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Original Article

IMPACT OF SOLVENT TYPES ON ANTIMICROBIAL ACTIVITIES OF ROOT EXTRACT OF *CAESARIA TOMENTOSA*

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ABSTRACT

Objective: The study deals with the antimicrobial activity of five solvent extracts of roots of *Caesaria tomentosa* against seven microorganisms: two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis), three* gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimrium)* and two fungal strains *(Candida albicans* and *Aspergillus niger).*

Methods: Roots of *C. tomentosa* were extracted with different solvents(n-hexane, ethanol, chloroform, acetone and water) and were subjected to antibacterial as well as antifungal screening by Well Diffusion Method. The Minimum Inhibitory Concentration(MIC) was also performed by two-fold dilution.

Results: The maximum inhibition zone at 50µg/ml concentration of n-hexane was 32 mm. These indicate that some active substances in *C. tomentosa* dissolved in varying degrees in the five solvents. The MIC for n-hexane was 1.6 µg/ml for *S. aureus* leading to a conclusion that the n-hexane extract was found to be the most potent.

Conclusion: All extracts were very effective against *S. aureus.* As for the solvents, the n-hexane extract had the best inhibitory effect among five solvents tested.

Keywords: Antimicrobial activity, Disc diffusion, Minimum Inhibitory Concentration

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INTRODUCTION

Bacteria have evolved numerous defences against the antimicrobial agent and drug resistance in pathogen is on rise. This is due to rapid development of multi-drug resistance, limited anti-bacterial spectrum and adverse effects of available anti-microbial agents. This necessitates the search for new antimicrobials with diverse structures and novel mechanism of action.

Plants have been used as a rich source of many natural products used for the treatment of diseases and their secondary metabolite constituents are the sources of important modern drugs such as atropine, codeine, digoxin, morphine, quinine and vincristine.

In ancient times South American and Asian countries used different *Casearia* species as traditional medicines. *Casearia* (family *Salicaceae*) has huge pharmacological importance with studies proving that the crude extracts and isolated compounds from this genus showed hypoglycemic, antioxidant, antiulcer, anti-inflammatory activities, and antimicrobial as well as anti-snake venom property [1].

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 [2-4].

The literature survey revealed that *C. tomentosa* is still an under explored species [5]. The aim of this study was to study the impact of solvent types on antimicrobial activities of root extract of *C. tomentosa.*

MATERIALS AND METHODS

Chemicals required

n-hexane, chloroform, ethanol, acetone and distilled water.

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 the blender.

Preparation of extracts

Ten grams of dried root powder of *C. tomentosa* was placed in five 500-ml Erlenmeyer flasks. Subsequently, a 200-ml each of five solvents, namely: n-hexane, chloroform, ethanol, acetone and water were separately placed accordingly. After addition of each solvent, the corresponding mixtures which had a final concentration of 5%, were shaken vigorously for 72 hr. The crude extracts were filtered under vacuum and filtrates were evaporated to dryness [6].

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 (S. aureus) (ATCC 6538P)* and *Bacillus subtilis (B. subtilis)(ATCC 6633)* and three gram-negative bacteria-*Escherichia coli (E. coli) (ATCC 35218), Pseudomonas aeruginosa (P. aeruginosa) (ATCC 19429)* and *Salmonella typhimurium (S. typhimurium) (ATCC 23564)*. Two fungal strains, *Candida albicans(C. albicans) (NCIM No.10231)* and *Aspergillus niger (A. 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 subcultured for 24-48 h at 37 °C and 25 °C respectively. All strains were procured from National chemical laboratory (NCL) Pune.

Antimicrobial agents

Ciprofloxacin (10 µg/ml) for antibacterial studies and metronidazole (10 µg/ml) were included in the study as standard reference drugs

Antimicrobial activity

The extracts of roots *C. tomentosa* was subjected to antibacterial as well as antifungal screening by Well Diffusion Method (Cup Plate Method) [7]. Mueller Hinton Agar/Broth(Hi Media) and Sabouraud's Dextrose Agar/Broth(Hi Media) were used as the seed medium for the antibacterial and antifungal screening respectively.

The Minimum Inhibitory Concentration (MIC) was performed by two-fold dilution of the test extract in the respective medium under

sterile conditions [8-11]. The inoculums were 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 around the well size. These studies were performed in triplicate and mean values were tabulated to check the effectiveness of the procedure. The MIC was determined by the turbidimetric method by measuring the Optical Density at 600 nm using Elico colorimeter (Filter No. 60).

Statistical analysis

The data recorded during the course of the investigation were statistically analysed by two-way classification method (Anova: two factor without replication) and the conclusion was drawn on the basis of analysis of variance technique. The calculated value of F was compared with the tabulated value at 5% and 1% level of significance for appropriate degrees of freedom.

