measuring the diameter of zone of inhibition in term of millimeter with a calibrated scale.

Discussion

In the TLC-based qualitative antioxidant assay using DPPH assay, *Hibiscus tiliaceus* L**.** showed the free radical scavenging properties indicated by the presence of strong yellow spot on a purple background on the TLC plate.

The ethanol extract of *Hibiscus tiliaceus* L**.** exhibited a significant inhibition of DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of 86.5µg/ml where as the IC_{50} value of the standard was (15.00µg/ml) ascorbic acid. Based on this, it could be concluded that it might possess antioxidant activity.

Plant containing Quercetagetin-7-arabinosylgalactoside, a flavonoid has been used extensively to treat infectious disease[7] .The flavone baicalein is reported to be largely responsible for antimicrobial effects[8]. Flavonoidrich plant extracts from species of *Hypericum*[9], *Capsella*[10] and *Chromolaenahave*[10] been reported to possess antibacterial activity. Many other phytochemical preparations with high flavonoid content have also been reported to exhibit antibacterial activity[11-19]. It has been reported that sponins have potent antimicrobial activity[20].

The antibacterial activity was assessed against 6 pathogenic bacterial strains (both gram positive and gram negative) at the dose of 250 µg/disc and 500 µg/disc and the results were compared with the

activity of the positive control kanamycin (30 µg/disc). The extract showed activity against three strains of bacteria *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative), *Salmonella paratyphi* (gram negative). The zone of inhibition varies within the ranges of 9 mm and 12 – 15 mm at the dose of 250 µg/disc and 500 µg/disc respectively. The highest zone of inhibition was found against *Staphylococcus aureus* (15mm) which is comparable to the positive control kanamycin (25mm). As it showed activity against E. coli and S. *aureus* the results support the traditional use of this plant as a remedy of biliousness, abdominal pains and throat problems. Based on this, it could be concluded that it might possess antimicrobial activity.

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Blank Solution	1st reading	2nd reading	Average
	.719	.719	.719

Table: 1 Absorbance of blank solution

Concentration	1st reading	2nd reading	Average	% inhibition
1.57 µg/ml	0.713	0.705	0.709	1.39
3.13 µg/ml	0.71	0.63	0.67	6.82
6.25 µg/ml	0.562	0.560	0.564	21.56
12.5 µg/ml	0.391	0.395	0.393	45.34
25 µg/ml	0.22	0.22	0.22	69.40
50 µg/ml	0.093	0.081	0.087	87.90
100 µg/ml	0.053	0.049	0.051	92.91
200 µg/ml	0.046	0.044	0.045	93.74
400 µg/ml	0.047	0.041	0.044	93.88

Table:2 Absorbance of ascorbic acid at different concentration

Concentration	1st reading	2nd reading	Average	% inhibition
1.57 µg/ml	0.694	0.696	0.695	3.4
3.13 µg/ml	0.681	0.681	0.681	5.285
6.25 µg/ml	0.660	0.664	0.662	7.93
12.5 µg/ml	0.597	0.599	0.598	16.83
25 µg/ml	0.508	0.509	0.509	29.07
50 µg/ml	0.374	3.372	0.373	48.12
100 µg/ml	0.342	0.342	0.342	52.43
200 µg/ml	0.340	0.342	0.341	56.19
400 µg/ml	0.290	0.290	0.290	59.53

