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Antimicrobial and antioxidant activities of *Blumea lanceolaria* (Roxb.)

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Medicinal plants are being used for the treatment of several ailments by the local tribes in Mizoram, North East India. The present study was focused to analyze the antibacterial and antioxidant activities of a widely used traditional medicinal plant, *Blumea lanceolaria.* Total phenol content was found to be high in the ethanolic extract of leaf (33.91 mg GAE/g DW) in comparison to root and stem of the plant. Reducing activity was measured by potassium ferricyanide reducing (FRAP) assay and found to be highest in the methanolic extract of root (39.29 mg GAE/g DW). Methanolic extracts of root showed highest reducing activity of superoxide dismutase (9.4 SOD units/ mg protein) and ascorbic acid oxidase (1.52 ascorbic acid oxidase units/mg proteins). A positive correlation was obtained between total phenol content and antioxidant activities of the extracts. The screening of the antibacterial activity of different extracts was conducted by using agar well diffusion test against three human bacterial pathogens. Ethanol extracts of stem, root and leaf showed promising results against *Staphylococcus aureus* with high (10 to 12 mm) inhibition zone as compared to standard antibiotics (2 to 10 mm). This study concludes that *B. lanceolaria* has high antioxidant and antibacterial activities and could be used as a potent plant in the treatment of a variety of ailments.

Key words: *Blumea lanceolaria*, ethno-medicine, antimicrobial activity, phenolic content, antioxidant activity.

INTRODUCTION

Several new antibiotics are being produced annually by pharmacological industries, whereas resistance to these drugs by micro-organisms has also increased gradually. The mortality rates in the hospitals are increasing due to new infection caused by multi-drug resistant bacterial strains (Cohen, 1992). Moreover, the use of synthetic drugs can also have serious side-effects and are ineffective for sustainable disease management (Sydney, 1980; Cunha, 2001). Hence, it is necessary to search the Replacement of synthetic antimicrobials with natural products that can inhibit the resistance mechanisms.

Plant extracts have great potential as antimicrobial compounds and are being used for the treatment of infections caused by resistant microbes (Silvia et al., 2013; Nascimento et al., 2011). About 80% of world population depends exclusively on plants for treating diseases (Khali et al. 2007; Vermani and Garg, 2002). Phenolic compounds improve the quality of plants by hindering oxidative degradation of lipids. Phenols comprise the largest group of plant secondary metabolites and have multiple

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biological effects, including antioxidant property. A correlation between the antioxidant capacity and phenolic content shows the importance of phenolic compounds (Vega-Galvez et al., 2007; Wojdylo et al., 2007). Medicinal herbs contain diverse classes of compounds such as polyphenols, tocophenols, alkaloids, tannins, carotenoids, terpenoids, etc.. Flavanoids and phenolic acids are particularly attractive as they are known to exhibit anticarcinogenic, antiviral, anti-inflammatory and antiallergic properties (Khali et al., 2007; Kraus et al., 1981). Antimicrobial compounds in plant extracts can lead to novel drug discovery against infectious diseases (Salwa et al., 2011).

Ethno-medicine has found a special place in lives of the people living in rural Mizoram. Due to the topography of the state, local people cannot avail all the modern medical facilities (Sharma et al., 2001). Thus, majority of the population is still following traditional methods of treatment to cure various diseases by using medicinal plants. *B. lanceolaria* (Roxb.) is a unique folkloric medicinal plant used by the native of Mizoram and is found in Thailand and Africa as well. A decoction of the leaves is taken orally to treat stomach ulcers, dysentery and wounds (Rai and Lalramnghinglova, 2010). More than 70 constituents have been isolated from the genus *Blumea*, including flavonoids, monoterpenes, sesquiterpenes, acetylenic thiophenes, triterpenoids, xanthenes, diterpenes, and essential oils. Blumealactones A, B, and C isolated from *B. balsamifera* exhibited antitumor activities against Yoshida sarcoma cells in tissue culture (Chen et al., 2009, Yasuo et al. 1988). Two acetylenic thiophenes, 63 and 64, isolated from *Begonia obliqua* showed antifungal activity against *Epidermophyton floccosum* and *Pleurotus ostreatus* (Chen et al., 2009; Ahmed and Alam, 1995). The essential oil of Blumea lanceolaria (Roxb.) Druce was analyzed by Dung et al., (1991) and methyl thymol (95%) was found to be its main constituent. As per the available literature, there is not much experimental evidence with regard to antimicrobial activities, total phenolic content, reducing activity and free radical scavenging activities on *B. lanceolaria*.