RESULTS

The results of present study revealed that all extracts were very effective against *S. aureus* showing maximum inhibition zone at 50µg/ml concentration of n-hexane extract was 32 mm among the five bacterial strains (table 1).

The results also indicated that the extracts of the roots of *C. tomentosa* showed significant antifungal activity against both *A. niger* and *C. albicans*. (table 2) Generally, the overall impact of extracts against the seven microorganisms was clearly shown where the effects of extracts increase with the increase of extract concentrations.

Table 1: Antibacterial activity of different solvent extracts by well diffusion method

***C. tomentosa* extract**	**Extract concentration (µg/ml)**	**Diameter of zone of inhibition in mm**									
		S. aureus		***B. subtilis***		***P. aeruginosa***		***E. coli***		***S. typhimurium***	
		Mean	***SEM***	**Mean**	***SEM***	**Mean**	***SEM***	**Mean**	***SEM***	**Mean**	***SEM***
n-hexane	25	18	±0.0	10	±0.5	13	±0.5	8	±0.0	6	±0.5
	50	32	±0.5	23	±0.5	28	±0.0	18	±0.0	14	±0.5
Ethanol	25	12	±0.5	11	±0.0	7	±0.5	8	±0.5	6	±0.0
	50	23	±0.0	17	±0.0	11	±0.0	15	±0.5	13.5	±0.0
Chloroform	25	15	±0.0	11	±0.5	10	±0.5	14	±0.0	7	±0.5
	50	26	±0.5	18	±0.5	23	±0.0	22	±0.5	17	±0.5
Acetone	25	13	±0.0	10	±0.0	11	±0.0	8	±0.0	9	±0.0
	50	21	±0.5	24	±0.5	18	±0.5	14	±0.5	11	±0.5
Water	25	13	±0.5	10	±0.0	8	±1.5	11	±0.5	7	±1.5
	50	18	±0.5	17	±0.5	16	±0.0	16	±0.0	11	±0.0
Standard	10	18	±0.5	27	±0.5	32	±0.0	24	±0.0	22	±0.5

n=3, data is given in mean±SEM(standard error of mean).

Table 2: Antifungal activity of different solvent extracts by well diffusion

***C. tomentosa* extract**	**Extract concentration (µg/ml)**	**Diameter of zone of inhibition in mm**			
		A. niger		***C. albicans***	
		Mean	***SEM***	**Mean**	***SEM***
n-hexane	25	18	±0.0	21	±0.5
	50	24	±0.0	26	±0.0
ethanol	25	10	±0.5	8	±0.0
	50	14	±0.5	9	±0.0
chloroform	25	14	±0.0	16	±0.5
	50	17	±0.5	19	±0.0
acetone	25	13	±1.5	9	±1.5
	50	20	±0.0	15	±0.0
Water	25	17	±0.0	12	±0.0
	50	21	±0.0	18	±0.5
standard	10	32	±0.5	28	±0.5

n=3, n=3, data is given in mean±SEM(standard error of mean)

In statistical analysis it was seen that the calculated F value is higher in almost all cases than the tabulated F value at 5% and 1% level of significance indicating that there is significant variation seen in between the strains (both antibacterial and antifungal) as well as different solvents types used for present study (table 3, 4,5 and 6 of statistical analysis).

Table 3: Statistical analysis of the antibacterial activity of solvent extract (25micro gram/ml)

	S. aureus	***B. subtilis***	***P aeuginosa***	***E. coli***	***S. typhimurium***
n hexane	18	10	13	8	6
Ethanol	12	11	7	8	6
Chloroform	15	11	10	14	7
Acetone	13	10	11	8	9
water	13	10	8	11	7
Anova: Two-Factor Without Replication					
SUMMARY	*Count*	*Sum*	*Average*	*Variance*	
Row 1	5	55	11	22	
Row 2	5	44	8.8	6.7	
Row 3	5	57	11.4	10.3	

Row 4		5	51	10.2	3.7	
Row 5		5	49	9.8	5.7	
Column 1		5	71	14.2	5.7	
Column 2		5	52	10.4	0.3	
Column 3		5	49	9.8	5.7	
Column 4		5	49	9.8	7.2	
Column 5		5	35	7	1.5	
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	20.96	4	5.24	1.382586	0.284151	3.006917
Columns	132.96	4	33.24	8.770449	0.000602	3.006917
Error	60.64	16	3.79			
Total	214.56	24				

Sum of squares (SS), the degree of freedom (df), mean of squares (MS), Probability Valve (P-value)

Table 4: Statistical analysis antibacterial activity of solvent extract (50 microgram/ml)

