Research & Reviews in Biotechnology & Biosciences **ISSN No: 2321-8681**
Website: www.biotechjournal.in **Research**
Volume: 8, Issue: 2, Year: 2021 **PP: 158-168**
DOI: https://doi.org/10.5281/zenodo.5775475 **Peer Reviewed Journal**

TO SCREEN AND ANALYZE THE ANTIMICROBIAL ACTIVITY OF TISSUES EXTRACT FROM *HIBISCUS ROSA SINESIS*

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Article History

Received: 16/11/2021
Accepted: 04/12/2021

Article ID: RRBB/115

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Abstract

The present study has been design for the determination of antimicrobial activities from different tissues of *Hibiscus rosa sinesis*. It was determined against different microorganisms and to check different tissue extract against the phytochemicals activity such as flavonoids, tannins, saponin, glycosides, phenolic compounds, protein, amino acid, alkaloids and oils from the different tissues of *Hibiscus rosa sinesis*. Extracts showed increasing antibacterial property with increase in the extraction concentration. Maximum activity was observed in *Enterobacter aerogenes* against methanolic leaf extract of *Hibiscus rosa sinesis* at concentration 800mg/ml and it showed maximum zone of inhibition (59.3 mm), *M. luteus* showed zone of inhibition at 800 mg/ml concentration is (39 mm). *Salmonella typhi in* methanolic leaf extract show maximum zone of inhibition at 800mg/ml concentration with zone of inhibition 46.3 mm, while *B. megatarium* shows Minimum zone of inhibition 38.6 mm in leaf extract at same concentration i.e. 800mg/ml, *Salmonella abony* shows 24.8 mm inhibition zone at concentration 800mg/ml along with *coccobacilli* also shows some amount of antibacterial activity at concentration of 800 mg/ml with inhibition zone (35.3 mm) in leaves extract of Hibiscus. Microorganism *B.subtilis*, *Pseudomonas aeuroginosa* didn't show any zone of inhibition in any different concentration it may be resistant to methanolic leaf extract of *H.rosa sinesis.*

Keywords: Antimicrobial activity, Methanolic leaf extract, *Hibiscus rosa, Zone of inhibition*

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1. Introduction

Nature has been a source of medicinal agents for thousands of years and a striking number of modern drugs have been isolated from natural source, many based on their use in traditional medicines or phytomedicines. Over the years, World Health Organization (WHO) advocated traditional medicines as safe remedies for aliments of both microbial and non-microbial origins. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry. Some antibiotics have become almost archaic because of drug resistant and consequently new drugs must be sought, for which herbal treatment is one possible way to treat diseases caused by multi drug resistant bactepotential [1]. Since plants have coevolved with pathogens, they understandably have also developed the chemical protection pathways against the parasitic organisms. Therefore, it is reasonable to expect a verity of plant compounds with specific as well as general antimicrobial activity and antibacterial potential Resistance towards reveling antibiotics having become widespread among bacteria and fungi, new class of antimicrobial substances are urgently required. There are several studies which reveal the presence of such compounds with antimicrobial properties in various plant parts[1],[2]. Hibiscus (*Malvaceae*) is a genus of herbs, shrubs,and trees; its 250 species are widely distribute dintropical and subtropical regions of the world. About 40species occur in India. Microorganisms are the most important pathogens causing severe morbidity and fatal infections in humans. The range of pathogenic bacteria is wide and so is the variety of diseases caused by them Hence, many efforts have been exploited to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants.[3].

2. Material and Methods:

2.1 Collection of plant materials: The Flower/Leaves of *Hibiscus rosa sinesis* were identified collected from the area of botanical garden of Agriculture university, Anand Gujarat. Fresh flowers /Leaves were washed under running tap water followed by distilled water and plant material is dried under sunlight. The powder of flowers/Leaves was prepared using clean mortar and pestle

2.2 Collection of test culture: A total of 8 isolates belonging to different bacterial species were collected from the microbiology and biotechnology laboratory of Shri PM Patel institute of P.G Studies and Research in Science college, Anand, SP University. Among them Gram negative bacteria and Gram positive bacteria. Bacterial species /genus is isolated and identified from the garden soil of APMS campus that is *coccobacilli* gram positive bacteria. The pure culture of bacteria were maintained at 4◦C on nutrient agar slant.