The present study was undertaken to evaluate the antimicrobial and antioxidant activities of different plants parts of *B. lanceolaria.* This study also assesses the correlation between *in-vitro* antioxidant activity of plant extracts and their total phenolic content.

MATERIALS AND METHODS

Collection and preparation of samples

B. lanceolaria (Roxb.) was collected from Dampa Tiger Reserve forest, Mamit district (23°42'N 92°26'E) of Mizoram in the month of June, 2014. The fresh leaf, stem and root samples were washed thoroughly 2 to 3 times with distilled water, cut into small pieces of 2 to 3 cm length using sterile blade and dried in hot air oven at 37°C for 72 to 96 h. The dried plant materials were ground to fine powder and stored in air tight dark bottles at room temperature. 10 g of each plant materials were extracted by mixing with distilled water (100 ml), 60% methanol (100 ml) and 95% ethanol (100 ml) in a waterbath at 40°C for 30 min. Extracts were filtered through Whatman No.1 paper filter and collected. Ethanol and methanol extracts were concentrated to dryness in a soxhlet apparatus at 60°C for 30 min and aqueous extracts were freeze dried. All extracts were stored at 4°C until further analysis (Wijeratne et al., 2006; Hatamnia et al., 2013).

Determination of antimicrobial activity

Strains and media

Three human pathogenic micro-organisms – *S. aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 2453) and *Escherichia coli* (MTCC 739) were collected from Microbial Type Culture Collection (MTCC), Chandigarh, India and used to test the antimicrobial activity of the plant extracts. All bacteria strains were grown in Nutrient broth at 37°C.

Antibacterial assay of known antibiotics

The test bacteria from the stock cultures were spread on the surface of the solidified Nutrient agar using sterile L spreader. After drying the agar surface, antibiotic discs of streptomycin (10 µg), tetracycline (30 µg), erythromycin (15 µg), chloramphenicol (30 µg), norfloxacin (10 µg) (Hi Media, Mumbai, India) were placed on the agar plate and incubated at 37°C for 24 h. Antibiotic sensitivity of each bacterial pathogen was evaluated by measuring the inhibition zone (mm) around each antibiotic disc.

Antimicrobial activity of plant extracts

Evaluation of the antibacterial activity of the crude ethanolic, methanolic and aqueous extracts of *B. lanceolaria* parts was determined by the agar well diffusion method of Silvia et al. (2013), Shoba et al., (2014). Inoculum of the bacterial strains (10^8 CFU/ml) was spread using sterile spreaders into 90 mm petri dishes with Mueller–Hinton agar. Wells of 6 mm were cut with help of sterile cork borer and filled with 50 µL of 30 mg/ml of solvent extracts. Empty wells were used as negative control. The Petri dishes were incubated for 3 h at room temperature for complete diffusion of the samples (Das et al., 2010; Moller, 1966). After initial incubation samples were incubated at 37 ± 1°C for 24 h. Antibacterial activity was evaluated by measuring the inhibition zone.

Estimation of total phenolic content

Preparation of standard solution

Gallic acid was used as the standard which represents the phenolic compounds in the plant extracts. Aliquots of 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 ml from the Gallic acid stock solution (100 mg/ml) were taken in 6 different 10 ml volumetric flask. To each flask, 2.5 ml of 1N Folin-Ciocalteu reagent and 2 ml of 20% sodium carbonate were added. The mixture was allowed to stand for 15 min and the volume was made up with water to get a concentration ranging from 2.5 to 25 µg/ml. The absorbance was measured at 760 nm against reagent blank. A standard calibration curve was prepared by plotting absorbance Vs concentration and it was found to be linear over this concentration range (Singleton and Rossi, 1965).

Preparation of plant extracts

100 µl of extract was taken in a sterile test tube to which 900 µl of double distilled water along with 500 µl of Folin Ciocalteau phenol reagent were added. The sample was shaken continuously and

Figure 1. Antibacterial activity of *B. lanceolaria* plant extracts against human pathogens.

incubated at room temperature for 10 min. Three ml of Sodium carbonate (20% W/V) was added to the sample, mixed and incubated for 10 min with addition of 10 ml of double distilled water. Similar procedure was carried out for the rest of different extracts with different temperature and time. The concentration of total phenol in the test sample was determined by extrapolation of the calibration curve (Singleton and Rossi, 1965).

