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Antimicrobial activity evaluation and phytochemical screening of *Bauhinia racemosa* L.A possible alternative in the treatment of multidrug-resistant microbes

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Abstract –
The bark of *Bauhinia racemosa* are reported to have great medicinal value. The present study was carried out to evaluate the antimicrobial activities and phytochemical evaluation of methanol, ethanolaqueous, acetone and petroleum etherextract of *Bauhinia racemosa* (Caesalpiniaceae) stem bark. Phytochemical screening of the plant bark reveals the presence of carbohydrates, alkaloids, phenols, steroids and tannins. The methanol, ethanol, aqueous, acetone and petroleum ether extracts of bark of *B. racemosa* Lamk. prepared and antimicrobial activity were studied by agar well diffusion method against enteric bacterial pathogens such as E. coli, S. aureus, B. subtilis, P. aeruginosa and fungi A. niger and C.albicans. The methanol extracts showed broad-spectrum antimicrobial activity against all tested microorganisms. The results obtained in the present study indicate that*Bauhinia racemosa* can be a potential source of natural antimicrobial agents.

Key words -*Bauhinia racemosa*, Antimicrobial activity, bacteria, microorganisms

Introduction
Natural medicines have been used to boost health since the time of immemorial and the success of modern medical science largely depends on drugs originally obtained from natural resources. In the past, a large number of antimicrobial compounds were discovered from synthetic and natural products for the treatment and control of infectious agents (Shriram and Khare 2018).

Infectious diseases are the world's leading cause of premature deaths, killing almost 50 000 people every day. Infections due to a variety of bacterial etiologic agents, such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae are most common. With the continuous use of antibiotics, microorganisms have become resistant, in addition to these problems; antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut, mucosal microorganism, immunosuppression and allergic reactions has created immense clinical problem in the treatment of infection diseases. Therefore, there is a need to search for new potential effective biocompounds against pathogenic bacteria and fungi.
(Khaled and Monica, 2013).

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The plant *Bauhinia racemosa* Lam. commonly known as the bidi leaf tree, is a rare medicinal species of flowering shrub with religious significance which belongs to the family Caesalpiniaceae. It is a small crooked tree with drooping branches that grows 3–5 metres (10–16 ft) tall and flowers between February and May. It is native to tropical Southeast Asia.
In Hindu families it is customary to exchange leaves of the Aapta tree on the Hindu festive day of Dussehra. An act known as exchanging gold pointing to the special significance of the plant on that particular day. This is also why the tree is often referred to as Sonpatta.
Almost all parts of the plant viz., leaves, flowers and stem bark of this plant carry many medicinal properties and used in traditional medicinal system for the treatment of several diseases.
The plant is used in the treatment of headache, fever, skin diseases, tumors, blood diseases, dysentery, and diarrhoea. Chemical constituents such as ß-sitosterol, ß-amyrin, Stilbene (resveratrol), flavonols (kaempferol and quercetin), two coumarins (scopoletin and scopolin) were also present in the plant. [(Chavan and Kadam.2012),(Kumar et.al, 2005)].

Materials and Methods

Plant materials

The bark of *B. racemosa* Lamk. was collected from the different areas of Dhule region of Maharashtra, India. The plant material was identified and authenticated from theDepartmentof Botany SSVPS's Dr. P. R. Ghogrey Science college Dhule, Maharashtra, India.

Extraction of plant materials

The shade dried and powdered material of barkof *Bauhinia racemosa*L. was extracted by continuous hot extraction methodusing methanol, ethanol, aqueous, acetone and petroleum ether solvents respectively. The extracts were concentrated in a rotary evaporator under vacuum at room temperature.

Phytochemical screening

All the extracts obtained by continuous hot extraction method were screened for the presence of different chemical constituents like alkaloids, glycosides, flavonoids, saponins, tannins, steroids, resins, oil and fats by using standard protocol give in reference books and pharmacopeia.

Test microorganisms

Bacterial and fungal strain used for testing included pure cultures of pathogenic bacteria like. Staphylococcus aereus, Bacillus subtilis and two are gram negative viz. Pseudomonas aeruginosa, Escherichia coli. Two species of fungi viz. Aspergilus niger, Candida albicans.These were obtained from the Department of Microbiology, Dr. P. R. Ghogrey Science College Dhule, Maharashtra, India.

Preparation of test organisms suspension

The test organisms were maintained on slants of medium containing nutrient agar (2.5 gm/ 10ml) and sub cultured once a week. The slants incubated at 370C for 24 hrs. and stored under refrigeration. The inoculums was 1x 108 cells/ml.

