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Evaluation of antimicrobial activity of solvent extracts and essential oil of fruits of *Cipadessa baccifera* (Roth.) Miq

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Abstract
In the present study we aimed to evaluate the antimicrobial activity of solvent extracts and essential oil of fruits 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 fruit samples were pulverized in an electric blender and the powdered material was stored in air tight containers for further analyses. The fruit samples were subjected to sequential extraction using Soxhlet apparatus, and extracted exhaustively in organic solvents such as, hexane, chloroform, methanol and water. The essential oil from fruit sample was extracted in Clevenger apparatus. The sequential extracts and essential oil of fruits 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 delineated both the essential oil and crude extracts of fruits of *C. baccifera* showed significant anti-bacterial potential against *Escherichia coli, Pseudomonas cepacia, Shigella flexneri, Streptococcus gordonii* and *Propionibacterium acnes*. Significant anti-fungal activity of the oil was observed against *Candida glabrata*, while the crude extracts were effective against *Candida albicans.* The broad spectrum of anti-bacterial activity of fruits of *C. baccifera* discovered in our study contributes scientific validity for its usage in treatment of dysentery, skin disorders and wound healing in traditional medicines.

Keywords: *Cipadessa baccifera*, Fruits, Antibacterial, Antifungal, Essential oil

1. Introduction
Essential oils (EOs) are aromatic volatile liquids secreted by oil secreting cells, glandular hairs or secretion ducts in different parts of the plants and stored in secretory cells, cavities, canals, epidermal cells or glandular trichomes. They are biosynthesized in plants through secondary metabolism giving it a characteristic odour, flavour and different colors ranging in shades of pale yellow to emerald green and sometimes even blue to dark brownish red [1,2], Essential oils are natural, complex hydrophobic mixtures containing from a dozen to several hundred components. The major components identified primarily include terpenes and terpenoids. Terpenes are made of different combinations of 5 carbon isoprene units and are classified into monoterpenes, sesquiterpenes, diterpene, and triterpene [3, 4]. A variety of other compounds found in the essential oils may include saturated and unsaturated hydrocarbons, aldehydes, esters, ethers, ketones, oxides, phenols, alcohols, sulphur and nitrogen containing compounds, coumarins and homologs of phenylpropanoids. The antimicrobial property of EOs known for centuries has found important applications today in diverse commercial products such as dental root canal sealer [5], antiseptics[6], show significant antifungal [7,8], antiviral [9,10], and antioxidant activities [11].

Infectious diseases caused by microorganisms is one of the leading causes of mortality worldwide which is a nagging challenge and is of great concern to the scientific community even to this day. Microorganisms are one of the oldest of creatures on this planet to have successfully evolved, adapted and survived all the vagaries of nature since millions of years [12].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 [13].

The discovery of antibiotics no doubt revolutionized medicine, drastically bringing down the morbidity and mortality rates due to infectious diseases. However, it led to rampant 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 phenomenon that has serious public health concern with economic and social implications [14]. As a consequence, 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 [15]. 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 [12].

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 [16,17]. Evaluation of the antimicrobial potency of ethnomedicinal plants such as *Cipadessa baccifera* which has been widely used in the treatment of dysentery, skin and wound infections etc… is relevant in this context[18]. With this background, the present study was designed to conduct with the main purpose of evaluation of antimicrobial activity of solvent extracts and essential oil of fruits of *C. baccifera* (Roth.) Miq.

2. Materials and Methods

2.1 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 [19,20].Further, the identification was authenticated by National Ayurveda and Dietetics Research Institute, Bangalore; vide voucher specimen number, RRCBI-8971.

2.2 Sample processing

The samples such as fruits 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 fruits were separated and shade dried for 20 days. The dried fruits were pulverized in an electric blender and the powdered material was stored in air tight containers for further analyses.

2.3 Sequential extraction

Dry and coarsely powered fruits of *C. baccifera* were subjected to sequential extraction using Soxhlet apparatus, and extracted exhaustively in organic solvents such as, hexane, chloroform, methanol and water[21]. Then the solvents were filtered and concentrated to dryness under pressure using rotary vacuum evaporator. The fruit extracts were air dried to remove the solvents completely, then sealed and stored at 4°C in a refrigerator for further studies.