Measurement of reducing activity

Ferric reducing activity of plant extracts was determined according to Li et al. (2008). 1 ml of different extract solution was mixed with 2.5 ml potassium buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. The reaction was stopped by adding 2.5 ml trichloroacetic acid. Further, 2.5 ml of distilled water and 0.5 ml 0.1% of ferric chloride were added and allowed to incubate for 30 min. at room temperature. Absorbance was measured at 593 nm. The absorbance obtained was expressed as gallic acid equivalent in mg per gm of dry weight (GAE/g) using gallic acid standard curve (Rao et al., 2013).

Estimation of enzymatic antioxidants

Assay of superoxide dismutase

0.5 ml of plant extracts were mixed separately with 1 ml of 125 mM sodium carbonate, 0.4 ml of 25 µM nitro blue tetrazolium (NBT) and 0.2 ml of 0.1 mM EDTA in a test tube. The reaction was initiated by the addition of 0.4 ml of 1 mM Hydroxylamine hydrochloride in the mixture. Absorbance was measured at 560 nm using spectrophotometer (Agilent Technologies, cary 60, USA) at 5 min. interval. SOD activity was expressed as units /mg protein and unit of SOD was expressed as amount of enzyme required for inhibiting the reduction of NBT by 50% (Beauchamp and Fedovich, 1976).

Assay of Ascorbic acid oxidase

0.1 ml of the plant extracts were added to 3 ml solution of ascorbic acid (8.8 mg in 300 ml phosphate buffer, pH 5.6). The change in absorbance was observed at 265 nm and measured at 30 s intervals for 5 min. One enzyme unit was expressed as to 0.01 OD change / minute/mg protein (Oberbacher and Vines, 1963).

Statistical analysis

All the experiments were performed statically in triplicate by using Microsoft Excel XP 2007 and expressed as mean ± SD (standard deviation). The correlation between the antioxidant capacities and total phenolic contents was analyzed using the simple linear regression, and the correlation coefficient (R^2) was calculated by using statistical package for the social sciences (SPSS) software version 16.0. ANOVA (analysis of variance, $P < 0.05$) was also performed using SPSS statistical software package version 16.0. Furthermore, the differences in means were contrasted using Duncan's new multiple range test following ANOVA.

RESULTS

Evaluation of antibacterial activity of plant extracts

The antimicrobial potential of the extracts of root, stem, leaves of *B. lanceolaria* was compared based on their zone of inhibition against the gram positive (*S. aureus*) and gram negative bacteria (*P. aeruginosa* and *E. coli*). Both ethanolic (12.1 mm ± 0.28) and methanolic (8.1 mm ± 0.28) extracts of root showed the highest antibacterial activity against *S. aureus*. Among gram negative bacteria, ethanolic extract showed more inhibition towards *E. coli* (8.1 mm ± 0.28). Ethanol and methanol extracts of leaf of *B. lanceolaria* showed the highest antibacterial activity against *S. aureus* strain. Highest zone of inhibition was achieved in case of ethanol extract (12 mm ± 0.00). Among gram negative bacteria, ethanolic extract of leaf showed more inhibition towards *P. aeruginosa* (7 mm ± 0.0) zone of inhibition. Methanolic extract of leaf too showed more zone of inhibition towards *P. aeruginosa* among gram negative bacteria (3.6 mm ± 0.28). Methanolic extract did not show any inhibition toward *E. coli* Figure 1. Ethanol and methanol extracts of stem showed the same results as obtained with extracts from leaf and root, showing highest antibacterial activity against *S. aureus*. Highest zone of inhibition was achieved

Table 1. Antibacterial activity of *B. lanceolaria* against human pathogen.

