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Phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali)

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Abstract *Eurycoma longifolia* Jack is a well-known traditional plant of Malaysia. It is popularly recognised as 'Tongkat Ali'. The present study deals with the preliminary phytochemical screening and determination of antimicrobial activity of methanol, acetone, ethyl acetate, chloroform and petroleum ether extracts of the stem and root of *E. longifolia*. The extracts were tested against two gram positive bacteria, three gram negative bacteria and one fungus by the disc diffusion method at various concentrations (12.5–200 μg/μl). The results revealed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, protein and cardiac glycosides in the extracts. Stem extracts were found to be a rich source of phytochemicals as compared to the root extracts. All the extracts exhibited dose dependent antimicrobial activity, however, highest antibacterial activity was observed against gram positive bacteria by both stem and root extracts. Nevertheless, stem extracts were more potent than root extracts against *Bacillus cereus* and *Staphylococcus aureus*. Merely, ethyl acetate extract of the stem showed moderate activity against gram negative bacteria, *Pseudomonas aeruginosa* and high activity against fungus, *Aspergillus niger*. The preliminary studies on *E. longifolia* extracts exhibited their antimicrobial potential which could be exploited further as future antimicrobials for pharmaceutical treatment, natural therapies, food preservation and cosmetic applications.

1. Introduction

Since antiquity, medicinal plants have been representing a rich source of antimicrobial agent (Kane et al., 1950). Unfortunately, in recent years, antimicrobial resistance has become a major public health concern globally. It had been reported that over 70% of pathogens found in US hospitals acquired resistance for at least one antibiotic resulting in mortality of more than 14,000 patients annually from nosocomial infections

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(Rezai and Weinstein, 2010). In order to solve antimicrobial resistance issue, drivers of resistance and possible solutions have been listed for future approaches. One of the effective approaches could be the discovery and development of new antimicrobial agents that have clinical significant importance from natural resources. It is crucial to discover new antimicrobial agents in the pharmaceutical pipeline in order to replace currently available antimicrobials.

Malaysia is rich in natural resources of more than 20,000 species of angiosperms, 600 species of ferns and 1082 species (15%) are reported to have medicinal properties (Jantan, 2004; Kulip, 2003; Ong et al., 2011). One of the famous traditional tropical plants of Malaysia is *Eurycoma longifolia* Jack (Tongkat Ali) indigenous to South-East Asian countries and has been scientifically proven to posses medicinal and healing properties (Bhat and Karim, 2010). The effectiveness of *E. longifolia* is attributed to various bioactive constituents present in the plant, including quassinoids, alkaloids, glycosides, eurycomanol, eurycomanone, etc. (Bedir et al., 2003; Bhat and Karim, 2010). Previous studies on *E. longifolia* reported various important pharmacological properties such as antimalarial, antitumor, anticancer, anti-diabetic, aphrodisiac, anxiolytic and anti-parasitic activities (Rashid et al., 2009). The present knowledge on antimicrobial activity of *E. longifolia* is limited and there is still a wide gap which needs to be explored further against various other pathogenic microbes (Bhat and Karim, 2010; Farouk and Benafri, 2007; Tzar et al., 2011). Thus, the present study was carried out to determine antimicrobial activity of different extracts of *E. longifolia* stem and root at various concentrations against some pathogenic bacterial and fungal species. The selected pathogens such as *Aspergillus niger*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* have not been evaluated in previous researches. To the best of our knowledge no antifungal study is available pertaining to stem extract of *E. longifolia*. Also, antimicrobial activity of petroleum ether, chloroform and ethyl acetate extracts of root and stem is reported for the first time in this paper.

2. Materials and methods

2.1. Plant materials

The stems and roots of *E. longifolia* were procured from Delima Jelita Herbs Pvt. Ltd. from Alor Setar, Kedah, Malaysia. The company is a giant supplier of herbal raw materials in Malaysia. The raw material was obtained in the powdered form, prepared by wood powder grinding machine before drying under the shade. The commercialised plant materials were forest produce of Sik district, Kedah, Malaysia as indicated on the package.