2.4 Essential oil extraction

About 100 g each of the powered fruit samples was subjected to hydrodistillation for 10 hours in a Clevenger apparatus [22]. The extracted oil samples were collected by solubilizing in hexane. Hexane was then allowed to evaporate completely at room temperature. The process of hydrodistillation extraction was repeated several times; the oil obtained was pooled and stored in vials at 4ºC in a refrigerator for further analyses.

2.5 Antimicrobial activity

2.5.1 Test microorganisms

The sequential extracts, and the essential oil from fruits 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.

2.5.2 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 [23].

Further, based on the zone diameter the antimicrobial activity of standard antibiotic ciprofloxacin against bacteria was expressed as resistant (ZOI is $\leq$ 15 mm), intermediate (ZOI is between 16-20 mm) and sensitive/susceptible (ZOI is $\geq$ 21 mm) and for Ketoconazole against fungi was expressed as resistant (ZOI is $\leq$ 22 mm), intermediate (ZOI is between 23-29 mm) and sensitive/susceptible (ZOI is $\geq$ 30 mm) [23, 24]. 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.

2.5.3 Minimum Inhibitory Concentration (MIC) assay

Minimum inhibitory concentration (MIC) was determined by modified resazurin assay using microtiter-plate technique described by Sarker [25]. 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.

3. Results

3.1 Antimicrobial activity of sequential fruit extracts of *C. baccifera*

The hexane extract of the fruit of *Cipadessa baccifera* inhibited *Bacillus cere*us with highest inhibition zone of 23 mm. While comparatively smaller zones of inhibition in the range of 15-17 mm were obtained for *Escherichia coli*, *Shigella flexneri*, *Streptococcus gordonii* and *Corynebacterium diphtheriae* in hexane extract of fruit.

The bacterial strains *Escherichia coli*, *Bacillus cereus*, *Propionibacterium acnes*, *Pseudomonas aeruginosa* and *Pseudomonas cepacia* were found to be susceptible to the chloroform extract of the fruit with zones of inhibition ranging between 15-17 mm.

The methanolic extract of fruit exhibited significant anti-bacterial activity with highest zone of inhibition against *Propionibacterium acnes* (22 mm) followed by *Escherichia coli* (21 mm). While *Shigella flexneri*, *Bacillus cereus*, *Pseudomonas cepacia*, *Streptococcus gordonii* and *Corynebacterium diphtheriae* showed susceptibility to methanolic extract with intermediate zones of inhibition (16-19 mm). The highest zones of inhibition were obtained in the aqueous extract of the fruit of *C. baccifera* against *Escherichia* coli (20 mm) and *Bacillus cereus* (19 mm). Anti-bacterial activity of aqueous extract of fruit against *Pseudomonas cepacia* (16 mm) and Propionibacterium *acnes* (16 mm) was recorded with ZOI in the intermediate range.

The aqueous extract of fruit *of C. baccifera* was found to be effective against *Candida albicans*. Whereas there was no zone of inhibition against *Candida glabrata* in the aqueous extract of fruit. There was no significant anti-fungal activity observed in hexane, chloroform and methanol extracts of fruit of *C. baccifera*.

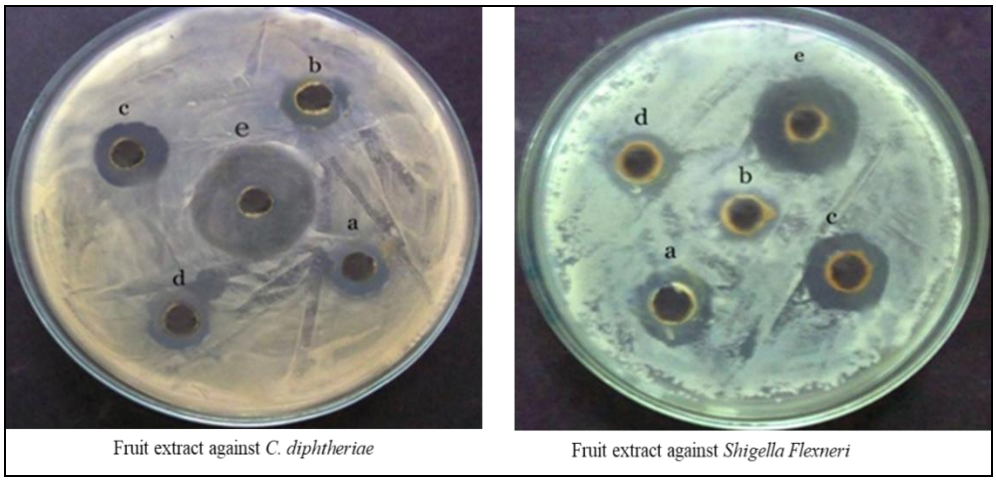
The solvent extracts of fruit of *C. baccifera* showed potent antimicrobial activity against *Escherichia coli*, *Bacillus cereus* and *Propionibacterium acnes*.

