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Original Research Article

Leucas aspera (Willd.) L.: Antibacterial, antifungal and mosquitocidal activities

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ABSTRACT

The various organic extracts from the leaves of *Leucas aspera* were screened for their antibacterial, antifungal and larvicidal activities against selected bacterial, fungal strains and mosquito larvae of *Culex quinquefasciatus*. Antimicrobial activity was carried out using the disc-diffusion method and MIC of the extract was tested by the broth microdilution method. The results revealed that all the extracts showed antibacterial and antifungal activities against selected microbes at 1, 2.5, 5 and 10 mg/disc concentrations. The maximum zone of inhibitions were recorded in dichloromethane (DCM) leaf extract of *L. aspera* (Willd.) L. against *S. aureus* (23.4 ± 2.90 mm), *E. coli* (20.3 ± 1.56 mm), *B. subtilis* (17.1 ± 2.04 mm), *P. aeruginosa* (16.5 ± 1.05 mm), *P. vulgaris* (16.1 ± 2.56 mm) and *K. pneumonia* (15.1 ± 3.66 mm) at 10 mg/disc concentration. The DCM extract of *L. aspera* (Willd.) L. exhibited significant growth inhibition against *T. viride* (29.2 ± 2.00 mm), *C. albicans* (24.4 ± 0.80 mm), *A. flavus* (22.8 ± 0.36 mm) and *E. floccosum* (19.5 ± 2.17 mm). The minimum inhibitory concentration (MIC) ranges between 75.5-425.5 µg/mL and 125-425 µg/mL against bacterial and fungal pathogens, respectively. In addition, DCM extracts of *L. aspera* (Willd.) L. showed 100% larvicidal activity against *C. quinquefasciatus* at 1000 ppm concentration. The biological activities could contribute to the medicinal properties of the plants, and also provide more scientific authentication of traditional medicinal plants to fight against the various infectious diseases.

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1. Introduction

Medicinal plant extracts have been used conventionally to cure various infectious diseases caused by bacteria and fungi (Pavunraj et al., 2014; Mohammadhosseini, 2017; Mohammadhosseini et al., 2017a). The medicinal properties of plants have been extensively investigated, due to their strong pharmacological activities, low toxicity and economic feasibility (Ignacimuthu et al., 2009; Mohammadhosseini, 2016). Many scientific investigations have been reported about the efficacious and chemotherapeutic role of medicinal plants in the treatment of diverse diseases (McGaw et al., 2013). Medicinal plants are a sign of many traditional claims concerning the value of natural products in health care (Nair et al., 2005). A wide range of plants are used as raw drugs which possess various medicinal properties (Verma, 2016). With the increase in resistance and the global awareness, the effective life span of any antibiotic is limited. In this regard, new sources especially plant are being intensely investigated (Mohamad et al., 2011). Researchers have immense interests in screening and evaluating medicinal plants for development of new therapeutics (Srivastava et al., 2011; Mohammadhosseini et al., 2017b). Recently, the methanol extract of *Anogeissus acuminata*, *Azadirachta indica*, *Bauhinia variegata*, *Boerhaavia diffusa*, *Punica granatum*, *Soymida febrifuga*, *Terminalia chebula*, *Tinospora cordifolia* and *Tribulus terrestris* were evaluated against 11 bacteria from Gram positive and

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negative stains; all the plants showed antibacterial activities; among them *A. acuminata*, *P. granatum* and *S. febrifuga* showed maximum activity (Mishra et al., 2017). The antimicrobial and antifungal activities of plant extracts may reside in an array of components, including aldehyde, phenolic compounds, olefinic acid and alkaloids (Eliza et al., 2009; Tchinda et al., 2017).

Mosquitoes are medically most important and responsible for transmitting the vector-borne diseases, parasites and pathogens which continue to have a devastating impact on human beings, public hygiene and ecological perspectives (Govindarajan et al., 2008). Mosquitoes under the genus *Culex* are the vectors of encephalitis and filariasis (WHO, 2010). Lymphatic filariasis affects 120 million people in 73 countries in Africa, India, Southeast Asia and Pacific Islands. These diseases cause high levels of morbidity and mortality. India alone contributes about 40% of global filariasis burden and the estimated annual economic loss is about 720 corers (Hotez et al., 2004).

