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Original Research

Antimicrobial and Antioxidant activity of *Anaphalis lawii* (Hook.f.) Gamble

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Abstract

The present study was undertaken to investigate antimicrobial and antioxidant activities of leaf and flower extracts of *Anaphalis lawii* (Hook.f.) Gamble (Compositae). The powdered leaves and flowers were extracted with methanol in soxhlet extraction assembly. The antimicrobial activity of leaf extract (LE) and flower extract (FE) were determined against a panel of 9 bacteria and 2 fungi by Agar well diffusion method. Antioxidant activity of LE and FE was screened by DPPH free radical scavenging, Ferric reducing and Metal chelating activity. Total phenolic content and flavonoid content of extracts was determined by Folin-Ciocalteu method and Aluminium chloride colorimetric estimation method respectively. Both LE and FE caused inhibition of test bacteria and fungi in a concentration dependent manner. Gram positive bacteria were more susceptible to LE and FE when compared to Gram negative bacteria. On comparing with *Candida albicans*, susceptibility to extracts were found higher in *Cryptococcus neoformans*. The LE and FE dose dependently scavenged DPPH free radical with IC_{50} value of 6.01 and 5.20µg/ml respectively. An increase in the absorbance of reaction mixture with increase in concentrations of extracts showed reducing potential of LE and FE in ferric reducing assay. In metal chelating assay, both LE and FE caused chelation of ferrous ion with IC_{50} value of 180.98 and 152.89µg/ml respectively. The phytochemical analysis revealed the presence of flavonoids, saponins and tannins in both the extracts. Total phenolic and flavonoid contents were high in FE than LE. Overall, FE showed potent antioxidant and antimicrobial activity when compared with LE and it could be related to the high phenolic and flavonoid content in FE. Further studies on isolation of active principles from extracts and testing their bioactivities are to be carried out.

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INTRODUCTION

The idea of healing potential of plants was well accepted before the discovery of therapeutic products from microbes. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the treatment of various maladies. Antibacterial therapy with antibiotics is going through a crisis due to the rapidly increasing development of resistance in microorganisms to existing agents. Higher plants have been shown to be a potential source of anti-infective agents on screening of plant extracts and natural products for antimicrobial activity. Besides antimicrobial properties against human pathogens, these extracts or compounds possess activity against plant pathogens andare also serving drug discovery from natural products for isolation of lead compounds (Rios and Recio, 2005; Ojala *et al*., 2000). Plants synthesize a huge number of aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Most of these substances are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defense mechanisms; give

characteristic odor and flavor and color (Cowan, 1999).

Free radicals are produced continuously by the normal metabolism of oxygen and some cell mediated immune functions of the body. There is a dynamic balance between amount of free radical generation and antioxidants to quench or/and scavenge them and protect the body. Natural defenses of the body (enzymatic, non-enzymatic or dietary origin), when overwhelmed by an excessive generation of free radicals and other reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide results in oxidative stress in which cellular and extracellular macromolecules such as proteins, lipids and nucleic acids suffer from oxidative damage causing tissue injury (Chung *et al*., 2006; Bektasoglu *et al*., 2006). Oxidative stress has been implicated in the pathology of several diseases and conditions such as Diabetes, Atherosclerosis, Alzhemier's disease, Parkinsonism, Cardiovascular diseases, Inflammatory conditions, Neonatal diseases, Cancer and Aging (Shirwaikar *et al*., 2006). ROS are highly reactive oxidizing agents belonging to the group of free radicals and are compounds with one or more unpaired electrons (Bhutia *et al*., 2006). Antioxidants are substances that inhibits or delay oxidative damage when present in small quantities compared to an oxidizable substrate. Antioxidants can help in disease prevention by effective quenching free radicals or inhibiting damage caused by them. Endogenous antioxidants such as ascorbic acid, vitamin E and others present in extracellular fluids act as a primary defense system that protects against oxidative damage. In pathophysiological conditions, however, there is an extra need for exogenous antioxidants from food and medicinal plants (Chatterjee *et al*., 2005).