2.2. Preparation of extracts

Briefly 100 g of each powdered sample (stems and root) was soaked separately in 500 ml of petroleum ether, chloroform, ethyl acetate, acetone and methanol for 24 h at 40 °C on water bath. The obtained extract was filtered by using Whatman No. 1 filter paper. Each filtrate was concentrated under reduced pressure on a rotary evaporator till golden viscous mass was obtained. Finally, the prepared extracts were stored at 4 °C for further analyses.

2.3. Phytochemical screening

The extracts were analysed for the presence of phenolic compounds, flavonoids terpenoids, saponins, alkaloids, cardiac glycosides and protein (Raaman, 2006).

2.3.1. Ferric chloride test

Each extract (50 mg) was dissolved in 5 ml of distilled water and few drops of 5% ferric chloride were added. Bluish black colour indicated the presence of phenolic compounds.

2.3.2. Alkaline reagent test

Few drops of sodium hydroxide were added into the extracts to give intense yellow colour. The disappearance of colour after addition of dilute hydrochloride acid showed the presence of flavonoid.

2.3.3. Salkowski's test

The extract (0.5 mg) was added with few ml of chloroform followed by concentrated sulphuric acid to form a layer. Reddish brown colour at the interface indicated the presence of terpenoids.

2.3.4. Froth test

Each extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. The development of two cm layer of foam indicated the presence of saponins.

2.3.5. Wagner's test

About 50 mg of extracts was stirred with few ml of dilute hydrochloric acid and filtered. Then, few drops of Wagner's reagent were added at the side of the test tube. The formation of reddish-brown precipitate showed the presence of alkaloids.

2.3.6. Keller-Kiliani's test

A small amount of extract (50 mg) was treated with 2 ml of glacial acetic acid containing one drop of 5% ferric chloride, followed by addition of 1 ml of concentrated sulphuric acid. A brown ring at interface is characteristic of cardenolide deoxy sugar. Appearance of the violet ring below the brown ring and greenish ring in acetic acid layer indicated the presence of cardiac glycosides.

2.3.7. Biuret test

Each extract (50 mg) was diluted with distilled water and treated with Biuret reagent. The appearance of pink colour indicated the presence of protein.

2.4. Antimicrobial study

2.4.1. Microorganism

The antimicrobial properties of *E. longifolia* Jack were investigated against one fungal species; *A. niger*, three gram

negative bacterial strains; *Escherichia coli*, *Salmonella virchow*, *P. aeruginosa* and two gram positive bacterial strains; *B. cereus*, *S. aureus*. A total of six pathogenic microorganisms were obtained from the culture collection of Microbiology Laboratory, Universiti Malaysia Kelantan, Campus Jeli, Kelantan, Malaysia.

2.4.2. Inoculation of microorganism

The pure cultures of *microorganisms* were streaked onto nutrient agar plate (Merck) and incubated at 37 °C for 24 h. The well-isolated colonies were aseptically transferred to nutrient broth (Merck) and again incubated at 37 °C for 24 h. The optical densities of incubated bacteria were measured using a UV spectrophotometer (Shimadzu, Japan) at 600 nm wavelength. The desired optical density (OD) was obtained as 0.45–0.55, represented 0.5 McFarland standards (10^8 CFU/mL). The turbidity of each pathogen was adjusted to the desired range by dilution if OD values were higher than the standards or otherwise incubated again if OD values were less than the standards.

2.4.3. Sample preparation

Antimicrobial activity of the extracts was tested at various concentrations ranging from 12.50–200.00 μg/μl. The stems and roots extracts of *E. longifolia* from five selected solvents were weighed and dissolved in DMSO to prepare stock solution of 200.00 μg/μl concentrations. The same stock solution has been utilised to get desired concentrations of 100.00 μg/μl, 50.00 μg/μl, 25.00 μg/μl and 12.50 μg/μl by the serial dilutions method using equation, $c_1v_1 = c_2v_2$, where c = concentration and v = volume.

