INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)
ISSN: 2231-6078
3(2): April.-June.: (2013), 636-340

Research Article

In-vitro antibacterial activity in leaf extract of *Hibiscus rosa-sinensis* against known pathogens

Leena Verma[1], Shweta Sao[1], Yogesh K. Deshmukh[1], Dilendra[1] and Pankaj K. Sahu[2]

[1]Department of Life Science, Dr. C.V. Raman University, Kota, Bilaspur, India
[2]Department of Botany, Dr. C.V. Raman University, Kota, Bilaspur, India

Abstract

Hibiscus rosa-sinensis Linn. belong to family Malvaceae (mallow family) plants parts shows medicinal properties. In present work four extract were prepared for antibacterial test i.e., aqueous, methanol, ethanol, and chloroform. In present study of paper disc method all extract shows best result against *Bacillus, Pseudomonas, CoNS, Enterococci, Enterobacter*, and *E. coli*, but *Bacillus* shows highest zone in methanol and aqueous extract and *Enterobacter* shows highest zone in ethanol and chloroform extract. In agar well diffusion method, all extract shows highest zone of inhibition in *Achromobacter* compare than other bacteria. The extracts of the *Hibiscus* are proved to have potential antibacterial activity.

Key words: Antibacterial activity, leaf extract, zone of inhibition, *Hibiscus rosa-sinensis*

Introduction

Hibiscus rosa-sinensis belongs to family Malvaceae, deciduous shrubs with dark green leaves, can grow up to 15 feet tall in frost-free areas [1,2]. The Hibiscus plant has been used to treat hypertension, lice, diabetes, cancer, for gall bladder attacks, skin afflictions, dry coughs, toxin removal, to lower cholesterol, and as a laxative and to reduce fevers. It also used as a purgative and for diarrhea, inflammations, prostate and menstrual problems, burns, boils, ear and toothaches, asthma, as an anti-inflammatory, and for tumors, hematomas and trauma. The three major effective agents present in *Hibiscus* are delphinidin (delphinidin chloride), esculetin (cichorigenin), cyanidin (cyanidine, cyanidol). *Hibiscus* flowers contain gossypetin, anthocyanin, and glycoside hibiscin[3].

Materials and Methods

1) **Sample collection**-: The bacterial samples were taken from CIMS (central institute of medical science). The bacterial samples are *Achromobacter, Bacillus, Klebsiella, CoNS (coagulase negative staphylococcus), Enterobacter, Enterococci, Pseudomonas, Proteus, Staphylococcus aureus, E. coli.*
2) **Plant collection**-: Plant was collected from Kota and University campus of C. V. Raman University, Kota, Bilaspur (C.G.). The leaf and flower of plant Were taken and then rinsed in running tap water, few leaves and flowers are shade dry in room for 6-7 days and few in oven dry for 4-5 days and then crushed with the help of mortar-pestle and make powder form for different extract preparation which is used for the practical.
3) **Extract preparation**-: For this practical five types of extract prepared they are-

a) **Aqueous and Ethanol Extract**: 100gm powder of fresh, shade dry and oven dry leaf was dipped in 400ml distilled water in a conical flask and left for 7 days with occasional shaking. Filtered off using sterile filter paper (Whattman no. 1) into a clean conical flask. The extracts obtained were then stored in a refrigerator at 4°C for antibacterial activity test [4].
b) **Methanol Extract**: 50gm powder of fresh, shade dry and oven dry leaves sequentially extracted by shaking for 2 hours on Wrist Action Shaker after overnight soaking in 150 ml of relevant solvent. After filtration, samples were rinsed with additional 3 x 60 ml portions of the solvent. Combined filtrates were dried at room temperature under electric fan. The extracts were stored in the refrigerator at 4°C until required [5].
c) **Chloroform Extract**: 10 gm powder of fresh, shade dry and oven dry leaf was dipped in 100ml distilled water in a conical flask and left for 5 days. Filtered off using sterile filter paper (Whattman no. 1) into a clean conical flask. The extracts obtained were then stored in a refrigerator at 4°C for antibacterial activity test[6].