2.3 Extraction of aqueous and organic components:

2.3.1 Cold extraction - A total of 5 g of dried *Hibiscus rosa sinesis* flower was soaked in 50 ml of cold water in a conical flak and kept on shaker for 24 hours. After 24 hour the solution was centrifuged with 10000 rpm for

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5 minutes by centrifuge tube and filtered through musclin cloth in sterile test tube. The extract stored in air tight bottle in refrigerator at 4 C. [1],[3]

2.3.2 Hot extraction - A total of 5 g of dried *Hibiscus rosa sinesis* flower was soaked in 50 ml of Hot water in a conical flak then mixture was boiled for 30 minute and kept on shaker for 24 hours. After 24 hour the solution was centrifuged with 10000 rpm for 5 minutes by centrifuge tube and filtered through musclin cloth in sterile test tube. The extract stored in air tight bottle in refrigerator at 4 C[1],[3]

2.3.3 Solvent extraction - 5 g of dried *Hibiscus rosa sinesis* flower was placed in 50 ml of Organic solvent methanol in plugged conical flask. After that was kept in a rotary shaker for 24 hour. After 24hour filter out the mixture with whatman filter paper no. 1 Then filtered solution centrifuged at 10000 rpm for 5 minutes. The supernant was collected and stores in air tight bottle in refrigerator 4°C. [1].

2.3.4 Extraction concentration – 500 mg/ml, 600 mg/ml, 700 mg/ml and 800 mg/ml.

i.e. 5 g dried flower and leaves in 10 ml of Organic solvent (Methanol)gives 500 mg/ml.

6 g dried flower and leaves in 10 ml methanol gives 600 mg/ml.

7 g dried flower and leaves in 10 ml methanol gives 700 mg/ml.

8 g dried flower and leaves in 10 ml methanol gives 800 mg/ml.

2.4 Antimicrobial assay - Agar well diffusion method is used to evaluate antibacterial activity. Autoclaved sterile Nutrient agar were prepared and well of 8 mm were cut using sterile cup borer and 0.3 ml culture was spread with different bacterial cultures The cut well was filled with 100 microliters of both aqueous and solvent extracts of flower/leaves separately. The bacterial culture plates were kept for incubation at 37 C for 24 hours. the zone of inhibition was calculated by measuring the diameter of the zone around the well in millimeters (mm). [1],[3].

2

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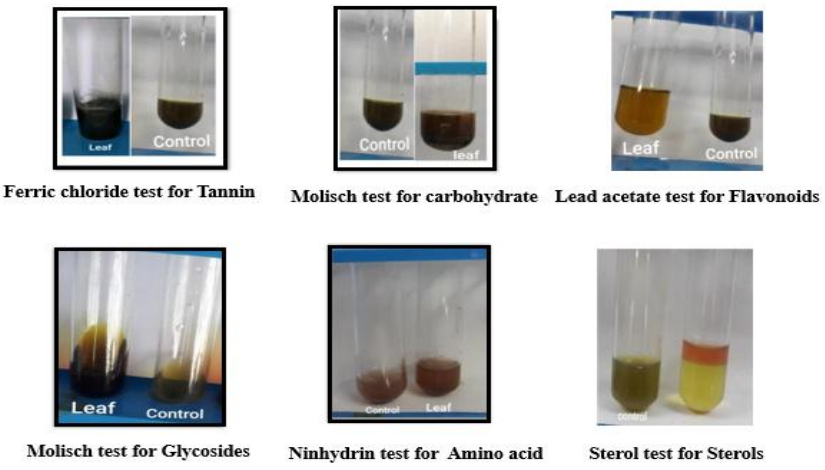

Figure 1: Profile of Bioactive compounds

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Phytochemical profiling:

From the plant extract different phytochemical test were perform like Detection of carbohydrates & Glycosidees by Molisch`s test, Detection of phenolic compounds (Tannin) by Ferric chloride test, Detection of alkaloids (Mayer`s test), Detection of fixed oils by Copper sulphate test, Detection of Saponins by foam test , Detection of Flavonoids by Sodium hydrixude test, Detection of amino acids and proteins by Ninhydrin test, Detection of sterol by Sterol test.[4],[5].

