WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 14, 778-785. Research Article ISSN 2277– 7105

SCREENING OF ANTIMICROBIAL ACTIVITY OF CAESARIA TOMENTOSA

Gauri Pai Angle*[1] and Yogita Sardessai[2]

[1]Assistant Professor, Department of Pharmacognosy P.E.S's Rajaram and Tarabai Bandekar College of Pharmacy, Ponda-Goa.

[2]Associate Professor, Goa College of Pharmacy, Panjim –Goa.

Article Received on
08 Sep. 2017,
Revised on 27 Sep. 2017,
Accepted on 18 Oct. 2017
DOI: 10.20959/wjpr201714-9974

***Corresponding Author**
Gauri Pai Angle
Assistant Professor, Department of Pharmacognosy P.E.S's Rajaram and Tarabai Bandekar College of Pharmacy, Ponda-Goa.

ABSTRACT

The present study was carried out to investigate the antimicrobial effect of the ethanolic extract of rootsand bark of ***Caesaria tomentosa.*** The antimicrobial activity of the extractswere studied against five bacterial and two fungal strains by Well Diffusion Methodand Broth Dilution method. Broad spectrum activity was seen against both gram-positive (*Staphylococcus aureus* and *Bacillus subtilis)* and gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimrium).* Among all the bacterial strains *Staphylococcus aureus*showed the highest activity. The antifungal study revealed a significant activity against both *Candida albicans*and antifungal *Aspergillus niger.*

KEYWORDS: Antimicrobial, Well Diffusion, Ethanolic.

1. INTRODUCTION

In recent years, antibiotic resistance has become a serious and widespread problem in developing countries and it is aresult of inappropriate usage, abuse and over prescription of antibiotics causingadverse drug reactions and mortality.[1,2] Global emergence of resistant bacteria is the result of ineffectiveness of current antibiotics and drugs causing treatment failure.[3] Hence there has been a growing interest in using alternative therapy and the therapeutic use of natural products specially medicinal plants.[4,5]

Casearia is one of the genus of *Salicaceae* family with huge pharmacological importance. In South American and Asian countries, different *Casearia* species have been used as folk

medicines since ancient times. The pharmacological studies proved that the crude extracts and isolated compounds from this genus showed hypoglycemic, antioxidant, antiulcer, anti-inflammatory activities, antimicrobial as well as anti-snake venom property.[6] *Casearia tomentosa,* it is a small tree up to 50-80 cm girth and 7 m tall. Its common name is *Chilla*. Different parts of *C. tomentosa* is traditionally claimed for its medicinal importance like in ulcers, dropsy, fissures, colic pain in the abdomen, malarial fever, tonsillitis pain, wounds, and in severe bone fractures as a plaster.[7-9]

The literature survey revealed that *Casearia tomentosa* is still an under explored species with a diverse range of folk uses.[10] The aim of this study was to screen theantibacterial and antifungal activity of *C. tomentosa*.

2. MATERIALS AND METHODS

2.1 Collection of plant species: The roots of*C. tomentosa* were collected from wild forests of North Goa. The collected plant material was air-dried under shade and dried roots were crushed into powder by mechanical blender.

2.2 Preparation of ethanolic extracts: The rootsand bark of *C. tomentosa* were collected, washed and dried in shade. The dried roots and bark were powdered (300 gm) and exhaustively extracted separately by maceration with ethanol (95%) for three days. After three days, ethanol layer was decanted off. The process was repeated for three times. The solvent from the total extract was distilled off and the concentrate was evaporated to a syrupy consistency using rotary vacuum evaporator (25 rpm; 60°C) and then evaporated to dryness.[11]

2.3 Antimicrobial Susceptibility Testing

2.4 Microorganisms: In the present study, ethanolic extract of the roots of *C. tomentosa* was tested for antimicrobial activity by well diffusion method. Five bacterial strains used included two gram-positive- *Staphylococcus aureus (ATCC 6538P)* and *Bacillus subtilis (ATCC 6633)* and three gram-negative bacteria -*Escherichia coli (ATCC 35218), Pseudomonas aeruginosa (ATCC 19429)* and *Salmonella typhimurium (ATCC 23564)*. Two fungal strains, *Candida albicans (NCIM No.10231)* and *Aspergillus niger (NCIM No.10864)* were used. All the bacterial strains and fungal strains were maintained on Nutrient Agar and Saboraud's Dextrose Agar respectively and were freshly sub cultured for 24-48 hrs at 37^{o}C and 25^{o}C respectively.

