Short Communication

ANTIBACTERIAL ACTIVITY OF THE METHANOLIC EXTRACT OF *HIBISCUS SABDARIFFA* LEAVES AND FRUITS

ABSTRACT

Hibiscus ~~*Sabdariffa*~~ *sabdariffa* (Indian elder, family: Malvaceae) is a species of hibiscus, found naturally in Malaysia, India, Africa and Australia. It is cultivated wild areas on village outskirts and wasteland in Malaysia, Perak State. The parts of the plant are used for diuretic, mild laxative, cholerectic, hypotensive, lowering blood pressure and treatment for cardiac and nerve diseases and cancer. So far no phytochemical and biological investigation has been carried out on this endangered and rare species, though several ethnomedical uses of the plant exist. The present study aimed to carry out antibacterial properties of the methanolic extract of ~~*Hibiscus*~~ *H. sabdariffa* leaves and fruits. The crude methanolic extract of ~~*Hibiscus*~~ *H. sabdariffa* leaves and fruits showed better antibacterial activity against ~~gram~~ Gram negative bacteria compared to ~~gram~~ Gram positive bacteria. However, the standards showed better activity with lower concentration when compared to both the extracts against the entire organism.

KEYWORDS: *Hibiscus sabdariffa*, antibacterial, disc-diffusion method

1. Introduction

The importance of medicinal plants and traditional health systems in solving the health care problems of the world are gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin [1]. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Natural remedies that~~,~~ may have been used for many thousands

of years by the human race must be appropriately catalogued to ensure that vital ethno medical information is not lost forever [1].

Medicinal plants represent a rich source of antimicrobial agents. There is also an urgent need to search for a new antimicrobial compounds with novel mechanisms of action because there have been an alarming increase in the incidence of new infections diseases, as well as the development of resistance to the antibiotics in current clinical trials [2].

The parts of the plant of *H.~~ibiscus~~ sabdariffa*[A1] used for diuretic, mild laxative, cholerectic, hypotensive, lowering blood pressure and treatment for cardiac and nerve diseases and cancer [3]. All the part of ~~*Hibiscus*~~ *H. sabdariffa* has its own medicinal uses. The seed extracts were found to have the highest antioxidant activity and strongest radical-scavenging activity of all plants tested. Infusions of the leaves are regarded as diuretic, cholerectic, febrifugal and hypotensive**,** decreasing the viscosity of the blood and stimulating intestinal peristalsis. Some benefits of the petals are it can reduce the thickness or viscosity of blood, helps the digestive process, prevent inflammation of the urinary tract and kidney, filters toxins in the body, prevent Vitamin C deficiency and blood circulation [4].

Although *H~~ibiscus~~. sabdariffa* has traditionally been used for the treatment of various diseases, ~~However so far there are~~ no biological activities were reported till to date.~~though the plant is used traditionally for the treatment of various diseases~~. Hence, in the present study we ~~are~~ were interested to carry out a detail antibacterial investigations on ~~of *Hibiscus sabdariffa*~~ this important plant.

2. Materials and methods

2.1. Collection and authentication of plant materials

The leaves and fruits of *H.~~ibiscus~~ sabdariffa* were collected from Manjoi village, Ipoh, Perak, Malaysia and authenticated by botanist (Fig. 1). The collected leaves and fruits were washed thoroughly in distilled water to remove contaminants; it was chopped into small pieces and dried under shade.

Fig 1: Leaves and Fruits of *Hibiscus Sabdariffa*

2.2. Extraction of *H.~~ibiscus~~ sabdariffa* leaves and fruits

The dried leaves and fruits of *H.~~ibiscus~~ sabdariffa* were coarsely powdered and separately subjected to extraction by maceration in methanol at room temperature with occasional shaking for seven days. The macerate was filtered and the filtrate was dried at low temperature (40-50°C) under vacuum. The extracts were stored in air-tight containers in a refrigerator at 4°C until further use.

2.3. Qualitative phytochemical screening

The methanolic extracts obtained were tested for the following qualitative chemical tests for the identification of various phytoconstituents [5,6].

2.3.1. Tests for Alkaloids

1. Dragendorff's test: [A2]To the extract, 1 ml of Dragendorff's reagent was added. An orange red precipitate indicates the presence of alkaloid.

2. Wagner's test: To the extract, Wagner's reagent was added. Reddish brown precipitate indicates the presence of alkaloid.
3. Mayer's test: To the extract, 1 or 2 ml of Mayer's reagent was added. A dull white precipitate indicates the presence of alkaloid.
4. Hager's test: To the extract, 3 ml of Hager's reagent was added. Yellow precipitate indicates the presence of alkaloid.

