ORIGINAL RESEARCH PAPER

Volume-7 | Issue-2 | February-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITY OF HIBISCUS ROSA-SINENSIS, TABERNAEMONTANA DIVARICATA AND NYCTANTHES ARBORTRISTIS LEAF EXTRACT

Microbiology

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ABSTRACT

The antibacterial activities of medicinal plant were evaluated *in vitro* against human pathogenic microorganisms by Kirby-Bauer agar diffusion method. Methanol and acetone extract of *Hibiscus rosa-sinensis, Tabernaemontana divaricata, and Nyctanthes arbortristis* were prepared and its antimicrobial property was evaluated against *S. aureus, E. coli* and *P. aeruginosa*. Different concentrations (25%, 50%, 75% and 100%) of fresh leaves extracts of all selected plants were used to screen antimicrobial activity. The results revealed that the methanol extracts of plant leaves have high antimicrobial activities on all test organisms (range of inhibition, 7- 20 mm) as compare to acetone extracts of plant leaves in same concentration. The antimicrobial activity against *S. aureus,* with maximum inhibitory zone at 100% was shown in methanol extract of *H. rosa-sinensis* and *T. divaricata,* whereas against *Escherichia coli* maximum inhibitory zone was shown in methanol extract of *T. divaricata* and against *Pseudomonas aeruginosa* maximum inhibitory zone was shown in methanol extract of *T. divaricata* and *N. arbortristis.* Results concluded that these plants contain high amount of secondary metabolites in their leaves extract which enhance its antimicrobial property against human pathogenic microorganisms.

KEYWORDS

Hibiscus rosa-sinensis, Tabernaemontana divaricata, and Nyctanthes arbortristis, Leaf Extract, Antibacterial activity, Agar-well diffusion

1. Introduction

Nature has provided the treasure of inherent therapy to cure all ailments of mankind. There is no doubt that plants are a repository of potentially useful chemical compounds which serve as drugs, and provide newer leads for modern drug synthesis [1]. The plant *Hibiscus rosa-sinensis* (H. rosa-sinensis) belongs to the family Malvaceae. It is a highly potential, functional and valuable medicinal plant, which has been reported in the ancient medicinal literature with beneficial effects in various disorders of humans. This is a tropical shrub, with large, glossy green leaves and spectacular trumpet shaped flowers. Its medicinal values have been mentioned in traditional folk medicines for variety of diseases. Recent studies shown that *H.rosa-sinensis* extract from different part of plant has significant protective effects [2], thus these plants have great medicinal potential for the therapy of infection. Traditionally the flowers can be used as anti asthmatic agents [3]. The genus Hibiscus (Malvaceae) comprises about 275 species in the tropics and sub-tropics [4]. Flowers of *Hibiscus tiliaceus L.* are widely used for birth control and for treating skin infections [5]. Leaves and flowers of selected Hibiscus species are used in traditional medicine. Information on their antioxidant, antityrosinase and antibacterial activities is insufficient [6].

T. Divaricata (family: Apocynaceae) commonly known as crepe flower, crepe jasmine (chandani) in India, is an evergreen shrub to 6 feet (1.8 cm). It is used in Chinese, ayurvedic and thai traditional medicine for the treatment of fever, pain and dysentery. The most common medicinal use of its crude extract involves antimicrobial action against infectious diseases such as syphilis, leprosy and gonorrhoea as well as its antiparasitic action against worms, dysentery, diarrhoea and malaria [7]. The plant is also used as a tonic to the brain, liver, and spleen. It is reported that plant extract possesses antinociceptive, antioxidant, anti-inflammatory, and reversible acetyl cholinesterase inhibition activities.

