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Study of Anti-Bacterial Activity of Allophylus Serratus Roxby (Kurtz) Leaves Extract.

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Abstract:

Researchers studied the antimicrobial effects of extracts from the leaves of Allophylus serratus to see how they perform against Staphylococcus aureus, a common bacterium. To begin, they gathered leaves of the plant, dried them, and powdered them for extraction using methanol. Screening of the extract revealed that it contained alkaloids, terpenoids, and saponins—chemical compounds known for their biological activities.

When it came to testing antimicrobial activity, the extract showed some ability to inhibit bacterial growth. The Minimum Inhibitory Concentration (MIC), or the lowest concentration at which the bacteria stop growing, was found to be 31.2 µg/mL. However, when comparing it to Metronidazole—a well-known antibiotic—Metronidazole was far more potent, showing significant bactericidal effects at just 7.8 µg/mL. The extract from Allophylus serratus only displayed strong bactericidal effects at much higher doses, up to 250 µg/mL. While this suggests the extract has potential, its ability to kill bacteria is mainly effective at higher concentrations. These findings pave the way for further research to pinpoint the specific compounds responsible for its antimicrobial effects and to explore its full potential. It's a step forward, but more work is needed to determine how it could be used practically.

Keywords- Allophylus serratus leaves, Staphylococcus aureus, Minimum Inhibitory Concentration, Metronidazole

1. INTRODUCTION:

Allophylus serratus (Roxby) Kurtz, commonly known as Tippani in Hindi, is a medicinal plant belonging to the Sapindaceae family [1][2]. It is an evergreen, low-branching small tree or a large shrub, typically growing to a height of 3-6 meters. This plant is distributed across India, Sri Lanka, and Southeast Asia. Within India, it can be found in regions such as Andhra Pradesh, West Bengal, Arunachal Pradesh, Tripura, Odisha, Karnataka, Tamil Nadu, and Kerala. Additionally, it thrives in scrub forests across areas like Mahabaleshwar, Kolhapur, Satara, Borbet, Barki, Manoli, Panhala, and Patgaon in Kolhapur District. (Kero et al. 2015)

Nearly all parts of the A. serratus plant hold significant medicinal value and have been traditionally utilized to address various health issues. These include reducing fever, soothing rashes, enhancing lactation, treating colic, alleviating stomach aches, and serving as an anti-ulcer remedy. (15, 16).

This research article highlights various aspects of Allophylus serratus, a plant belonging to the Sapindaceae family.

The accurate classification of Allophylus serratus is outlined below.

Ingredients Profile:

- Kingdom: Plantae
- Phylum: Magnoliophyta
- Class: Magnoliatae
- Order: Sapindales
- Family: Sapindaceae
- Genus: Allophylus
- Species: Allophylus serratus Kurtz.

Fig no. 1: *Allophylus serratus* leaves

Numerous studies have shown that various parts of this plant possess pharmacological properties, including anti-inflammatory, anti-osteoporotic, anti-ulcer, antiviral, and antibacterial activities.[3] These therapeutic effects are attributed to the diverse phytochemicals found in A. serratus. Therefore, conducting preliminary phytochemical screenings and antioxidant activity tests is crucial in discovering new sources of medically significant compounds, such as flavonoids, alkaloids, phenolic compounds, saponins, tannins, steroids, and terpenoids.[4]

Uses of plant:

a. Plants possess numerous pharmacological and ethnobotanical properties. Among their ethnobotanical applications, they are notably used as anti-inflammatory agents.[5][6]
b. Plants are commonly utilized as carminative drugs.
c. The extracts derived from plants are used as a treatment for elephantiasis.
d. Parts of the plants are utilized for treating edema and fractured bones.
e. Plants are utilized for treating ulcers, healing wounds, and addressing gastrointestinal disorders.

f. Plants are employed in managing dyspepsia and anorexia.
g. Plant extracts are utilized to treat diarrhea. The fruits are sweet, edible, nutritious, and serve as a tonic.
h. The fruit's flesh is consumed to address tapeworm infections, while crushed leaves are utilized to treat fever.
i. The leaves, when mixed with quicklime, are used to treat stomach ulcers.
j. The leaves and bark are used to treat elephantiasis, as well as for their anti-ulcer properties and to alleviate piles.

