

In Vitro Anti – Bacterial Activity Of Hibiscus Sabdariffa L Stem in Acetone Extract

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Abstract:

The present investigation envisages anti-bacterial activity of Hibiscus sabdariffa L stem in invitro condition. The extraction was carried out by Soxhlet apparatus using acetone as solvent. The antibacterial activity was measured by well diffusion method as prescribed by Alice and Sivaprakasam (1966) using test bacteria such as Bacillus megaterium, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Salmonella typhimurium. The zone of inhibition was recorded in cm. A maximum zone of inhibition was recorded (2.53cm) against Pseudomonas aeruginosa indicates stem extract possess a considerable number of bactericidal compounds as compared to control – acetone solvent. After Pseudomonas aeruginosa, Salmonella typhimurium shows a recognizable zone of inhibition as (2.5) cm followed by Escherichia coli (2.17 cm). Hence crude extract of acetone reflects a good bactericidal property; may be due to presence some phytochemicals.

Keywords: Hibiscus sabdariffa L, Bacillus megaterium, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium.

1. Introduction:

Hibiscus sabdariffa L, traditional, medicinal plant in India commonly called Roselle, cultivated in tropical and subtropical regions of world as it is originated from West Africa, belong to the family Malvaceae. In Marathi it is known as Aambadi, with enormous medicinal properties for making jams, sauces, jellies, syrups. etc, as well as a flavouring agent for several drinks and considered most important plant in Ayurveda, Tribal medicines, Siddha and Unani medicines.

Hibiscus sabdariffa L an annual or perennial shrub, growing to the height of 1-2 meters with reddish or green coloured stem, with deeply lobed leaves, alternate sided. Calyx of this plant has more importance as it contains a large number of phytochemical compounds, used as a beverage. Plant has diuretic, cardiac, mild laxative and nerve toxic abilities providing health benefits. The plant is enriched with enormous phytochemicals such as sitosterol, quercetin, protocatechuic acid, hydroxycitric acid, caffeic acid, sinapic acid, saponins, triterpenoids. etc. Therefore plant possess anti diabetic activity Arellano. et.al (2004), anti-cancer activity Chiu. et.al (2015), Renal and diuretic activity Laikangbam.R. and Devi. M. D. (2012),

Hypo- lipidemic activity Guardiola (2014), anti- obesity activity with huge therapeutic applications; hence an attempt was made to study anti-bacterial activity in acetone extract of stem *Hibiscus sabdariffa* L in-vitro condition.

2. Material and Methods:

Hibiscus sabdariffa L was collected from Shivaji University, Kolhapur campus in front of Department of Botany, during January 2024. The collected plant sample was brought to the Agrochemicals and Pest Management laboratory where initially it was washed with tap water followed by distilled water for 2-3 times. Soon after, stem of *Hibiscus sabdariffa* was cut into small pieces, and dried in shade for 1-2 days. Later transferred to electric oven for 2 days at 60° C. Then dried samples were powdered in domestic grinder to fine powder. The fine powder of 15 gm was subjected to extraction. The extraction was carried out by Soxhlet's apparatus using acetone as solvent. Anti-bacterial activity was measured by well diffusion method provided by Allice and Sivaprakasam (1966) using test bacteria such as *Bacillus megaterium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. The species were procured from Vivekanand College, Kolhapur and maintained in Nutrient Agar media. The inoculum was prepared by with saline condition. The inoculated petri plates were incubated at 48 hours and zone of inhibition was measured (cm) in triplicates and compared with acetone solvent as control.

3. Result And Discussion:

Present screening of natural products for new antimicrobial agents is prime importance in medical field - pharmacology due to presence of active ingredients against bacteria is the need of the hour. The crude acetone extract of *Hibiscus sabdariffa* L against bacteria was shown in Table. I figure 1. Among the five test bacteria, maximum zone of inhibition was recorded against *Pseudomonas aeruginosa* (2.53 cm) as compared to control acetone. *Pseudomonas aeruginosa* is a gram negative, rod shaped aerobic, facultative, anaerobic bacteria causes diseases in plants, animals including human being. Again, it infects phyllospheres, causes crown gall diseases in ornamentals, sometimes fire blight in apples or specks and cankers of tomatoes, etc. Hence crude extract of acetone proves to be inhibited growth of *Pseudomonas aeruginosa*; a parallel result was published by Nagaraja.et.al (2008) in *Barringtonia acutangula* in alcoholic extract and Sivan Kumar and Anna Sheba L. (2019) in methanolic extract of leaves of *Hibiscus sabdariffa*. *Salmonella typhimurium* a non-pathogenic bacterium in plants, but causes typhoid fever in human beings. Still some plant tissues have been reported to cause disease like symptoms showing Culerosis (Schikora. et.al 2008; Berger.et.al. 2011) shows considerable zone of inhibition in acetone stem extract against *Salmonella typhimurium* 2.50 cm (Table I, figure 1).

The bacterium *Bacillus megaterium*, pathogenic causes Bacteraemia and Meningitis in human beings Sometimes Anthrax, T.B, Leptospirosis etc. along with tract which induces rice resistance against Spikelet rot disease (SRD) Tingcheng. et.al (2020) and in *Camellia sinensis* by Usha Chakraborty (2006). Our investigation reveals crude extract of *H. sabdariffa* shows sensitivity to *Bacillus megaterium* with a good zone of inhibition as 2.33 cm (Table I). A parallel result was reported by Nagaraja and Shaikh (2012) in *Anacardium occidentale* L and Nagaraja (2011) ethanolic extract of *Mallotus Philippines's* Muell against *Bacillus megaterium*.