	S. aureus	***B. subtilis***	***P aeuginosa***	***E. coli***	***S. typhimurium***
n hexane	32	23	28	18	14
Ethanol	23	17	11	15	13.5
Chloroform	26	18	23	22	17
Acetone	21	24	18	14	11
water	18	17	16	16	11

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	5	115	23	53
Row 2	5	79.5	15.9	20.55
Row 3	5	106	21.2	13.7
Row 4	5	88	17.6	27.3
Row 5	5	78	15.6	7.3
Column 1	5	120	24	28.5
Column 2	5	99	19.8	11.7
Column 3	5	96	19.2	42.7
Column 4	5	85	17	10
Column 5	5	66.5	13.3	6.2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	216.96	4	54.24	4.83638	0.009483	3.006917
Columns	307.96	4	76.99	6.864913	0.002043	3.006917
Error	179.44	16	11.215			
Total	704.36	24				

Table 5: Statistical analysis antifungal activity of solvent extract (25micro gram/ml)

	A. niger	***C. albicans***
n hexane	18	21
Ethanol	10	8
Chloroform	14	16
Acetone	13	9
water	17	12

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Row 1	2	39	19.5	4.5
Row 2	2	18	9	2
Row 3	2	30	15	2
Row 4	2	22	11	8
Row 5	2	29	14.5	12.5
Column 1	5	72	14.4	10.3
Column 2	5	66	13.2	28.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	130.6	4	32.65	5.141732	0.070899	6.388233
Columns	3.6	1	3.6	0.566929	0.493371	7.708647
Error	25.4	4	6.35			
Total	159.6	9				

Table 6: Statistical analysis antifungal activity of solvent extract (50 micro gram/ml)

	A. niger	*C. albicans*
n hexane	24	26
Ethanol	14	9
Chloroform	17	19
Acetone	20	15
water	21	18

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	2	50	25	2
Row 2	2	23	11.5	12.5
Row 3	2	36	18	2
Row 4	2	35	17.5	12.5
Row 5	2	39	19.5	4.5
Column 1	5	96	19.2	14.7
Column 2	5	87	17.4	38.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	186.6	4	46.65	7.346457	0.039625	6.388233
Columns	8.1	1	8.1	1.275591	0.321863	7.708647
Error	25.4	4	6.35			
Total	220.1	9				

Different organic solvents extracts have different phyto-constituents in different amounts and that is why there is differential inhibition of the bacterial and fungal strains. As for the solvents, the n-hexane extract had the best inhibitory effect among five solvents tested (n-hexane, ethanol, chloroform, acetone and water). The MIC for n-hexane was 1.6 µg/ml for *S. aureus* (tables 7) leading to a conclusion that the n-hexane extract was found to be the most potent among all extracts.

Table 7: Extract MIC of test microorganisms

Microorganisms	n-hexane	Ethanol	chloroform	Acetone	Water
S. aureus	1.6	3.12	12.5	12.5	12.5
B. subtilis	3.12	12.5	25	3.12	25
P. aeruginosa	3.12	12.5	12.5	25	25
E. coli	3.12	25	3.12	12.5	25
S. typhimurium	12.5	12.5	3.12	25	25
A. niger	3.12	12.5	25	25	3.12
C. albicans	3.12	3.12	3.12	12.5	3.12

DISCUSSION

In last few years, there has been a dramatic rise in search for natural products with antimicrobial properties because they offer a hope to find new drugs or drug leads which have a promising antimicrobial activity with lesser side effects to human beings.

The results of phytochemical screening of leaves and aerial parts of *C. tomentosa* revealed the presence of active phytoconstituents such as alkaloids, glycosides, steroids, saponins, flavonoids, terpenoids and tannins etc [12].

According to previous studies, it was found that roots were having better antimicrobial potential as compared to other parts of the plant [7].

Out of these phytoconstituents, flavonoids are one of the largest group of phenolic compounds, numerous reports support their use as a primary antioxidant and possess antimicrobial, anti-inflammatory, anti-allergic, anticancer activities etc [13]. Saponins are a special class of glycosides which have soapy characteristics and possess antibacterial, hypolipidemic, antidiabetic, and anticancer activity [16]. Plant-derived alkaloids are the biggest class of phytochemical and exhibit many therapeutic effects like antioxidant, analgesics, muscle relaxant, antibiotics, anticancer and also responsible for antiprotozoal, cytotoxic and antimicrobial properties [14, 15].

The presence of secondary metabolites including alkaloids, saponins, flavonoids in the plant may be are responsible for its potential antibacterial activity [17].