4) **Antibacterial test**-: Two types of method were used for the test.

a) **Paper Disc Diffusion Method**: In this method the test compounds, i.e. the flower extract and leaf extract were introduced into a disc 0.5 mm and then allowed to dry. Thus the disc was completely saturated with the test compound. Then these discs were placed directly on the surface of Muller Hinton agar plates, swabbed with the test organism.
b) **Agar Well Diffusion Method:** In Muller Hinton media add few ml of culture and poured in the plates after solidify wells of 5 mm were cut with the help of cork borer. The cut wells were then filled with 20 ml of both leaf and flower extracts separately[7].

*** Corresponding Author**
Email : leenaver1989@gmail.com

Results and Discussion

Plant *Hibiscus rosa-sinensis* was used for study of antibacterial activity test. In this study four types of extract prepared i.e., aqueous extract, methanol extract, ethanol extract, chloroform extract of leaves which is fresh, shade dry and oven dry. All extract is used for antibacterial test which include two types of method i.e., paper disc method and well diffusion method and in this study the practical was done triplet. **Figures** 1-4 representing the zone of inhibition of *Bacillus, Enterococci, CoNS* and *Enterobacter* of leaf extract against human pathogenic bacteria. **Figure** 5-7 representing the zone of inhibition of *Bacillus, Achromobacter* and *Enterococci* against human pathogenic Bacteria.

Paper disc method-

Aqueous extraction- The results clearly showed that aqueous extractions of leaf in fresh leaf extract get highest zone against *Bacillus, Enterococci, CoNS* and *Enterobacter* , (35 ± 0.1), (30 ± 1.7), (25 ± 0.1), and (30 ± 0.4) respectively but in shade, dry extract (31 ± 0.1), (24 ± 0.1), (30 ± 0.1), and (29 ± 0.7) and in oven, dry extract (20 ± 0.6), (25 ± 0.6), (27 ± 0.3), and (27 ± 0.2), respectively.

Methanol extract- The results clearly showed that methanol extractions in fresh leaf extract get highest zone against *Bacillus, Enterococci, CoNS* and *Enterobacter,* (30 ± 0.2), (25 ± 0.4),(26 ± 0.8), and (27 ± 0.1) respectively but in shade, dry extract (33 ± 0.1), (23 ± 0.4), (28 ± 0.3), and (26 ± 0.3), and in oven dry extract (18 ± 0.4), (19 ± 0.4), (22 ± 0.6), and (24 ± 0.1) respectively. Table 1 & 2 showing antibacterial activity of aqueous, methanol, ethanol & Chloroform extract in leaf in paper disc method.

Ethanol extract- The results clearly showed that ethanol extractions of in fresh leaf extract get highest zone against *Bacillus, Enterococci, CoNS* and *Enterobacter,* (25 ± 0.4), (19 ± 0.4),(21 ± 0.5), and (30 ± 1.3) respectively but in shade dry extract (29 ± 0.1), (21 ± 0.2), (18 ± 0.3), and (26 ± 0.7) and in oven dry extract (15 ± 0.7), (17 ± 0.3), (17 ± 0.1), and (22 ± 0.5) resp.

Chloroform extract- The results clearly showed that chloroform extractions in fresh leaf extract get highest zone against *Enterobacter, Enterococci, CoNS* and *Bacillus,* (28 ± 0.9) (11 ± 0.4), (17 ± 0.7), and (21 ± 0.2), respectively but in shade dry extract (26 ± 0.5) (21 ± 0.7), (19 ± 0.4), and (24 ± 0.5), and in oven dry extract (23 ± 0.7) (15 ± 0.1), (15 ± 0.1), and (20 ± 0.8), resp.

Well diffusion method- Table 3 & 4 showing antibacterial activity of aqueous, methanol, ethanol & Chloroform extract in leaf well diffusion method.