2.4.4. Disc diffusion assay

The petroleum ether, chloroform, ethyl acetate, acetone and methanol extracts of *E. longifolia* stems and roots were screened for antimicrobial activity by using the disc diffusion method (Zaidan et al., 2005). In the assay each inoculum suspension (10^8 CFU/mL) was spread evenly over the entire nutrient agar surface by sterile collection swab. Then, discs of diameter 6 mm were sterilized at 121 °C for 15 min and loaded with prepared positive control (ampicillin, 20 μg/μl) and extract solutions of *E. longifolia* at various concentrations. The impregnated discs were dried for 3–5 min and dispensed onto the surface of the inoculated plates with flamed forceps. Each disc was pressed down firmly to ensure complete contact with nutrient agar surface. The discs were placed suitably apart and not relocated once contacted with the agar surface. The plates were then labelled and incubated at 37 °C for 24 h for both bacteria and fungus (Espinel-Ingroff et al., 2007; Zaidan et al., 2005). The results were measured and expressed in terms of zone of inhibition (ZI) of bacterial and fungal growth around each disc in millimetres as low activity (1–6 mm), moderate activity (7–10 mm), high activity (11–15 mm), very high activity (16–20 mm), no activity (−) (Parveen et al., 2010). The antimicrobial index (AI) was calculated by using equation (Bhat and Abdul Khalil, 2010).

$$\text{Antimicrobial index} = (1 - D_a/D_b) \times 100$$

where D_a is the diameter of growth zone in the test plate and D_b is the diameter of growth zone in the control plate.

2.5. Statistical analysis

Statistical technique of Duncan's multiple range test (DMRT) was used with the assistance of SPSS Version 16 for the assessment of mean comparisons. The results were expressed in terms of mean ± standard deviation. All data presented are mean values of triplicate measurements (n = 3), obtained from three separate runs; unless stated otherwise.

3. Results and discussion

3.1. Phytochemical screening

The results of phytochemical screening of extracts revealed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, protein and cardiac glycosides in the stem and root extracts of *E. longifolia* (Table 1). Particularly, methanol, ethylacetate and chloroform extracts of *E. longifolia* were good sources of different classes of compounds. This indicates that these solvents are effective to isolate active biological compounds due to their high polarity. Flavonoids were detected in chloroform, ethyl acetate, acetone and methanol extracts of both stem and root parts of plant except petroleum ether extract. Flavonoids belong to the group of polyphenolic compounds and are typically known for health promoting properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties (Aiyelaagbe and Osamudiamen, 2009). They exist widely in the plant kingdom and displayed positive correlation between increased consumption of flavonoids and reduced risk of cardiovascular and cancer diseases (Yang et al., 2001). Correspondingly, these extracts also tested positive for phenolic compounds. The phenolic compounds are aromatic secondary metabolites that impart colour, flavour and associated with health benefits such as reduced risk of heart and cardiovascular diseases (Alothman et al., 2009; Bhat et al., 2011). According to Aliyu et al. (2009) phenolic compounds account for most of the antioxidant activities in plants. All the extracts of *E. longifolia* except acetone extract have been detected for the presence of terpenoids, although saponins were altogether absent in all stem and root extracts. Terpenoids such as triterpenes, sesquiterpenes and diterpenes have been referred to as antibiotics, insecticidal, anthelmintic and antiseptic in pharmaceutical industry (Duke, 1992; Parveen et al., 2010). The alkaloid was observed only in petroleum ether, chloroform and ethyl acetate extracts of the stem. However, the absence of alkaloid in roots is quite unexpected contrary to the previous researches (Kuo et al., 2003). The absence of alkaloid in the current study may be the consequence of different geographical locations in which soil minerals and environmental factors have great influence on phytochemical contents of the plant (Borokini and Ayodele, 2012). Alkaloids have been reported to possess analgesic, antispasmodic and bactericidal, antimalarial and analgesic activities (Okwu and Okwu, 2004; Oomah, 2003). Moreover, cardiac glycosides commonly used to treat congestive heart failure and cardiac arrhythmia, were discovered in all the extracts of the stem except petroleum ether extract, whereas, none of the root extracts indicated their presence (Hollman, 1985). All the prepared extracts of the stem showed the presence of protein except petroleum ether and chloroform extract, while roots of *E. longifolia* found totally lacking in

Table 1 Phytochemical analysis of *E. longifolia* root and stem extracts.