Anaphalis lawii (Hook. f.) Gamble is a wide spread, very white and tall herb belonging to the family Compositae. It is distributed in Western Ghats, Coorg, Bababudan hills of Karnataka, Brahmagiris, hills of Coimbatore, N. Nilgiris, Anamalais, Pulneys and hills of Tinnevelly, at 5000-7000 ft. Leaf margins flat, not folded back except the upper once of the scape, which are closely pressed and ascending; leaves linear oblong or oblanceolate, very white-wooly, 1-3.5 inch long, 0.3 inch broad; heads 0.2-0.3 inch broad, in broad corymbs of many branches; bracts white, limb ovate, acute; achenes minute (Gamble, 1993). The whole plant is air-dried, powdered, and consumed with food as Kayakalpa by the Malasars of the Velliangiri hills in the Western Ghats of Nilgiri Biosphere Reserve, India (Raghupathy *et al*., 2008). The present study was undertaken to investigate antimicrobial and antioxidant potential of leaf and flower extract of *A. lawii*.

MATERIALS AND METHODS

Collection and Identification of Plant

The plant *A. lawii* was collected at a place called Talakaveri, Karnataka in the month of September 2012 and identified by Prof. Rudrappa D, Department of Botany, SRNMN College of Applied Sciences, Shivamogga, Karnataka. Voucher specimen (SRNMN/MB/Al-001) was deposited in the department herbaria for future reference.

Extraction

The leaves and flowers were separated from plants, washed well to remove adhering matter, dried under shade and powdered using blender. 100 gram of powdered leaf and flower material was subjected to soxhlet extraction and extracted with methanol. The extracts were filtered through 4-fold muslin cloth followed by Whatman No. 1 and concentrated in vacuum under reduced pressure and dried in the desiccator (Kekuda *et al*., 2012).

Phytochemical Analysis

The leaf extract (LE) and flower extract (FE) were tested for the presence of phytochemicals namely alkaloids, flavonoids, tannins, saponins, glycosides and terpenoids by standard tests (George *et al*., 2010; Mallikarjuna *et al*., 2007).

***Test for Tannins*:** About 0.5g of the extract was stirred with 10 ml of distilled water and filtered. 5% ferric chloride reagent was added to the filtrate. A Blue-black precipitate indicates the presence of tannin.

***Test for Saponins*:** 0.5g of the extract was dissolved with 5 ml of distilled water and filtered. Persistent frothing observed when the filtrate was shaken vigorously indicates the presence of saponins.

***Test for Terpenoids*:** 0.5g of extract was dissolved with 5 ml of chloroform and filtered. 10 drops of acetic anhydride was added to the filtrate followed by two drops of concentrated acid. Presence of pink colour at the interphase was an indication of the presence of terpenoids.

***Test for Flavonoids*:** Few pieces of magnesium metal were added to 5ml of the extract and concentrated hydrochloric acid was carefully added. The formation of orange or crimson

colourwas taken as evidence of the presence of flavonoids.

***Test for Glycosides*:** 0.5g of the extract was dissolved in 2 ml of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown coloration at the interphase indicates the presence of a steroidal ring of glycoside.

***Test for Alkaloids*:** 5ml of 1% aqueous hydrochloric acid was added to 5 g of the extract and warmed in a steam bath while stirring. It was filtered and the filtrate was used to test for alkaloid. i) 1 ml of the filtrate was treated with a few drops of Dragendorff's reagent. Formation of a reddish -brown turbid dispersion or precipitate indicates the presence of alkaloid. ii) 1 ml of the filtrate was treated with a few drops of Mayer's reagent. Formation of creamy turbid dispersion indicates the presence of alkaloid.