Test organism	Extracts(mm) ± SD									Antibiotics				
	Root			Leaf			Stem			N	T	E	C	S
	Met	Eth	DW	Met	Eth	DW	Met	Eth	DW					
S. aureus	8.1±0.28^{a}	12.1±0.8^{a}	0.0±0.0	6.0± 0.50^{a}	12.0±0.00^{a}	0.0±0.0	7.0±0.0^{a}	10.1±0.28^{a}	0.0±0.0	7.0±0.0^{a}	10.1±0.28^{a}	3.0±0.0^{a}	8.1±0.28^{a}	2.1± 0.28a
P. aeruginosa	4.0±0.0^{bc}	6.0±0.50^{c}	0.0±0.0	3.6±0.28^{bc}	7.0±0.0^{bc}	0.0±0.0	0.0±0.0^{d}	5.1±0.28^{bd}	0.0±0.0	4.0± 0.50^{bd}	10.1±0.28^{a}	3.1±0.28^{a}	5.1±0.28^{bd}	2.0±0.0^{a}
E. coli	4.0±0.0^{bc}	8.1±0.28^{d}	0.0±0.0	0.0± 0.0^{bd}	6.0±0.50^{bc}	0.0±0.0	6.0±0.5^{e}	9.0± 0.0^{ce}	0.0±0.0	5.1±0.28^{ce}	8.0±0.50^{b}	0.0±0.0^{b}	0.0±0.0^{ce}	2.0±0.5^{a}

The values are the average of three replicates and are expressed as mean ±S.D. Mean (±SD) followed by the same letter(s) in each column are not significantly different at P<0.05 using Duncan's new multiple range test. DW - Aqueous extract, Eth - Ethanol extracts, Met - Methanol extract, N- Norfloxcin, T- Tetracycline, E-Erythromycine, C- Chloramphenical, S- Streptomycin.

in case of ethanol (10.1 mm ± 0.28) and methanol (7 mm ± 0.0) extracts. Among gram negative bacteria, methanolic extract did not show any inhibition toward *P. aeruginosa*. Ethanolic extract showed inhibition towards *P. aeruginosa* with inhibition zone of 5.1 mm ± 0.28. Both ethanolic and methanolic extracts showed more inhibition towards *E. coli* (9 mm ± 0.00 and 6 ± 0.50 mm, respectively) when it comes to gram negative bacteria (Table 1). Aqueous extract from all plant parts did not show any inhibition zone.

Antibiotic susceptibility test

Bacterial pathogens were tested for their antibiotic sensitivity against five standard antibiotics. All pathogens were found to be sensitive particularly against tetracycline, showing highest zone of inhibition (*S. aureus* – 10 mm, *P. aeroginosa* – 10 mm and *E. coli* – 8 mm). Norfloxin and chloramphenicol were also found to inhibit most of the pathogens. *E. coli* alone was found to be resistant against chloramphenicol and erythromycin. Streptomycin showed inhibition against all pathogenic bacteria but also exhibit least inhibition zones (2 mm) for assessing susceptibility (Table 1).

Total phenolic content

The phenolic components were extracted from leaf, stem and root by three different solvents viz. water, methanol and ethanol. The total phenol concentration was expressed as gallic acid equivalent /g/dry wt. Phenolic contents ranged from 21.4 to 33.9 mg gallic acid equivalent/g/dry wt. Ethanol extracts of leaf showed the highest amount of phenolic content (33.91mg/ml/gw) while the lowest content was observed in aqueous extract of stem (21.4 mg/ml/gw) (Table 2).

Measurement of reducing activity

In vitro antioxidant activity of the plant extract is shown in (Table 2). Methanolic extract of root has the highest reducing activity of 39.29 mg equivalent to Gallic acid (GAE)/g while aqueous extract of stem has least reducing activity of 20.58 mg GAE/g DW.

Correlation between antioxidant capacity and total phenolic content

The correlation between antioxidant capacity and total phenolic content of extracts of *B. lanceolaria* is shown in Figure 2. There was a positive linear correlation between antioxidant capacity and total phenolic content with R^2=0.63. This indicate positive linear correlations between total phenolic compounds and antioxidant capacity supported by scatter diagram showing linear path diagonally from bottom left hand corner to the top right. Correlation was plotted by using SPSS 16.0 software.

Estimation of enzymatic antioxidants

Superoxide dismutase and ascorbic acid oxidase are important antioxidant defense in plants. Methanolic extract of root possessed highest SOD and ascorbic acid oxidase activity with 9.4 SOD units/ mg protein and 1.52 ascorbic acid oxidase units/mg proteins as compared to other extracts (Table 3). It is also evident from the tabulated results that ethanolic, methanolic and aqueous extracts of the roots have more SOD and ascorbic acid oxidase activity than other parts. The least activity was observed in aqueous extracts of all the plant parts used in the study.

