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Short Communication

Pharmacognostic Evaluation and Antimicrobial Activity of Root of *Careya arborea*

Bandenawaz Ramadurga[1,*], Rakesh Kumar Jat[2], Shrishailappa Badami[3]

ABSTRACT

Background: *Careya arborea* is known for its traditional medicinal properties and reported for potent antitumor, antioxidant, hepatoprotective and many other activities. Its stem bark, leaves and fruits were studied biologically, but so far its root has not been studied. **Aim:** The aim of the present study is to standardize the root of *Careya arborea* and its extracts pharmacognostically and also to screen its extracts for their antimicrobial activities against several bacteria and fungi using standard procedures. **Materials and Methods:** Loss on drying, extractive and ash values, fluorescence and phytochemical analysis of the root and its extracts were studied using standard procedures. Antimicrobial activity was carried out by determining minimum inhibitory concentration. **Results:** Among all the extracts, the successive ethyl acetate extract was found to be the most active with lowest MIC values against *L. acidophilius*, *S. aureus*, *C. freundii, P. aeruginosa and M. luteus*. The successive chloroform extract was also found to be highly active against *P. aeruginosa* and fungi, *M. furfur* and *C. albicans*. **Conclusion:** The results are helpful in standardizing the root of the plant and since several of the root extracts possess antimicrobial properties, there is a need to isolate its constituents.
Key words: *Careya arborea*, Standardization, Antibacterial, Antifungal.

INTRODUCTION

Careya arborea Roxb. (family: Barringtoniaceae) is a handsome small to medium sized deciduous tree found in India sub Himalayan tract from Jammu eastwards to West Bengal, Madhya Pradesh, Tamilnadu, Karnataka, Sri Lanka and Andaman Islands. Most of its parts are of medicinal value.[1] Its root is used in tuberculosis and skeletal fracture and in Ayurveda, bark and root are used in Vata and Kapha.[2] Several steroids, saponins, flavonoids, tannins and alkaloids have been isolated from its various parts.[1-7] Its CNS depressant, antidiarrheal, anticonvulsant, wound healing, gastroprotective, antioxidant, hepatoprotective, antitumor and other biological activities are well known.[8,9]Antimicrobial activity of its fruits,[10] leaves[11] and stem bark extracts[12] have been reported. Though the root is also used traditionally, so far it has not been studied biologically.

Antimicrobial resistance is presently a threat worldwide to human and animal health. The plant drugs, due to their diverse phytoconstituents and a wide variety of secondary metabolites derived from them, are useful as alternative strategies to control infectious diseases and to avoid resistance.[13] Hence, in the present study we were interested in screening antimicrobial activity of root of *Careya arborea*. Along with this, to standardize the root and its extracts, pharmacognostic evaluation was also carried out.

MATERIALS AND METHODS

Chemicals and Media

Mueller Hinton broth, Brucella broth, Nutrient broth, Brain heart infusion broth and Sabouraud Chloramphenicol Agar were obtained from Micro Master Laboratories and resazurin dye and ciprofloxacin were obtained from Himedia, India. All other solvents and chemicals used were of standard grades.

Collection, authentication and extraction

The root of the plant *Careya arborea* was collected from Haridravati village, Hosanagar, Shimoga dist., Karnataka State, India in the month of March 2016. The plant was authenticated by Dr. S. Rajan, Survey of Medicinal Plants and Collection Unit, Ooty, Tamilnadu, India (voucher no.8570), where voucher specimens are preserved. The root was shade dried, powdered and Soxhlet extracted (300 g) with methanol (2.5 lts) for 12 h. The extract was concentrated under reduced pressure at 50-60˚C in a rotary evaporator, yielding a brown residue 59.25 g, 19.75%. The root powder was also extracted (350 g) in a Soxhlet apparatus successively with petroleum ether, chloroform, ethyl acetate and methanol (1.5 lts each) for 12 h. The extracts were concentrated. Yields, successive petroleum ether extract, yellowish semisolid residue, 2.94 g, 0.84%, chloroform extract,

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yellowish brown solid, 1.47 g, 0.42%, successive ethyl acetate extract, brown solid, 3.47 g, 0.99% and successive methanol extract, brown solid, 22.9 g, 6.54%. The extracts were stored in a closed vessel at 4°C in a refrigerator till further use.