Antibacterial Activity of LE and FE

In order to screen susceptibility of bacteria to LE and FE, Agar well diffusion method was employed (Kekuda *et al*., 2012). Antibacterial activity was tested against a panel of nine bacteria that included three Gram positive bacteria namely *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus subtilis* and six Gram negative bacteria namely *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigellaflexneri*, *Vibrio cholerae*, *Xanthomonascampestris* and *Klebsiellapneumoniae*. The test bacteria were inoculated into test tubes containing sterile Nutrient broth (Peptone 5g; Beef extract 3g; Sodium chloride 5g; Distilled water 1,000 ml) and incubated for 24 hours at 37 ^{o}C. The broth cultures were aseptically swabbed on the sterile Nutrient agar (Peptone 5g; Beef extract 3 g; Sodium chloride 5g; Agar 20 g; Distilled water 1,000 ml) plates uniformly. Using a sterile cork borer, wells of 0.6 cm diameter were punched in the inoculated plates and 0.2ml of extract (20 mg/ml of 10% DMSO), standard (Chloram-phenicol, 1mg/ml of sterile distilled water) and control (DMSO, 10%) were filled into the respectively labelled wells. The plates incubated for 24 hours at 37 ^{o}C and the zones of inhibition formed around the wells were measured. The experiment was repeated two times and the mean value was obtained.

Antifungal Activity of LE and FE

The antifungal efficacy of LE and FE were tested against two human pathogenic fungi *Candida albicans* and *Cryptococcus neoformans* by Agar well diffusion method (Kekuda *et al*., 2012). The test fungi were inoculated into test tubes containing sterile Sabouraud dextrose broth (Peptone 10g; Dextrose 40g; Distilled water 1,000 ml) and incubated for 24 hours at room temperature. The broth cultures were inoculated aseptically by swabbing uniformly on the sterile Sabouraud dextrose agar (Peptone 10 g; Dextrose 40g; Agar 20g; Distilled water 1,000 ml) plates followed by punching wells of 0.6cm diameter using sterile cork borer. 0.2 ml of extract (20 mg/ml of 10% DMSO), standard (Fluconazole, 1mg/ml of sterile distilled water) and control (DMSO, 10%) were filled into the respectively labelled wells. The plates incubated for 48 hours at room temperature and the zones of inhibition formed around the wells were measured. The experiment was repeated two times and the mean value was obtained.

Antioxidant Activity of LE and FE

DPPH Free Radical Scavenging Assay

The radical scavenging efficacy of different concentrations of LE, FE and ascorbic acid (standard) were evaluated by mixing equal volume (2ml) of 1,1-diphenyl-1-picrylhydra (DPPH) solution (0.002% in methanol) and extracts/standard (2.5-200µg/ml of methanol) in clean and labelled test tubes. The tubes were incubated in dark at room temperature for 30 minutes and the absorbance was measured at 517nm in UV-Visible spectrophotometer. The absorbance of DPPH control was noted. The scavenging activity (%) of each concentration of extracts and standard was calculated using the formula: A_0-A_1/A_0 x 100 where A_0 is absorbance of control and A_1 is absorbance of test (extract/standard). The concentration of extract required to inhibit 50% of free radicals (Inhibitory concentration, IC_{50}) was calculated for each extract (Kekuda *et al*., 2011).

Ferric Reducing Assay

The reducing power of LE, FE and tannic acid (standard) was determined by employing the method of Kekuda *et al*. (2011). Briefly, different concentrations of extracts and standard (5-200µg/ml of methanol) in 1ml of methanol were mixed with 2.5ml of phosphate buffer (pH 6.6), 2.5ml of potassium ferricyanide (1%) and incubated at 50 ^{o}C for 20 minutes in water bath. Afterwards, 2.5ml of trichloroacetic acid (10%) was added to each tube followed by addition of 0.5ml of ferric chloride (0.1%). The absorbance was measured at 700nm after 10 minutes. An increase in the absorbance with increase in concentration of extracts/standard indicated increasing reducing power.

Metal Chelating Activity

The chelating effect of various concentrations (5-200µg/ml) of LE, FE and EDTA (standard) were determined according to the protocol of Dinis *et al*. (1994). The Fe^{+2} was monitored by measuring the formation of ferrous iron- ferrozine complex. The extracts were mixed with 2mM $FeCl_2$ and 5mM ferrozine at a ratio of 10:1:2, shaken and left for 10 minutes at room temperature. The absorbance of the resulting solution was measured at 562nm. A lower absorbance of the reaction mixture indicated a higher Fe^{+2} chelating ability. The capability of extracts to chelate the ferrous iron was calculated using the formula: chelating effect (%) = [1 - (absorbance of sample/absorbance of control)] x 100%.