Leucas aspera (Willd.) L. Lamiaceae as an ethnomedicinal plant, is commonly known as 'Thumbai' and distributed throughout India possessing promising therapeutic properties. Traditionally, the whole plant is taken orally for analgesic (Saundane et al., 2000), antipyretic (Gupta et al., 2011), hepatoprotective (Raju and Rao, 2010), antirheumatic and anti-inflammatory (Srinivasan et al., 2001) treatments. The leaf juice is used as an external application for psoriasis, chronic skin eruption and painful swelling (Anonymous, 1994; Chopra et al., 1996). Its anti-inflammatory activity has been shown in rats, through prostaglandin inhibition (Goudgaon et al., 2003). The entire plant is also used as an insecticide (Bagavan et al., 2009a). The whole plant of *L. aspera* (Willd.) L. is reported to contain oleanolic acid, ursolic acid and sitosterol (Mangathayaru et al., 2005). *L. aspera* (Willd.) L. used for as medicinal properties *viz.*, anti-inflammation, antipyretic, antiseptic and anti-snake venom (Singh and Gindha, 2017). The present study was carried out to screen hexane, dichloromethane (DCM), acetone and aqueous extracts from the leaves of *L. aspera* (Willd.) L. against selected bacterial and fungal strains as well as fourth instar mosquito larvae of *C. quinquefasciatus*.

2. Experimental

2.1. Collection and identification of plant materials

The fresh and healthy leaves of *L. aspera* (Willd.) L. were collected from Thandarai Village, Kanchipuram District, Tamil Nadu, India during the period of June, 2013. Plant specimen was identified by authentic plant taxonomist. A voucher specimen [UM-Zool - H10] has been preserved and kept in the Department of Zoology, University of Madras for further authentication.

2.2. Preparation of leaf extracts

The collected leaves were shade dried at room temperature and ground in a manual mill. One kilogram powder was extracted with 3 L of hexane (1:3 w/v) for 72 hours. The extract was filtered through a Buchner funnel with Whatmann number 1 filter paper and the residue was dried. The filtrate was evaporated to dryness under reduced pressure (500 mm Hg) using rotary vacuum evaporator at 40 ºC. The remains of the plant material were extracted using DCM, acetone and water sequentially in a similar manner using cold percolation method by Pavunraj et al. (2016). The yields of different extracts obtained from *L. aspera* (Willd.) L. were hexane (8 g), DCM (12 g), acetone (10 g) and aqueous (11 g). The crude extracts were stored at 4 ºC for further use.

2.3. Microorganisms

The following common standard strains were used for screening of antibacterial and antifungal activities: Bacteria-*Bacillus subtilis* (MTCC 3053), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus vulgaris* (MTCC 1771) and *Klebsiella pneumoniae* (ATCC 15380) along with fungi including *Aspergillus flavus* (MTCC 1344), *Candida albicans* (MTCC3018), *Epidermophyton floccosum* (MTCC 7880) and *Trichoderma viride* (MTCC No. 164).

2.4. Reference drugs

Streptomycin and ciprofloxacin were used as positive controls; they were dissolved in dimethyl sulfoxide (10% DMSO) and stored at -20 ºC.

2.5. Antibacterial and antifungal assays

Antimicrobial activity was carried out using disc diffusion method described by Dube et al. (2017). Accordingly, Petri plates were poured with 20 mL of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai) for bacteria and potato dextrose agar (PDA) for fungi. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The experiments were conducted at four different concentrations of the crude extracts (1, 2.5, 5 and 10 mg per disc) with three replicates. The loaded discs were placed on the surface of the medium and left for 30 min. Negative control was prepared using respective solvent. Streptomycin (10 mg/disc) was used as positive control for antibacterial and ciprofloxacin for antifungal tests. The plates were incubated for 24 h at 30 ºC for bacterial and 48 h at for fungal strains. Zone of inhibition was recorded in millimetres (mm).

2.6. Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was determined by microbroth dilution assay (Al-Bayati, 2008). Culture of each test organism was inoculated on Petri plates containing Muller Hinton Agar medium and Potato Dextrose Agar medium; dichloromethane leaf extract was placed into the wells over a concentration range of 25-500 µg/mL. The plates were incubated at 30 ºC. Minimum inhibitory concentration (MICs) was determined after 24 h for the bacteria and 48 h for fungi. MIC was determined as the least concentration of the leaf extract of *L. aspera* (Willd.) L. that inhibited the growth of the test microorganisms.