Pharmacognostic evaluation

The dried root powder of *Careya arborea* was used for pharmacognostic evaluations. Loss on drying, alcohol and water soluble extractive values and fluorescence analysis by treating the powder with various reagents and observing under ordinary light and UV light (long, 360 nm and short, 254 nm) for possible color variations[14,15] were determined. The extracts were also observed for their color variations under ordinary and ultraviolet rays. Ash values such as total ash, acid insoluble ash, water-soluble ash and sulfated ash were determined according to the Indian Pharmacopoeia.[16] The extracts of *Careya arborea* were subjected to preliminary phytochemical screening for the detection of various plant constituents present using standard procedures.[15]

Antimicrobial activity

The crude methanol extract, successive petroleum ether, chloroform, ethyl acetate and methanol extracts were evaluated by MIC for antibacterial activity against *C. freundii, E. coli, P. aeruginosa, S. aureus, L. acidophilus, S. marcescens* and *M. luteus* and for antifungal activity against *C. albicans, C. glabrata, C. krusei, M. furfur, A. niger, R. oryzae* and *Mucor* sp at different concentrations. The MIC values of test substances were compared with the activity of standard antibiotic. Standard procedures were followed.[17,18] The microbial strains and their sources are shown in Table 1.

Antibacterial activity

Preparation and standardization of stock cultures

A day prior to the experiment, a loopful culture of *E. coli, P. aeruginosa, S. aureus, S. marcescens* and *M. luteus* were grown in NB at 37°C for 24 h. The culture of *L. acidophilus,* was grown on MHA. The cultures were adjusted to 0.17 absorbance at 600 nm (corresponding to approximately 10^8CFU/ml and 0.5 McFarland Standard), using a spectrophotometer and further diluted to a concentration of approximately 10^5 CFU/ml.

Preparation of resazurin and standard antibiotic solution

The stock resazurin solution was prepared by dissolving 2.7 mg in 4 ml of sterile saline. Further, working solution was prepared by dissolving 1 ml of stock solution in 5 ml of sterile saline. The standard antibiotic *i.e.,* ciprofloxacin solution at 1% concentration was prepared in sterile distilled water.

Preparation of test samples

Test samples crude methanol and successive petroleum ether, chloroform, ethyl acetate and methanol extracts were prepared at 10 mg/ml concentration by dissolving 10 mg of test sample in 1 ml of MHB. Sample were mixed using cyclomixer for 5 min and sonicated for 5-10 min. Samples were mixed thoroughly before using for experiment.

Determination of MIC

Experiments were performed in triplicate under aseptic conditions. A volume of 50 µl respective sterile MHB was added to all 96 wells except first three wells of the microtitre plate $A_1B_1C_1$ to which only 100 µl test product was added. From first three wells (A_1B1C_1) of plate, 50 µl of the test product was double diluted till $A_{12}B_{12}C_{12}$. To the wells containing test material 10 µl bacterial suspension of approximately 10^5 CFU/ml (10^6 CFU/ml for *S. mutans* and *L. acidophilus* and *P. acne*) was added. A growth control (bacterial cell suspension + 50 µl broth medium) from G_1 to G_{12} and broth control (only broth medium 50 µl) from H_1 to H_{12} was kept. A positive control that consists of ciprofloxacin was also placed in the plate. The plates were incubated at 37°C for 48 h. After incubation, 10 µl of working solution of resazurin was added to all wells. The plates were wrapped with aluminum film and incubated at 37°C for 1 h. The color change was then assessed visually. Any color change from purple to pink or colorless was recorded as positive (growth). The lowest concentration at which there is no color change occurred was taken as the MIC value.

Antifungal activity

Preparation and Standardization of Stock cultures

C. albicans, C. glabrata, C. krusei, A. niger, R. oryzae and *Mucor* sp cultures were subcultured on SCA from glycerol stock. Plates of *Candida*

Table 1: Microbial strains and their sources.

S. No.	Tested Strain	Strain No.	Equivalent No.	Source
	Bacteria			
1	*Citrobacter freundii*	NCTC 9750	ATCC 8090	National Collection of Type Cultures, United Kingdom.
2	*Escherichia coli*	NCTC 12923	ATCC 8739	National Collection of Type Cultures, United Kingdom.
3	*Pseudomonas aeruginosa*	NCIM 2862	ATCC 15442	National Collection of Industrial Microorganisms, India.
4	*Staphylococcus aureus*	NCTC 10788	ATCC 6538	National Collection of Type Cultures, UK
5	*Lactobacillus acidophilus*	NCIM 2660	-	National Collection of Industrial Microorganisms, India.
6	*Serratia marcescens*	NCIM 2919	-	-do-
7	*Micrococcus luteus*	NCIM2103	-	-do-
	Fungi			
8	*Candida albicans*	NCPF 3179	ATCC 10231	National Collection of Pathogenic Fungi, UK
9	*Candida glabrata*	MTCC 3019	ATCC 90030	Microbial Type Culture Collection, India
10	*Candida krusei*	MTCC 9215	-	-do-
11	*Malassezia furfur*	MTCC *1374	-	-do-
12	*Aspergillus niger*	NCPF 2275	ATCC 16404	National Collection of Pathogenic Fungi, UK
13	*Rhizopus oryzae*	MTCC 262	-	Microbial Type Culture Collection, India
14	*Mucor sp*	MTCC 3340	-	-do-