Total Phenolic Content of LE and FE

The Total Phenolic Contentof LE and FE were estimated by employing the method of Kekuda *et al*. (2011) with minor modifications. A dilute concentration of extract (0.5 ml) was mixed with 0.5 ml diluted Folin-Ciocalteu reagent (1:1) and 2 ml of sodium carbonate (7%). The mixtures were allowed to stand for 30 minutes and the absorbance was measured colorimetrically at 765nm. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000 µg/ml). The concentration of total phenolic compounds was determined as µg Gallic acid equivalents (GAE) from the graph.

Total Flavonoid Content of LE and FE

Aluminium chloride colorimetric estimation method was employed to determine total flavonoid content of LE and FE. A dilute concentration of extract of LE and FE (0.5ml) were mixed with 0.5ml of methanol, 4ml of water, 0.3ml of $NaNO_2$ (5%) and incubated for 5 minutes at room temperature. After incubation, 0.3ml of $AlCl_3$ (10%) was added and again incubated at room temperature for 6 minutes. Later, 2ml of 1M NaOH and 2.4ml of distilled water were added and the absorbance was measured against blank (without extract) at 510nm using UV-Vis spectrophotometer. A calibration curve was constructed using different concentrations of Catechin (0-120 µg/ml) and the flavonoid content of LE and FE was expressed as µg Catechin equivalents (CE) from the graph (Zhishen *et al*., 1999).

Statistical Analysis

All data were expressed as mean±Standard deviation of the number of experiments (n=3). Past software version 1.92 was used. The IC_{50} values were calculated by Origin 6.0 software.

RESULTS

Phytoconstituents Detected in LE and FE

The preliminary phytochemical analysis showed the presence of flavonoids, saponins and tannins in both the extracts. Terpenoids were detected in FE. Alkaloids and glycosides were not detected in LE and FE.

Total Phenolic and Flavonoid Content of LE and FE

Total phenolic and flavonoid content of extracts were expressed as µg GAE/mg and µg CE/mg of extract respectively. The content of total phenolics and flavonoids in LE and FE is shown in Table 1. Both phenolic and flavonoid contents were high in FE when compared to LE.

Table 1: Flavonoid and Total Phenolic content of leaf and flower extract.

Extract	Total Phenolic content (µg GAE/mg extract)	Flavonoid (µg CE/mg extract)
Leaf	175.0±0.05	10.0±0.03
Flower	227.5±0.01	25.0±0.05

Antibacterial Activity of LE and FE

The result of inhibitory activity of LE and FE against Gram positive and Gram negative bacteria is shown in Table 2. The presence of zone of inhibition around the well was considered positive. The extracts were found to cause marked inhibition of bacteria in a dose dependent manner. Among bacteria, Gram positive bacteria have shown higher sensitivity to extracts when compared to Gram negative bacteria except *X. campestris*. FE caused marked inhibition of test bacteria than LE. The inhibition caused by extracts was lesser than that of standard antibiotic. Inhibition of Gram positive bacteria by antibiotic was higher when compared to Gram negative bacteria. DMSO did not cause any inhibition of bacteria.

Table 2: Antibacterial activity of leaf and flower extract.