Phyto-chemicals	Methanol extract		Acetone extract		Ethyl acetate extract		Chloroform extract		Petroleum ether extract	
	Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root
Alkaloids	–	–	–	–	+	–	+	–	+	–
Phenolic compounds	+	+	+	+	+	+	+	+	–	–
Flavonoids	+	+	+	+	+	+	+	+	–	–
Terpenoids	+	+	–	–	+	+	+	+	+	+
Saponins	–	–	–	–	–	–	–	–	–	–
Cardiac glycosides	+	–	+	–	+	–	+	–	–	–
Proteins	+	–	+	–	+	–	–	–	–	–

+ = present; – = absent.

protein content. Typically, Proteins are the huge group of macromolecules and act as antibiotic and antimicrobial agents. Plants defend themselves against microbial pathogens by various defence responses including production of antimicrobial proteins which are small molecular mass antimicrobial peptides (Walter, 2012; Garćıa-Olmedo et al., 2001).

For instance, the root part exhibited the presence of phenolic acid, flavonoids, terpenoids and protein while the stem part comprised of all the tested phytochemicals except saponins. Among all the tested extracts of stem and roots, petroleum ether extract has the lowest number of phytochemicals present. The ethyl acetate extract of the stem was found rich source of phytochemicals as compared to the other extracts, whereas, in case of root, methanol extract was the best source of phytochemicals.

3.2. Antimicrobial activity

3.2.1. Root

The antimicrobial activity of *E. longifolia* root extracts is shown in Tables 2 and 3. All the root extracts were found active against tested gram positive bacteria; *B. cereus* and *S. aureus*; while, totally inactive against gram negative bacteria; *E. coli*, *P. aeruginosa*, *S. virchow* and fungus; *A. niger* (Table 2). However, previous studies carried out by Tzar et al. (2011) on aqueous root extract, exhibited antifungal activity against other fungi of *Candida* species. This could be attributed to the presence of certain active compounds which may be absent in root extracts of the current study; like alkaloids (Feng et al., 2007). Also, low concentrations or selectivity of active principles towards a particular fungus could also be one of the reasons of inactivity. The selective activity of drugs towards certain bacteria might be due to the presence of lipopolysaccharide in outer membrane of gram negative bacteria, which acts as a permeability barrier and restricts diffusion of active compounds through its lipopolysaccharide covering (Niv and Yechiel, 2005). Unlike gram negative bacteria, gram positive bacteria allow the direct contact of the extract constituents with the phospholipid bilayer of the cell membrane, causing either enhanced ion permeability, leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems (Zhao et al., 2001). Among all the gram negative bacterial strains, *P. aeruginosa* demonstrated highest resistance, since after treatment with amphicillin (20 μg/μl), it showed no positive response. This was particularly due to the resistance developed by the bacterial strain with time due to drug exposure or mutation (David, 2002). Among gram positive bacteria, the chloroform root extract of *E. longifolia* showed highest activity (ZI of 11.67 ± 1.53 mm) against *S. aureus* followed by acetone extracts (ZI of 11.00 ± 1.00 mm) at 200 μg/μl, indicated by comparatively lower AI (17 and 21) relative to other extracts, respectively (Tables 3 and 5. The low AI of the extract corresponds to high antimicrobial susceptibility relative to the control. These findings were corroborated with the close ZI values of the extracts (chloroform and acetone at 200 μg/μl) with the control (ZI of 14.00 ± 1.00). Conversely, in the previous findings on the root extracts none of the prepared root extracts (methanol, ethanol, acetone and aqueous) were found to be active against *S. sureus* (Farouk and Benafri, 2007). This could be explained that antibacterial actions are related to their chemical components in the crude extracts (Varahalarao and Kaladhar, 2012; Sekar et al., 2012). Moderate activity was exhibited by petroleum ether, ethyl acetate and methanol extracts of root against *S. aureus* at various concentrations (Table 3).

Based on the Duncan's multiple range test, acetone and ethyl acetate extracts of root were not significantly different

Table 2 Antimicrobial activity of *E. longifolia* stem and root extracts.

Micro-organism	Methanol extract		Acetone extract		Ethyl acetate extract		Chloroform extract		Petroleum ether extract	
	Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root
E. coli	+	–	–	–	–	–	–	–	–	–
P. aeruginosa	–	–	–	–	+	–	–	–	–	–
S. virchow	–	–	–	–	–	–	–	–	–	–
B. cereus	+	+	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+	+
A. niger	–	–	–	–	+	–	–	–	–	–

+ = present; – = absent.

Table 3 Antimicrobial activity of *E. longifolia* root extracts at various concentrations.