2.7. Larvicidal bioassay

Larvicidal bioassay of the various crude extracts was screened at 1000 ppm concentration against 4th instar larvae of *C. quinquefasciatus* a common vector of filariasis. The larvae were obtained from laboratory-established colony. Twenty-five larvae were released into 500 mL glass beakers containing 250 mL of tap water. The experiments were carried out at 26 ± 2 ºC. Five replicates for each concentration were maintained under the laboratory conditions along with untreated control. Mortality of larvae was recorded up to 96 h. The percent mortality was calculated by using Abbott's formula (Abbott, 1925) according to the following formula (equation 1).

$$\text{Corrected mortality} = \frac{\text{\% mortality in treatment} - \text{\% mortality in control}}{100 - \text{\% mortality in control}} \times 100 \quad \text{(Eqn. 1)}$$

2.8. Statistical analysis

The data related to zone of inhibition activity was analysed using one-way ANOVA. Significant differences between treatments were determined using Tukey's HST multiple range tests ($P \leq 0.05$).

3. Results and Discussion

Different phytochemicals *viz.*, alkaloids, glycosides, flavonoids, saponins, tannins and terpenoids were present in *L. aspera* (Willd.) L. (Das et al., 2011). It's responsible for their biological activity against tested organisms.

3.1. Antibacterial activity

In the present study, different crude extracts were obtained from the leaves of *L. aspera* (Willd.) L. and evaluated against six bacterial strains. Among the tested extracts, DCM extract revealed a broad spectrum of antibacterial activity against *B. subtilis* (17.1 ± 2.04 mm), *S. aureus* (23.4 ± 2.90 mm), *E. coli* (20.3 ± 1.56 mm), *P. aeruginosa* (16.5 ± 1.05 mm), *P. vulgaris* (16.1 ± 2.56 mm) and *K. pneumonia* (15.1 ± 3.66 mm) at 10 mg/disc. Acetone extract inhibited the growth of *B. subtilis* (15.3 ± 1.85 mm), *S. aureus* (21.8 ± 2.61 mm), *E. coli* (17.5 ± 1.25 mm), *P. aeruginosa* (14.0 ± 0.57 mm), *P. vulgaris* (13.4 ± 0.50 mm) and *K. pneumonia* (14.3 ± 0.68 mm) at the concentration of 10 mg/disc. The reference drug (Streptomycin) showed zone of inhibition ranged between 22.4 to 27.1 mm under the same conditions. The activity of DCM extract of *L. aspera* (Willd.) L. leaves found to be more pronounced than the hexane,

Table 1

Antibacterial activity of hexane, DCM, acetone and aqueous leaf extracts of *Leucas aspera* (Willd.) L.