DISCUSSION

Resistance of bacteria to antimicrobials has become a major concern to clinical and public health sectors. New strategies for antibiotic discovery or new alternatives to control bacterial infection by evading fast become a major concern to clinical and public health sectors. New strategies for antibiotic discovery or new alternatives to control bacterial infection by evading fast evolving resistance

Table 2. *In vitro* antioxidant activity and total phenolic content of *B. lanceolaria* plant extracts.

Solvent	Plant parts	Total phenol Content (mg GAE/g DW)	In vitro antioxidant activity - FRAP method (mg GAE/g DW)
Methanol	Stem	23.18±0.32^{a}	29.00±0.02^{a}
	Root	31.40±0.14^{bc}	39.29±0.05^{bc}
	Leaf	29.2±0.18^{bde}	33.72±0.03^{bc}
Dist. water	Stem	21.40±0.14^{adfg}	20.58±0.05^{bde}
	Root	31.60±0.12^{bcehi}	37.72±0.02^{bcfg}
	Leaf	25.63±0.01^{adfgjk}	31.86±0.15^{adfhi}
Ethanol	Stem	23.96±0.12^{acfhjkm}	21.72±0.07^{bdehjk}
	Root	22.67±0.01^{adfhjlmo}	25.58±0.25^{bdfhjkm}
	Leaf	33.91±0.02^{bcfhilnp}	31.15±0.32^{adfhiln}

The values are the average of three replicates and are expressed as mean ±S.D.
Mean (±SD) followed by the same letter(s) in each column are not significantly different at P<0.05 using Duncan's new multiple range test.

resistance are highly desirable. Plants are known to have therapeutic values to treat various infections and diseases for centuries. Plants can produce complex mixtures of different compounds, some of which are being reported to have high antimicrobial activity against several important clinical pathogens (Vilas, 2013). The antibacterial activity of the tested ethanol and methanol extracts of different parts of *B. lanceolaria* was more effective against the growth of Gram-positive compared to the Gram-negative bacteria. Gram negative bacteria have a lipopolysaccharide outer membrane through which entry of molecules is achieved based on their size and shapes. Most of the compounds present in aqueous and methanolic extracts probably could not pass through the outer membrane to reach their site of action resulting in less inhibition (Fernandez-Agullo et al., 2013; Kavak et al., 2010).

There was significant antibacterial activity in ethanol extract of all the plant parts tested (Table 1). The zone of inhibition measured with ethanolic extract of root and leaf was highest against *S. aureus* which was more than any of the known antibiotics used in this study. However, tetracycline showed more inhibition compared to plant extracts against gram negative bacteria. Though, *B. lanceolaria* ethanol and methanol extracts were showing significant inhibition of gram negative bacteria as compared to few other antibiotics used in the present study. Plants with high phenol content are important for food industry as it can inhibit oxidative degradation of lipid and improve the quality and nutritional value of food (Kahkonen et al., 1999). The highest concentration of phenol was found in ethanolic extracts of root. The concentration of the phenolic compounds is controlled by different factors like environment, development stage, type of solvent used and the degree of polymerization of phenolic (Fratianni et al., 2007; Fernandez-Agullo et al., 2013). Phenolic compounds can stabilize and delocalize the unpaired electron, chelate metal ions, protect against microbial infections and act as a good source of antioxidant (Kahkonen et al., 1999; Boo et al., 2012). In vitro antioxidant activity of methanolic extracts of root *B. lanceoria* has highest reducing activity, suggesting that *B. lanceoria* extracts act as an electron donor which can react with free radicals to stop the radical chain reaction (Huda-Faujan et al., 2009).

Reactive oxygen species (ROS) can lead to many oxidative damages causing degenerative diseases such as atherosclerosis, heart diseases, aging and cancer (Finkel and Holbrook, 2008; Madhavi et al., 1996). Medicinal plants are considered to play a beneficial role in health sector by curing and preventing various ailments and diseases. The health promoting activities of antioxidants from plants can be attributed to reduce the potential effects of ROS. However, synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene needs to be replaced with natural antioxidants as they also possess potential health risks (Oyaizu, 1986; Safer and Al-Nughamish, 1999; Li et al., 2008).