Aqueous extraction- The results clearly showed that aqueous extractions in fresh leaf extract get zone against *Bacillus, Achromobacter,* and *Enterococci,* (21 ± 0.4), (16 ± 0.7), and (23 ± 0.3) respectively but in shade dry extract (18 ± 0.1), (14 ± 0.1), and (21 ± 0.1) and in oven dry extract (12 ± 0.1), (14 ± 0.3), and (20 ± 0.6) resp. (Table 3)

Methanol extraction- The results clearly showed that methanol extractions of in fresh leaf extract get zone against *Bacillus, Achromobacter,* and *Enterococci* (18 ± 0.7), (12 ± 0.5), and (20 ± 0.1) respectively but in shade dry extract (13 ± 0.3), (11 ± 0.2), and (20 ± 0.5) and in oven dry extract (11 ± 0.1), (0.0 ±0.0), and (17 ± 0.3) resp. (Table 3)

Ethanol extraction- The results clearly showed that ethanol extractions of leaf in fresh leaf get zone against *Bacillus, Achromobacter,* and *Enterococci* , (15 ± 0.2), (7 ± 0.1), and (17 ± 0.3) respectively but in shade dry extract (9 ± 0.1), (12 ± 0.5), and (16 ± 0.1) resp. In oven dry extract (8 ± 0.1), (11 ± 0.4), and (14 ± 0.5) resp. (Table 4)

Chloroform extraction- The results clearly showed that chloroform extractions of leaf in fresh leaf get zone against *Enterococci, Bacillus,* and *Achromobacter* (21 ± 0.3), (18 ± 0.5), (14 ± 0.2), and respectively but in shade, dry extract (18 ± 0.1) (12 ± 0.3), and (15 ± 0.3), resp. In oven dry extract (17 ± 0.4) (9 ± 0.1), and (0.0 ± 0.0) resp. (Table 4)

The methanol extracts of Hibiscus exhibited higher antibacterial activity against *Bacillus, S. aureus, Pseudomonas* and *Klebsiella*[8]. Flower extract contain phenolics compounds like tannins that are very good antimicrobial agent. Thus it may be summarized that the class of natural compounds must exhibit the antibacterial activity. The metabolites have been shown to be responsible for various therapeutic activities of medicinal plants [9, 10]. In present study the paper disc method methanol extract shows best result against *Bacillus, Pseudomonas, CoNS, Enterococci, Enterobacter, E.coli,* and *Achromobacter* but *Bacillus* get highest zone of inhibition. Aqueous extract shows best result against *Bacillus, Pseudomonas, CoNS, Enterococci, Enterobacter, E. coli, and Achromobacter* but *Bacillus* get highest zone of inhibition. Ethanol extract shows best result against *Bacillus, CoNS, Enterobacter,* and *E.coli,* but *Enterobacter* get highest zone of inhibition. Chloroform extract shows best result against *Bacillus, Cons, Enterococci, Enterobacter,* and *E.coli,* but *Enterobacter* get highest zone of inhibition. Present study represent agar well diffusion method, methanol extract shows highest zone of inhibition in *Achromobacter* compare than other bacteria. Aqueous extract shows highest zone of inhibition in *Achromobacter* compare than other bacteria. Ethanol extract shows highest zone of inhibition in *Enterococci* compare than other bacteria and

chloroform extract shows highest zone of inhibition in *Enterococci* compare than other bacteria.

Acknowledgement

Authors are thankful to Vice Chancellor & Registrar, Dr. C.V. Raman University, Kota, Bilaspur (C.G.) for their blessing and inspiration.

Table 1- Antibacterial activity of aqueous and methanol extract of *Hibiscus* in paper disc method