N. arbortristis commonly known as Harsinger or Night jasmine, is a well-documented plant belonging to the family Oleaceae. It is a native of India, distributed wild in sub-Himalayan region and also found in Indian garden as ornamental plant. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, enlargement of spleen, diaphoretic diuretic [8]. The whole plant is used for treatment of cancer, root for fever, sciatica, anorexia; bark as expectorant, leaf for control fever, diabetes and as cholagogue, diaphoretic and anthelmintic. Various extracts of the plant is used to treat arthritis [9, 10] malaria, intestinal worms tonic, laxative, antitrypanosomal, antiinflammatory and antioxidant activity [11].

The present study has been designed to determine the role of leaves in *H. rosa-sinensis*, *T. Divaricata* and *N. arbortristis* extract in the in vitro antibacterial activity against human pathogens viz., Gram positive bacteria [Staphylococcus aureus (S. aureus)] and Gram negative bacteria [Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa)] and to compare their maximum inhibitory activity with each other as well as their ability with commercially available antibiotics.

2. Material and Methodology

Collection and processing of Plant Material

Fresh and healthy leaves of *H. rosa-sinensis, N. arbortristis* and *T. divaricata* were collected from village Koni, Bilaspur district, Chhattisgarh, India and brought to the laboratory for further analysis. The leaves were washed thoroughly under tap water and then with 2% Mercuric chloride. After washing leaves were shade dried for 10-15 days. The dried plant leaves were crushed into fine powder with the help of pestle mortar. Finally the fine powder were stored in air tight containers at room temperature for 3-5 days.

Preparation of Methanol and Acetone Leaf Extracts of *H. rosa-sinensis, N. arbortristis* and *T. divaricata*

50 g of dried leaf powder of each plant were soaked separately in 100 ml of methanol and acetone separately in flask. These flasks were allowed to stand for 3-5 days for extraction. The extracts were collected and stock solutions of conc. 50 mg/10ml were prepared.

Test Organisms

Three microorganisms were used in this study as test organisms which comprises clinical isolates of three bacteria (*Escherichia coli, Staphylococcus aureus and Pseudomonas)* were obtained from the microbiology department of Govt. E. Raghvendra Rao Postgraduate Science College, Bilaspur (C.G.). The cultures of bacteria were sub-cultured on nutrient agar slants respectively.

Screening the antibacterial activity of methanol and acetone extracts of *H. rosa-sinensis, T. divaricata and N. arbortristis* and *against Escherichia coli, Staphylococcus aureus and Pseudomonas*

The antibacterial activities of methanol and acetone extracts were determined by Kirby-Bauer agar diffusion method as described by [12] *against E coli, S. aureus and P. aeruginosa.* The culture plates having test organisms were allowed to solidify and punched with a sterile cork borer (7.0 mm diameter) to make open wells which were then filled with 0.05 ml of the extract of *H. rosa-sinensis, T. divaricata, and N.*

arbortristis with 25%, 50%, 75% and 100% concentration in each plate separately having test organism. The plates were incubated at 37°C for 48 hours. The zones of inhibition were measured and recorded. The pure solvent was used as control. The plates were incubated at 37±20 °C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction for all the three replicates and the average values were tabulated [13].

3. Results and Discussion

The result clearly shows that methanol and acetone leaf extract of *H. rosa-sinensis, T. divaricata and N. arbortristis* justified itself as a potent antibacterial agent.

The methanolic extracts of all the three plants which have been selected for present study showed significant growth inhibition of test bacteria's at different concentrations (25%, 50%, 75% , 100%) as compared to acetone leaf extract of the three plants.

Table 1, graph 1a & 1b and fig 1 shows that methanol extract of both *H. rosa-sinensis, T. divaricata* were found to be most efficient growth inhibitors against *S. aureus* with inhibition zone of (19.0 Mm at 100%) as compared to *N. arbortristis* with inhibition zone of (15.0 Mm at 100%). The acetone leaf extract of *H. rosa-sinensis* was alone found to be most efficient growth inhibitor against *S. aureus* with inhibition zone of (15.0 Mm at 100%) as compared to *T. divaricata* and *N. arbortristis* where both have inhibition zone of (13 Mm at 100%).

