Evaluation of Antimicrobial Activity of Solvent Extracts of Bark of *Cipadessa baccifera* (Roth.) Miq.

Kavitha. K. R.[1], Jyothsna. B. S.[2], Keshamma. E[3]
[1]*Registrar and HOD, Department of Botany, UG & PG studies, Nrupathunga University, Bengaluru, Karnataka, India*
[2]*Associate Professor, Department of Botany, UG & PG studies, Nrupathunga University, Bengaluru, Karnataka, India*
[3]*Assistant Professor, Department of Biochemistry, Maharani Cluster University, Bengaluru, Karnataka, India*

Abstract* - In the current study we aimed to evaluate the antimicrobial activity of solvent extracts bark of *Cipadessa baccifera* (Roth.) Miq. The plant samples for investigation were collected from Thavarekere and Savandurga, Magadi Taluk, Bengaluru Rural district, and outskirts of Bengaluru, and identification was authenticated by National Ayurveda and Dietetics Research Institute, Bangalore; vide voucher specimen number, RRCBI-8971. The dried bark samples were pulverized in an electric blender and the powdered material was stored in airtight containers for further analyses. The bark samples were subjected to sequential extraction using Soxhlet apparatus, and extracted exhaustively in organic solvents such as, hexane, chloroform, methanol and water. The sequential extracts of bark of *C. baccifera* (Roth) Miq. were subjected to evaluation of antimicrobial activity against diarrhoea, skin, wound and oral infections causing selected pathogens, including 07 Gram positive, 06 Gram negative bacteria and 06 fungal strains. Results discovered that the solvent extracts of bark of *C. baccifera* exhibited significant anti-bacterial activity against *Escherichia coli, Bacillus cereus, Propionibacterium acnes, Streptococcus gordonii and Corynebacterium diphtheriae.* Furthermore, solvent extracts of the bark showed anti-fungal activity against *Candia glabrata* and *Fusarium.

***Index Terms* - *Cipadessa baccifera*, Bark, Sequential extraction, Antimicrobial, Antifungal.**

I.INTRODUCTION

The struggle between man and microbes has been going on since times immemorial. Probably one of most successful forms of therapy used to control infectious diseases, recorded in the history of human existence has been the use of antimicrobials from medicinal plants indicating that plants were the first weapons used against microbes. Even today traditional medicines used for treatment of infectious maladies include scores of plants like, barberry (*Arctostaphylos uva-ursi)* and cranberry juice (*Vaccinium macrocarpon)* for urinary tract infections, while lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tee tree (*Melaleuca alternifolia*) are used as broad-spectrum antimicrobial agents.[1]

The discovery of antibiotics no doubt transformed medicine, drastically bringing down the morbidity and mortality rates due to infectious diseases. However, it led to flourishing misuse, indiscriminate or inappropriate use of commercial antibiotics. This resulted in the development of antibiotic resistance in bacterial pathogens against many microbial infections, an alarming portent that has serious public health concern with economic and social consequences.[2] As a concern, the choices of antibiotic treatment against the already existing or multidrug resistant bacterial infections are becoming limited, resulting in high morbidity and increased mortality rates.[3] The prevalence of many highly resistant clinical isolates such as, *Staphylococcus aureus, Streptococcus pyogenes, Mycobacterium tuberculosis, Streptococcus pneumoniae, Haemophilus influenzae, Pseudomonas aeruginosa, Klebsiella pneumoniae* etc. have been reported in the last few decades.[4]

Considerable number of studies conducted on the antimicrobial activity of medicinal plants indicates that they are a promising source of potent antimicrobials which include secondary metabolites such as saponins, tannins, phenols, alkaloids,

flavonoids, sesquiterpenes lactones, terpenoids and esters. Hence plants have been successfully used worldwide in traditional medicines to treat several diseases and infections.[5,6] Evaluation of the antimicrobial potency of ethnomedicinal plants such as *C. baccifera* which has been widely used in the treatment of dysentery, skin and wound infections etc… is relevant in this context.[7] With this background, the present study was designed to conduct with the main purpose of evaluation of antimicrobial activity of solvent extracts of bark of *C. baccifera* (Roth.) Miq.