Test bacteria	Zone of inhibition in cm					
	Leaf Extract(mg/ml)		Flower Extract (mg/ml)		Standard	Control
	50	25	50	25		
S. aureus	2.7±0.03	2.5±0.03	2.9±0.03	2.7±0.03	3.6±0.03	0.0±0.0
B. cereus	2.2±0.01	1.2±0.03	2.8±0.09	2.2±0.01	3.4±0.05	0.0±0.0
B. subtilis	2.7±0.03	2.2±0.06	3.0±0.09	2.8±0.09	3.8±0.01	0.0±0.0
P. aeruginosa	2.5±0.03	2.2±0.03	2.5±0.01	2.3±0.09	2.9±0.03	0.0±0.0
E. coli	1.1±0.09	0.0±0.0	1.3±0.03	0.8±0.03	2.5±0.09	0.0±0.0
S. flexneri	0.8±0.03	0.0±0.03	0.9±0.03	0.0±0.00	2.2±0.03	0.0±0.0
V. cholerae	1.7±0.01	0.9±0.01	2.3±0.05	1.3±0.01	2.5±0.09	0.0±0.0
X. campestris	3.2±0.03	2.5±0.01	3.5±0.09	3.0±0.01	3.2±0.01	0.0±0.0
K. pneumoniae	2.0±0.00	1.7±0.03	2.3±0.01	1.9±0.03	2.9±0.01	0.0±0.0

Antifungal Activity of LE and FE

Antifungal activity of different concentrations of LE and FE against *C. albicans* and *C. neoformans*is shown in the Table 3. Both LE and FE inhibited test fungi dose dependently. Among fungi, *C. neoformans* was inhibited to high extent than *C. albicans*. FE showed high inhibition of test fungi than LE. *C. albicans* was unaffected by LE. Standard antibiotic caused marked inhibition of fungi when compared to LE and FE. There was no inhibition recorded in case of control (DMSO).

Table 3: Antifungal activity of leaf and flower extract.

Test bacteria	Zone of inhibition in cm					
	Leaf Extract (mg/ml)		Flower Extract (mg/ml)		Standard	Control
	50	25	50	25		
C. neoformans	1.1±0.03	0.8±0.01	1.2±0.03	0.8±0.09	1.5±0.03	0.0±0.0
C. albicans	0.0±0.00	0.0±0.00	0.8±0.01	0.0±0.0	1.1±0.09	0.0±0.0

DPPH Radical Scavenging Activity of LE & FE

Free radical scavenging capacities of the LE and FE, measured by DPPH assay are shown in Figure 1. The scavenging efficacy of extracts was dose dependent and was high in FE when compared to LE. The FE, at concentrations 100 and 200 µg/ml scavenged DPPH to more extent when compared with ascorbic acid. The LE, FE and ascorbic acid were able to reduce the stable free radical DPPH to the yellow colored diphenylpicrylhydrazine with an IC_{50} of 6.01, 5.20 and 2.27µg/ml, respectively.

Ferric Reducing Activity of LE and FE

In order to examine the reducing power, the reduction of Fe^{3+} to Fe^{2+} was investigated in the presence of extracts and standard (tannic acid) and the result is shown in Figure 2. The absorbance of the reaction mixtures (at 700nm) was found to increase with the concentration of extracts and standard and is indicating reducing potential. When compared to LE, the reducing activity was found to be higher in FE. The reducing potential of LE and FE were lesser than tannic acid as revealed by higher absorbance.

Metal Chelating Activity of LE and FE

The chelation of ferrous ions by LE and FE was estimated and the result is shown in Figure 3. The formation of the Fe^{2+}-ferrozine complex was not completed in the presence of extracts, indicating that the extracts chelate the iron. FE was found to have high chelating activity than LE and the chelating effect of LE and FE were not marked when compared to EDTA. The absorbance of Fe^{2+}-ferrozine complex decreased dose-dependently with IC_{50} value for LE, FE and EDTA being 180.98, 152.89 and 17.02 µg/ml respectively.