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

Microorganisms	Std.	Zone of inhibition (mm)			
		Solvent extracts of Fruit			
		HE	CE	ME	AE
Causative agents of diarrhea					
Escherichia coli	16±0.35	17±0.23	16±0.42	21±0.3	20±0.13
Shigella flexneri	30±0.22	16±0.25	13±0.16	19±0.43	15±0.66
Bacillus cereus	34±0.32	23±0.53	17±0.3	16±0.49	19±0.51
Causative agents of skin and wound infections					
Propionibacterium acnes	14±0.67	16±0.75	22±0.71	16±0.74	14±0.67
Pseudomonas aeruginosa	14±0.76	15±0.72	13±0.60	-	14±0.76
Pseudomonas cepacia	14±0.7	15±1.25	17±0.15	16±0.25	14±0.7
Causative agents of oral infections					
Streptococcus gordonii	15±0.2	11±0.30	17±0.91	12±1.23	15±0.2
Streptococcus mutans	14±1.21	13±0.13	12±1.35	12±0.75	14±1.21
Corynebacterium diphtheriae	15±1.34	14±0.3	17±0.53	15±0.63	15±1.34
Candida albicans	12±0.38	10±0.21	12±0.81	17±0.65	12±0.38
Candida glabrata	-	-	13±0.75	10±0.42	-
Fusarium	-	-	11±0.61	08±0.54	-

Mean ± SD; Std- Ciprofloxacin for bacteria and Ketoconazole for fungi; HE - Hexane Extract; CE - Chloroform 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



Fruit extract against *C. diphtheriae* Fruit extract against *Shigella Flexneri*

Fig 1: Antimicrobial activity of sequential extracts of fruit extracts of *C. baccifera*

3.2 Minimum Inhibition Concentration (MIC) fruit extracts of *C. baccifera*

The MIC of the sequential hexane, chloroform, methanol and aqueous extracts of leaves of *C. baccifera* was assessed and the results are presented in Tables 2.

The least MIC of 62.5 and 125 µg/mL were obtained in both hexane and methanolic extracts of fruit of *Cipadessa baccifera* for *Escherichia coli* and *Shigella flexneri* respectively. While *Bacillus cereus* was found susceptible to a MIC of 250 µg/mL of aqueous, hexane and chloroform extracts of fruit. The methanolic extract of fruit followed by hexane showed significant anti-bacterial activity against *Propionibacterium acnes* at MIC of 62.5 and 125 µg/mL respectively. Whereas, *Pseudomonas cepacia, Streptococcus gordonii* and *Corynebacterium diphtheriae* were susceptible in MIC of 125 µg/mL of both hexane and methanolic extracts of fruit. While the least MIC of 250 µg/mL was obtained in the aqueous and hexane extract for *Streptococcus mutans.*

Significant anti-candidal activity was observed against *C. albicans* in MIC of 125 µg/mL of hexane and aqueous extracts of fruit. The anti-fungal activity of the different solvent extracts of the fruit against *Candia glabrata* was not significant and also there was no significant difference among their MIC values. However, *Fusarium* was found to be susceptible in MIC of 500 µg/mL of hexane and methanolic extracts of the fruit.