Table 2, graph 2a and 2b and fig 2 shows that methanol extract of *T. divaricata* was alone found to be most efficient growth inhibitor against *E.coli* with inhibition zone of (20mm at 100%) as compared to *H. rosa-sinensis* and *N. arbortristis* which have inhibition zone of (15mm and 14mm at 100%) respectively. The acetone extract of H. rosa-sinensis was alone found to be most efficient growth inhibitor against

E.coli with inhibition zone of (15mm at 100%) as compared to *T. divaricata* and *N. arbortristis* where both have inhibition zone of (14 mm at 100%)

Table.1. Zone of inhibition of leaves extracts against *Staphylococcus aureus*

Plant Extract	Dilution of Plant Extract.(µl/Ml)								
	Acetone extract				Methanol extract				Positive Control
Conc.	25	50	75	100	25	50	75	100	
Extract	Zone of Inhibition (Mm)								
Hibiscus rosa-sinensis (Gudhal)	9.0	11.0	12.0	15.0	10.0	13.0	17.0	19.0	Nil
Tabernaemontana divaricata (Chandani)	8.0	11.0	12.0	13.0	9.0	12.0	16.0	19.0	Nil
Nyctanthes arbortristis (Harshingar)	9.0	11.0	13.0	13.0	9.0	11.0	14.0	15.0	Nil

Graph. 1. Zone of inhibition of leaves extracts against *Staphylococcus aureus*

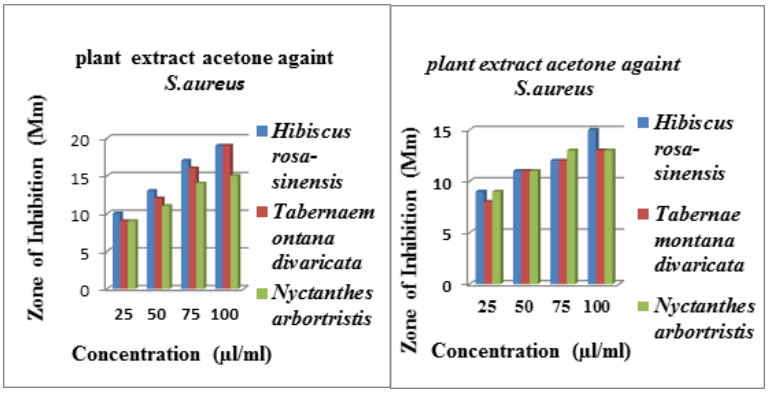


Fig 1. Inhibition zone photographs of Gram-positive bacteria *Staphylococcus aureus based on agar well diffusion assay for the various extracts of leaves*

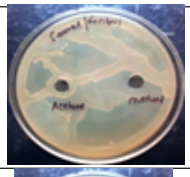
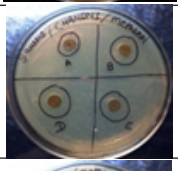
	ACETONE EXTRACT	METHANOL EXTRACT	POSITIVE CONTROL
Hibiscus rosa-sinensis (Gudhal)			
Tabernaemontana divaricata (Chandani)			
Nyctanthes arbortristis (Harshingar)			

Table.2. Zone of inhibition of leaves extracts against Escherichia coli

Plant Extract	Dilution of Plant Extract.(µl/Ml)								
	Acetone extract				Methanol extract				Positive Control
Conc. Extract	25	50	75	100	25	50	75	100	
	Zone of Inhibition (Mm)								
Hibiscus rosa-sinensis (Gudhal)	8.0	12.0	14.0	15.0	9.0	11.0	13.0	15.0	Nil
Tabernaemontana divaricata (Chandani)	7.0	12.0	14.0	14.0	8.0	10.0	17.0	20.0	Nil
Nyctanthes arbortristis (Harshingar)	7.0	11.0	12.0	14.0	7.0	10.0	12.0	14.0	Nil