Solvent Used	(mg/disc)	Microorganisms tested					
		B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***P. vulgaris***	***K. pneumonia***
		Zone of inhibition (mm)					
Hexane	1	8.0 ± 1.52^{b}	09.8 ± 1.06^{b}	8.70 ± 1.01^{bc}	7.5 ± 1.32^{b}	8.8 ± 0.11^{bc}	7.7 ± 0.40^{b}
	2.5	10.5 ± 0.5^{bc}	13.4 ± 1.10^{bc}	12.8 ± 0.64^{c}	8.0 ± 1.15^{b}	10.6 ± 0.50^{bc}	9.3 ± 0.36^{bc}
	5	11.0 ± 0.57^{bc}	16.4 ± 0.50^{c}	15.8 ± 1.61^{cd}	10.0 ± 1.52^{bc}	11.8 ± 1.0^{bc}	11.0 ± 0.57^{bc}
	10	14.3 ± 0.40^{c}	17.3 ± 2.80^{c}	16.4 ± 1.50^{cd}	11.3 ± 0.68^{bc}	12.5 ± 0.76^{bc}	12.4 ± 0.80^{bc}
DCM	1	12.2 ± 1.0^{c}	17.6 ± 1.80^{c}	14.0 ± 2.08^{cd}	11.5 ± 0.28^{bc}	9.8 ± 1.50^{b}	9.7 ± 1.53^{b}
	2.5	15.6 ± 1.70^{cd}	18.8 ± 2.53^{cd}	16.6 ± 1.74^{cd}	13.2 ± 2.50^{c}	12.6 ± 0.69^{bc}	11.5 ± 1.60^{bc}
	5	16.8 ± 2.94^{cd}	21.3 ± 2.53^{d}	18.5 ± 1.27^{d}	15.2 ± 1.0^{cd}	14.8 ± 2.12^{c}	13.6 ± 2.25^{bc}
	10	17.1 ± 2.04^{cd}	23.4 ± 2.90^{d}	20.3 ± 1.56^{d}	16.5 ± 1.05^{cd}	16.1 ± 2.56^{c}	15.1 ± 3.66^{c}
Acetone	1	11.0 ± 1.52^{bc}	14.6 ± 2.41^{bc}	12.5 ± 1.44^{c}	10.3 ± 0.66^{bc}	9.3 ± 0.26^{bc}	8.5 ± 0.28^{b}
	2.5	13.0 ± 0.57^{c}	17.8 ± 1.00^{c}	13.0 ± 2.08^{c}	12.7 ± 1.32^{c}	11.5 ± 1.04^{bc}	9.6 ± 0.98^{bc}
	5	14.5 ± 0.28^{cd}	19.3 ± 2.37^{d}	16.5 ± 1.80^{cd}	13.6 ± 1.46^{c}	12.6 ± 1.72^{bc}	12.4 ± 1.0^{bc}
	10	15.3 ± 1.85^{cd}	21.8 ± 2.61^{d}	17.5 ± 1.25^{d}	14.0 ± 0.57^{c}	13.4 ± 0.50^{c}	14.3 ± 0.68^{bc}
Aqueous	1	8.2 ± 0.64^{b}	8.60 ± 1.25^{b}	6.5 ± 0.95^{b}	8.6 ± 0.80^{b}	7.4 ± 0.50^{b}	7.2 ± 0.69^{b}
	2.5	10.1 ± 0.63^{bc}	11.2 ± 1.61^{b}	7.7 ± 0.60^{bc}	9.5 ± 0.10^{bc}	9.7 ± 0.75^{b}	9.3 ± 0.95^{bc}
	5	11.3 ± 1.56^{bc}	15.4 ± 1.02^{bc}	08.0 ± 0.10^{bc}	11.8 ± 0.90^{bc}	10.1 ± 0.32^{bc}	10.6 ± 0.68^{bc}
	10	12.3 ± 1.01^{c}	16.2 ± 2.25^{c}	11.7 ± 1.73^{c}	13.0 ± 0.35^{c}	11.2 ± 1.10^{bc}	12.2 ± 0.61^{bc}
*Streptomycin	1	15.5 ± 1.55^{cd}	19.3 ± 2.13^{cd}	21.1 ± 2.00^{de}	19.5 ± 2.78^{cd}	16.4 ± 2.30^{cd}	17.7 ± 2.81^{c}
	2.5	17.8 ± 2.30^{d}	21.4 ± 1.85^{d}	22.3 ± 2.20^{de}	22.3 ± 2.61^{cd}	18.1 ± 3.00^{cd}	19.7 ± 2.87^{cd}
	5	20.2 ± 2.53^{de}	24.2 ± 2.68^{de}	23.6 ± 2.57^{de}	25.2 ± 3.07^{d}	21.2 ± 3.63^{cd}	23.2 ± 3.52^{d}
	10	22.4 ± 2.20^{e}	26.1 ± 3.04^{e}	25.8 ± 3.02^{de}	27.1 ± 2.67^{de}	23.5 ± 3.19^{d}	24.5 ± 2.77^{d}
#DMSO		0.0 ± 0.0^{a}					

The mean ± SD followed by same letter do not differ significantly using Tukey's test $P \leq 0.05$.
Zone of inhibition includes the diameter of the disc (6 mm); - no zone formation.
*Standard antibiotics for reference control;
#Dimethyl sulfoxide 10%, for negative control.

acetone and aqueous extracts against all the tested microorganisms. In comparison, the aqueous extract showed less pronounced antibacterial activity at 1 mg/disc. DMSO did not reveal any activity (Table 1).

