International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 08-10-2021, Accepted: 23-10-2021, Published: 09-11-2021
Volume 6, Issue 6, 2021, Page No. 185-188

Comparative phytochemical study of total compound of *Hibiscus rosa-sinesis* and *Tageteserecta* and their antimicrobial activity

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Abstract
The study was carried out to identify the phytochemicals present in total compound of the flowerof *Hibiscus rosa-sinesis*and *Tageteserecta.* The antimicrobial sensitivity of the total compound was checked against Gram Negative Bacteria (*Escherichia coli*) and Gram Positive Bacteria (*Staphylococcus aureus).*Solvent (Acetone) extract of both the samples were tested for different phytochemical test. In case of solvent extract of total compound of both samples S1 and S2, sample1 *(Hibiscus rosa-sinesis*) showed the better antimicrobial activity result then sample2 (*Tageteserecta*) against both microorganism *Staphyllococcusaureas (gram +ve)* and *Escherichia coli (gram-ve).*

Keywords: *Hibiscus rosa-sinesis*, *Tageteserecta*, solvent extract, total compound, antimicrobial activity, phytochemical analysis

Introduction
Resources from plants have always been an integral part for the mankind from the very beginning. After the basic needs are fulfilled human have also found that plants can be used for curing diseases [1].
It is found that among all the plant species more than 30% plants are beneficial for us due to its medicinal property. Among 250,000 higher plants almost 80,000 are medicinal plants. Drugs are obtained from all the different parts of the plant, even from the excretory products such as latex, gum and resins [2]. The nature has gifted us with lots of botanical wealth. The natural phytochemicals which we get from plant plays an important role in management of much different human health condition which includes Cancer too. Phytochemical compound mainly helped in protecting human from pathogenic microorganisms. Many non-nutritive chemicals of the plants have the property to prevent from diseases. These chemicals are produce by the plant for its self-prevention but recent researches had found these phytochemicals are preventingin nature for humans from different diseases [3]. It is seen that World Health Organization has recommended the assessment of traditional plant for diabetes treatment [4].
The bioactive constituents consisting of medicinal values are alkaloids, tannins, flavonoid, phenolic compounds that have specific physiological action on human body [5]. Nature has given many medicinal agents which are useful to prevent human being from fungal and microbial diseases too, even many modern drugs are made from natural sources. The botanists and mycologists are attracted towards the antimitotic derived from plants since they are easily available in the nature as well as ecofriendly and are safe too. Hence natural products are a great source of distinctive molecules and act as a great conserver for new potential drugs as well as for examination of fungal and microbial biology. Hence many fungal and bacterial infections are treated by natural plants product rather than synthetic [6].

Selection of plant
Hibiscus rosa-sinesis
*Hibiscus rosa-sinesis*belongs to the Malvaceaefamily, it is a shorn shrub which is cultivated in tropical regions and are used as ornamental plant. This flower varies in different color but the red one is use for medicinal purpose. It is seen that the leaves and the flowers act as hair growth promoters and even helps in ulcer healing. The flowers are observed to be useful in treating arterial hypertension. In India various herbal products for hair growth encompass the extract of various parts of the flower [7]. Further it is seen that ethanol extract of Hibiscus rosa-sinesis possesses anti-diabetic activity in hyperlipidemia rat models, therefore it can be used as supportive factor for the preexisting treatment of diabetes in hyperlipidemia condition [4].

Fig 1: *Hibiscus rosa-sinesis* (S1)

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Malvales
Family: Malvacea
Genus: Hibiscus
Species: Rosa-sinensis

***Tageteserecta* (S2)**

Targeteserecta belongs to the Asteraceaefamily which consist about almost 50 different species of annual or perennial non-woody plant. It is indigenous to Mexico and hotter parts of America and in the tropical and subtropical regions which includes India and Bangladesh [5]. It is most commonly used in the preparation of cosmetics, in medicines and is even used as ornamental plant. It varies in different color and fragrance. We get a strongly aromatic essential oil from *Tageteserecta*. The leaves are been observed to be useful against piles, kidney disease, muscular ache, ulcers and wounds[9]. The flowers are reported to be effective in fevers, epileptic fits, astringent, carminative, stomachic, scabies and liver troubles and also useful in eye diseases. It also possess activities such as Anti-bacterial activity, Anti-microbial activity, hepatoprotective activity, Insecticidal activity, Mosquitocidal activity, Nematicidal activity, Wound healing activity, Anti-oxidant and Analgesic activity, Larvicidal activity [8,10].

Fig 2: *Tageteserecta* (S2)

Kingdom: Plantae
Division: Tracheophyta
Class: Magnoliopsida
Order: Asterales
Family: Asteraceae
Genus: Tagetes
Species: Erecta

This present study was designed to extract total compound with solvent (Acetone) from *Hibiscus rosa-sinesis*and *Tageteserecta* and their phytochemical analysis and to also perform antimicrobial activity of the total compounds on Gram+ve And Gram –ve Bacteria.