Bacteria	Aqueous extract			Methanol extract		
	Fresh	Shade dry	Oven dry	Fresh	Shade dry	Oven dry
Bacillus	35 ± 0.1	31 ± 0.1	20 ± 0.6	30 ± 0.2	33 ± 0.1	18 ± 0.4
Proteus	15 ± 0.5	10 ± 0.3	7 ± 0.2	13 ± 0.7	12 ± 0.2	0.0 ± 0.0
Pseudomonas	16 ± 1.1	31 ± 0.2	23 ± 0.4	20 ± 0.1	28 ± 0.7	25 ± 0.2
Achromobacter	30 ± 0.7	28 ± 0.3	25 ± 0.3	25 ± 0.2	21 ± 0.1	18 ± 0.2
S. aureus	16 ± 0.8	14 ± 0.6	10 ± 0.5	19 ± 0.5	15 ± 0.1	11 ± 0.7
CoNS	25 ± 0.1	30 ± 0.1	27 ± 0.3	26 ± 0.8	28 ± 0.3	22 ± 0.6
Enterococci	30 ± 1.7	24 ± 0.1	25 ± 0.6	25 ± 0.4	23 ± 0.4	19 ± 0.4
Klebsiella	27 ± 0.2	25 ± 0.8	20 ± 0.5	20 ± 0.3	14 ± 0.6	10 ± 0.5
Enterobacter	30 ± 0.4	29 ± 0.7	27 ± 0.2	27 ± 0.1	26 ± 0.3	24 ± 0.1
E. coli	27 ± 1.4	15 ± 0.9	21 ± 0.3	21 ± 0.6	29 ± 0.6	20 ± 0.2

(Mean ± SD in mm)

Table 2- Antibacterial activity of Ethanol and Chloroform extract in paper disc method

Bacteria	Ethanol extract			Chloroform extract		
	Fresh	Shade dry	Oven dry	Fresh	Shade dry	Oven dry
Bacillus	25 ± 0.4	29 ± 0.1	15 ± 0.7	21 ± 0.2	24 ± 0.5	20 ± 0.8
Proteus	9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	8 ± 0.1	6 ± 0.1	0.0 ± 0.0
Pseudomonas	8 ± 0.1	20 ± 0.1	13 ± 0.4	0.0 ± 0.0	20 ± 0.7	17 ± 0.6
Achromobacter	0.0 ± 0.0	18 ± 0.8	20 ± 0.5	0.0 ± 0.0	21 ± 0.9	18 ± 0.4
S. aureus	17 ± 0.3	15 ± 0.6	14 ± 0.9	14 ± 0.2	11 ± .03	9 ± 0.1
CoNS	21 ± 0.5	18 ± 0.3	17 ± 0.1	17 ± 0.7	19 ± 0.4	15 ± 0.1
Enterococci	19 ± 0.4	21 ± 0.2	17 ± 0.3	11 ± 0.4	21 ± 0.7	15 ± 0.1
Klebsiella	18 ± 0.4	12 ± 0.1	10 ± 0.3	9 ± 0.1	15 ± 0.6	13 ± 0.5
Enterobacter	30 ± 1.3	26 ± 0.7	22 ± 0.5	28 ± 0.9	26 ± 0.5	23 ± 0.7
E. coli	15 ± 0.7	26 ± 0.3	23 ± 0.9	16 ± 0.5	26 ± 0.2	20 ± 0.8

(Mean ± SD in mm)

Table 3 Antibacterial activity of Aqueous and Methanol extract in well diffusion method

Bacteria	Aqueous extract			Methanol extract		
	Fresh	Shade dry	Oven dry	Fresh	Shade dry	Oven dry
Bacillus	21 ± 0.4	18 ± 0.1	12 ± 0.1	18 ± 0.7	13 ± 0.3	11 ± 0.1
Achromobacter	16 ± 0.7	14 ± 0.1	14 ± 0.3	12 ± 0.5	11 ± 0.2	0.0 ±0.0
Enterococci	23 ± 0.3	21 ± 0.1	20 ± 0.6	20 ± 0.1	20 ± 0.5	17 ± 0.3

(Mean ± SD in mm)

Table 4- Antibacterial activity of Ethanol and Chloroform extract in well diffusion method

Bacteria	Ethanol extract			Chloroform extract		
	Fresh	Shade dry	Oven dry	Fresh	Shade dry	Oven dry
Bacillus	15 ± 0.2	9 ± 0.1	8 ± 0.1	18 ± 0.5	15 ± 0.3	0.0 ± 0.0
Achromobacter	7 ± 0.1	12 ± 0.5	11 ± 0.4	14 ± 0.2	12 ± 0.3	9 ± 0.1
Enterococci	17 ± 0.3	16 ± 0.1	14 ± 0.5	21 ± 0.3	18 ± 0.1	17 ± 0.4