2.5 Antimicrobial Activity

The ethanolic extract of roots and barkof *C. tomentosa*was subjected to antibacterial as well as antifungal screening by Well Diffusion Method (Cup Plate Method) and Broth Dilution method (Tube Dilution Method).[12-15]Mueller Hinton Agar/Broth and Sabouraud's Dextrose Agar/Broth were used as the seed medium for the antibacterial and antifungal screening respectively. The Minimum Inhibitory Concentration was performed by two-fold dilution of the test extract in the respective medium under sterile conditions.[13, 15] The inoculum was verified by streaking on specific medium for colony identification and purification. Appropriate controls were maintained.

The plates were observed visually and the diameter of zones was measured using mm scale. The activity was indicated by the presence of clear zones aroundthe well size. The bioassay was repeated thrice and the mean was recorded to check the effectiveness of the procedure. The MIC was determined by turbidimetric method by measuring the Optical Density at 600 nm using Elico colorimeter (Filter No. 60). The MIC results were further reinforced by determining viable counts using pour plate method. These studies were performed in triplicate and mean values were tabulated.

3. RESULTS AND DISCUSSION

The ethanolic extracts of the roots and bark of *C. tomentosa* were subjected to antimicrobial studies, results reveal that both extracts are active against all five pathogenic bacterial strains and two fungal strains. The zones of inhibition for bacterial strains were determined and results were shown in Table-I, II (Refer Figure1 and 2).

The gram positive *Staphylococcus aureus* is more sensitive than all other bacterial strains (*Bacillus subtilisEscherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimrium).* Highest inhibitory activity was seen against *S. aureus* (zone of inhibition 23 and 18 mm for root and bark respectively) while the weakest activity was demonstrated against *P. aeruginosa* (zone of inhibition 11 and 9 mm respectively).

The ethanolic extract of *C. tomentosa* showed a significant decline in growth of all bacterial cultures which was indicating by decreasing absorbance values with negligible growth at concentration of 500 µg/ ml. (Refer Table III).

The results also indicated that the ethanolic extract of the roots and bark of C. *tomentosa* showed significant antifungal activity against both *Aspergillus niger* and *Candida albicans*. (Refer Tables II and IV). It was observed that as the concentration of the ethanolic extract of the roots increased, there was significant inhibition seen in the growth of the antifungal cultures and indicates that MIC value lies between 31.25 - 62.5 µg/ ml.

The results of phytochemical screening of *C. tomentosa* leaves extract have revealed the presence of active phytoconstituents such as alkaloids, glycosides, steroids, saponins, flavonoids, terpenoids and tannins etc.[16] Out of these, alkaloids and terpenoids present in this plant were reported to possess activities like antioxidant, analgesics, muscle relaxant, antibiotics, anticancer and also responsible for antiprotozoal, cytotoxic and antimicrobial properties.[17,18]

This could be probable mechanism of action of antimicrobial activity of *C. tomentosa*. However, further investigations of the phytochemical screening of the barks and roots are required to find out, which active ingredient is responsible for this antimicrobial activity.

3.1 Tables.

Table. I: Antibacterial activity of ethanolic extract of the rootsand bark of *Caesaria Tomentosa* by well diffusion method.

SAMPLE	DIAMETER OF ZONE OF INHIBITION IN MM				
	E.coli	***P.aeruginosa***	***S.aureus***	***B.subtilis***	***S.typhimurium***
Root Extract	15	11	23	17	13.5
Bark Extract	12	9	18	15	10
Ciprofloxacin	17	27	32	24	22

Concentration of the extract is 25mg/ml

Solvent control (DMSO) did not exhibit any zone

Table. II: Antifungal activity of ethanolic extract of the roots and barks *Caesaria Tomentosa* bywell diffusion method.

SAMPLE	DIAMETER OF ZONE OF INHIBITION IN MM	
	Aspergillus niger	***Candida albicans***
Root Extract	14	9
Bark Extract	12	6
Nystatin	35	32

Concentration of the extract is 25mg/ml

Solvent control (DMSO) did not exhibit any zone

Table. III: Effect of ethanolic extract of the roots *Caesaria tomentosa* on bacterial cultures by tube dilution method. (values are optical density - 600 nm).

Concentration µg/ ml	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*
30	0.78	0.69	0.71	0.49	0.61
31.25	0.75	0.64	0.69	0.47	0.58
35	0.75	0.66	0.68	0.45	0.57
40	0.72	0.58	0.63	0.43	0.56
45	0.66	0.55	0.61	0.39	0.54
50	0.61	0.52	0.59	0.37	0.47
55	0.59	0.50	0.55	0.35	0.45
60	0.58	0.49	0.52	0.32	0.30
62.5	0.62	0.47	0.54	0.32	0.32
70	0.54	0.53	0.49	0.26	0.27
80	0.50	0.49	0.47	0.23	0.24
125	0.42	0.29	0.40	0.23	0.20
250	0.23	0.11	0.16	0.19	0.13
500	0.12	0.11	0.13	0.17	0.11

Table IV: Effect of ethanolic extract of the roots *Caesaria Tomentosa* on bacterial cultures by broth dilution method.