Therefore in today's scenario, medicinal plants as a resource of medicine has become more important where oxidative stress is found to be one of the major causes of health hazards (Padmaja et al., 2011). Enzymes such as superoxide dismutase catalyze the dismutation of superoxide (O^{2-}) into oxygen and hydrogen peroxide. Ascorbic acid oxidase is also an important enzyme often regarded responsible for the first line of defense against various oxidative stresses (Nicholas, 1996).

Conclusion

In the present study, methanolic extracts of root was

Table 3. Enzymatic antioxidant activity of the *B. lanceolaria* medicinal plant extracts.

Solvent	Plant parts	SOD units/ mg protein	Ascorbic acid oxidase units/ mg protein
Methanol	Stem	8.2 ± 0.60^{a}	1.05 ± 0.04^{a}
	Root	9.4 ± 0.67^{bc}	1.52 ± 0.09^{ab}
	Leaf	7.6 ± 0.56^{bde}	$0.091 \pm .01^{bcd}$
Dist. water	Stem	4.5 ± 0.21^{bdfg}	0.65 ± 0.05^{abef}
	Root	5.8 ± 0.32^{bdfgi}	0.92 ± 0.10^{abef}
	Leaf	3.3 ± 0.14^{bdfhjk}	0.88 ± 0.06^{abef}
Ethanol	Stem	7.3 ± 0.70^{adehjlm}	1.25 ± 0.14^{abef}
	Root	8.5 ± 0.62^{acfhjlmo}	1.26 ± 0.17^{abef}
	Leaf	6.2 ± 0.66^{bdfhilnp}	0.98 ± 0.08^{abef}

The values are the average of three replicates and are expressed as mean ±S.D. Mean (±SD) followed by the same letter(s) in each column are not significantly different at $P<0.05$ using Duncan's new multiple range test.

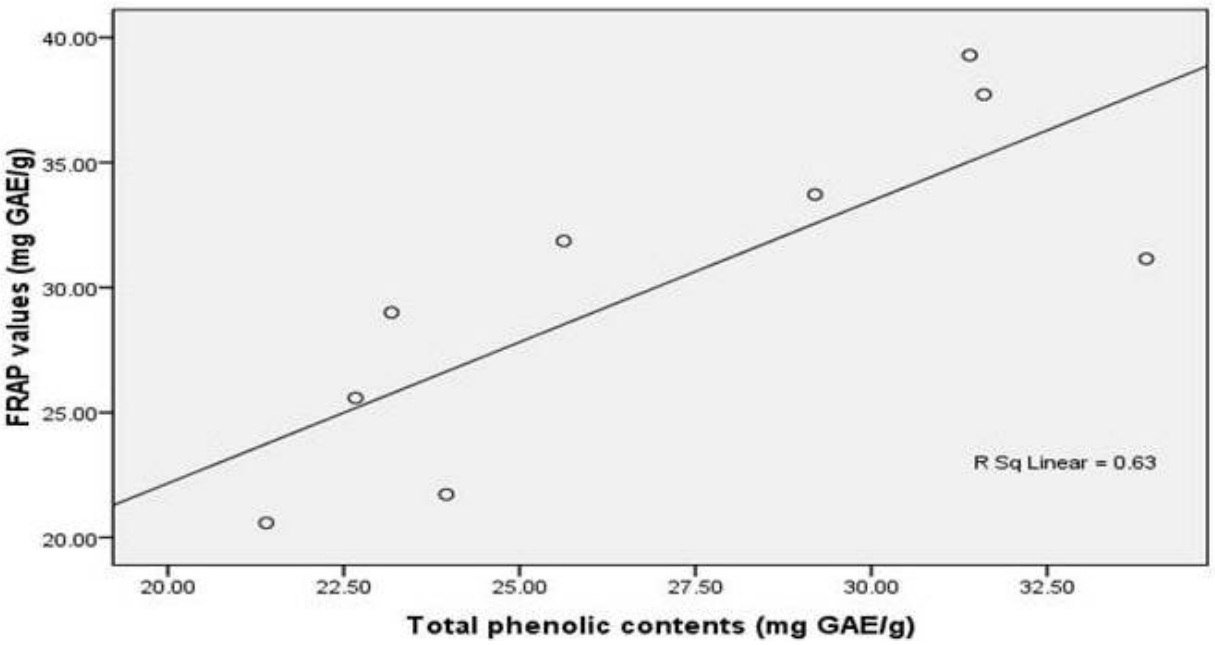

Figure 2. Correlation between FRAP values and total phenolic contents.
GAE: Gallic acid equivalents.