sp. were incubated at 28°C for 48 h and other fungi were incubated at room temperature (30°C) for seven days. *M. furfur* was subcultured on Dixon's agar plate and incubated for 3-days at 37°C. In 10 ml of sterile saline, a loopful of fungal conidia was dissolved and total numbers of conidia were adjusted to 10^6CFU/ml (0.5 McFarland standards) by counting under microscope using haemocytometer and fungal suspension was diluted in order to get 10^3 CFU/ml.

Preparation of resazurin and standard antibiotic solution

The stock resazurin solution was prepared as in antibacterial studies. The standard antibiotic *i.e.,* ketoconazole solution at 1% concentration was prepared in sterile distilled water.

Preparation of test samples

Test samples, crude methanol, successive petroleum ether, chloroform, ethyl acetate and methanol extracts were prepared at 10 mg/ml concentration by dissolving 10 mg of test sample in 1 ml of MHB. Samples were mixed using cyclomixer for 5 min and sonicated for 5-10 min. Samples were mixed thoroughly before using for experiment.

Determination of MIC

Experiments were performed in triplicate under aseptic conditions. A volume of 50 µl sterile RPMI was added to all 96 wells except first three wells of the microtitre plate $A_1B_1C_1$ to which only 100 µl test product was added. In first three wells ($A_1B_1C_1$) of plate, 50 µl of the test product was double diluted till $A_{12}B_{12}C_{12}$. To the wells containing test material 10 µl conidial suspension of approximately 10^3 CFU/ml was added. A growth control (bacterial cell suspension + 50 µl broth medium) from G_1 to G_{12} and broth control (only broth medium 50 µl) from H_1 to H_{12} was kept. A positive control that consists of the ketoconazole (standard antibiotic) was placed in separate plate. The plates were then incubated at 28°C for 48 h. MIC of *M. furfur* conducted in Dixon's broth and plates were incubated at 37°C for 3-4 days. After the incubation, 10 µl of working solution of resazurin was added to all wells. The plates were wrapped with aluminum film and incubated for 1 h. The color change was then assessed visually. Any color change from purple to pink or colorless was recorded as positive growth. The lowest concentration at which there is no color change occurred was taken as the MIC value.

RESULTS

Pharmacognostic evaluation

The moisture content, alcohol soluble and water soluble extractive values (average of three determinations) of the root of *Careya arborea* were found to be 5, 13.6 and 12.8 percentages, respectively. The effects of both ordinary and ultraviolet lights on fluorescence properties of dried root powder and for extracts are recorded in Tables 2 and 3. The total, acid insoluble, water soluble and and sulphated ash values for the root powder of *Careya arborea* were found to be 2.5, 2, 2.33 and 2.5 (average of three determinations), respectively.

Phytochemical analysis

The qualitative phytochemical analysis showed the presence of carbohydrates, glycosides, saponins, flavonoids, phenolics, tannins, phytosterols and triterpenoids in the crude methanol extract. Flavonoids, phytosterols, triterpenoids, fixed oils and fats were present in the successive petroleum ether extract. Flavonoids, phytosterols and triterpenoids were present in the successive chloroform, Carbohydrates, glycosides and flavonoids in the successive ethyl acetate and Carbohydrates, glycosides, saponins, flavonoids, phenolics and tannins in the successive methanol extracts. The results from the above chemical tests are summarized in Table 4.

Table 2: Fluorescence analysis of the root powder of *Careya arborea*.

Sl. No	Solvent added to root powder	Observation* under		
		Ordinary	UV Short wave	UV Long wave
	Distilled water	Orange	Green	Green
	Chloroform	Very light yellow	Colorless	Colorless
	1N NaOH in water	Dark brown	Dark brown	Dark brown
	1N NaOH in methanol	Light yellow	Light green	Light green
	10 % HCl	Light orange	Light yellow	Very light green
	10 % H_2SO_4	Light orange	Light yellow	Very light green

* The observation was based on three determinations

Table 3: Data showing the fluorescence analysis of different extracts of the root powder of *Careya arborea*.