Concentration (µg/ ml)	30	35	40	45	55	62.5	65	70	80
Aspergillus niger	+	+	+	+	+	-	-	-	-
Candida albicans	+	+	+	+	+	+	-	-	-

+ positive indicating presence of growth -negative indicating absence of growth

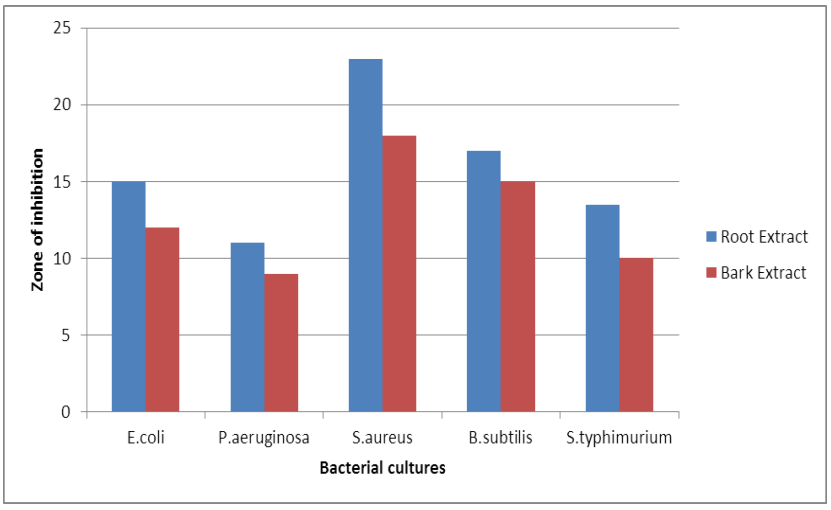

Fig 1: Comparison of antibacterial activity of ethanolic extract of the rootsand bark of *Caesaria Tomentosa* by well diffusion method.

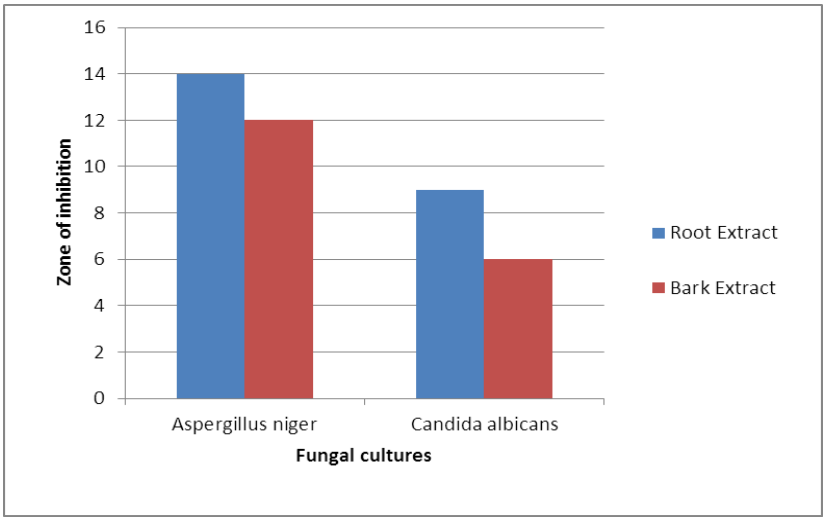

Fig. 2: Comparison of anti fungal activity of ethanolic extract of the rootsand bark of *Caesaria Tomentosa* by well diffusion method.

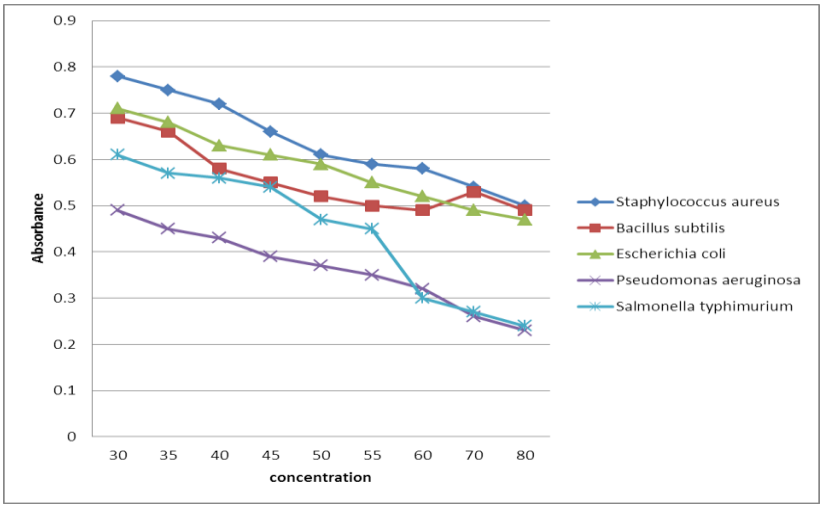

Fig. 3: Effect of concentration of ethanolic extract of the roots *Caesaria Tomentosa* on the growth of bacterial culrures.