3. Result

3.1 Antimicrobial activity: In the present investigation antibacterial patterns for methanolic leaf extracts of *Hibiscus rosa sinesis* for different concentrations 100mg/ml, 500mg/ml to 800mg/ml were observe Extracts showed increasing antibacterial property with increase in the extraction concentration. Maximum activity was obtained in*Enterobacter aerogenes* in methanolic leaf extract of *Hibiscus rosa sinesis* at concentration of 800mg/ml which showed maximum zone of inhibition (59.3mm), *M.luteus* also showed zone of inhibition (39mm) at 800 mg/ml concentration. *Salmonella typhi* methanolic leaf extract show zone of inhibition at 800mg/ml concentration (46.3mm), while *B. megatarium* shows Minimum zone of inhibition 38.6mm in leaf extract at concentration 800mg/ml, *Salmonella abony* shows 24.8mm inhibition zone at

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Table 1. ***Hibiscus rosa sinesis*** **Leaf extract for concentration mg/ml.**

Sr.no	Microorganism	Zone of inhibition (mm) [Leaf]			
		Control(C) (99.9% Methanol)	Concentration mg/ml	Experiment(E) (Solvent extract Leaf)	E-C
1	M.luteus	-	500	40mm	40mm
			600	35.6mm	35.6mm
			700	36.6mm	36.6mm
			800	39mm	39mm
2	E.coli	-	500	-	-
			600	-	-
			700	22mm	22mm
			800	21mm	21mm
3	Soil Coccobacilli	-	500	36.3mm	36.3mm
			600	34mm	34mm
			700	36.3mm	36.3mm
			800	35.3mm	35.3mm
4	Pseudomonas aeruginosa	44.6mm	500	-	-
			600	-	-
			700	-	-
			800	-	-
5	B.subtilis	23.5mm	500	22mm	-1.5mm
			600	21.5mm	-2mm
			700	13mm	-10.5mm
			800	23mm	-0.5mm
6	B.megatarium	-	500	34.3mm	34.3mm
			600	33.6mm	33.6mm
			700	34mm	34mm
			800	38.6mm	38.6mm
7	Enterobacter aerogenes	-	500	29mm	29mm
			600	32.5mm	32.5mm
			700	44.3mm	44.3mm
			800	59.3mm	59.3mm
8	Salmonella abony	24.5mm	500	49.3mm	24.8mm
			600	43.6mm	19.1mm
			700	46mm	21.5mm
			800	49.3mm	24.8mm
9	Salmonella typhi	-	500	42mm	42mm
			600	44.6mm	44.6mm
			700	46mm	46mm
			800	46.3mm	46.3mm

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concentration 800mg/ml along with *coccobacilli* also shows some amount of antibacterial activity at concentration of 800mg/ml with inhibition zone (35.3 mm) in leaves extract of Hibiscus. Microorganism *B.subtilis*, *Pseudomonas aeuroginosa* may be resistant to methanolic leaf extract of *H.rosa sinesis*.(Graph1 & Table 1). Although we have also tried Hot and Cold water extract of *Hibiscus rosa sinesis* with doses 100 and 500 mg/well, but did not find any notable inhibition of bacterial growth (data not shown) . In the present study, we have shown only the susceptibilities of clinical bacterial isolates to 100mg, 500mg to 800mg of the plant extracts of *H.rosa sinesis* per well . Application of higher doses of the extracts prepared from selected medicinal plants in studying antibacterial activity by by agar well diffusion and disc-diffusion method have also been reported. (23,26 & 27)

