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Evaluation of Antimicrobial Activity of Solvent Extracts and Essential Oil of Leaves of *Cipadessa baccifera* (Roth.) Miq.

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A B S T R A C T

The present study was designed to conduct with the main purpose of evaluation of antimicrobial activity of solvent extracts and essential oil of leaves 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 leaf samples were pulverized in an electric blender and the powdered material was stored in air tight containers for further analyses. The leaf 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 leaf sample was extracted in Clevenger apparatus. The sequential extracts and essential oil of leaf 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. Results revealed that essential oils and crude solvent extracts leaves of *Cipadessa baccifera* showed significant anti-bacterial potential against diarrhoea, skin wound and oral pathogens inhibiting both Gram positive, Gram negative and fungal species. The broad spectrum of anti-bacterial activity 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.

Introduction

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

(Emad, 2011). 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 (Heinrich *et al.*, 2004).

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 (Neu, 1992). 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 (Aminov, 2010). 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 (Emad, 2011).

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 (Tiwar and Singh, 2004, Lewis and Ausubel, 2006). 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. (Jeevan *et al.*, 2004) is relevant in this context. 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 leaves of *C. baccifera* (Roth.) Miq.

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 (Saldanha *et al.*, 1976, Saldanha and Cecil, 1996).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 leaves 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 plant parts *viz.*, leaves, bark, fruits and roots were separated and shade dried for 20 days.

The dried plant parts were pulverized in an electric blender and the powdered material was stored in air tight containers for further analyses.

Sequential extraction

Dry and coarsely powered plant parts of *C. baccifera* were subjected to sequential extraction using Soxhlet apparatus (Raman, 2006). Each of the plant part was extracted exhaustively in organic solvents such as, hexane, chloroform, methanol and water. 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.

Essential oil extraction

About 100 g each of the powered samples was subjected to hydrodistillation for 10 hours in a Clevenger apparatus (Clevenger, 1928). 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.

Antimicrobial activity

Test microorganisms

The sequential extracts of leaf, bark, fruit, root and the essential oil from leaves, fruits and roots 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 (NCCLS, 2002). 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) (CLSI, 2009; NCCLS, 2002). 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 (2007).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.

Results and Discussion

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

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

The hexane extract of the leaf showed significant anti-bacterial activity with highest zone of inhibition against *Pseudomonas cepacia* (21 mm). While *Escherichia coli, Shigella flexneri, Bacillus cereus,* and *Propionibacterium acnes* were inhibited with zones of inhibition in the intermediate range of 16-19 mm.

The chloroform extract of leaf showed potent activity against *Pseudomonas cepacia* (20 mm). While *Escherichia coli, Shigella flexneri, Bacillus cereus, Pseudomonas aeruginosa, Streptococcus gordonii* and *Corynebacterium diphtheriae* were inhibited, recording inhibition zones of 16-19 mm.

Escherichia coli was found to be most susceptible to the methanolic extract of the leaf with an inhibition zone of 23 mm. Significant zones of inhibition were observed for *Bacillus cereus* (21 mm), *Propionibacterium acnes* (20 mm), *Pseudomonas cepacia* (20 mm), *Pseudomonas aeruginosa* (18 mm), Streptococcus *gordonii* (20 mm) and *Corynebacterium diphtheriae* (16 mm) in the methanolic extract of leaf.

In the aqueous extract of leaf, a significant zone of inhibition was observed against *Escherichia coli* (21 mm) followed by *Bacillus cereus* (20 mm). Inhibition of *Propionibacterium acnes, Pseudomonas aeruginosa, Pseudomonas cepacia, Streptococcus gordonii, Streptococcus mutans*

and Corynebacterium diphtheriae in aqueous extract of leaf, with comparatively smaller zones of inhibition in the range of 15-19 mm were also obtained.

The anti-fungal activity of aqueous extract of leaf against *Candida albicans* and hexane extract against *Candida glabrata* with inhibition zone of 16 mm for both was observed. Whereas the anti-candidal activity of chloroform and methanol extracts of the leaf was not significant. Also *Fusarium* was not effectively inhibited by the solvent extracts of the leaf.