Graph. 2. Zone of inhibition of leaves extracts against Escherichia coli

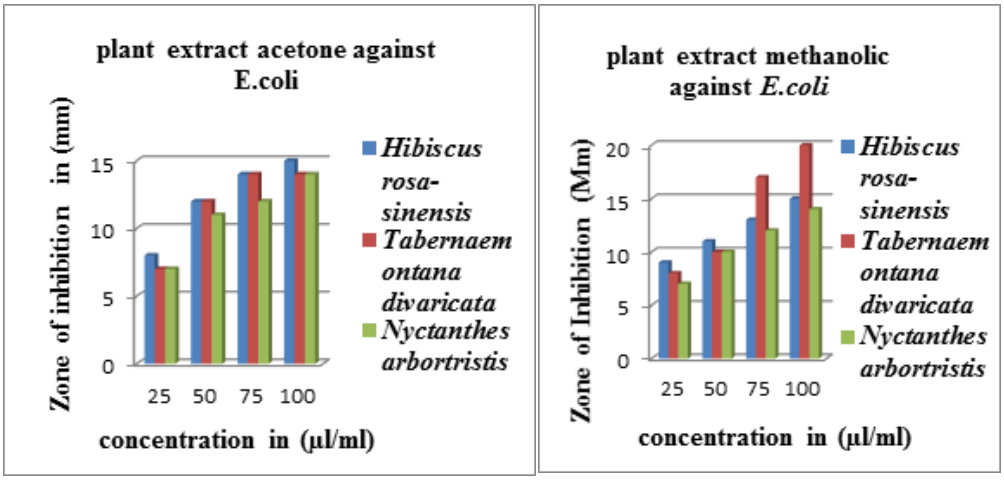


Fig 2.. Inhibition zone photographs of Gram-negative bacteria *Escherichia coli* based on agar well diffusion assay for the various extracts of leaves

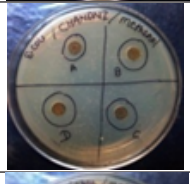
	ACETONE EXTRACT	METHANOL EXTRACT	POSITIVE CONTROL
Hibiscus rosa-sinensis (Gudhal)			
Tabernaemontana divaricata (Chandani)			
Nyctanthes arbortristis (Harshingar)			

Table.3. Zone of inhibition of leaves extracts against *Pseudomonas aeruginosa*

Plant Extract	Dilution of Plant Extract.(µl/Ml)								
	Acetone extract				Methanol extract				Positive Control
Conc.	25	50	75	100	25	50	75	100	
Extract	Zone of Inhibition (Mm)								
Hibiscus rosa-sinensis (Gudhal)	6.0	11.0	13.0	14.0	9.0	10.0	10.0	12.0	Nil
Tabernaemontana divaricata (Chandani)	8.0	9.0	11.0	15.0	9.0	10.0	13.0	16.0	Nil
Nyctanthes arbortristis (Harshingar)	7.0	10.0	12.0	15.0	8.0	11.0	13.0	16.0	Nil