2.3.2. Tests for Carbohydrates

1. Molisch test: To the extract, 1 ml of α-naphthol solution was added and conc. sulphuric acid was added along the sides of test tube. Purple or reddish violet colour at the junction between the two liquids indicates the presence of carbohydrates.
2. Fehling's test: To the extract, equal quantities of Fehling's solution A and B was added. Upon heating gently, a brick red precipitate indicates the presence of carbohydrates.
3. Benedict's test: To 5 ml of Benedict's reagent, 8 drops of solution under test was added, mixed and the mixture was boiled vigorously for two minutes and cooled. A red precipitate indicates the presence of carbohydrates.

2.3.3. Tests for Proteins

1. Biuret test: To the extract, 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulfate solutions were added. A violet color indicates the presence of proteins.
2. Xanthoproteic test: To the extract, 1 ml of concentrated nitric acid was added, a white precipitate formed, it was boiled and cooled. Then, 20% of sodium hydroxide or ammonia was added. Orange color indicates the presence of aromatic amino acids.
3. Lead acetate test: To the extract, 1 ml of lead acetate solution was added. A white precipitate indicates the presence of proteins.

2.3.4. Test for Amino Acids

Ninhydrin test: Two drops of freshly prepared 0.2% ninhydrin reagent was added to the extract and heated. Development of blue colour indicates the presence of proteins, peptides or amino acids.

2.3.5. Tests for Steroids and Sterols

1. Libermann Burchard test: The test extract was dissolved in 2 ml of chloroform in a dry test tube. Ten drops of acetic anhydride and 2 drops of concentrated sulfuric acid were added. The solution becomes red, then blue and finally bluish green in color indicating the presence of steroids.
2. Salkowaski test: The extract was dissolved in chloroform and an equal volume of conc. sulfuric acid was added. Bluish red to cherry red color is observed in chloroform layer, whereas the acid layer assumes marked green fluorescence indicating the presence of steroids.

2.3.6. Tests for Glycosides

1. Legal test: The extract was dissolved in pyridine and sodium nitroprusside solution added to it and made alkaline. Pink red or red color indicates the presence of glycosides.
2. Baljet test: To the extract, sodium picrate solution was added. Yellow to orange color indicates the presence of glycosides.
3. Borntrager's test: Few ml of dilute sulfuric acid was added to the test solution. Boiled, filtered and extracted the filtrate with ether or chloroform. The organic layer was separated and treated with ammonia. Pink, red or violet color indicates the presence of glycosides.

4. Keller Killiani test: Sample was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of conc. sulfuric acid. At the junction, reddish brown color was formed, which gradually becomes blue indicating the presence of glycosides.

2.3.7. Test for Flavonoids

Shinoda test: To the extract, magnesium turnings were added, followed by the addition of conc. hydrochloric acid. A red color indicates the presence of glycosides.

2.3.8. Tests for Tannins

1. To the extract, ferric chloride was added. Dark blue or greenish black color indicates the presence of tannins.
2. To the extract, potassium dichromate solution was added. A precipitate indicates the presence of tannins.

2.3.9. Test for Triterpenoids

In the test tube, 2 or 3 granules of tin was added and dissolved in 2 ml of thionyl chloride solution. Then, test solution was added. Production of pink colour indicates the presence of triterpenoids.

2.3.10. Tests for Fixed Oils

1. Spot Test: A small quantity of extract was pressed between two filter papers. Oil stains on paper indicate the presence of fixed oils.
2. Saponification Test: To the extract, few drops of 0.5 N alcoholic potassium hydroxide were added along with a drop of phenolphthalein. The mixture was heated on a water bath for 1–2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils.

2.4. Antimicrobial screening

2.4.1. Test microorganisms

A panel of four common pathogenic microorganisms were used in the study, which includes two ~~gram~~Gram-positive bacteria (*Streptococcus mutans* and *Staphylococcus aureus*[A3]) and two ~~gram~~Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*[A4]).

2.4.2. Disc-diffusion method

A suspension of the tested microorganisms was uniformly swabbed on agar. Sterile blank discs were individually impregnated with different concentration of extracts (1000, 500, 250 µg/ml) and placed onto the inoculated agar plates [2]. The plates were inverted and incubated at 37 ºC for 24 h. The antimicrobial activity was measured by measuring diameter of the resulting zone of inhibition against the tested organisms. The test for positive control and negative control were performed in duplicate.

3. Results and discussion

The nature of *H.~~ibiscus~~ sabdariffa* leaves and fruits extracts and yields were mentioned in Table 1.