Micro-organisms	Conc. (μg/μl)	ZI (mm)					Ampicillin (20 μg/μl)
		Methanol extract	Acetone extract	Ethyl acetate extract	Chloroform extract	Petroleum ether extract	
B. cereus	200.0	10.00 ± 1.00	7.00 ± 0.00	10.67 ± 0.58	9.33 ± 0.58	9.67 ± 0.58	12.67 ± 2.89
	100.0	8.67 ± 0.58	7.00 ± 0.00	9.67 ± 1.15	8.00 ± 0.00	8.33 ± 0.58	
	50.0	–	–	2.67 ± 4.62	–	–	
	25.0	–	–	–	–	–	
	12.5	–	–	–	–	–	
S. aureus	200.0	9.00 ± 1.00	11.00 ± 1.00	9.33 ± 0.58	11.67 ± 1.53	10.00 ± 1.00	14.00 ± 1.00
	100.0	6.33 ± 5.51	10.00 ± 1.73	9.33 ± 0.58	8.33 ± 0.58	9.00 ± 0.00	
	50.0	–	9.00 ± 1.73	8.67 ± 0.58	–	8.00 ± 1.00	
	25.0	–	6.67 ± 5.78	8.33 ± 0.58	–	5.00 ± 4.36	
	12.5	–	6.00 ± 5.20	8.00 ± 0.00	–	2.33 ± 4.04	

ZI were expressed as mean ± standard deviation of three replicates. Low activity (1–6 mm), moderate activity (7–10 mm), high activity (11–15 mm), very high activity (16–20 mm), no activity (–).

Table 4 Antimicrobial activity of *E. longifolia* stem extracts at various concentrations.

Micro-organisms	Conc. (μg/μl)	ZI (mm)					Ampicillin (20 μg/μl)
		Methanol extract	Acetone extract	Ethyl acetate extract	Chloroform extract	Petroleum ether extract	
E. coli	200.0	3.33 ± 5.77	–	–	–	–	32.00 ± 1.00
	100.0	–	–	–	–	–	
	50.0	–	–	–	–	–	
	25.0	–	–	–	–	–	
	12.5	–	–	–	–	–	
P. aeruginosa	200.0	–	–	9.33 ± 0.58	–	–	Resistant
	100.0	–	–	8.67 ± 0.58	–	–	
	50.0	–	–	–	–	–	
	25.0	–	–	–	–	–	
	12.5	–	–	–	–	–	
B. cereus	200.0	14.00 ± 1.00	11.33 ± 1.53	12.33 ± 0.58	11.00 ± 0.00	10.33 ± 1.53	12.67 ± 2.89
	100.0	12.00 ± 0.00	10.33 ± 0.58	11.00 ± 0.00	9.67 ± 1.53	8.67 ± 0.58	
	50.0	11.67 ± 1.15	9.67 ± 0.58	10.00 ± 0.00	8.67 ± 1.15	8.33 ± 0.58	
	25.0	10.67 ± 0.58	8.33 ± 0.58	9.00 ± 0.00	7.67 ± 0.58	7.67 ± 1.15	
	12.5	9.67 ± 0.58	7.67 ± 0.58	8.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	
S. aureus	200.0	17.00 ± 6.56	12.00 ± 0.00	14.00 ± 1.73	14.33 ± 4.93	13.00 ± 1.00	14.00 ± 1.00
	100.0	13.00 ± 1.00	10.33 ± 0.58	12.67 ± 1.53	10.00 ± 1.00	11.00 ± 2.00	
	50.0	13.33 ± 4.16	9.67 ± 0.58	11.67 ± 1.53	9.00 ± 0.00	9.67 ± 0.58	
	25.0	15.33 ± 9.29	8.33 ± 0.58	9.67 ± 0.58	8.33 ± 0.58	10.00 ± 0.00	
	12.5	12.00 ± 2.65	7.67 ± 0.58	9.33 ± 0.58	7.33 ± 0.58	8.67 ± 1.15	
A. niger	200.0	–	–	11.00 ± 1.73	–	–	17.00 ± 0.00*
	100.0	–	–	9.00 ± 1.00	–	–	
	50.0	–	–	–	–	–	
	25.0	–	–	–	–	–	
	12.5	–	–	–	–	–	