Table: 3 Absorbance of *Hibiscus Tiliaceus* L. at different concentration

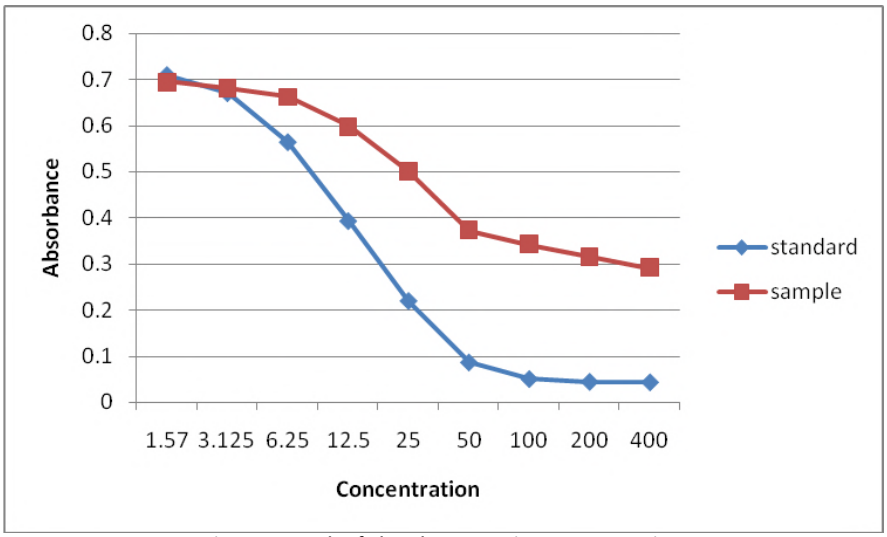

Figure 1: Graph of absorbance against concentration

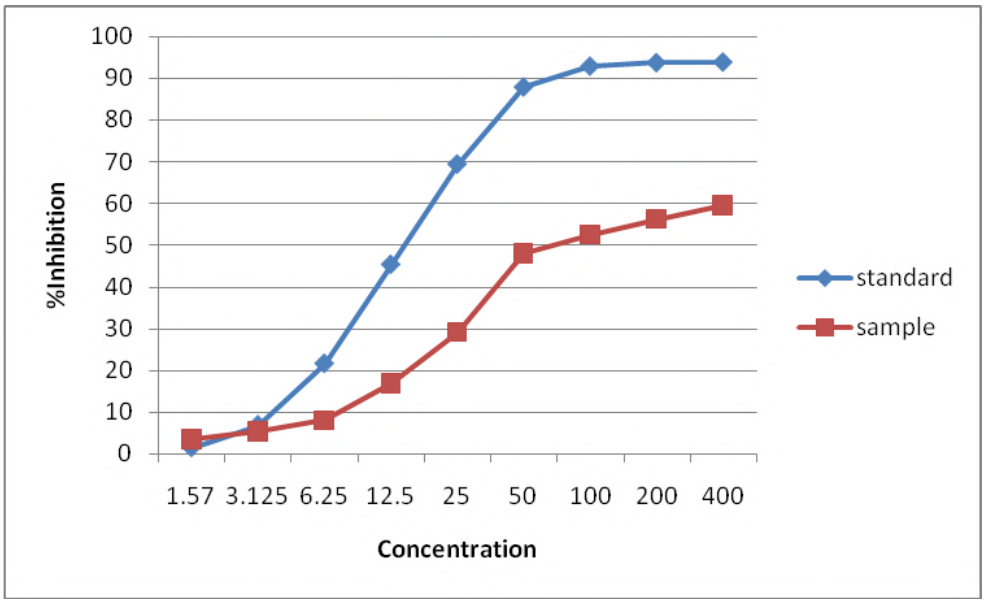

Figure 2 : Graph of % inhibition against concentration

Bacterial Strains	Diameter of Zone of Inhibition in mm									
	Hibiscus tiliaceus L									
	Blank	Kanamycin (30 µg/disc)			250 µg/disc			500 µg/disc		
		1st	2nd	Average	1st	2nd	Average	1st	2nd	Average
Gram positive Bacteria										
Staphylococcus aureus	0	23	27	25	8	10	9	14	16	15
Streptococcus pyogens	0	22	24	23	0	0	0	0	0	0
Gram Negative Bacteria										
Salmonella paratyphi	0	18	22	20	8	10	9	12	14	13
Shigella dysenteriae	0	24	26	25	0	0	0	0	0	0
Salmonellatyphi	0	21	19	20	0	0	0	0	0	0
Escherichia coli	0	20	20	20	8	10	9	10	14	12

Table: 4 In vitro antibacterial activity of ethanol extract