Material and Methods:

Plant material:

Healthy fresh leaves of Allophylus serratus were collected from Local field of Chandgad taluka, Kolhapur in month of January 2025. The leaves were identified and authenticated by Dr. A.V. Patil, head of department of Botany, Yashwantrao Chavan Collage of Halkarni, Tal.Chandgad, Dist. Kolhapur. Mature leaves were collected during dry and pleasant weather.

These leaves were shade-dried for three weeks, then coarsely powdered for further research and analysis.

Fig.No.2: Powder Form *of Allophylus Serratus*.

Preparation of extract:

The leaf of the aegles marmelos plant were washed under running water to remove foreign substance, later thoroughly dried under shade and finely powdered and further used for preparing the herbal extract. Extraction was carried out by Soxhlet extraction method. The fine powder of green leaf of aegles marmelos was packed tightly in the Soxhlet extractor separately. A 250ml of methanol was used as a solvent for extraction. This process was evaporator, evaporated to dryness under reduced pressure at 60 C to get the solid product.[7][8]

Fig. No3: Soxhlet extraction

Phytochemical screening:

- Detection of alkaloids:

Test	Procedure	Observations
Dragendroff's/ Kraut's test	Few mL filtrate + 1-2 mL Dragendorff's reagents	A reddish-brown precipitate
Hager's test	Few mL filtrate + 1-2 mL Hager's reagents	A creamy white precipitate
Mayer's test	Few mL filtrate + 1-2 drops of Mayer's reagent (Along the sides of test tube)	A creamy white/yellow precipitate
Wagner's test	Few mL filtrate + 1-2 drops of Wagner's reagent (Along the sides of test tube)	A creamy white/yellow precipitate

- Detection of Terpenoides:

Test	Procedure	Observations
Salkowski's test	Filtrate+ 2ml of chloroform + few drops of conc. H2SO4 (Shaken well and allowed to stand)	Reddish brown colour at interface

- Detection of Saponins:

Test	Procedure	Observations
Foam test	1ml extract was treated with 1% lead acetate solution.	Formation of white precipitates indicates the presence of saponins.

MIC (Minimal Inhibitory Concentration) of Allophylus leaves extract

Experimental procedure:

The anti-microbial activity of the synthesized test sample was evaluated by the resazurin assay method (Sarker et al. 2007; Valsalam et al. 2019a, b).[9][10]

The assay was prepared by dissolving 270 mg of resazurin in 40 mL of sterile distilled water. A vortex mixer was used to make sure that the solution was homogenous and very well dissolved. 96- Well plate under aseptic conditions was used to carry out the studies.[11]

A sterile 96-well plate was labeled. 100 µL volume of different concentration sample solution (7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000µg/ mL of Dimethyl Sulphoxide, DMSO) was pipetted first into the well of the plate. Then, 50 µL of nutrient broth was added to all different wells and subsequently diluted. To each well, 10 µL of resazurin indicator solution was added. After this, 10 µL of fungal or bacterial suspension was added to every well.

Metronidazole (7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000µg/mL) was used as a standard control. Using a cling flm, every plate was covered loosely to avoid the dehydration of microbes. Then, the plate was incubated for 18–24 h at 37 °C and color change was then studied visually. Any color variations from blue to pink or colorless were denoted as positive and the absence of color change was indicated as negative. The lowest concentration of the sample at which the color change was observed was considered as the minimum inhibitory concentration (MIC) value. And the absorbance of the plate was measure at 600nm by using ELISA reader.[12]

The percentage of Inhibition was calculated by following formula:

% of Inhibition = Control- Test/Control*100

Effects of compound against S.aureus:

SR NO	**SAMPLE CODE**	**Concentration(µl/ml)**	**Absorbance at 600nm**			**Mean**	**% Of Growth Inhibition.**
			Test 1	**Test 2**	**Test 3**		
1	**Growth C**		1.603	1.603	1.603	1.603	NG
2	**Standard**	7.8	NG	NG	NG	NG	NG
	Metronidazole	15.6	1.538	1.538	1.538	1.538	4.05%