showing highest reducing, superoxide dismutase and ascorbic acid oxidase activities indicating that roots of *B. lanceolaria* are a rich source of antioxidants (Table 3). Phenolic compounds such as flavonoids, tannins and phenolic acids are known to contribute in antioxidant capacities of plants (Cai et al., 2004). The correlation coefficient (R^2) between FRAP values and total phenolic contents for the different parts of medicinal plant extracts was 0.63 (Figure 2). A positive linear correlation between the antioxidant capacities and the total phenolic content of *B. lanceolaria* extracts indicates that phenolic compounds are major contributors of antioxidant activities of the plant. This result was in concordance with previous studies (Cai et al., 2004; Li et al., 2013). The findings of the present study demonstrated the potential of *B. lanceolaria* extracts as rich source of antioxidant and significant antibacterial activity.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

REFERENCES

Ahmed VU, Alam N (1995). New antifungal bithienylacetylenes from *Blumea obllqua*. J. Natl. Prod. 58:1426-1429.

Beauchamp C, Fedovich BC (1976). Superoxide dismutase: Impproved assay and an assay applicable to acrylamide gel. Anal. Biochem. 10:276-287.

Boo HO, Hwang SJ, Bae CS, Park SH, Heo BG, Gorinstein S (2012). Extraction and characterization of some natural plant pigments. Ind. Crop Prod. 40:129-135.

Cai YZ, Luo Q, Sun M, Corke H (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 74:2157-2184.

Chen M, Jin H, Zhang W, Yan S, Shen Y (2009). Chemical constituents of plants from the genus *Blumea.* Chem. Biodivers. 6:809-817.

Cohen ML (1992). Epidemiology of drug resistance: implications for a post-antimicrobial era. Science 257:1050-1055.

Cunha BA (2001). Antibiotics side effects. Med. Clin. N. Am. 85:149-185.

Das K, Tiwari RKS, Shrivastava DK (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: current methods and future trends. J. Med. Plants Res. 4:104-111.

Dung NX, Loi DT, Huang DT, Leclercq PA (1991). Chemical Composition of the Oil of *Blumea lanceolaria* (Roxb.) Druce from Vietnam. J. Essent. Oil Res. 3:285-286.

Fernandez-Agullo A, Pereira E, Freire MS, Valentao P, Andrade PB, Gonzalez AJ, Pereira JA (2013). Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. Ind. Crop Prod. 42:126-132.

Finkel T, Holbrook NJ (2008). Oxidants, oxidative stress and the biology of ageing. Nature 408(6809):239-47.

Fratianni F, Tucci M, De-Palma M, Pepe R, Nazzaro F (2007). Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L) Fiori). Food Chem. 104:1282-1286.

Hatamnia AA, Abbaspour N, Darvishzadeh R (2013). Antioxidant activity and phenolic profile of different parts of Bene (*Pistacia atlantica* subsp. *kurdica*) fruits. Food Chem. 62:155-61.

Huda-Faujan N, Noriham A, Norrakiah A, Babji AS (2009). Antioxidant activity of plants methanolic extracts containing phenolic compounds. Afr. J. Biotechnol. 8(3):484-489.

Kahkonen MP, Hopia AI, Vuorel HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M (1999). Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. J. Agric. Food Chem. 47:3954-3962.

Kavak DD, Altiok E, Bayraktar O, Ulku S (2010). *Pistacia terebinthus* extract as a potential antioxidant, antimicrobial and possible glucuronidase inhibitor. J. Mol. Catal. B: Enzym. 64:167-171.

Khali MY, Moustafa AA, Naguib NY (2007). Growth, phenolic compounds and antioxidant activity of some Medicinal plants grown under organic farming condition. World J. Agric. Sci. 3:451-457.

Kraus W, Cramer R, Sawitzki G (1981). Tetranotripenoids from seeds of *Azardirachta indica.* Phytochemistry 20:117-120.