(Mean ± SD in mm)

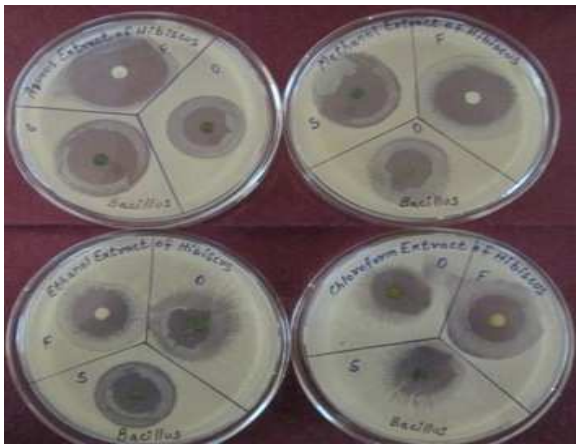

Fig 1: Zone of inhibition of *Bacillus*

Fig 2: Zone of inhibition of *Enterococci*

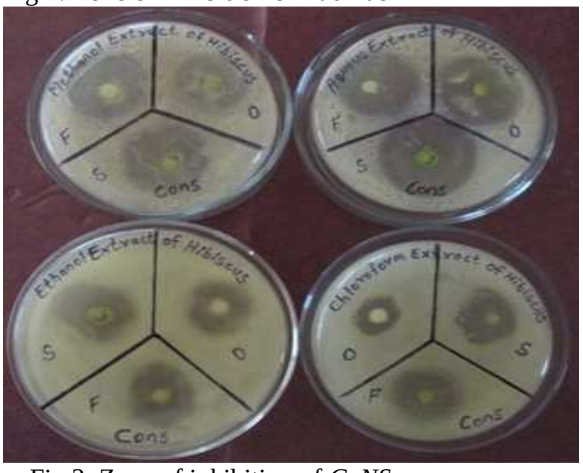

Fig 3: Zone of inhibition of *CoNS*

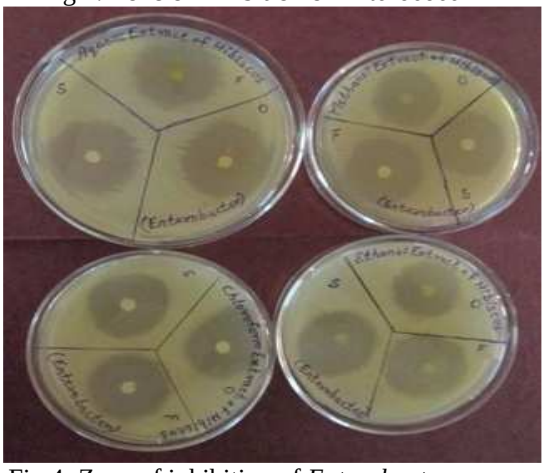

Fig 4: Zone of inhibition of *Enterobacter*

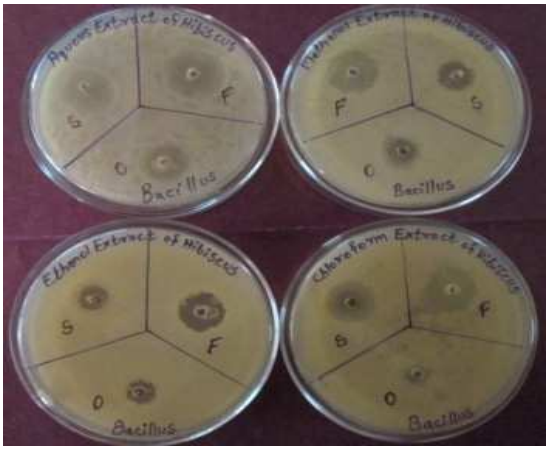

Fig 5: Zone of inhibition of *Bacillus.*

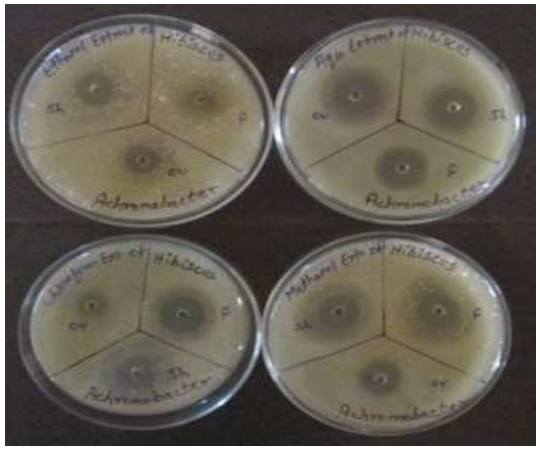
Fig 6: Zone of inhibition of *Achromobacter*

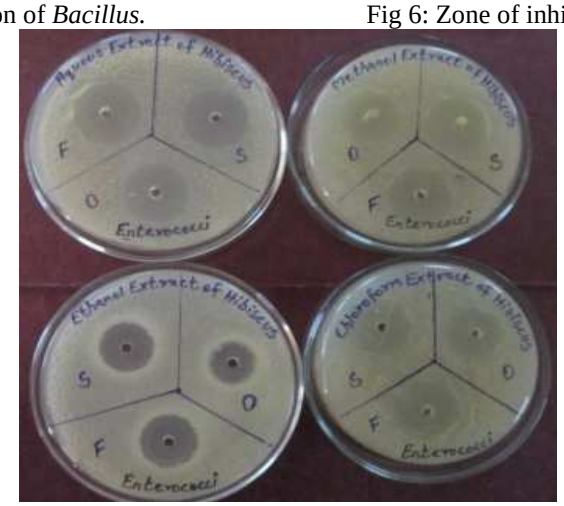

Fig 7: Zone of inhibition of *Enterococci*

References

1. Lawton, B. P. (2004). *Hibiscus: Hardy and Tropical Plants for the Garden*. Timber Press. p. 36.
2. Lee, D. W. (2007). *Nature's Palette: the Science of Plant Color*. University of Chicago Press. p. 183.
3. Bussmann R. W., Gilbreath G. G., Solio J., Lutura M., Lutuluo R., Kunguru K., Wood N., Mathenge S. G. (2006). Plant use of the Maasai of Sekenani Valley, Maasai Mara, Kenya. *Journal of Ethnobiology and Ethnomedicine*, 2, 22.