3.2 Phytochemical analysis

Pytochemical test done by various methods. Flower showed the presence of Tannin, Carbohydate, Saponin and Sterol. While leaves showed presence of Tannin, Flavonoid and Sterol. The Profile of this Bioactive compounds is shown in Table 2. & Figure 1 The antibacterial activity of plant extracts depends on the available bioactive secondary metabolites in the plant part. which may not only be developmental stage specific but also organ and tissue specific. It has been reported that hibiscus possess alkaloid, flavonoids, tannin and phenols (28) **.** From the preliminary screening, it has been identified that methanol extract of the hibiscus exhibits phytochemical property which may be due to the presence of biologically active compounds in hibiscus whose activity are enhanced in the presence on methanol. Furthermore, methanol has strong extraction capacity which could be helpful in extracting greater number of active constituents responsible for antibacterial activity.(30)

Table 2. Phytochemicals Test in methanolic extracts of hibiscus leaves.

Sr No.	Phytochemical test	Observation	Result
1	Tannin : Ferric Chloride Test	Gives dark green color	Present
2	Carbohydrates : Molish's Test	Gives a violet ring	Absent
3	Saponins : Foaming Test	Gives layer of foam	Absent
4	Flavonoids :Sodium Hydroxide Test	Gives Yellow color	Present
5	Glycosides :Molish's Test	Gives violet ring	Absent
6	Alkaloids Mayers Test	Gives white or creamy precipitate	Absent
7	Amino Acid : Ninhydrin Test	Gives Purple color	Absent
8	Sterol test	Upper layer turns to red and sulphuric layer showed yellow with green fluorescence	Present

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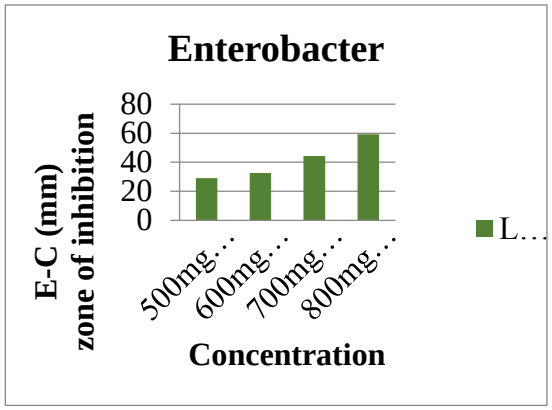