II. MATERIALS AND METHODS

Collection of plant material

The plant samples for investigation were collected from Thavarekere and Savandurga, Magadi Taluk, Bengaluru Rural district, and outskirts of Bengaluru. The plant under study was identified as *C. baccifera* (Roth) Miq. as per Flora of Hassan (1976) and Flora of Karnataka (1996) by Saldana.[8,9] Further, the identification was authenticated by National Ayurveda and Dietetics Research Institute, Bangalore; vide voucher specimen number, RRCBI-8971.

Sample processing

The samples such as bark of *C. baccifera* were collected in clean and sterile polythene bags for various analyses. The collected samples were washed thoroughly in running tap water to remove dust and soil particles and were blotted dry. Healthy and infection free bark was separated and shade dried for 20 days. The dried bark park of *C. baccifera* was pulverized in an electric blender and the powdered material was stored in air tight containers for further analyses.

Sequential extraction

Dry and coarsely powered bark part of *C. baccifera* were subjected to sequential extraction using Soxhlet apparatus, and extracted exhaustively in organic solvents such as, hexane, chloroform, methanol and water.[10] Then the solvents were filtered and concentrated to dryness under pressure using rotary vacuum evaporator. The extracts were air dried to remove the solvents completely, then sealed and stored at 4°C in a refrigerator for further studies.

Antimicrobial activity

Test microorganisms

The sequential extracts of bark part of *C. baccifera* were evaluated for their antimicrobial activity against selected pathogens causing diarrhoea, skin, wound and oral infections. The diarrhoea causing pathogens include, Gram positive bacteria, *Bacillus cereus* NCIM 2155, Gram negative bacteria *viz., Escherichia coli* NCIM 2343, *Shigella flexneri* NCIM 5265 and *Salmonella abony* NCTC 5080. The skin and wound infections causing pathogens include Gram positive *Propionibacterium acnes* ATCC 11827, *Nocardia asteroides* MTCC 274 and *Staphylococcus aureus* MTCC 96 and Gram negative *Pseudomonas cepacia* NCIM 5089, *Pseudomonas aeruginosa* MTCC 741 and *Candida* sp. such as, *Candida krusei* MTCC 9215 and *Candida parapsilosis* MTCC 6510. The pathogens causing oral infections selected were Gram positive *Streptococcus gordonii* MTCC 2695, *Streptococcus mutans* MTCC 497 and *Corynebacterium diphtheriae* NCIM 5212 and fungal sp., *Candida albicans* ATCC 10231, *Candida glabrata* MTCC 3019 and *Fusarium* NCIM 894. In addition, antimicrobial activity was evaluated against Gram negative, *Klebsiella pneumoniae* NCIM 2719 and fungal strain, *Aspergillus niger* NCIM 501. These microorganisms were procured from American Type Culture Collection (ATCC), National Collection of Industrial Microorganisms (NCIM), National Culture of Type Cultures (NCTC) and Microbial Type Culture Collection (MTCC) Institutes.

Determination of zone of inhibition (ZOI)

The standard protocols of Clinical and Laboratory Standards Institute (CLSI) and National Committee for Clinical Laboratory Standards (NCCLS) for screening of antimicrobial activity of the sequential plant extracts and essential oils by agar well diffusion method were followed. The stock solution concentration of 10 mg/mL of solvent extracts and essential oils were prepared in DMSO. The stock concentration of 1 mg/mL of antibiotics Ciprofloxacin and Ketoconazole were prepared and used as positive controls for bacteria and fungi respectively. The test was carried out in triplicate.[11,12]

Further, based on the zone diameter the antimicrobial activity of standard antibiotic ciprofloxacin against bacteria was expressed as resistant (ZOI is ≤ 15 mm),

intermediate (ZOI is between 16-20 mm) and sensitive/susceptible (ZOI is ≥ 21 mm) and for Ketoconazole against fungi was expressed as resistant (ZOI is ≤ 22 mm), intermediate (ZOI is between 23-29 mm) and sensitive/susceptible (ZOI is ≥ 30 mm).[11,12] The sensitivities of the microorganism species to the plant extracts were determined by measuring the size of inhibitory zones (including the diameter of well) on the agar surface and values <8 mm were considered as not active against microorganisms.