The agar well diffusion assay indicated potent anti-bacterial activity of leaf extracts against *Escherichia coli, Bacillus cereus, Propionibacterium acnes Pseudomonas cepacia* and *Streptococcus gordonii* with significant zones of inhibition.

Minimum Inhibition Concentration (MIC)

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.

Among the leaf extracts of *Cipadessa baccifera* the MIC of 62.5 µg/mL of methanolic and aqueous extracts for *Escherichia coli* was significant, indicating their anti-bacterial potential. The anti-bacterial activity of chloroform extract against *Shigella flexneri*, aqueous and methanolic extract against *Bacillus cereus* was found to be potent, indicated by the significantly low MIC of 125 µg/mL.

Significant antibacterial activity of methanolic extracts of leaf with MIC of 62.5 µg/mL, followed by both aqueous and hexane extracts with MIC of 125 µg/mL were observed against *Propionibacterium acnes*. In the case of *Pseudomonas cepacia* the least MIC of 31.5 µg/mL was obtained in hexane extract of the leaf followed by MIC of 62.5 µg/mL in chloroform extract. All the solvent extracts of leaf at MIC of 250 µg/mL were found to effectively inhibit *Pseudomonas aeruginosa.*

The MIC of methanolic and chloroform extracts of leaf for *Streptococcus gordonii* was the least at 125 µg/mL. All three solvent extracts viz., aqueous, chloroform and hexane showed antibacterial activity against *Streptococcus mutans* with significant MIC of 250 µg/mL. While *Corynebacterium diphtheriae* was effectively inhibited in 250 µg/mL MIC of chloroform and hexane extracts of leaf. Significant antifungal activity of aqueous extract of leaf against *Candida albicans* was seen at MIC of 250 µg/mL. Whereas, the inhibition of *Candida glabrata* in hexane extract of leaf and *Fusarium* in aqueous extract was found to be potent at MIC of 500 µg/mL.

Antimicrobial activity of essential oils of leaves of *C. baccifera*

Significant antibacterial activity of leaf oil at MIC of 125 µg/mL and intermediate inhibition zones of 16 mm, 17 mm and 20 mm were observed against *Shigella flexneri, Bacillus cereus and Streptococcus abony* respectively (Table 15-16). The inhibition of *Escherichia coli* by essential oil of leaf was not significant. The leaf oil exhibited effective antibacterial activity against *Propionibacterium acnes, Pseudomonas cepacia, Pseudomonas aeruginosa* and *Nocardia asteroides* with zones of inhibition in the range of 13-16 mm (Table 15). However, MIC value of 125 µg/mL of leaf oil observed against *Propionibacterium acnes, Pseudomonas cepacia* was significant and for *Nocardia asteroids* it was less significant at 250 µg/mL. The maximum inhibition zone of 21 mm and the least MIC of 62.5µg/mL were obtained in the leaf oil for *Streptococcus gordonii*. Significant antibacterial activity was also seen against *Corynebacterium*

diphtheriae (ZOI=16 mm MIC 125 µg/mL) followed by *Streptococcus mutans* (ZOI=15 mm; MIC=250 µg/mL). The anti-fungal activity of leaf oil was significant against *Candida glabrata* recording the highest zone of inhibition (21 mm) and least MIC value of 62.5µg/mL. However, no significant activity was noted against *Staphylococcus aureus, Klebsiella pneumoniae, Candida krusei, Candida albicans, Candida parapsilosis* and *Fusarium*(Table 3).

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 leaf 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 (Aboaba, 2001).

The results of antimicrobial activities of leaf extracts of *C. baccifera* revealed that leaf extract exhibited strong antimicrobial activities. The high total phenolic and flavonoid content in the leaf along with presence of alkaloids, saponins and tannins could explain the strong antimicrobial potential of the leaf. As reported by Briskin (2000), 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 leaf, of *C. baccifera*. The methanolic extract of leaf exhibited potent inhibitory effect which reveals that the bioactive compounds of leaf are better extracted with polar solvents such as methanol than the non-polar solvents.