4. Akueshi, C.O., Kadiri, E. U., Akueshi, S. E., Agina, B., Ngurukwem (2002). Antimicrobial potentials of *Hyptis sauvedens* Poit (Lamiaccae). *Journal of Botany*, 15, 37-41.
5. Sunday E. Atawodi (2005). Comparative in vitro trypanocidal activities of petroleum ether, chloroform, methanol and aqueous extracts of some Nigerian savannah plants, *African Journal of Biotechnology*, 4 (2), 177-182.
6. Kareem, S. O., Akpan, I., Ojo, O. P. (2008). Antimicrobial Activities of *Calotropis procera* on Selected Pathogenic Microorganisms. *African Journal of Biomedical Research*, 11(1), 105-110.
7. Ruban P, Gajalakshmi K. (2012). In vitro antibacterial activity of *Hibiscus rosa-sinensis* flower extract against human pathogens. *Asian Pacific Journal of Tropical Biomedicine*, 399-403.
8. Gothandam, K. M., Aishwarya, R., Karthikeyan S. (2010). Preliminary screening of antimicrobial properties of few medicinal plants. *Journal of Phytology*, 1-6.
9. Scalbert AC. (1991). Antimicrobial properties in tannins. Phytochem, 30, 3875–3883.
10. Padmaja M, Sravanthi M, Hemalatha K.P.J. (2011). Evaluation of antioxidant activity of two Indian medicinal plants. *Journal of Phytology*, 3(3), 86–91.