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

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

Mean ± SD; Std-Ciprofloxacin for bacteria and Ketoconazole for fungi; HE - Hexane Extract; CE - Chloroform 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

2.3 Antimicrobial activity of essential oils of Fruits of *C. baccifera*

The essential oil of fruit of *Cipadessa baccifera* exhibited potent anti-bacterial activity against the *Shigella flexneri* and *Bacillus cereus* with inhibition zones of 17 mm and 15 mm respectively and MIC of 125 µg/mL for both bacteria. With smaller zones of inhibition, *Escherichia coli* and *Salmonella abony* were inhibited by MIC of 500 and 250 µg/mL respectively of the fruit oil (Table 16). The anti-bacterial activity of fruit oil against *Propionibacterium acnes* (19 mm) *Pseudomonas cepacia* (15 mm) was significant with MIC values of 62.5 µg/mL and 125 µg/mL respectively.

Table 3: Zone of inhibition of leaf oils of *C. baccifera*

Microorganisms	Std.	MIC (µg/mL) (mm)
		Fruit oil
Causative agents of diarrhea		
Escherichia coli	16±0.35	14±0.43
Shigella flexneri	30±0.22	17±0.32
Bacillus cereus	34±0.32	15 ± 0.72
Salmonella abony	35±1.22	12±0.22
Causative agents of skin and wound infections		
Propionibacterium acnes	27±0.60	19±0.80
Pseudomonas cepacia	23±0.71	15±0.3
Pseudomonas aeruginosa	37±0.56	11±0.12
Nocardia asteroides	39±0.83	13±0.13
Causative agents of oral infections		
Streptococcus gordonii	35±0.13	21± 0.4
Streptococcus mutans	36±0.52	12±0.17
Corynebacterium diphtheriae	32±0.51	14±0.62
Candida albicans	26±0.43	-
Candida glabrata	14±0.31	20 ±0.21
Fusarium	13±0.22	19 ±0.17

Mean ± SD; Std- Ciprofloxacin for bacteria and Ketoconazole for fungi; -: ZOI <10 mm
S. aureus, K. pneumoniae, C. krusei, C. parapsilosis, and Aspergillus niger were not inhibited

The inhibition of *Pseudomonas aeruginosa* was not significant. While the MIC of fruit of oil for *Nocardia asteroides* was 250 µg/mL. The anti-bacterial activity fruit oil against *Streptococcus gordonii* recorded the highest ZOI of 21 mm and significantly low MIC of 62.5 µg/mL followed by *Corynebacterium diphtheriae* with ZOI of 14 mm and MIC of 250 µg/mL. The fruit oil showed anti-fungal activity against *Candida glabrata* and *Fusarium* with pronounced inhibition zones of 20 mm and 19 mm respectively. The MIC of fruit oil against *Candida glabrata* was significant at 125 µg/mL, when compared to that for *Fusarium* (Table 3).

2.4 MIC of essential oils of Fruits of *C. baccifera*

The MIC of fruit oils of *Cipadessa baccifera* for the tested microorganisms ranged from 62.5 to over 1000 µg/mL (Table 4). The MIC nd fruit essential oil was significant against *Streptococcus gordonii* (62.50 µg/mL). Further, the MIC of fruit oil was significantly low at 62.50 µg/mL for *Propionibacterium acne*s and 125 µg/mL for *Bacillus cereus* and *Shig*ella *flexneri.* The fruit oil exhibited anti-bacterial activity against *Pseudomonas cepacia* with significant MIC of 125 µg/mL. The anti-bacterial activity of fruit oil for *Shigella flexneri*, *Propionibacterium acnes*, *Pseudomonas cepacia* was reflected by their significantly low MIC values.

The MIC of fruit essential oil against *Fusarium* was high and insignificant although its ZOI was in the susceptible intermediate range. The MIC values for the essential oils for the filamentous fungi indicate that they were not effectively inhibitory against the fungal strains at low concentration. However, among the *Candida* species, *Candida glabrata* was most sensitive, while rest of the strains was not inhibited by the essential oils.