Graph. 3. Zone of inhibition of leaves extracts against *Pseudomonas aeruginosa*

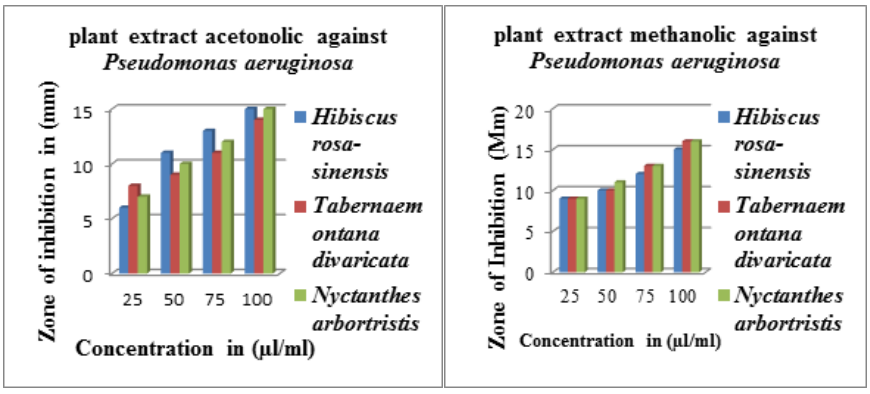


Fig.3... Inhibition zone photographs of Gram-negative bacteria *Pseudomonas aeruginosa* based on agar well diffusion assay for the various extracts of leaves

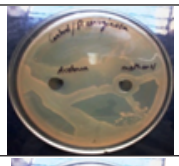
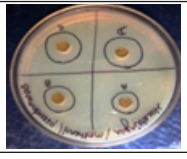
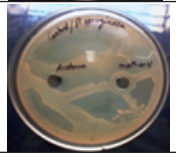
	ACETONE EXTRACT	METHANOL EXTRACT	POSITIVE CONTROL
Hibiscus rosa-sinensis (Gudhal)			
Tabernaemontana divaricata (Chandani)			
Nyctanthes arbortristis (Harshingar)			

Table 3, Graph 3a and 3b and fig 3 shows that methanol extract of both *T. divaricata* and *N. arbortristis* were found to be most efficient growth inhibitors against *P. aeruginosa* with inhibition zone of (16 mm at 100%) as compared to *H. rosa-sinensis* which have inhibition zone of (12mm at 100%) . The acetone extract of both *T. divaricata* and *N. arbortristis* were found to be most efficient growth inhibitors against *P. aeruginosa* with inhibition zone of (15 mm at 100%) as compared to *H. rosa-sinensis* which have inhibition zone of (14mm at 100%) .

The data supports the hypothesis that some common plant leaves have an inhibitory effect on the growth of certain food, soil and water borne pathogens in tissue culture. The results suggest that methanol as well as acetone leaves extract of *H. rosa-sinensis, T. divaricata, and N. arbortristis* produced significant antimicrobial effects. The antimicrobial activity against Staphylococcus *aureus,* with maximum inhibitory zone was shown in methanol extract of *H. rosa-sinensis* and *T. divaricata,* whereas against *Escherichia coli* maximum inhibitory zone was shown in methanol extract of *T. divaricata* and against *Pseudomonas aeruginosa* maximum inhibitory zone was shown in methanol extract of *T. divaricata* and *N. arbortristis* which shows similarity with the results of previous works [14, 15, 16].

It is clear from the findings that *H. rosa-sinensis* leaves contain important constituents that confer its antibacterial activity and may be used in treating pathological conditions caused particularly by *S. aureus* and *E. coli.* A number of previous studies reported that *H. rosa-sinensis* contains flavonoids, cyanidin, querecetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin, ascorbic, citric, tartaric and oxalic acid [17]. Flavonoids especially are known to be effective antimicrobial agent against a wide array of microorganisms. The activity is attributed to their ability to complex with extra cellular and soluble proteins and with bacterial cell wall [18]. *T. divaricata* and *N. arbortristis* revealed a significant scope to develop a novel broad spectrum of antibacterial herbal formulation and can be used for food preservation, pharmaceutical, alternative medicine and natural therapy. Phenol compounds and tannins in ethanolic extract of leaves are found to be active against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and, *E. coli.* Antimicrobial evaluation of extract of leaves against numerous Gram positive and Gram negative strains revealed that, *Pseudomonas aeruginosa, E. coli* and *Staphylococcus aureus* were more sensitive to methanolic extract [19, 16].

Acknowledgement

The authors like to express their gratefulness to The Principal and head of the Department Of Microbiology, Government E. Raghvendra Rao P.G. Science College, Bilaspur (C.G.) for the assistance during experimental work.

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