Sl. No	Name of the Extract	Observation* under		
		UV Short wave	UV Long wave	Visible Light
	Successive			
	Petroleum ether	Brown	Greenish yellow	Yellow
	Chloroform	Brown	Greenish brown	Light brown
	Ethyl acetate	Dark brown	Greenish brown	Yellowish brown
	Methanol	Dark brown	Dark black	Brown
	Crude Methanol	Dark brown	Dark black	Brown

* The observation was based on three determinations".

Antibacterial activity

Among all the extracts, the successive ethyl acetate extract was found to be most active with lowest MIC values against *L. acidophilius* (MIC 31.25 µg), *S. aureus* (MIC 62.5 µg), *C. freundii, P. aeruginosa* and *M. luteus* (MIC 125 µg, Table 5). The successive chloroform extract was found to be highly active against *P. aeruginosa* with MIC value of 15.65 µg. The successive petroleum ether and crude methanol extracts have shown medium activity against *P. aeruginosa* with MIC value of 62.5 µg. The successive chloroform and methanol extracts have also shown medium activity against *C. freundii* and *P. aeruginosa,* respectively with MIC value of 125 µg. Except these, the extracts have shown low activity against the remaining organisms. However, the standard Ciprofloxacin was highly active when compared to all the extracts.

Antifungal activity

The successive chloroform extract of the plant *Careya arborea* root was found to be most active against *M. furfur* and *C. albicans* with MIC values of 31.25 and 62.5 µg, respectively (Table 6). The successive petroleum ether extract was also found to be active against these two organisms and *C. krusei* with MIC value of 62.5 µg. The successive ethyl acetate and methanol extracts and the crude methanol extract have also shown activity against *M. furfur* with MIC value of 62.5 µg. *M. furfur* was found to be the most susceptible to all the extracts. *C. albicans* was also found to be susceptible to all the extracts with MIC values ranging from 62.5 to 250 µg. *A. niger* was found to be the next susceptible with all the extracts except the successive methanol showing MIC value of

Table 4: Preliminary Phytochemical tests for the root extract of *Careya arborea*.

Sl. No	Test	Extract				
		Crude	Successive			
		Methanol	Pet ether	Chloroform	Ethyl acetate	Methanol
1	**Test for carbohydrates**					
	a. Molisch's test	+	-	-	+	+
2	**Test for Glycosides**					
	Keller-Killiani test	+	-	-	+	+
3	**Test for Saponins**					
	a. Foam test	+	-	-	-	+
4	**Test for Alkaloids**					
	Mayer's test	-	-	-	-	-
	Dragendroff's test	-	-	-	-	-
5	**Test for Flavonoids**					
	Alkaline reagent test	+	+	+	+	+
6	**Test for Phenolics and Tannins**					
	Ferric chloride test	+	-	-	-	+
	Test for Tannins	+	-	-	-	+
7	**Test for Phytosterols and Triterpenoids**					
	Leiberman-Burchard's test	+	+	+	-	-
	Salkowaski test	+	+	+	-	-
8	**Test for fixed oils and fats**					
	a. Oily spot test	-	+	-	-	-

(+) Present, (-) Absent, the observation was based on three determinations

Table 5: MIC of the extracts of *Careya arborea* against selected bacteria.

Sl. No	Sample Name	Conc. Range (µg)	MIC (µg)*						
			C. freundii	*E. coli*	*P. aeruginosa*	*S. aureus*	*L. acidophilus*	*S. marcescens*	*M. luteus*
	Crude								
1	Methanol	1000 – 0.488	500	500	62.5	500	500	500	250
	Successive								
2	Petroleum Ether	1000 – 0.488	250	500	62.5	500	1000	500	250
3	Chloroform	1000 – 0.488	125	500	15.65	500	>1000	500	250
4	Ethyl acetate	1000 – 0.488	125	500	125	62.5	31.25	500	125
5	Methanol	1000 – 0.488	250	500	125	500	1000	500	250
6	Ciprofloxacin	1000 – 0.488	<0.488	<0.488	<0.488	<0.488	<0.488	<0.488	<0.488

* MIC value is expressed as mean of triplicate, $n = 3$

Table 6: MIC of the extracts of *Careya arborea* against selected fungi.