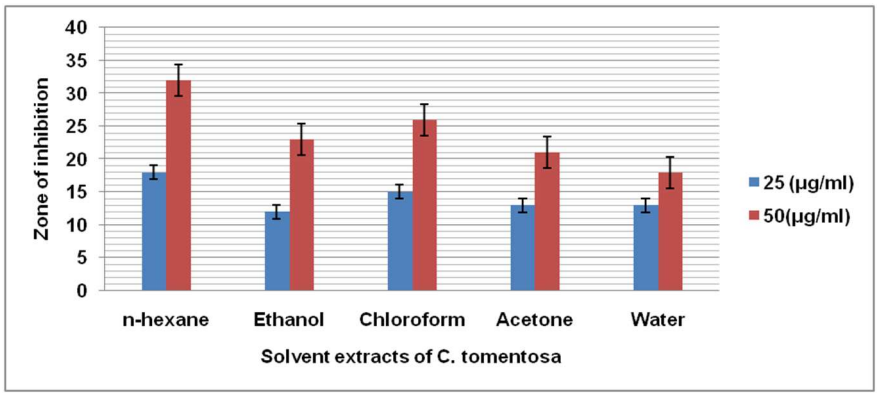

Fig. 1: Comparison of antibacterial activity of different solvent extracts against *S. aureus* and the values are expressed as mean±SEM; n=3

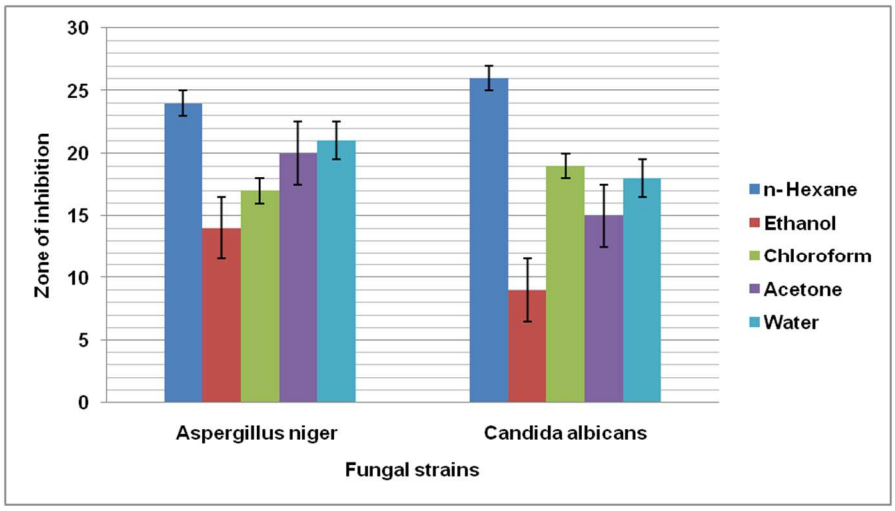

Fig. 2: Comparison of antifungal activity of different solvent extracts and the values are expressed as mean±SEM; n=3

The reason for a greater activity of hexane extract could be attributed to the non-polar nature of the solvent which may be responsible for the extraction of a wide range of phytoconstituents that potentiates the activity of extract [18]. Thus, the greater the phytochemical load, the greater the activity of a plant extract.

On correlating the results an inference can be drawn that presence of the majority of phytochemicals and large quality of non-polar compounds in hexane extract may be responsible for its prominent activity against all the bacterial and fungal strain. The present study not only suggests the strong antimicrobial potential of *C. tomentosa* roots, but also suggests that extracting solvent plays a crucial role for evaluating the antimicrobial activity of medicinal plants.

CONCLUSION

The results of this study revealed a correlation between traditional therapeutic use and the *in vitro* antibacterial activity, a broad spectrum anti-microbial activity of root extract of *C. tomentosa* extracts against both Gram-positive, Gram-negative bacteria as well as fungi, with variable degrees of sensitivity. Many phytochemical investigations carried on the rhizomes and roots of *C. tomentosa* had led to the steroids, triterpenoids, alkaloids, fats, sterols, and phytol. The above antimicrobial activity may be attributed to the presence of these bioactive constituents.

More importantly, the results indicated that n-hexane extract of *C. tomentosa* was more effective against *S. aureus* followed by chloroform, ethanol, acetone and water extracts, respectively. More work on the antimicrobial activity of this and similar such extracts can be instrumental in discovery and development of novel lead bioactive molecules from natural products

The above activity has been reported for the first time of the roots *C. tomentosa*.

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AUTHORS CONTRIBUTIONS

Ms. Gauri Pai Angle corresponding author for this publication, working as Assistant Professor has designed the study, performed the experiments, interpreted the results and worked on the manuscript; Dr. Yogita Sardessai has guided the study and co-wrote the paper. Both authors have discussed the results and written the manuscript.

CONFLICT OF INTERESTS

Authors declare no conflict of interest

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