4. CONCLUSIONS

The present study establishes that the ethanolic extract of the roots and bark of C. *tomentosa* shows antibacterial studies as well as antifungal activity. The phytochemical investigation in previous studies had led to the isolation of steroids, triterpenoids, alkaloids, fats and phytol sterols. The above antimicrobial activity may be attributed to the presence of these bioactive constituents in the bark and roots of the plant. The results revealed significant activity of *C. tomentosa* and suggesting its use as natural antimicrobial agent.

The above activity has been reported for the first time from ethanolic extract of the roots and bark of C. *tomentosa*.

REFERENCES

1. Simmons K, Islam, MR, Rempel H, Block G et al. Antimicrobial Resistance of Escherichia fergusonii Isolated from Broiler Chickens. Journal of food protection, 2016; 79: 929-938.
2. Wikaningtyas P, Sukandar EY. The antibacterial activity of selected plants towards resistant bacteria isolated from clinical specimens. Asian Pacific Journal of Tropical Biomedicine, 2016; 6: 16-19.
3. Djeussi DE, Noumedem JA, Seukep JA et al. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC complementary and alternative medicine, 2013; 13: 1.
4. Abew B, Sahile S, Moges F. In vitro antibacterial activity of leaf extracts of Zehneria scabra and Ricinus communis against Escherichia coli and methicillin resistance Staphylococcus aureus. Asian Pacific Journal of Tropical Biomedicine, 2014; 4: 816-820.
5. Khomarlou N, Aberoomand A, Lashgari AP, Hakakian A et al. Evaluation of antibacterial activity against multidrug-resistance (mdr) bacteria and antioxidant effects of the ethanolic extract and fractions of chenopodium album (sub sp striatum). International Journal of Pharmaceutical Sciences and Research, 2017; 8(9): 3696-3708.
6. Li Xia, Qiang Guo, Pengfei Tu, Xingyun Chai. The genus Casearia: a phytochemical and pharmacological overview. Phytochem Rev., 2015; 14: 99-135.
7. Rao, KT, Sreedevi K, Veerabhadrappa A, Chetty MK et al. Indian Journal of Plant Sciences, 2014; 3(1): 136-144.
8. Adhikari BS, Babu, MM, Saklani, PL, Rawat, GS. Ethnobotanical Leaflets, 2010; 14(1): 46-83.
9. Maurya SK, Seth A. International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(5): 172-177.
10. Tyagi R, Shukla A, Shukla RK. Phytochemical screening and pharmacological evaluation of different extracts of plant casearia tomentosa leaves. Int J Pharm Pharm Sci., 2017; 9(4): 166-171.
11. Sardessai Y, Pai Angle G, Joshi A, Carvalho S, Bhobe M. Antimicrobial activity of methanolic extract of the rhizomes of costus igneus. J Pharm Chem Biol Sci., 2014; 2(3): 176-185.
12. Kumar S, Kamraj M. Antimicrobial activity of Cucumis anguria L. By agar well diffusion method. Botany Research International, 2011; 4(2): 41-42.

13. Farrukh, R, Zargar MA, Akhtar A, Tasduq SA. Antibacterial and Antifungal activity of Thymus sepeyllum. Botany Research International, 2012; 5(2): 36-39.
14. GbadamosiIT. Evaluation of Antibacterial activity of six Ethnobotanicals used in the treatment of Infectious diseases in Nigeria. Botany Research International, 2012; 5(4): 83-89.
15. Harsha VS. In Vitro Antibacterial Activity of Amaranthus spinosus Root Extracts. Pharmacophore, 2011; 2(5): 266-270.
16. Shukla A, Tyagi R, Vats S, Shukla RK.Total phenolic content, antioxidant activity and phytochemical screening of hydroalcoholic extract of Casearia tomentosa leaves. Journal of Chemical and Pharmaceutical Research, 2016; 8(1): 136-141.
17. Nagalinga S, Sasikumar CS, Cherian KM. Extraction and phytochemical screening of active compounds.Asian J. Pharm. Clin. Res., 2012; 5: 179-181.
18. Shukla A, Vats S, Shukla RK. Proximate composition, nutritive value and evaluation of anti oxidant potential of stem of Dracaena reflexa Lam. Int. J. Pharm. Sci., 2014; 6(11): 360-364.