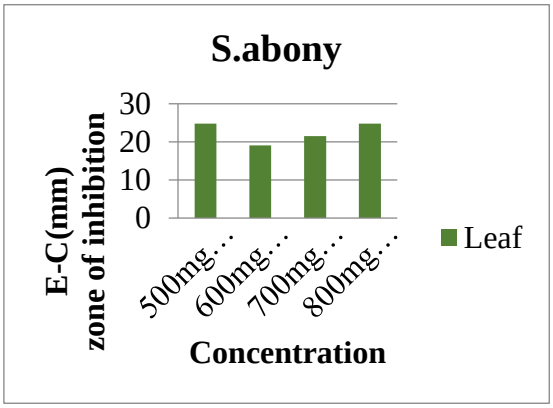

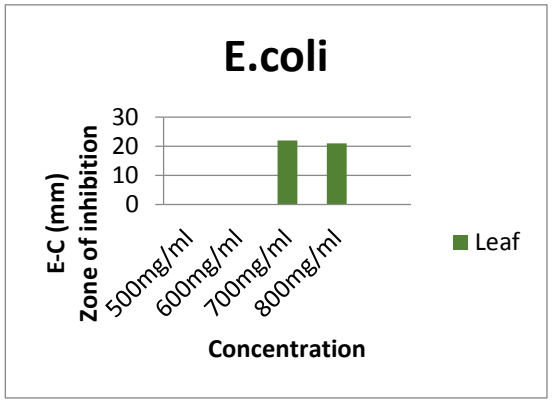

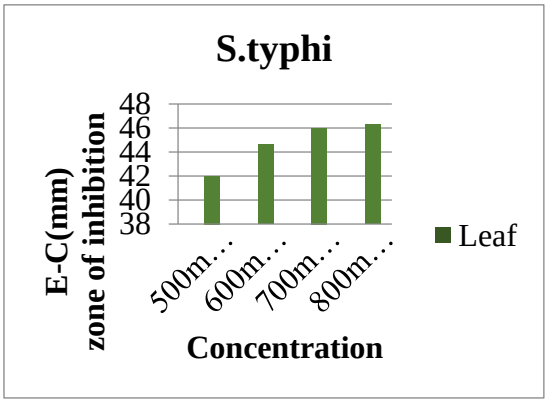

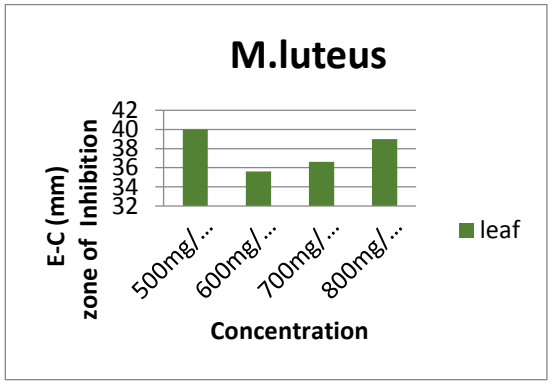

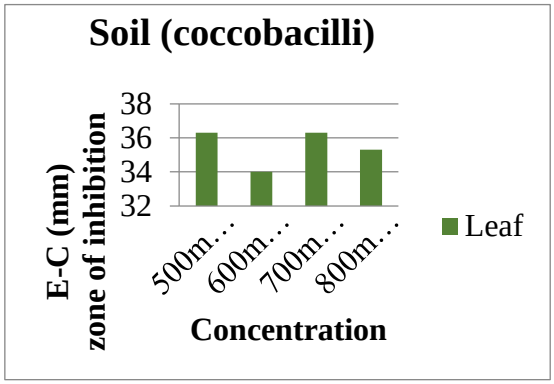

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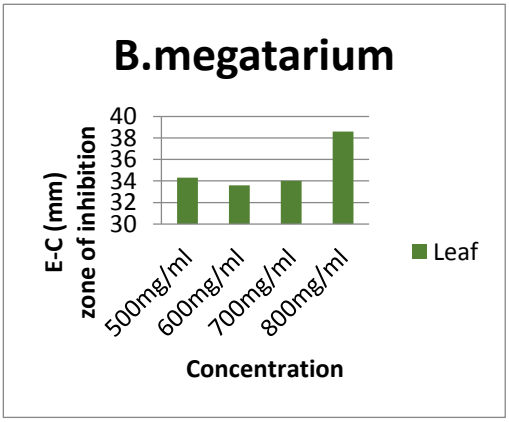

Graph 1: Graphical representation of antimicrobial activity

Conclusion

Result were encouraging but precise assessment is utterly necessary before being situated in practice as well as the most active extracts can be subjected to isolation of the therapeutic antimicrobials extract. Hence *Bacillus megatarium, coccobacilli* and *Salmonella abony* shows minimum antibacterial activity. other bacteria such as *Escherichia coli, Pseudomonas aerogenes* and *Bacillus subtilis* may be resistant to tissues extract of *Hibiscus rosa sinesis.*

Based on the result, we may conclude methanolic tissues extract of *Hibiscus rosa sinesis* contain Phytochemical compounds with antibacterial activities against the antibacterial study bacteria by using Leaves of *Hibiscus rosa sinesis* and we may consider that the leaves shows better antibacterial and undergo secondary pharmacological evaluation.

The maximum antibacterial activity shown by *Salmonella typhi Enterobacter aeruginosa, Micrococcus luteus* in leaves acitivity of *H. rosa sinesis.* Pharmacology and toxicology of *H.rosa sinesis* that should be further studied to determine how it can be utilized to treat bacterial infection.

Funding

No

Ethical issue

No

Conflict of interest

no

Acknowledgments: NIL

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