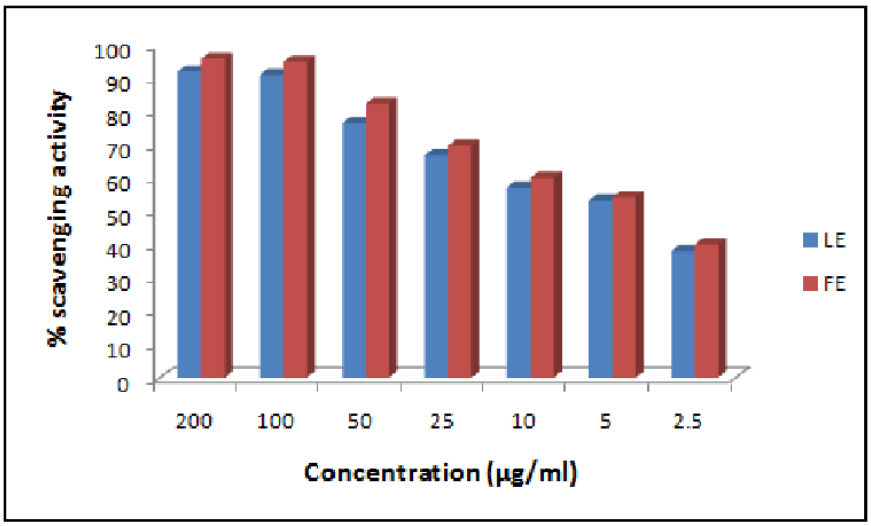

Figure 1: DPPH free radical scavenging activity of LE and FE.

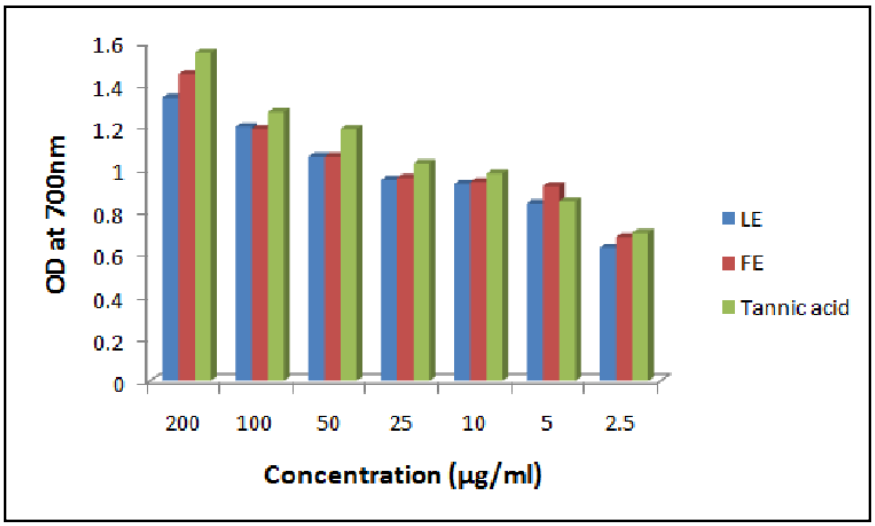

Figure 2: Ferric reducing activity of LE and FE.

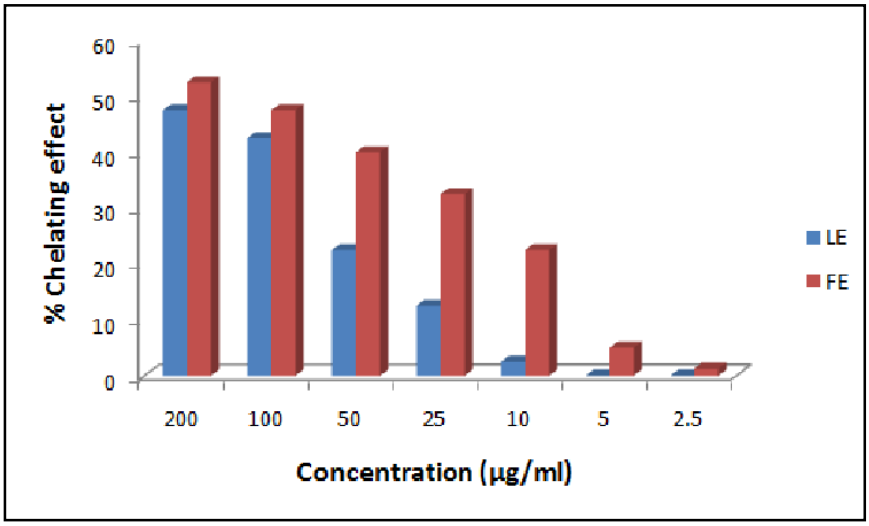

Figure 3: Metal chelating activity of LE and FE.