Sl. No.	Sample Name	Conc. Range (µg)	MIC (µg)*						
			C. albicans	*C. glabrata*	*C. krusei*	*M. furfur*	*A. niger*	*R. oryzae*	*Mucor* sp.
1	**Crude** Methanol	1000 – 0.488	125	250	250	62.5	125	125	125
Successive									
2	Petroleum Ether	1000 – 0.488	62.5	500	62.5	62.5	125	250	250
3	Chloroform	1000 – 0.488	62.5	250	250	31.25	125	>1000	>1000
4	Ethyl Acetate	1000 – 0.488	250	125	125	62.5	125	>1000	>1000
5	Methanol	1000 – 0.488	125	500	250	62.5	>1000	125	250
6	Ketoconazole	1000 – 0.488	<0.488	0.976	0.976	<0.488	15.62	15.62	<0.488

* MIC value is expressed as mean of triplicate, $n = 3$

125 µg. The crude methanol extract was found to be most active against all the organisms with MIC values ranging 62.5 to 250 µg. The successive methanol extract was also found to be active against all the organisms except *A. niger* and *C. glabrata* with MIC values of 62.5 to 250 µg. The ethyl acetate extract has shown activity against all the extracts except *R. oryza* and *Mucor* sp with MIC values of 62.5 to 250 µg. Except these, the extracts were found to be least active against the remaining organisms. However, the standard ketoconazole was highly active when compared to all the extracts.

DISCUSSION

Plants are untapped sources of chemotypes, used in many countries traditional medicine. The increasing incidence of drug resistant pathogens around the world has made the scientists to screen plant derived substances for antimicrobial activity.[13] The plant *Careya arborea* is well known for its traditional biological used around the world, especially in India. Kumar *et al*.[12] have tested the stem bark extract of the plant for antimicrobial activity and the extracts exhibited potent activity. Similarly the leaves extracts also exhibited the activity.[10-11] Hence, in the present study, the antimicrobial activity of the root extracts was carried out. The successive ethyl acetate extract was found to be the most active against *L. acidophilius*, *S. aureus*, *C. freundii, P. aeruginosa and M. luteus* and the chloroform extract was found to be highly active against *P. aeruginosa* and fungi, *M. furfur* and *C. albicans*. Flavonoids, phytosterols and triterpenoids present in the successive chloroform extract and glycosides and flavonoids present in the successive ethyl acetate extract are responsible for the potent antimicrobial activity observed. Further studies are required to establish the same. The pharmacognositcal evaluation of the root and extracts carried out in the present study helps in establishing standards for the identification.

CONCLUSION

The results obtained in the present study are helpful in pharmacognostical standardization of the root of *Careya arborea* and several of its extracts exhibited potent antibacterial and antifungal activities, which are in concordance with the results obtained for the antimicrobial activity of its leaves and bark.[10-12] Further studies are warranted to isolate its active constituents responsible for its antimicrobial activity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

C: Centigrade; **CFU:** Colony Forming Units; **CNS:** Central Nervous System; **h:** Hour; **MHA:** Mueller Hinton Agar; **MHB:** Mueller Hinton Broth; **MIC:** Minimum Inhibitory Concentration; **NB:** Nutrient Broth; **RPMI:** Roswell Park Memorial Institute Medium; **SCA:** Sabouraud Chloramphenicol Agar; **UV:** Ultra Violet.

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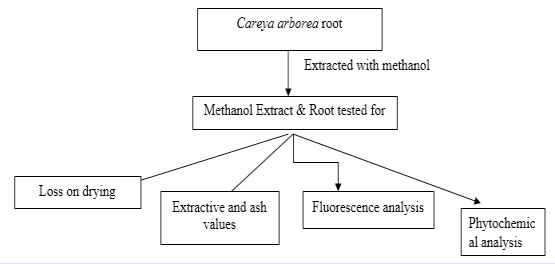
GRAPHICAL ABSTRACT



SUMMARY

- In the present study, *Careya arborea* root and its extracts were standardized pharmacognostically by determining Loss on drying, extractive and ash values, fluorescence and phytochemical analysis and its extracts were screened for their antimicrobial activities against several bacteria and fungi by determining minimum inhibitory concentrations. Among all the extracts, the successive ethyl acetate extract was found to be the most active with lowest MIC values against *L. acidophilius, S. aureus, C. freundii, P. aeruginosa* and *M. luteus*. The successive chloroform extract was also found to be highly active against *P. aeruginosa* and fungi, *M. furfur* and *C. albicans*.

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