Table 4: Minimum Inhibitory Concentration (MIC) of essential oil of fruits of *C. baccifera*

Microorganisms	Std.	Zone of Inhibition (mm)
		Fruit oil
Causative agents of diarrhea		
Escherichia coli	62.5	500
Shigella flexneri	62.5	**125**
Bacillus cereus	15.62	**125**
Salmonella abony	500	250
Causative agents of skin and wound infections		
Propionibacterium acnes	500	**62.50**
Pseudomonas cepacia	31.25	**125**
Pseudomonas aeruginosa	15.62	1000
Nocardia asteroides	31.25	250
Causative agents of oral infections		
Streptococcus gordonii	7.81	**62.50**
Streptococcus mutans	62.5	500
Corynebacterium diphtheriae	1000	250
Candida albicans	31.25	1000
Candida glabrata	15.62	**125**
Fusarium	0.97	1000

Mean ± SD; Std-Ciprofloxacin for bacteria and Ketoconazole for fungi; HE - Hexane Extract; CE - Chloroform 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

4. 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 &aqueous extracts, and essential oils of fruits 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 [26].

The results of antimicrobial activities of fruit extracts of *C. baccifera* revealed that fruit extract exhibited strong antimicrobial activities. The high total phenolic and flavonoid content in the fruit along with presence of alkaloids, saponins and tannins could explain the strong antimicrobial potential of the leaf. As reported by Briskin [27], 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 fruit, of *C. baccifera.* In the fruit, methanol followed by hexane extracts showed significant antimicrobial activity. This indicates that bioactive-antimicrobial molecule in fruit may be both polar and non-polar in nature. The variation in antimicrobial activity in different solvent extracts of fruit 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 [28, 29].

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 fruit extracts of *C. baccifera.* These findings are consistent with results of previous studies on *C. baccifera* and other species of Meliaceae [30-32].

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*., where Gram positive bacteria were inhibited effectively when compared to Gram negative and fungal pathogens by *C. baccifera* [32]. 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 [33]. 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, and Obeidat *et al.* [34, 35].

Antimicrobial potential of essential oils derived from plants is the basis of many applications especially in food preservation, aromatherapy and medicine [28]. The essential oils of fruits of *Cipadessa baccifera* were found to show varied degree of inhibition on the tested microorganisms. The antimicrobial study of essential oils of *C. baccifera* showed effective and broad spectrum antimicrobial activity wherein, the essential oil of fruit exhibited the moderate antimicrobial activity. Sesquiterpenes especially caryophyllenes are known to possess anti-inflammatory, anti-bacterial, anti-fungal and spasmolytic properties [36]. The antimicrobial activity in the fruit oil of *C. baccifera* could be attributed principally to the presence of a significant amount of 17.32% of sesquiterpene *viz.*, caryophyllene.

The root oil exhibited significant antibacterial activity against *Escherichia coli.* Diarrhoea causing pathogens were more susceptible to fruit oils. The skin and wound infections causing pathogens viz., *Propionibacterium acnes* and *Pseudomonas cepacia* were significantly inhibited by the fruit oils [37, 38].

In the present study, Gram positive bacteria were more susceptible to fruit essential oils of *C. baccifera* than the Gram negative bacteria, which is supported by the earlier researches on antimicrobial study of essential oils of various plants [39-40]. The differences in cell wall structure of Gram positive and Gram negative bacteria could be one of the possible reasons for higher antimicrobial activity of the essential oils of *C. baccifera* towards Gram positive bacteria [42].

The antimicrobial activity observed in the present study could possibly be explained by two modes of action. Firstly, the essential oil may disrupt the bacterial cell membrane resulting in the loss of ions, changes in membrane potential, disturbance of the proton pump, leading to the lysis of the bacteria. The second mechanism could involve the inhibition of production of amylase and protease which stops the toxin production, electron flow, thereby resulting in coagulation of the cell content as reported by Nazzaro [43]. Ultimately these events lead to bacterial cell death [44]. The anti-fungal activity of the oils was potent only against *Candida glabrata*, while the inhibition of other fungi was not significant. The mechanism of anti-fungal effect of essential oils is similar to bacteria as reported in earlier researches [1, 42].