ZI were expressed as mean ± standard deviation of three replicates. Low activity (1–6 mm), moderate activity (7–10 mm), high activity (11–15 mm), very high activity (16–20 mm), no activity (–), NR = not reported,
* ZI of positive control, nystatin against *A. niger* was cited from Oshi and Abdelkarim, 2013.

from each other against *S. aureus*, but they were both considerably different from methanol, chloroform and petroleum ether extracts with significant value of $P < 0.05$. This corresponds to comparable efficacy of acetone and ethyl acetate extract against *S. aureus*, may be attributed to similar phytochemical composition. Moreover, methanol, chloroform and petroleum ether extracts were appreciably different from each other, suggesting they have dissimilar antimicrobial properties. In view of concentration correlation, positive control was appreciably different from root extracts in all concentrations against *S. aureus*.

Apart from that, *E. longifolia* root extracts also reported moderate antimicrobial activities for the first time against *B. cereus* at concentrations of 100 μg/μl and above. The highest

activity was showed by ethyl acetate extract (10.67 ± 0.58 mm) against *B. cereus* at 200 μg/μl, supported by low AI (16) value (Tables 3 and 5). It was observed that ethyl acetate extract exhibited dose dependent increase in activity against *B. cereus*. Petroleum ether, chloroform, acetone and methanol extract exhibited moderate activity at various concentrations. Overall, the efficacy of *E. longifolia* root extracts against *S. aureus* was found more potent as compared to *B. cereus*, which was moderately active. Duncan's multiple range test of *E. longifolia* root extracts revealed that there were significant differences between extracts against *B. cereus* with $P < 0.05$. In contrast with *S. aureus*, methanol, petroleum ether and chloroform extracts were not significantly different from each other as indicated from antimicrobial activities. Also, ethyl acetate and chloroform extracts also did not vary considerably. Yet, acetone extracts were greatly differed from other extracts with relatively low ZI compared to others. The correlations between concentrations of root extracts against *B. cereus* showed that 50, 25 and 12.5 μg/μl of the extracts were not significantly different. This was firmly acceptable as there were no antimicrobial activities exhibited by the extracts at the said concentrations. However, positive control and extracts at 200 and 100 μg/μl were significantly different from each other as their activities vary broadly against *B. cereus*.

3.2.2. Stem

The antimicrobial activities of *E. longifolia* stem extracts were displayed in Tables 2 and 4. Stem extracts were found highly active against gram positive bacteria, *B. cereus* and *S. aureus*. Unlike roots, ethyl acetate and methanol extract of the stem exhibited moderate and low activity against ampicillin resistant gram negative bacteria, *P. aeruginosa* (ZI of 9.33 ± 0.58) and *E. coli* (ZI of 3.33 ± 5.77), respectively (Tables 2 and 4). This activity may be attributed to the presence of greater concentration of active compounds upon extraction with these solvents (Akharaiyi, 2011; Anyasor et al., 2010). Among all the tested extracts, merely ethyl acetate extract of the stem showed high activity (ZI of 11.00 ± 1.73) against *A. niger* at 200 μg/μl. These findings on stem extracts against *A. niger*, *P. aeruginosa* and *E. coli* are novel to the best of our knowledge. Moreover, the antimicrobial properties of stem extracts were observed to be superior with respect to the root extracts. This implies that the stem extracts possess more effective phytochemicals responsible for antimicrobial activities than root extracts, which was also supported by preliminary phytochemical screening (Akharaiyi, 2011).

All the stem extracts exhibited high to moderate activity against *S. aureus* at the lowest tested concentration of 12.5 μg/μl while methanol extract showed very high activity at 200 μg/μl concentration with ZI, 17.00 ± 6.56 mm and AI, −22. The extremely low value of AI with negative sign indicates higher antimicrobial susceptibility as compared to the control (Tables 4 and 5). This further suggests methanol extract at 200 μg/μl concentrations acted as better antimicrobial agent than amphicilin against *S. aureus*. At lowest concentration (12.5 μg/μl) most effective extract was determined as methanol followed by ethyl acetate, petroleum ether, acetone and chloroform extract. The results obtained in the present study were far superior as compared to earlier reports, where methanol extract exhibited moderate activity at 100 μg/μl. Also, petroleum ether, chloroform and ethyl acetate extracts of the stem were evaluated for the first time against *S. aureus*. All the extracts exhibited dose dependent increase in activity. This may be due to the increase in the amount of the active components in the crude extracts with concentration (Varahalarao and Kaladhar, 2012; Sekar et al., 2012). From Duncan's multiple range test, chloroform, petroleum ether and acetone extract were not significantly different from each other against *S. aureus* while, ethyl acetate and methanol extracts were considerably different with significant value $P < 0.05$. The correlations between concentrations indicated that positive control and extracts were considerably different from each other. Moreover, stem extracts at different concentrations, 100, 50 and 25 μg/μl were not significantly different from each other, indicating similar efficacy at different concentrations.