Antibacterial substances commencing from medicinal plants have been gradually reported from different regions of the world. Medicinal plants have provided a significant source of novel compounds as plants derived medicines and have made important contribution towards human health. The World Health Organization (WHO) has estimated that the plant crude extracts and their bioactive molecules are used as folk medicine in traditional therapies, about 80% of the world population. In the current report, hexane, acetone, DCM and aqueous extracts obtained from *L. aspera* (Willd.) L. leaves showed strong antibacterial activity against selected bacterial pathogens. It is shown that all the prepared extracts from the leaves of *L. aspera* (Willd.) L. were active against all bacterial strains at all the tested concentrations. DCM extract showed maximum zone of inhibition against *B. subtillis*, *S. aureus*, *E. coli*, *K. pneumonia*, *P. vulgaris* and *P. aeruginosa*. Among them, *S. aureus*, a pyrogenic bacterium, was known to play a significant role in invasive skin diseases including superficial and *Salmonella typhii*, which causes typhoid fever to human beings (Kidgell et al., 2002). *P. aeruginosa*, is an important microbial pathogen of nosocomial infection in humans. It is also responsible for community acquired infections associated with contaminated water, folliculitis, otitis and corneal ulcers (Dubois et al., 2008). The present findings coincide with the findings of Pavunraj et al. (2014) who reported that different organic solvent extracts such as hexane, acetone, DCM and aqueous leaf extracts of *Spilanthes acmella* (L.) showed antibacterial activity against the Gram-negative bacterial pathogens such as *E. coli*, *K. pneumonia* & *P. vulgaris* and Gram-positive bacterial pathogens such as *B. subtilis* and *S. aureus*. The present study is in agreement with the findings of Chew et al. (2012) reported that methanol extracts of *L. aspera* (Willd.) L. (root) showed the highest zone of inhibitions against *S. aureus* and *E. coli*, *K. pneumonia*, *S. typhimurium*, *S. choleraesuis* and *S. flexneri*. A research done with the crude hexane, chloroform and ethyl acetate extracts of *Pergularia daemia* leaves by Ignacimuthu et al. (2009) revealed that ethyl acetate extract showed growth inhibitory activity against *B. subtilis* (15 mm), *S. aureus* (17 mm) and *P. vulgaris* (20 mm) at 400 µg/disc. The minimum inhibitory concentrations (MIC) of the ethyl acetate extract were 500, 300 and 200 µg/mL for *B. subtilis*, *S. aureus* and *P. vulgaris* respectively. The present results also corroborate the findings of Mostafa et al. (2017) reported that ethanolic extracts from *Cuminum cyminum*, *Punica granatum*, *Syzygium aromaticum*, *Thymus vulgaris* and *Zingiber officinale* showed antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*.

3.2. Antifungal activity

Antifungal activity of organic and aqueous extracts from the leaves of *L. aspera* (Willd.) L. was assayed against four fungal strains. The obtained results revealed that all the extracts were found to be active against all the fungal strains. Among the extracts tested, DCM extract of *L. aspera* (Willd.) L. leaves were found to be most potent and exhibited the maximum zone of inhibitions against *T. viride* (29.2 ± 2.00 mm), *C. albicans* (24.4 ± 0.80 mm), *A. flavus* (22.8 ± 0.36 mm), *E. floccosum* (19.5 ± 2.17 mm) at 10 mg/disc than the other extracts (Table 2). The acetone extract was found to be moderately effective against the four tested fungal pathogens. In addition, the hexane and aqueous extract at a concentration of 10 mg/disc were relatively found to be less effective. Negative control (disc containing only DMSO) showed no zone of inhibition against the microorganisms. The reference drug (ciprofloxacin) produced zone of inhibitions against the tested microorganisms ranging from 24.3 to 31.4 mm. The bioactivity was directly proportional to the concentration of the extracts.