Similar results were reported by Thiruvanukarasu *et al.,* (2014) revealing the bioactive-antimicrobial molecules in the leaf to be polar in nature.

In the present investigation, the inhibition of *Propionibacterium acnes* by the leaf extracts of *C. baccifera* was observed to be significant when compared to the findings of earlier study which revealed insignificant antibacterial effect against *Propionibacterium acnes* by *A. indica* despite the plant being known as a potent antibacterial agent (Pratibha *et al.,* 2012). 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 by Deepika and Yash, (2013); Reddy *et al.,* (2013) and Thirunavukarasu *et al.,* (2014).

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.,* (2014) where Gram positive bacteria were inhibited effectively when compared to Gram negative and fungal pathogens by *C. baccifera*. 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 (Tortora *et al.*, 2001). 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 (2007) and Obeidat *et al.*, (2012).

Antimicrobial potential of essential oils derived from plants is the basis of many applications especially in food preservation, aromatherapy and medicine (Cowan, 1999). The essential oils 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 leaf exhibited the highest antimicrobial activity. Sesquiterpenes especially caryophyllenes are known to possess anti-inflammatory, anti-bacterial, anti-fungal and spasmolytic properties (Almeida *et al.*, 2005).

Table.1 Antimicrobial activity of sequential extracts of leaf extracts of *C. baccifera*

Microorganisms	Std.	Zone of inhibition (mm)			
		Solvent extracts of leaf			
		HE	CE	ME	AE
Causative agents of diarrhea					
Escherichia coli	16±0.35	19±0.48	16±0.42	23±0.22	21±0.71
Shigella flexneri	30±0.22	16±0.3	19±0.31	15±0.22	15±0.32
Bacillus cereus	34±0.32	17±0.3	18±0.41	21±2.1	20±0.33
Causative agents of skin and wound infections					
Propionibacterium acnes	27±0.60	18±0.59	15±0.53	20±033	19±0.81
Pseudomonas aeruginosa	23±0.71	14±0.61	14±0.70	18±072	15±0.69
Pseudomonas cepacia	37±0.56	21±1.08	20±0.64	20±042	16±0.31
Causative agents of oral infections					
Streptococcus gordonii	35±0.13	15±0.53	18±0.51	20±0.96	15±0.16
Streptococcus mutans	36±0.52	14±0.20	13±0.79	14±0.17	15±0.23
Corynebacterium	32±0.51	15±0.92	17±1.01	16±0.23	15±0.7
Candida albicans	26±0.43	15±0.35	13±0.68	14±0.61	16±0.51
Candida glabrata	14±0.31	16±0.22	-	10±0.11	08±0.6
Fusarium	13±0.22	11±0.74	12±0.84	14±0.12	14±0.41

Mean ± SD; Std- Ciprofloxacin for bacteria and Ketoconazole for fungi ; HE - Hexane Extract; CE - Choloform Extract ; ME - Methanol Extract; AE - Aqueous Extract ;

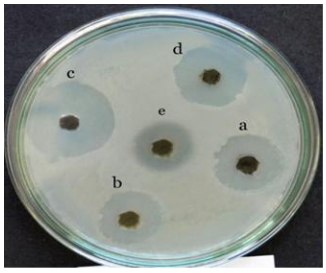
-: ZOI <10mm *S. abony, S. aureus, N. asteroides, K. pneumoniae, C. krusei, C. parapsilosis, and Aspergillus sp.* were not inhibited