Table 1. Yields and nature of methanolic extract of *Hibiscus sabdariffa* leaves and fruits

Hibiscus sabdariffa	**Quantity used for methanol extraction**		**Nature of the extracts**	**Yield (%)**
	Powder (g)	**Solvent (ml)**		
Leaves	100	250	Dark green residue	10.72
Fruits	100	250	Pinkish brown residue	7.46

Phytochemical analysis (Table 2) of crude methanolic extracts of *H.~~ibiscus~~ sabdariffa* leaves and fruits showed the presence of flavonoids, tannins, triterpenoids, fixed oils and absence of alkaloids, carbohydrates, proteins, amino acids, steroids, and glycosides.

Table 2. Qualitative phytochemical analysis of Methanolic extract of *Hibiscus sabdariffa* leaves and fruits

Phytoconstituents	Methanolic extract of Red rambutan peels	Methanolic extract of Yellow rambutan peels
Alkaloids	-	-
Carbohydrates	-	-
Proteins	-	-
Amino acids	-	-
Steroids and sterols	-	-
Glycosides	-	-
Flavonoids	+	-
Tannins	+	+
Triterpenoids	+	+
Fixed oils	+	+

+ Present, - Absent

Table 3. Antimicrobial activity of methanolic extract of *Hibiscus sabdariffa* leaves and fruits

S. No.	Organism used	Concentration in µg/ml						Control	Standard (Ciprofloxac in 5 µg/ml)
		Methanolic extract of *Hibiscus sabdariffa* leaves			Methanolic extract of *Hibiscus sabdariffa* fruits				
		1000	500	250	1000	500	250		
Gram positive bacteria									
1	*Streptococcus mutans*	-	-	-	-	-	-	-	9 mm
2	*Staphylococcus aureus*	11	9	7	9	-	-	-	32 mm
Gram negative bacteria									
3	*Escherichia coli*	10	8	6	9	6	6	-	16 mm
4	*Pseudomonas aeruginosa*	9	5	-	9	7	-	-	21 mm

No inhibition[A5]

The antibacterial activity of crude methanolic extract of *H.~~ibiscus~~ sabdariffa* leaves and fruits against *S.~~treptococcus~~ mutans~~s~~*, *S.~~taphylococcus~~ aureus*, *E~~scherichia~~. coli* and *P.~~seudomonas~~ aeruginosa* were presented in Table 3. The zone of inhibition produced by the crude methanolic extract of *H.~~ibiscus~~ sabdariffa* leaves against *S.~~taphylococcus~~ aureus* was 11, 9 and 7 mm, respectively at 1000, 500 and 250 µg/ml concentrations. The methanolic extract of *H.~~ibiscus~~ sabdariffa* fruits showed 9 mm zone of inhibition against *S.~~taphylococcus~~ aureus*. Both the extracts do not produced any zone of inhibition against *S.~~treptococcus~~ mutans*.

The methanolic extract of *H.~~ibiscus~~ sabdariffa* leaves and fruits showed ~~zone of~~ inhibition zone of 6-10 mm and 6-9 mm against *E.~~scherichia~~ coli*, ~~respectively. Also produced zone of~~

~~inhibition~~ and 5-9 mm and 7-9 mm against *P.~~seudomonas~~ aeruginosa* with the concentration of 500 and 1000 µg/ml, respectively

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The above results indicates that the crude methanolic extract of *H.~~ibiscus~~ sabdariffa* leaves and fruits showed better antibacterial activity against ~~gram~~ Gram negative bacteria than ~~gram~~ Gram positive bacteria. However, the standards showed better activity with lower concentration when compared to both the extracts against the entire organism. These results also well correlates with the previous study of Nwaiwu et al., 2012, [7] the crude extracts of *H.~~ibiscus~~ sabdariffa* seeds showed antimicrobial effect against three types of ~~gram~~ Gram negative bacteria's.

4. Conclusion

The phytochemical tests for the methanolic extracts of red rambutan peels gave positive results for flavonoids, tannins, triterpenoids and fixed oils. In the methanolic extracts of yellow rambutan peels all the constituents were also present except for flavonoids. The crude extracts of *H.~~ibiscus~~ sabdariffa* leaves and fruits showed antimicrobial activity to *S.~~taphylococcus~~ aureus*~~-~~, a ~~gram~~ Gram positive bacterium~~a~~, and~~-~~ *E.~~scherichia~~ coli*~~-~~ and *P.~~seudomonas~~ aeruginosa* (~~gram~~ Gram negative bacteria) ~~. This activity is~~ might be due to the presence of phytochemicals.

5. References

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