Minimum Inhibitory Concentration (MIC) assay

Minimum inhibitory concentration (MIC) was determined by modified resazurin assay using microtiter-plate technique described by Sarker.[13] Each plate had a set of controls; the column with positive control contained the broad spectrum antibiotics Ciprofloxacin for bacteria and Ketoconazole for fungi, whilst the negative control column had all solutions except test extracts and sterility control that is, a column with all solutions with the exception of the bacterial/fungal solution adding 10 µL of nutrient broth instead. The plates were incubated for 18 to 24 hours at 37°C at 100% relative humidity. The change in colour of resazurin dye was observed and assessed visually. Any change in colour from purple to pink to colorless was recorded as positive result. The lowest concentration prior to which the positive color change occurred was taken as the MIC value for that particular test sample against the tested bacteria and fungi. The average of three values was taken to be the MIC of the test sample and the bacterial/fungal strain.

III. RESULTS

Antimicrobial activity of sequential bark extracts of *C. baccifera*

The antimicrobial activity of the sequential hexane, chloroform, methanol and aqueous extracts of bark of *C. baccifera* was assessed and the results are presented in Tables 1.

Significant anti-bacterial activity of hexane extract of bark of *C. baccifera* was observed against *Bacillus cereus* with a zone of inhibition of 20 mm, followed by *Escherichia coli* (19 mm), *Streptococcus gordonii* (16 mm) and *Pseudomonas cepacia* (15 mm). The highest zone of inhibition was obtained in the chloroform extract of the bark part of *C. baccifera* against *Propionibacterium acnes* (28 mm), followed by *Pseudomonas cepacia* (20 mm), *Corynebacterium diphtheriae* (20 mm) and *Escherichia coli* (19 mm). The methanolic extract of the bark showed anti-bacterial activity against *Escherichia coli* (21 mm), *Streptococcus gordonii* (20 mm), *Corynebacterium diphtheriae* (20 mm), *Shigella flexneri* (18 mm) and *Propionibacterium acnes* (17 mm) and *Bacillus cereus* (18 mm). The aqueous extract of the bark showed significant antibacterial activity with highest zone of inhibition against *Escherichia coli* (23 mm). While intermediate zones of inhibition were observed for *Corynebacterium diphtheriae* (16 mm), *Streptococcus gordonii* (16 mm), *Pseudomonas aeruginosa* (16 mm) and *Streptococcus mutans* (17 mm) in the aqueous extract of the bark.

The aqueous extract of the bark was found to be effective against *Candida albicans* with ZOI in the intermediate range of 16 mm. However, no significant anti-fungal activity was seen in hexane, chloroform, methanol extracts of bark.

Table 1: Antimicrobial activity of sequential extracts of bark extracts of *C. baccifera*

Microorganisms	Std.	Zone of inhibition (mm)			
		Solvent extracts of bark			
		HE	CE	ME	AE
Causative agents of diarrhea					
Escherichia coli	16±0.35	19±0.33	19±0.49	21±0.21	23±0.26
Shigella flexneri	30±0.22	12±0.82	13±0.18	18±0.19	15±0.71
Bacillus cereus	34±0.32	20±0.42	15±0.91	18±0.84	15±0.34
Causative agents of skin and wound infections					
Propionibacterium acnes	27±0.60	14±0.79	28±0.58	17±0.58	15±0.65
Pseudomonas aeruginosa	23±0.71	14±0.12	14±0.92	15±0.67	16±0.79
Pseudomonas cepacia	37±0.56	15±0.51	20±0.41	16±0.31	16±0.71
Causative agents of oral infections					