ISSN PRINT 2319 1775 Online 2320 7876
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Antimicrobial activity

The in-vitro antimicrobial activity of different bark extracts of *B. racemosa* Lamk. was determined by agar well diffusion methodin Mueller Hinton Agar (MHA) plates.The plant extracts were dissolved in distilled water at concentrated50 Fg/ ml streptomycin was used as reference antibiotic. Each platewas inoculated with 20Fl microbial suspension having concentration 1x 108 cells/ml. The 0.1 ml extract was added to each well. The plates containing bacteria were incubated at 37^0C for 24 hrs. and those containing fungi were incubated at 25^0C for 7 days. Positive antimicrobial activity was based on growth inhibition zone and compared with standard drug. The diameter of zone of inhibition surroundings each of the well was recorded. All the experiments were performed in triplicate.

Statistical analysis

Results were expressed as mean ± S.D. statistical significance was determined using analysis of student's t- test.

Results and Discussion

The phytochemical investigation of the various solvent extractof bark of *B. racemosa* Lamk. Presented in table 1. The resultsrevealed that the bark of *B. racemosa* Lamk. Showed the presence ofalkaloids,glycosides, carbohydrates, saponins, flavonoids,triterpenoids, anthroqunonine, phytobatalanine are present inmethanol, ethanol and aqueous extracts. The result of theantimicrobial activity of the different extracts of bark of *B. racemosa*Lamk. are presented in table 2. In the present investigation methanoland aqueous extracts of bark showed higher antibacterial activityagainst the test organisms which was greater than the standardreference antibiotic streptomycin. The methanolic extract of bark alsofound more inhibitory activity on gram negative and gram positivebacteria E. coli, P.aeruginosa, S. aureus and B.subtilis. The gram negativeactivity bacteria P. aeruginosa shows the higher activity in bothethanol and water extracts the antimicrobial activity of the ethanolwas higher than that of water extract.

Table 1.Phytochemical analysis of bark of *Bauhinia racemosa* Lamk

Sr.no.	PhytoConstituents	Chemicaltests	*B.racemosabark*extract				
			P.E	Met	Eth	Aq	Ac
1	Alkaloids	1.Mayer'stest	-	-	+	-	-
		2.Dragendroff'stest	-	+	-	+	-
		3.Wagner'stest	-	-	+	-	-
		4.Hagerstest	-	-	-	-	-
2	Carbohydrates	1.Molisch'stest	-	+	+	+	-
		2.Benedictstest	-	-	-	-	-
		3.Fehling'stest	-	-	-	-	-
3.	Glycosides	1.ModifiedBorntragers	-	+	+	+	+
		2.Legaltest	-	+	-	+	-
4	Saponins	1.Foamtest	-	+	-	+	-
		2.Frothtest	-	-	-	-	-
5	Triterpenes	1.Salkowskitest	-	-	+	+	-

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 Journal Volume 11, Iss 12, 2022

		2.LibermannBurchard	-	-	-	-	+
		3.Tschugajewtest	-	-	-	-	-
6.	Fats&Oil	1.Staintest	-	-	-	-	-
7	Tannins	1.AlkalineReagent	-	+	-	+	-
8	Flavanoids	1.Gelatintest	-	+	+	+	-
		2.Leadacetatetest	-	+	+	+	
		3.Shinodatest	-	+	+	+	+
		4.Zn-Hclreduction	-	+	+	+	
9	Photobatalin		-	+	+	-	+
10	Anthraqunonines		-	+	+	-	+

Table 2. In vitro antimicrobial activity of bark of *Bauhinia racemosa* Lamk.

Microorganisms	DiameterofZoneofinhibitioninmmofdifferentextractsofbark(2mg/ml)Mean±S.D.					Standardsreference Antibiotic(Streptomycin)
Bacteria	Pet.ether	Methanol	Ethanol	Aqueous	Acetone	
Escherichiacoli	12±2.12	15±1.33	14±1.13	14.3±1.11	11±2.11	14±1.11
Staphylococcusaureus	11±1.12	14.8±1.11	14±2.12	15±1.11	12±1.21	14.3±1.11
Bacilliussubtilis	13±1.23	15±1.15	13±1.21	15±1.43	13±1.32	13±1.12
P.aeruginosa	14±1.21	15±2.23	14±2.41	14±1.11	14±1.23	14±1.34
Fungi						
Candidaalbicans	11±1.11	12±0.12	12±3.33	13±1.24	12±1.11	14±1.13
Aspergillusniger	12±2.12	13±1.23	12±1.23	12±1.11	12±2.31	15±1.15

Conclusion

The plant extracts have shown appreciable antimicrobial activities comparable to the currently prescribed modern drugs tested. Accordingly, further studies on clinical efficacy trial, safety, toxicity and affordability analyses have to be instigated promptly, so as to head to the final step to synthesize precursor molecules for new effective antimicrobials.

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