Materials and method

Plant material

***Hibiscus rosa-sinesis* (S1) *and Tageteserecta* (S2)**

*Hibiscus rosa-sinesis*and *Tageteserecta* samples were collected from Nalbari District (26.3660° N, 91.3276° E) of Assam, India in the month of December 2020.It was skimmed off with a sharp knife from the plant and dried under sunlight and grinded by the help of mixer grinder to get powder form of sample.

Chemical

Bacteriological media used in this study were purchased from Hi-media laboratories and chemicals of phytochemical analysis were obtained from Sigma-Aldrich. Co., St. Louis, USA, Himedia laboratories and Sisco Research Laboratory, Mumbai, India. Solvent were obtained from Sisco Research Laboratory, Mumbai, India. Molisch's reagent, Fehling's reagent were obtained from Himedia laboratories, Mumbai, India. Known antimicrobial disk was obtained from Himedia laboratories, Mumbai, India.

Extraction of total compound from plant sample

Solvent extraction

Solvent extraction was done a method slightly modified from Franz Von Soxhlet in 1879. Acetone as solvent was used for the extraction. Extraction of total compound from both S1 and S2 were done by the solvent as Acetone (ratio 1:10) through Shoxlet apparatus (U R Biocoction SXA-43-6) for 24 to 30 hour. The solvent were then evaporated by the help of rotary evaporator to get powder form of total compound as extract. And then weighed 300 mg of powder form of solvent extract and added 6 ml of DMSO (20 % DMSO) and then dissolved completely into 24 ml of dist. water to (total 30 ml) get standard solvent extract solution of total compound (10mg/ml) and stored it at 4° C for further phytochemical analysis.

Pyotochemical screening

Preliminary assay were performed to detect the presence of various phytochemicals in the total compound of plant extract. Tests were performed for S1 and S2 with the standard (10mg/ml solvent extract (Acetone).

Test for steroid

In 2 ml of plant extract, 2 ml of chloroform and 2 ml of concentrated H_2SO_4 was added and shaken well. If Chloroform layer appeared red and acid layer greenish yellow fluorescent. It confirms the presence of steroids.

Test for terpenoids

2ml of each extracts were treated with Chloroform (1 ml) followed by a few drops of concentrated sulphuric acid. Formation of brown ring at the junction indicated the presence of terpenoids.*2.3.3.3 Test for flavonoid*

Few drops of 20% sodium hydroxide solution were added to 2mL of extracts. Formation of intense yellow color, which becomes colorless on addition of dilute hydrochloric acid, confirms the presence of flavonoids.

Test for saponin

Foam test

The extract was diluted with 20 ml of distilled water in a test tube and it was shaken by hand for 15 min. A layer of foam formed at the top of the test tube indicated the presence of Saponin.

Test for glycoside

Keller-Kiliani Test

In 2 ml plant extract, 2ml acetic acid, one drop of 2% $FeCl_3$ and concentrated H_2SO_4 were added. Reddish brown color ring appears at the junction of the two liquid layers indicates the presence of glycosides.

Test for tannins

Ferric chloride test

1 ml each of plant extract and 3-4 drops of ferric chloride is added. A transient greenish to the black color indicated the presence of Tannins.

Lead sub acetate test

1 ml of plant extract added with 3-4 drops of 1% of lead acetate solution. A creamy gelatinous precipitation indicates a positive test for Tannins.

Test for alkaloids

Mayer's reagent

It is used for the detection of alkaloids. 2-3 ml of plant extract, few drops of Mayers's reagent. It will produce a creamy white precipitation if alkaloids are present.

Picric acid test

2ml of plant extract is treated with few drops of 1% picric acid. A yellow precipitate indicates the presence of alkaloids.

Test for carbohydrate

Molisch test

Treat extract with few drops of molisch reagent. Add concentrated sulphuric acid slowly along the sides of the test tube. If purple to violet color ring appears it confirms the presence of carbohydrates.

Fehling's test

Fehling A and Fehling B reagents were mixed and few drops of the extract is added and boiled. A brick red colored precipitate of cuprous oxide formation confirms the presence of carbohydrates.

Anti-microbial activity test

For determining the antimicrobial activity media was prepared by Mueller Hinton Agar and distilled water in required proportions. The media was autoclaved and poured into the dishes (14X90 mm Tarson, Cat. No. 460090) and left for solidification.

After solidification of the media two particular bacteria *Staphyllococcusaureas* and *Escherichia coli* from pure culture in peptone water media and microorganisms were spreading by the help of swabs stick all over the Mueller Hinton Agar media. Paper disk were placed on to the solid media plate. Then 10 micro liter solvent (Acetone) extract of total compound for both sample 1 and 2 with different concentration (5mg/ml and 10mg/ ml) were poured on that paper disk with respect to known antibiotic disk. The dishes are then kept for overnight in incubator (U R Biocoction). The plates were then observed regularly after 24 hrs. and 48 hrs. Respectively and the diameter of zone of inhibition was then measured.

Statistical analysis

Statistical analysis was carried out in triplicates (n=3) and standard error (SE) was calculated.