The present work demonstrated that *L. aspera* (Willd.) L. crude extracts exhibited promising antifungal activity against *A. flavus*, *C. albicans*, *E. floccosum* and *T. viride*. The antifungal activity of the crude extracts was significantly higher in DCM extract and this is in agreement with the findings of Duraipandiyan and Ignacimuthu (2007) on *Trichophyton mentagrophytes* and *Epidermophyton floccosum*. Bi et al. (2008) observed that the DCM extracts of *Erigeron floribundus* exhibited a broad spectrum antifungal activity with MIC values ranging between 0.1 to 0.25 mg/mL against *Microsporum canis*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Scopulariopsis brevicaulis*. A similar result was reported by Mohana and Raveesha (2007) in which the antifungal activity of petroleum ether, benzene, chloroform, methanol and ethanol extracts of *Decalepis hamiltonii* were screened against *Fusarium solani* and *Aspergillus flavus*. In accordance with this study, the petroleum ether extract showed highly significant antifungal activity followed by benzene and chloroform extracts at 2000 µg/mL. In the present study, DCM extract of *L. aspera* (Willd.) L. has been found to exhibit a greater antifungal activity which justifies the earlier findings of Mangathayaru et al. (2005) and Akroum (2017) who stated that acetone extracts from *Punica granatum*, *Quercus suber* and *Vicia faba* showed antifungal activity against 7 pathogenic fungi.

3.3. Determination of MIC value

The lowest concentration of leaf extract at which no growth of microorganisms was observed on visual inspection, considered as the MIC value after incubating at 30 ºC for 24 and 48 h. Pellets formed on the bottom of wells were considered as growth of microorganisms even if the wells were clear of turbidity.

Table 2
Antifungal activity of hexane, DCM, acetone and aqueous leaf extracts of *Leucas aspera* (Willd.) L.

Solvents used	(mg/disc)	Name of the fungal species			
		A. flavus	***C. albicans***	***E. floccosum***	***T. viride***
		Mycelial growth inhibition (mm)			
Hexane	1	9.1 ± 1.00^{a}	9.8 ± 1.16^{b}	7.5 ± 0.86^{b}	13.3 ± 0.51^{b}
	2.5	12.8 ± 2.00^{bc}	13.4 ± 1.10^{bc}	8.6 ± 0.41^{b}	15.5 ± 1.04^{bc}
	5	17.5 ± 1.04^{c}	16.5 ± 0.55^{c}	9.5 ± 0.76^{bc}	23.2 ± 2.19^{c}
	10	19.9 ± 1.30^{c}	17.3 ± 2.80^{c}	11.6 ± 0.51^{bc}	25.0 ± 0.57^{c}
DCM	1	13.8 ± 1.92^{b}	14.5 ± 0.47^{bc}	11.3 ± 1.36^{bc}	16.5 ± 2.29^{bc}
	2.5	17.2 ± 1.41^{c}	19.3 ± 0.49^{c}	15.7 ± 0.75^{c}	18.5 ± 0.85^{bc}
	5	19.5 ± 0.50^{c}	21.3 ± 1.15^{d}	17.2 ± 2.61^{c}	25.5 ± 1.32^{c}
	10	22.8 ± 0.36^{c}	24.4 ± 0.80^{e}	19.5 ± 2.17^{c}	29.2 ± 2.00^{cd}
Acetone	1	10.4 ± 0.92^{b}	12.8 ± 0.75^{bc}	7.5 ± 1.32^{b}	16.0 ± 2.51^{bc}
	2.5	13.5 ± 1.32^{bc}	14.5 ± 0.72^{c}	10.8 ± 0.87^{bc}	18.3 ± 0.85^{bc}
	5	18.5 ± 0.76^{c}	19.4 ± 1.36^{c}	12.3 ± 1.01^{bc}	24.5 ± 0.76^{c}
	10	22.0 ± 0.76^{c}	21.2 ± 1.90^{cd}	14.3 ± 1.07^{c}	28.2 ± 1.44^{cd}
Aqueous	1	9.4 ± 0.80^{b}	11.8 ± 1.28^{b}	7.8 ± 1.03^{b}	14.0 ± 0.57^{bc}
	2.5	13.7 ± 1.69^{bc}	14.1 ± 0.60^{bc}	12.0 ± 0.72^{bc}	19.3 ± 1.40^{bc}
	5	15.5 ± 1.04^{bc}	17.4 ± 0.34^{c}	15.3 ± 0.85^{c}	22.6 ± 0.45^{c}
	10	20.2 ± 1.10^{d}	19.2 ± 1.03^{c}	17.5 ± 1.38^{c}	26.2 ± 0.46^{d}
*Ciprofloxacin	1	16.4 ± 0.50^{c}	17.5 ± 1.01^{c}	15.5 ± 0.76^{c}	17.5 ± 2.25^{bc}
	2.5	19.2 ± 2.42^{c}	21.9 ± 1.27^{cd}	17.7 ± 0.35^{c}	21.4 ± 1.00^{c}
	5	22.5 ± 1.32^{d}	23.7 ± 1.57^{e}	25.2 ± 1.50^{d}	27.5 ± 1.44^{c}
	10	24.3 ± 0.85^{de}	26.4 ± 1.31^{f}	28.0 ± 1.52^{e}	31.4 ± 2.41^{d}
#DMSO		0.0 ± 0.0^{a}			