Streptococcus gordonii	35±0.13	16±0.84	15±0.41	20±0.34	16±0.83
Streptococcus mutans	36±0.52	12±1.19	15±0.23	12±0.83	17±0.13
Corynebacterium diphtheriae	32±0.51	14±0.45	20±0.55	21±0.53	16±1.15
Candida albicans	26±0.43	11±0.32	08±0.82	13±0.52	17±0.40
Candida glabrata	14±0.31	12±1.11	-	-	-
Fusarium	13±0.22	-	14±0.86	12±0.27	10±0.43

Mean ± SD; Std- Ciprofloxacin for bacteria and Ketoconazole for fungi; HE - Hexane Extract; CE - Choloform Extract; ME - Methanol Extract; AE - Aqueous Extract; -: ZOI <10mm *S. abony, S. aureus, N. asteroides, K. pneumoniae, C. krusei, C. parapsilosis, and Aspergillus sp.* were not inhibited

Minimum Inhibition Concentration (MIC)

The MIC of the sequential hexane, chloroform, methanol and aqueous extracts of bark of *C. baccifera* was assessed and the results are presented in Tables 2. Among the solvent extracts of bark of *C. baccifera*, significant anti-bacterial activity of aqueous extract was seen for *E. coli* at MIC of 62.5 µg/mL. Least MIC of 250 µg/mL was obtained in the methanolic extract for *Shigella flexneri* and hexane extract for *Bacillus cereus.* The chloroform extract of bark showed potent inhibition of *Propionibacterium acnes* and *Pseudomonas cepacia* with least MIC of 125 and 62.5 µg/mL respectively. The different solvent extracts of the bark showed antibacterial activity against *Pseudomonas aeruginosa* at a higher MIC of 500 µg/mL.

The hexane and methanolic extracts of bark showed the least MIC of 250 µg/mL for *Streptococcus gordonii*. While MIC of aqueous, chloroform and hexane extracts against *Streptococcus mutans* was 250 µg/mL. Similarly, MIC of 250 µg/mL of chloroform and methanolic extracts of bark exhibited potent inhibition of *Corynebacterium diphtheria.* Significant anti-fungal activity against *Candida albicans* was observed in MIC of 62.5 µg/mL of aqueous extract of bark.

Table 2: Minimum Inhibitory Concentration (MIC) of sequential extracts of bark of *C. baccifera*

Microorganisms	Std.	Minimum inhibitory concentration (µg/mL) Solvent extracts of bark			
		HE	CE	ME	AE
Causative agents of diarrhea					
Escherichia coli	62.5	125	125	125	62.5
Shigella flexneri	62.5	500	500	250	500
Bacillus cereus	15.62	250	500	500	500
Causative agents of skin and wound infections					
Propionibacterium acnes	500	500	125	250	500
Pseudomonas aeruginosa	31.25	500	500	500	500
Pseudomonas cepacia	15.62	250	62.5	500	500
Causative agents of oral infections					
Streptococcus gordonii	7.81	250	500	250	500
Streptococcus mutans	62.5	250	250	500	250
Corynebacterium diphtheriae	1000	500	250	250	500
Candida albicans	31.25	1000	-	1000	62.5
Candida glabrata	15.62	1000	-	1000	1000
Fusarium	0.97	1000	1000	1000	1000

Mean ± SD; Std-Ciprofloxacin for bacteria and Ketoconazole for fungi; HE - Hexane Extract; CE - Choloform Extract; ME - Methanol Extract; AE - Aqueous Extract; -: MIC > 1000 µg/mL *S. abony, S. aureus, N. asteroides, K. pneumoniae, C. krusei, C. parapsilosis, and Aspergillus* were not inhibited.