5. Conclusion

In conclusion, the results of antimicrobial study revealed that both the essential oil and crude extracts of fruits of *C. baccifera* showed significant anti-bacterial potential against *Escherichia coli, Pseudomonas cepacia, Shigella flexneri, Streptococcus gordonii* and *Propionibacterium acnes*. Significant anti-fungal activity of the oil was observed against *Candida glabrata*, while the crude extracts were effective against *Candida albicans*. The broad spectrum of anti-bacterial activity of fruits of *C. baccifera* revealed in the present investigation gives scientific validity for its usage in treatment of dysentery, skin related disorders and wounds in traditional and folk medicines.

6. References

1. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of Essential oils: A Review. Food and Chemical Toxicology 2008;46:446-475.
2. Bassole IHN, Juliani HR. Essential Oils in combination and their Antimicrobial Properties. Molecules 2012;17:3989-4006.
3. Dorman HJD, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. Journal of. Applied. Microbiology 2000;88(2):308-16.
4. Rosangela DP, Vincenzo DF, Francesco V, Gianluigi M. In vitro antimicrobial activity of essential oils from mediterranean *Apiaceae, Verbenaceae* and *Lamiaceae* against foodborne pathogens and spoilage bacteria. Annals of Microbiology 2005;55(2):139-143.
5. Manabe A, Nakayama S, Sakamoto K. Effects of essential oils on erythrocytes and hepatocytes from rats & dipalitoyl phophatidylcholine-liposomes. Japanese Journal of Pharmacology 1987;44:77-84.
6. Cox SD, Mann CM, Markham JL. Interactions between components of the essential oil of *Melaleuca alternifolia*. Journal of Applied Microbiology 2001;91:492- 497.
7. Silva F, Ferreira S, Duarte A, Mendonca DI, Domingues FC. Antifungal activity of *Coriandrum sativum* essential oil, its mode of action against *Candida* species and potential synergism with amphotericin B. Phytomedicine, 2011;19(1):42-47.
8. Marongiu B, Piras A, Porcedda S, Falconieri D, Frau MA, Maxia A *et al*. Antifungal activity and chemical composition of essential oils from *Smyrnium olusatrum* L. (*Apiaceae*) from Italy and Portugal. Natural Product Research 2012;26(11):993-1003.
9. Astani A, Reichling J, Schnitzler P. Screening for Antiviral Activities of Isolated Compounds from Essential Oils. Evidence-Based Complementary and Alternative Medicine 2011, 253643-253651.
10. Vuko E, Dunkic V, Bezic N, Rusci M, Kremer D. Chemical composition and antiphytoviral activity of essential oil of *Micromeria graeca*. Natural Product Communications 2012;7(9):1227-1230.
11. Ortet R, Regalado EL, Thomas OP, Pino JA, Fernandez MD. Chemical composition and antioxidant activities of the essential oil from *Tornabenea Bischoffii* (*Apiaceae*). Natural Products Communications 2011;6(8):1179-82.
12. Emad MA. Plants: An alternative source for antimicrobials. Journal of Applied pharmaceutical science 2011;1(6):16-20.
13. Heinrich M, Barnes J, Gibbons S, Williamson EM. Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone, Edinbrugh 2004;1:245-252.
14. Neu HC. The crisis in antibiotic resistance. Science, 1992;257(5073):1064-73.
15. Aminov RI. A Brief History of the Antibiotic Era: Lessons Learned and Challenges for the Future. Frontiers in Microbiology 2010;1:134.
16. Tiwar S, Singh A. Toxic and sub-lethal effects of oleadrin on biochemical parameters of freshwater air breathing murrel, Chant punctatus (Bloch.). Indian Journal of Experimental Biology 2004;42:413-418.
17. Lewis K, Ausubel FM. Prospects of plant derived antibacterials. Natural. Biotechnology 2006;24:1504-1507.
18. Jeevan RA, Bhakshu LM, Venkata Raju RR. In-vitro antimicrobial activity of certain medicinal plants form Eastern Ghats, India, used for skin diseases. Journal of Ethnopharmacology 2004;90(2, 3):353-357.
19. Saldanha Cecil J. Flora of Karnataka. Department of Science and Technology India, Oxford and IBH publishing Co. Pvt. Ltd. Calcutta. New Delhi 1996.
20. Saldanha, Cecil J, Nicolson, Dan H. Flora of Hassan District Karnataka, India. Published for Smithsonian Institute and National Science foundation Washington