Klebsiella pneumoniae a non- motile, gram-negative bacteria cause pneumonia, blood stream infections and rhinoscleroma and ozena etc to human beings (nasopharynx and gastrointestinal tract) but non-pathogenic to plants. Present investigation shows the crude extract reveals a good inhibition zone against *Klebsiella pneumoniae* (2.20 cm) as compared to acetone extract. In different investigation aqueous extract of root and calyx of *H. sabdariffa*, reveals a marked antibacterial activity in that of stem Wong. SK et.al (2010). The potent bactericidal properties in the stem attributed to richness in its phytochemicals.

A least zone of inhibition was found against *Escherichia coli* (2.17cms) Table – I fig.1. Hence, acetone forms a good solvent, possess anti-bacterial potency against bacteria (Table – I). Similar findings were recorded in *Calophyllum inophyllum* stem in acetone extract (Nagaraja, 2013) and Swati Lade et.al (2022) in *H. sabdariffa* L flower extract.

However, the presence of bactericidal active compound and its isolation and absence of its toxicity is main importance and necessary to propose as an alternative approach to plant bacterial disease management.

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Table-1. Anti-bacterial activity of stem of *Hibiscus sabdariffa* L in acetone extract against test bacteria.

.Sr. No	Test Bacteria	Inhibition zone in cms				
		Control Acetone	1	2	3	Mean
1.	<i>Bacillus megaterium</i>	2.4	2.3	2.3	2.4	2.33
2.	<i>Escherichia Coli</i>	2.2	2.3	1.9	2.3	2.17
3.	<i>Klebsiella pneumoniae</i>	1.8	2.1	2.3	2.2	2.20
4.	<i>Pseudomonas aeruginosa</i>	1.6	2.5	2.6	2.5	2.53
5.	<i>Salmonella typhimurium</i>	2.4	2.7	2.4	2.4	2.5

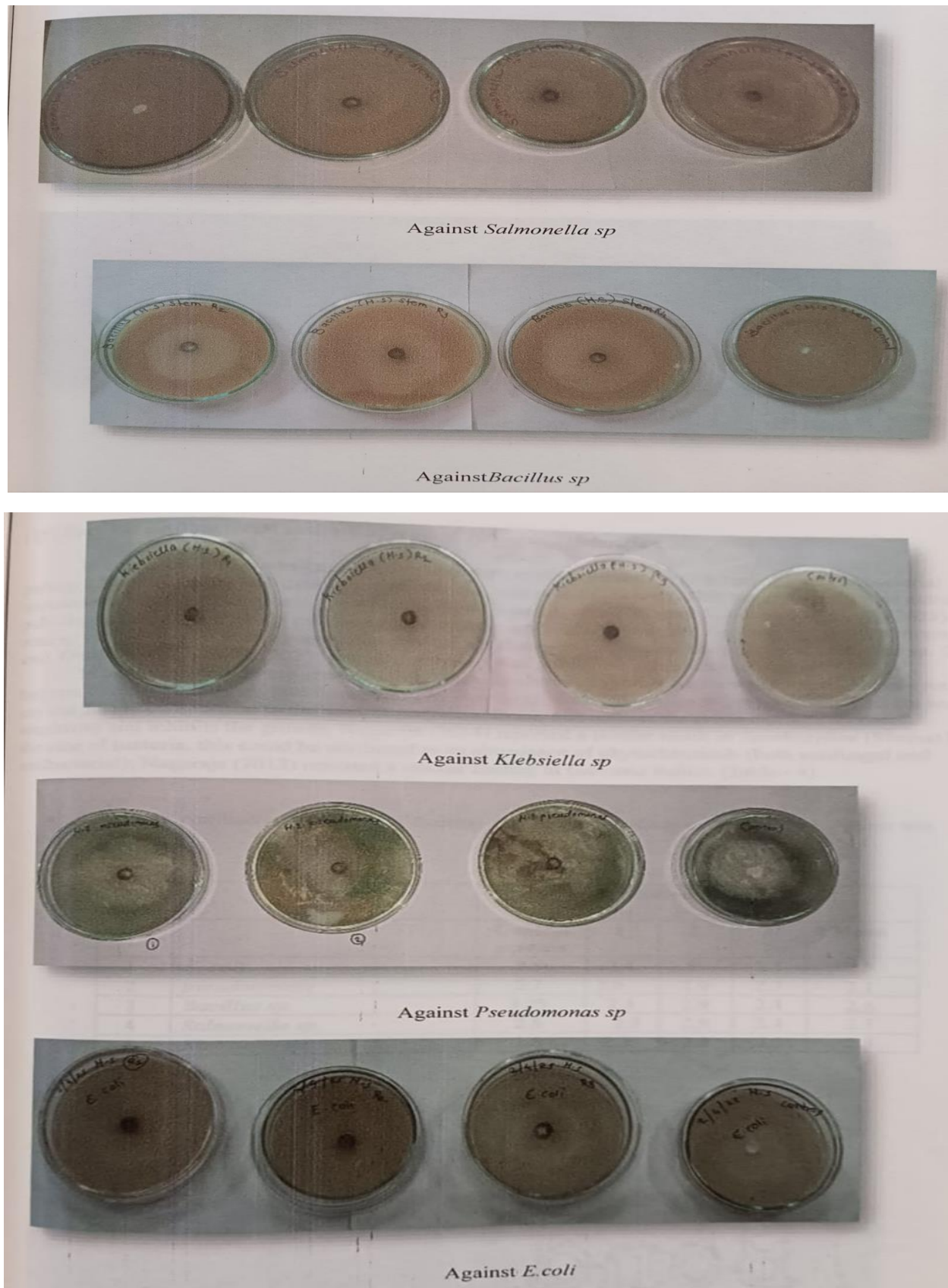


figure 1.