IV. DISCUSSION

Natural plant based antimicrobial compounds have enormous therapeutic potential as they do not cause side effects which are often associated with synthetic antimicrobials. The hexane, chloroform, methanol and aqueous extracts of bark parts of *C. baccifera* (Roth) Miq. were subjected to evaluation of antimicrobial activity against diarrhoea, skin, wound and oral infections causing selected pathogens, including 07 Gram positive, 06 Gram negative bacteria and 06 fungal strains. Previous studies have shown that antimicrobial potential could be due to the presence and distribution of phytochemicals such as flavonoids, phenolic compounds, tannins, coumarins, saponins and alkaloids.[14]

The results of antimicrobial activities of bark extracts of *C. baccifera* revealed that bark extract exhibited antimicrobial activities. The high total phenolic and flavonoid content in the bark along with presence of alkaloids, saponins and tannins could explain the strong antimicrobial potential of the bark. As reported by Briskin,[15] the combination of some of these phytochemicals could be responsible for the observed antimicrobial potential of the various solvent extracts. Considerable variation was observed in the degree of antimicrobial activity of the hexane, chloroform, methanol and aqueous solvent extracts of bark of *C. baccifera.* Aqueous followed by chloroform extracts showed significant antimicrobial activity in the bark of *C. baccifera*. This indicates that bioactive-antimicrobial molecule in bark may be polar to amphiphilic. Therese findings were in accordance with the findings reported by Thiruvanukarasu *et al*., revealing the bioactive-antimicrobial molecules in the bark to be polar in nature.[16] Moreover, according several researchers the variation in antimicrobial activity in different solvent extracts of bark, of *C. baccifera* could be attributed to the polar, non-polar nature of the bioactive compounds, insolubility or difference in degree of solubility of phytoconstituents in different solvents and their denaturation during extraction process.[17,18]

The causative agents of oral infection viz., *Streptococcus gordonii*, *Streptococcus mutans*, *Corynebacterium diphtheriae*, *Candida albicans*, *Candida glabrata* and *Fusarium* were also effectively inhibited by the extracts of *C. baccifera*. These findings are consistent with results of previous studies on *C. baccifera* and other species of Meliaceae.[16,19,20] The antimicrobial study results clearly indicate that the anti-bacterial activity was found to be more pronounced against the Gram positive bacteria followed by Gram negative bacteria and fungi. Five among the seven selected Gram positive pathogens, four of the six Gram negative and two of the six fungal pathogens were found to be effectively inhibited. Similar observations were reported by Thiruvanukarasu *et al*., wherein Gram positive bacteria were inhibited effectively when compared to Gram negative and fungal pathogens by *C. baccifera*.[16] This difference in sensitivity of the Gram positive and negative bacteria to the solvent extracts could be attributed to the inherent structural difference in their cell walls. The Gram negative bacteria possess an outer phospholipid membrane carrying the lipopolysaccharide component, which acts as a barrier to many antimicrobial agents including antibiotics due to its intrinsic nature of impermeability. However, the Gram positive bacteria are more susceptible due to its peptidoglycan cell wall which is not an effective permeability barrier.[21] In the present study significant anti-fungal activity was observed only against *Candida albicans* and *Fusarium* species. However, inhibition of *C. krusei*, *C. parapsilosis* and *Aspergillus niger* was not significant. *C. albicans* was less sensitive to plant extracts compared to Gram positive and Gram negative bacteria. This difference in susceptibility between eukaryotic cells of *C. albicans* and Fusarium and the prokaryotic cells of bacteria may be attributed to their difference in cell type which is in accordance with findings of antimicrobial studies carried out by Oskay and Sari,[22] and 23-Obeidat *et al*.[23]

V. CONCLUSION

In conclusion, the solvent extracts of bark of *C. baccifera* exhibited significant anti-bacterial activity against *Escherichia coli, Bacillus cereus, Propionibacterium acnes, Streptococcus gordonii and Corynebacterium diphtheriae.* Furthermore, solvent extracts of the bark showed anti-fungal activity against *Candia glabrata* and *Fusarium.*

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