DC. Amerind Publishing Co. Pvt. Ltd. New Delhi 1976.
21. Raman N. Phytochemical techniques. New India Publishing Agency. New Delhi 2006.
22. Clevenger JH. Apparatus for the determination of volatile oil. Journal of American Pharmaceutical Association 1928;17:346.
23. National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for antimicrobial susceptibility testing, 8th Informational Supplement. M100 S12, Villanova Pa, USA 2002.
24. Clinical Laboratory Standards Institute (CLSI) Performance standards for antimicrobial susceptibility testing; 19thinformational supplement. Document M100-S19. Clinical Laboratory Standards Institute, Wayne, PA 2009.
25. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 2007;42:321-324.
26. Aboaba OO, Efuwape BM. Antibacterial Properties of Some Nigerian Species. Bio Research. Communications, 2001;13:183-188.
27. Briskin DP. Medicinal Plants and Phytomedicines. Linking Plant Biochemistry and Physiology to Human Health. Plant Physiology 2000;124:507-514.
28. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews 1999;12(4):564-582.
29. Igbinosa OO, Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). African Journal of Pharmacy and Pharmacology 2009;3(2):58-62.
30. Deepika S, Yash P. Preliminary and Pharmacological Profile of Melia azedarach L.: An Overview. Journal of Applied Pharmaceutical Science 2013;3(12):133-138.
31. Reddy YRR, Kumari CK, Lokanatha O, Mamatha S, Reddy CD. Antimicrobial activity of *Azadirachta Indica* (neem) leaf, bark and seed extracts. International Journal of Research in Phytochemistry and Pharmacology, 2013;3:1-4.
32. Thirunavukarasu T, Santhanan LK, Tamilarasan M, Sivamani S, Sangeetha D, Rajesh TP. In vitro antimicrobial, antioxidant, haemolytic, thrombolytic activities and phytochemical analysis of *Cipadessa baccifera* leaves extract. International Journal of Phytomedicine 2014;6(1):109-114.
33. Tortora GJ, Funke BR, Case CL. Microbiology: An Introduction. Benjamin Cummings, San Francisco 2001.
34. Oskay M, Sari D. Antimicrobial screening of some Turkish medicinal plants. Pharmaceutical Biology 2007;45:176-181.
35. Obeidat M, Shatnawi M, Al-alawi M, Enas A, Hanee A, Masia A *et al*. Antimicrobial activity of crude extracts of some plant leaves. Research Journal of Microbiology, 2012;7:59-67.
36. Almeida LFR, Delachiave MEA, Marques MOM. Composition of the essential oil of rubim (*Leonurus sibiricus* L.- Lamiaceae). Revista Brasileira de Plantas Medicinais 2005;8:35-38.
37. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (*Azadirachta indica*). Current Science 2002;82(11):1336-1345.
38. Upadhyay RK, Dwivedi P, Ahmad S. Screening of Antibacterial Activity of Six Plant Essential Oils against Pathogenic Bacterial Strains. Asian Journal of Medical Sciences 2010;2(3):152-158.
39. Nostro A, Germano MP, D'Angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology 2000;30:379-384.
40. Nevas M, Korhonen AR, Lindström M, Turkki P, Korkeala H. Antibacterial efficiency of Finnish spice essential oils against pathogenic and spoilage bacteria. Journal of Food Protection 2004;67:199-202.
41. Preuss HG, Echard B, Enig M, Brook I, Elliott TB. Minimum inhibitory concentrations of herbal essential oils and monolaurin for Gram-positive and Gram-negative bacteria. Molecular and Cell Biochemistry 2005; 272:29-34.
42. Burt S. Essential Oils: their antibacterial properties and potential applications in food-a Review. International Journal of Food Microbiology 2004;94(3):223-253.
43. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of Essential Oils on Pathogenic Bacteria. Pharmaceuticals 2013;6(12):1451-1474.
44. Cox SD, Mann CM, Markham JL. Interactions between components of the essential oil of *Melaleuca alternifolia*. Journal of Applied Microbiology 2001;91:492-497.