The mean ± SD followed by same letter do not differ significantly using Tukey's test $P \leq 0.05$.
*Standard antibiotics for reference control; #Dimethyl sulfoxide 10%, for negative control

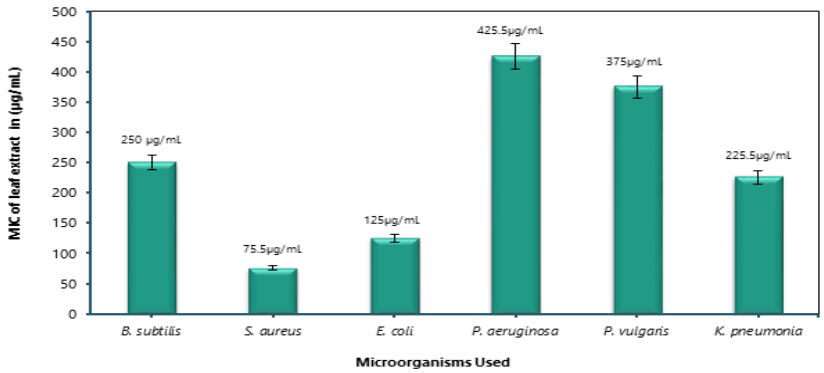

Fig. 1. MIC value of dichloromethane leaf extract of *Leucas aspera* (Willd.) L. against selected bacterial pathogens.

In the case of bacterial pathogens, the leaf extract had the highest and lowest antibacterial impacts with MIC values of 75.5 and 425.5 µg/mL against *S. aureus* and *P. aeruginosa*, respectively (Fig. 1). However, in the case of fungal pathogens, the extract showed the significant MIC value of 125 µg/mL against *T. vridie*, whereas the extract showed moderate MIC values against the other fungal pathogens (see Fig. 2). In the present findings corroborates with earlier findings of Sarkar et al. (2016) who reported that *Tridax procumbens* exhibited minimum inhibitory concentration for all the tested fungus (200-350 µg/mL) and for bacteria (10-20 mg/mL). Ethiopian medicinal plants showed minimum inhibitory concentration of 12.5 to 25 mg/mL concentration against bacteria and fungi (Bacha et al., 2016). Singh and Kumar (2013) stated that different part *viz.*, stem, root leaves and fruit extract from *Euphorbia hirta* showed promising antifungal and bacterial activity with MIC value of 0.039-1.25 mg/mL (fungi) and 0.078-1.25 mg/mL (bacteria).

3.4. Larvicidal activity

The larvicidal activity of hexane, DCM, acetone and aqueous extracts of *L. aspera* (Willd.) L. leaves were screened at a concentration of 1000 ppm and the respective results have been summarized in Fig. 3. It was observed that DCM extracts of *L. aspera* (Willd.) L. was found to have potent larvicidal activity against 4th instar larvae of *C. quinquefasciatus*. In fact, the DCM extract of *L. aspera* (Willd.) L. showed 100% larval mortality at 1000 ppm against *C. quinquefaciatus* followed by acetone (74.0%) and hexane (65.18%) extracts compared to control. On the other hand, the aqueous extract at 1000 ppm was found to be less effective versus 4th instar larvae of *C. quinquefasciatus*. Moreover, no mortality