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

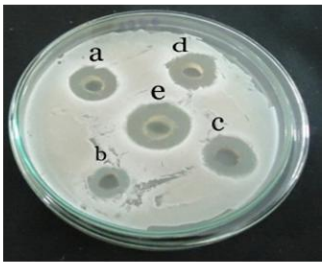
Microorganisms	Std.	Minimum inhibitory concentration			
		Solvent extracts of leaf			
		HE	CE	ME	AE
Causative agents of diarrhea					
Escherichia coli	62.5	125	500	**62.5**	**62.5**
Shigella flexneri	62.5	250	**125**	500	500
Bacillus cereus	15.62	500	500	**125**	**125**
Causative agents of skin and wound infections					
Propionibacterium acnes	500	125	500	**62.5**	125
Pseudomonas aeruginosa	31.25	250	250	250	250
Pseudomonas cepacia	15.62	**31.5**	**62.5**	125	500
Causative agents of oral infections					
Streptococcus gordonii	7.81	250	125	125	250
Streptococcus mutans	62.5	250	250	500	250
Corynebacterium diphtheriae	1000	250	250	500	500
Candida albicans	31.25	500	1000	1000	250
Candida glabrata	15.62	500	1000	1000	1000
Fusarium	0.97	1000	1000	1000	500

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

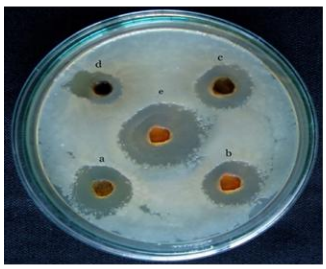
Fig.1 Antimicrobial activity of sequential extracts of leaf extracts of *C. baccifera*



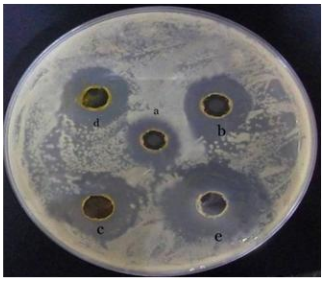
Leaf extract against *E. coli*



Leaf extract against *P. acnes*



Leaf extract against *P. cepacia*



Leaf extract against *B. cereus*

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

Microorganisms	Std.	Zone of Inhibition (mm)
		Leaf oil
Causative agents of diarrhea		
Escherichia coli	16±0.35	-
Shigella flexneri	30±0.22	16±0.12
Bacillus cereus	34±0.32	17±0.84
Salmonella abony	35±1.22	**20±0.63**
Causative agents of skin and wound infections		
Propionibacterium acnes	27±0.60	14±0.96
Pseudomonas cepacia	23±0.71	16±0.29
Pseudomonas aeruginosa	37±0.56	13±0.21
Nocardia asteroides	39±0.83	16±0.32
Causative agents of oral infections		
Streptococcus gordonii	35±0.13	**21±0.3**
Streptococcus mutans	36±0.52	15±1.2
Corynebacterium diphtheriae	32±0.51	16±1.09
Candida albicans	26±0.43	10 ±0.81
Candida glabrata	14±0.31	**21±0.56**
Fusarium	13±0.22	12 ±0.31

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 comparatively higher antimicrobial activity in the leaf oil of *C. baccifera* could be attributed principally to the presence of a significant amount of 17.32% of sesquiterpene *viz.*, caryophyllene.

Diarrhoea causing pathogens were more susceptible to leaf oils. The skin and wound infections causing pathogens *viz.*, *Propionibacterium acnes* and *Pseudomonas cepacia* were significantly inhibited by leaf oils. In the present investigation, the leaf essential oil of *C. baccifera* effectively inhibited the oral infection causing pathogens such as, *Streptococcus gordonii*, *Streptococcus mutans*, *Corynebacterium diphtheria* and *Candida glabrata*. Therese findings were in accordance with various other research studies reported in the literature (Biswas *et al.*, 2002; Upadhyay *et al.*, 2010).

In the present study, Gram positive bacteria were more susceptible to leaf 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 (Nostro, 2000; Nevas *et al.*, 2004; Preuss *et al.*, 2005). 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 (Burt, 2004).

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 (2013). Ultimately these events lead to bacterial cell death (Cox *et al.*, 2011). 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 by Burt, (2004) and Bakkali *et al.*, (2008).

Essential oils and crude solvent extracts leaves of *C. baccifera* showed significant anti-bacterial potential against diarrhoea, skin wound and oral pathogens inhibiting both Gram positive, Gram negative and fungal species. The broad spectrum of anti-bacterial activity 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.

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