		31.2	1.421	1.421	1.421	1.421	11.35%
		62.5	1.397	1.398	1.397	1.397	12.85%
		125	0.834	0.834	0.834	0.834	47.97%
		250	0.611	0.611	0.611	0.611	61.88%
		500	0.531	0.531	0.531	0.531	66.87%
		1000	0.422	0.422	0.422	0.422	73.67%
3	**Allophylus Serratus**	7.8	NG	NG	NG	NG	NG
		15.6	NG	NG	NG	NG	NG
		31.2	1.530	1.532	1.528	1.532	4.43%
		62.5	1.494	1.496	1.492	1.494	6.79%
		125	1.215	1.211	1.213	1.213	24.32%
		250	1.116	1.115	1.113	1.115	30.44%
		500	0.988	0.991	0.989	0.989	38.30%
		1000	0.881	0.879	0.877	0.879	45.16%

NG= No Growth

Results-

1)Minimum Inhibitory Concentration-

After specific incubation period the test sample **Allophylus Serratus** showing the minimum inhibitory concentration at **31.2µg/ml** against **S.aureus** as compared to standard

MBC- (MINIMAL BACTERIOCIDAL CONCENTRATION) OF ALLOPHYLUS LEAVES EXTRACT-

PROCEDURE-

After the MBC determination of the test sample, aliquots of 50 µL from all the wells which showed no visible bacterial growth were seeded on NA agar plates and incubated for 24 h at 28 °C.[13][14] When 99.9% of the bacterial population is killed at the lowest concentration of an anti-bacterial agent, it is termed as MBC endpoint. This was done by observing pre and post-incubated agar plates for the presence or absence of bacteria. [15]

SR.NO.	Sample	Concentration ug/ml	Strain	MBC
1	Metronidazole	7.8	S aureus	+
2		15.6	S aureus	++
3		31.2	S aureus	++
4		60.5	S aureus	+++
5		125	S aureus	+++
6		250	S aureus	++++
1	Allophylus serratus	7.8	S aureus	-
2		15.6	S aureus	--
3		31.2	S aureus	---
4		60.5	S aureus	-+
5		125	S aureus	-+
6		250	S aureus	++++

(+): Killing activity

(-): No killing activity

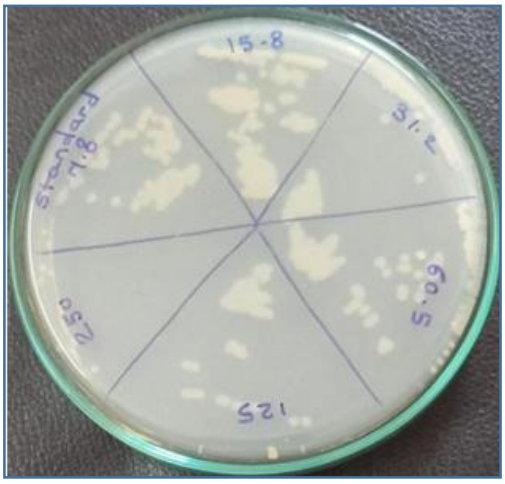

Fig 1: Metronidazole

Fig 2: Allophylus serratus

2) Minimum Bacteriocidal Concentration -

(Metronidazole):

The effectiveness increases with concentration. At higher concentrations (7.8 µg/ml and above), Metronidazole shows strong to very strong bactericidal activity against **S.aureus (Allophylus serratus):** The sample shows very little bactericidal activity at lower concentrations. Significant activity is only observed at the highest concentration (250 µg/ml), where it shows a very strong effect.[16][17]

2. Conclusion:

The antimicrobial activity of Allophylus serratus leaf extract was assessed in terms of its Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against Staphylococcus aureus. The results showed that the extract exhibited moderate antimicrobial effects, with the MIC determined to be 31.2 µg/mL. In comparison to Metronidazole, a standard antibiotic, the leaf extract demonstrated weaker antibacterial activity, as Metronidazole achieved a significant bactericidal effect at much lower concentrations (7.8 µg/mL).

The MBC results further corroborated this finding, with Allophylus serratus showing minimal bactericidal activity at concentrations up to 125 µg/mL, and significant bactericidal effects only emerging at 250 µg/mL. In contrast, Metronidazole displayed strong bactericidal activity across lower concentrations (as low as 7.8 µg/mL).

Although the extract from Allophylus serratus was effective at higher concentrations, its antimicrobial potential is considerably less potent compared to the standard antibiotic Metronidazole. This suggests that while the plant has some bactericidal properties, more research is needed to identify the specific compounds responsible for this activity and to enhance its efficacy. The findings underscore the need for further studies to explore the full antimicrobial potential of Allophylus serratus, with the possibility of developing it into a more potent therapeutic agent in the future.

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