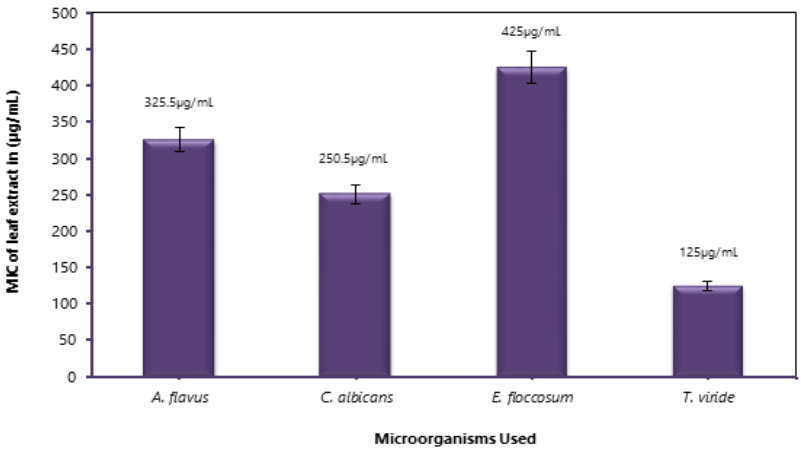

Fig. 2. MIC value of dichloromethane leaf extract of *Leucas aspera* (Willd.) L. against selected fungal pathogens.

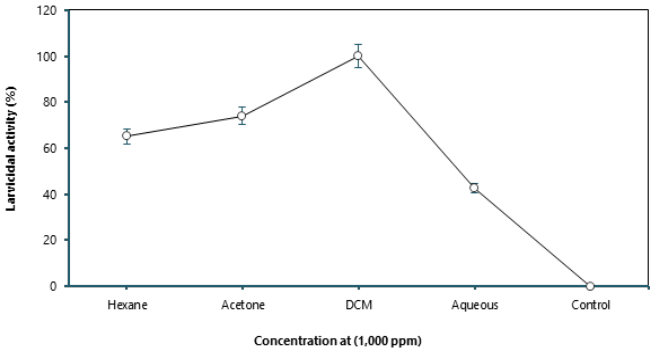

Fig. 3. Percentage larvicidal activity of various solvent extracts of *Leucas aspera* (Willd.) Linn. against 4th instar larvae of *C. quinquefasciatus* (Say).

was recorded in various replicates of control.

Plant products have been used traditionally by human communities in many parts of the world against the vectors and insect pests (Muthu et al., 2015). Application of these plant derivatives in mosquito control as an alternative of synthetic insecticides could diminish the environmental hazards and also reduce the overall cost of production (Baskar et al., 2017). The phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, ovipositional attractants and also have deterrent actions as being highlighted by many researchers (Rajkumar and Jebanesan, 2007; Bagavan et al., 2009b; Kamaraj et al, 2011; Baskar et al., 2017; Pavunraj et al., 2017).

The current work has shown that the DCM extract of *L. aspera* (Willd.) L. leaves exhibited hundred percent larvicidal activities against 4th instar mosquito larvae of *C. quinquefasciatus*. Our results accounted for greater activity as it was compared with an earlier study by Bosire et al. (2014) reported that dichloromethane: methanol (1:1) extract of *Millettia usaramensis* showed larvicidal activity against 4th instar larvae of *Aedes aegypti* with LC_{50} value of 167.0 µg/mL. Earlier, Anitha and Geethapriya (2012) reported that petroleum ether extract of *Lantana camara*, *Tridax procumbens* and *Datura stramonium* showed 100% larval mortality against *Aedes aegypti* at concentration of 1000 µg/mL. Bagavan et al. (2008) studied the larvicidal activity of hexane, chloroform and ethyl acetate extracts of *P. daemia* against mosquito larvae. They observed that ethyl acetate extract showed 100% mortality against *Anopheles subpictus* and *Cx. tritaeniorhynchus* at 1000 ppm concentration. Balaji et al. (2012) also reported that dichloromethane extract of *Roccella montagnei* showed high toxicity against the 3rd instar mosquito larvae of *C. quinquefasciatus*.

4. Concluding remarks

The present findings on various solvent extracts prepared from the leaves of *L. aspera* (Willd.) L. showed potential antibacterial, antifungal and mosquito larvicidal activities. DCM extract of *L. aspera* (Willd.) L. exhibited maximum zone of inhibition against bacteria (15-23 mm), fungi (19-29 mm) and 100% larvicdal activity against mosquitoes. The maximum biological activity of *L. aspera* (Willd.) L., due to present of active phytochemical of alkaloids, glycosides, flavonoids, saponins, tannins and terpenoids. Further studies on isolation and development of natural herbal formulation using bioactive fraction/constituent may provide promising products for control of bacteria, fungus and mosquito larvae.

Conflict of interest

The authors declare that we have no conflict of interest.

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