Table 5 Antimicrobial index of *E. longifolia* extracts at active concentrations.

Plant part	Micro-organisms	Conc. in μg/ml	Methanol extract	Acetone extract	Ethyl acetate extract	Chloroform extract	Petroleum ether extract
Root	*B. cereus*	200.0	21	44	16	26	24
		100.0	32	44	24	37	34
		50.0	–	–	79	–	–
	S. aureus	200.0	36	21	33	17	29
		100.0	55	29	33	41	36
		50.0	–	36	38	–	43
		25.0	–	52	41	–	64
		12.5	–	57	43	–	83
Stem	*B. cereus*	200.0	−11	11	3	13	19
		100.0	5	19	13	24	32
		50.0	8	24	21	32	34
		25.0	16	34	30	40	40
		12.5	24	40	34	42	42
	S. aureus	200.0	−22	14	0	−2	7
		100.0	7	26	10	29	21
		50.0	5	31	17	36	31
		25.0	−10	41	31	41	29
		12.5	14	45	33	48	38

The response of stem extracts towards *B. cereus* was slightly poor as compared to *S. aureus*. Nevertheless, moderate activity was observed by all the stem extracts against *B. cereus* at lowest concentration of 12.5 μg/μl and methanol extract was found more potent than the other four extracts. The most strong antibacterial activity was observed at the highest tested concentration of 200 μg/μl, where methanol extract (ZI of 14.00 ± 1.00 mm) was followed by ethyl acetate (ZI of 12.33 ± 0.58 mm), acetone (ZI of 11.33 ± 1.53 mm), chloroform (ZI of 11.00 ± 0.00 mm) and petroleum ether (ZI of 10.33 ± 1.53 mm) extract (Table 4). This was also represented by AI values at −11, 3, 11, 13 and 19, respectively (Table 5). The lower AI values as compared to other extracts evidenced either higher or nearly equal antimicrobial potential with respect to the positive control against *B. cereus*. Based on Duncan's multiple range test, chloroform, petroleum ether and acetone extracts were not significantly different from each other, whereas, methanol and ethyl acetate stem extracts were considerably different from other extracts against *B. cereus*, corresponding to the high activity exhibited by both methanol and ethyl acetate extracts than other extracts. The extracts of the stem at all concentrations were greatly different from each other against *B. cereus* suggesting variation in potency of extract with dosage.

In case of *P. aeruginosa* and *A. niger*, the interpretation of Duncan multiple range test indicated that ethyl acetate extract of the stem was significantly different from other four extracts, since, among all the prepared stem extracts, only ethyl acetate extract demonstrated activity against these microorganisms. The ethyl acetate extracts at 200 and 100 μg/μl concentrations were considerably different from each other as well as from other treatments of ethyl acetate extract, as evidenced from their strength at the tested concentrations (Table 4). All the stem extracts showed no responses against *S. virchow*.

4. Conclusions

The results of preliminary phytochemical screening suggest that both stem and root extracts of *E. longifolia* are good sources of beneficial phytochemicals. The antifungal activities of the prepared extracts indicated that stem extracts are more potent antimicrobial agent as compared to the root extracts. Among the various prepared extracts of stem, methanol and ethylacetate extracts were most effective against tested bacterial and fungal species. The statistical analyses performed with regard to antimicrobial activities of different extracts at various concentrations were corroborated with the present findings. Thus, further research is warranted to determine the efficacy of these extracts against various other pathogenic bacterial and fungal species. Moreover, *E. longifolia* obtained from various geographical locations could also be explored; to evaluate better potential of the plant as a source of antimicrobials. Also, there is call for isolation and identification of active principles of the plant extracts responsible for antimicrobial activity in order to develop future pharmaceuticals.

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