Li HB, Wong CC, Cheng KW, Chen F (2008). Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT-Food Sci. Technol. 41:385-390.

Li LSK, Gan RY, Song FL, Kuang L, Li HB (2013). Antioxidant capacities and total phenolic contents of infusions from 223 medicinal plants. Ind. Crop Prod. 51:289-298.

Madhavi DL, Deshpande SS, Salunkhe DK (1996). Food antioxidants: Technological, toxicological, health perspective. New York: Marcel Dekker.

Moller AJR (1966). Microbiological examination of root canals and periapical tissues of human teeth. Odontol. Tidskr 74:1-38.

Nascimento GGF, Locatelli J, Freitas PC, Silva GL (2011). Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Braz. J. Microbiol. 31:247-256.

Nicholas SO (1996). The Function and Metabolism of Ascorbic Acid in Plants. Ann. Bot. 78:666-669.

Oberbacher MF, Vines HM (1963). Estimation of ascorbic acid oxidase in plant tissue. National 197:1203-1205.

Oyaizu M (1986). Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jap. Soc. Nutr. Food Sci. 44:307-315.

Padmaja M, Sravanthi M, Hemalatha KPJ (2011). Evaluation of antioxidant activity of two Indian medicinal plants. J. Phytol. 3(3):86-91.

Rai PK, Lalramnghinglova H (2010). Ethnomedicinal plant resources of Mizoram, India: Implication of traditional knowledge in health care system. Ethnobot. Leafl. 14:274-305.

Rao SB, Jayanthi M, Yogeetha R, Ramakrishnaiah H, Nataraj J (2013). Free radical scavenging activity and reducing power of *Gnidia glauca* (Fresen.) Gilg. JAPS 3:203-207.

Safer AM, Al-Nughamish AJ (1999). Hepatotoxicity induced by the anti-oxidant food additive butylated hydroxytoluene (BTH) in rats: An electron microscopical study. Histol. Histopathol. 14:391-406.

Salwa AIE, Taha MB, Abdalla MAM (2011). Amendment of soil fertility and augmentation of the quantity and quality of soybean crop by using phosphorus and micronutrients. Int. J. Acad. Res. 3(2):10-127.

Sharma HK, Chhangte L, Dolui AK (2001). Traditional medicinal plants in Mizoram, India. Fitoterapia 72:146-161.

Shoba FG, Babu VA, Parimala M, Sathya J (2014). In vitro evaluation of antimicrobial activity of *Moringa oleifera* and *Momordica charantia* seeds. Int. J. Pharm. Sci. Res. 5(5):1988-1993.

Silvia M, Amorim ELC, Peixoto-Sobrinho TJS, Saraiva AM, Pisciottano MNC, Aguilar CN, Teixeira JA, Mussatto SI (2013). Antibacterial activity of crude methanolic extract and fractions obtained from *Larrea tridentata* leaves. Ind. Crop Prod. 41:306-311.

Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. 16(3):144-158.

Sydney S, Lacy RW, Bakhtiar M (1980). The Betalactam antibiotics Penicillin and Cephalosporin in perspective. London: Hodder and Stongton. P 224.

Vega-Galvez A, DiScala K, Rodriguez K, Mondaca RL, Miranda M, Lopez J, Perez-Won M (2007). Effect of air-drying temperature on physico-chemical properties, antioxidant capacity, colour and total phenolic content of red pepper (*Capsicum annuum*, L. var. Hungarian). J. Food Chem. 117:647-653.

Vermani K, Garg S (2002). Herbal medicine for sexually transmitted diseases and AIDS. J. Ethnopharmacol. 80:49-66.

Vilas MA (2013). Microbial pathogens and strategies for combating them: Science, Technology And Education. Formatex Research Center Publication, Spain.

Wijeratne SSK, Abou-Zaid MM, Shahidi F (2006). Antioxidant polyphenols in almond and its co-products. J. Agric. Food Chem. 54:312-318.

Wojdylo A, Oszmianski J, Czemerys R (2007). Antioxidant activity of phenolic compounds in 32 selected herbs. Food Chem. 55:940-949.

Yasuo F, Agustine S, Made S (1988). Sesquiterpene lactones from *Blumea balsamifera*. Phytochemistry 27:1109-1111.