Results and Discussion

The outcome of qualitative phytochemical analysis of total compound of *Hibiscus rosa-sinesis (S1) and Tageteserecta (S2)* are presented in Table 1. "+" sign indicates Presence and "-"sign indicates absence of compound in the total compound of solvent (Acetone) extract.

Table 1: Qualitative phytochemical analysis of total compound of *Hibiscus rosa-sinesis (S1) and Tageteserecta (S2).*

Tests	Marigold (acetone extract)			Hibiscus (acetone extract)	
Steroids	-			+	
Terpenoid	+			+	
Flavonoids	+			+	
Saponins	-			+	
Glycosides	-			+	
Tanins	lead acetate	-		Lead acetate	-
	Ferric chloride	+		Ferric chloride	+
Alkaloids		Mayer's	-	Mayer's	-
		Picric	-	Picric	-
Carbohydrates		Molish	+	Molish	+
		Fehling	+	Fehling	+

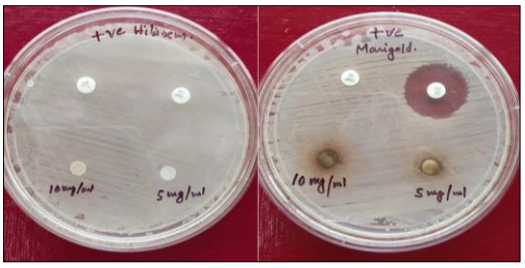


Fig 3: Solvent extract of *Hibiscus rosa-sinesis (S1) and Tageteserecta (S2)* and their comparative antimicrobial activity against *Staphylococcus aureus* (Gram positive bacteria).

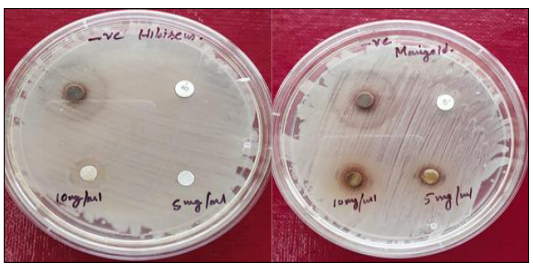
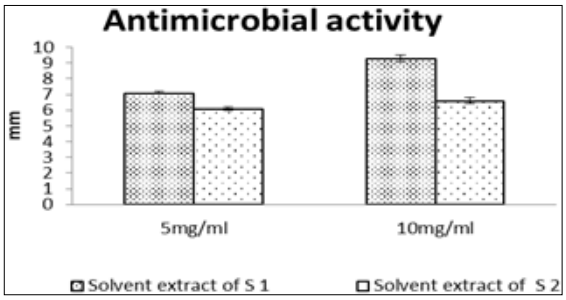
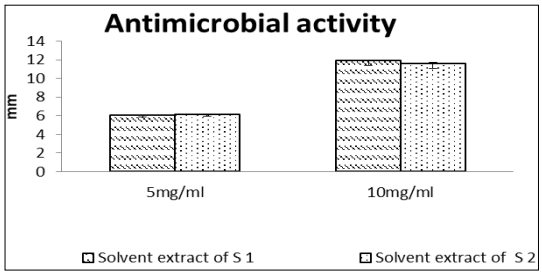


Fig 4: Solvent extract of *Hibiscus rosa-sinesis (S1) and Tageteserecta(S2)* and their comparative antimicrobial activity against *Escherichia coli* (Gram negative bacteria).

In this study the anti-microbial activity of total compound of solvent extract of *Hibiscus rosa-sinesis (S1) and Tageteserecta (S2)* were tested against *Staphyllococcusaureas* and *Escherichia coli.* As shown in Fig 3 zone of inhibition measured against *Staphyllococcusaureas* for solvent extract of S1 and S2 were 9.23 and 6.56 mm with 10mg/ml. The concentration of solvent extract (10 mg/ml) showed the best result for both sample against *Staphyllococcusaureas* (Graph 1).



Graph 1: Solvent extract of *Hibiscus rosa-sinesis (S1) and Tageteserecta (S2)* and their comparative antimicrobial activity against *Staphylococcus aureus*(Gram positive bacteria).



Graph 2: Solvent extract of *Hibiscus rosa-sinesis (S1) and Tageteserecta (S2)* and their comparative antimicrobial activity against *Escherichia coli* (Gram negative bacteria).

The zone of inhibition measured against *Escherichia coli* for solvent extract of S1 and S2 were 11.9 and 11.6 mm with 10mg/ml (Fig4). As shown in Graph 2 concentration of solvent extract (10 mg/ml) measured the best inhibition result for both the sample.

Conclusion

In case of solvent extract of total compound of both samples *Hibiscus rosa-sinesis (S1) and Tageteserecta(S2).Hibiscus rosa-sinesis*showed the better antimicrobial result then sample2 (*Tageteserecta*) against both microorganism *Staphyllococcusaureas (gram+ve)* and *Escherichia coli (gram –ve).*

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