DISCUSSION

Natural products with several applications are produced from the primary and secondary metabolism of living organisms (plants, animals and microorganisms). Among them, 50-60% are produced by plants and 5% have a microbial origin. Secondary metabolites, particularly antibiotics from microorganisms have exerted a major impact on the control of infectious diseases and other medical applications and the development of pharmaceutical industry. In recent decades, there is increasing number of reports on the development of resistance in microbes against almost all available antimicrobial agents. The major problem of multidrug resistance in Gram negative bacteria was in 1970s; later in 1980s the Gram positive bacteria became important, including methicillin resistant *S. aureus*, penicillin resistant *pneumococci* and vancomycin resistant *enterococci*. Hence, there is a need for the development of new antimicrobials from natural sources, particularly from plants. Many traditional medicinal plants have been used in the treatment of various health ailments. Interest in phyto-medicine has exploded during the last few years. Phytochemicals are considered to be less toxic and does not have side effects when compared with synthetic drugs. The medicinal properties of plants are due to the presence of complex chemical substances which are generally secondary metabolites (alkaloids, flavonoids, other phenolic compounds etc.) present in one or more parts (Demain and Sanchez, 2009; Berdy, 2005; Cowan, 1999; Laikangbam*et al*., 2009).Antimicrobial activities of tannins, flavonoids, saponins, terpenoids, alkaloids, steroids and glycosides have been well documented (Akiyama *et al*., 2001; Ruddock *et al*., 2011; Mandal*et al*., 2005; Singh and Singh, 2003; Paulo *et al*., 1992; Taleb-Contini*et al*., 2003; Nazemiyeh*et al*., 2008). The LE and FE of the plant *A. lawii* is found to possess most of the phytoconstituents such as tannins, saponins, flavonoids and terpenoids. The antimicrobial activity of extracts in this study could be chiefly due to the presence of these phytoconstituents.

Many methods have been developed for measuring the antioxidant capacity *in vitro*. One of the most widely used methods, which is based on quenching of stable free radicals, is DPPH assay. This assay uses commercially available and stable free radical 1,1-diphenyl-1-picrylhydrazil which is soluble in methanol. DPPH free radical has maximum absorption in methanol at 517nm and becomes a stable diamagnetic molecule on accepting an electron or hydrogen atom from antioxidant substances (Kaviarasan *et al*., 2007). It is a very useful compound to evaluate antioxidant potency of compounds. In DPPH test, the antioxidants reduce the DPPH radical to a yellow coloured compound, diphenylpicrylhydrazine, and the extent of reaction will depend on the hydrogen donating ability of compounds (Bondent *et al*., 1997). In this study, we have investigated the ability of varying concentrations of LE and FE to neutralize the free radical DPPH. In the presence of LE and FE, capable of donating hydrogen atom, its free radical nature is lost and hence the reduction in DPPH radical was determined by the decrease in its absorbance at 517nm. It was observed that the extracts at high concentrations showed significant decrease in the absorbance of DPPH radical. Although the scavenging abilities of the extracts were lesser than that of standard (ascorbic acid), it was evident that the extracts possess hydrogen donating ability and could serve as free radical scavengers or inhibitors, acting possibly as primary antioxidants (Chung *et al*., 2006).

The antioxidant activity of certain plant extracts have been related to their reducing potential. The reducing potential of LE and FE was evaluated using ferric reducing assay. The reducing potency is generally associated with the presence of substances called reductones, which exert antioxidant action by breaking the free radical chains, via hydrogen atom donation. Reductones are reported to prevent peroxide formation, by reacting with certain precursors of peroxides. In this assay, the presence of reductants in the samples would result in the reducing of Fe^{+3} to Fe^{+2} by donating electron. The amount of Fe^{+2} complex can be measured by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance indicates an increase in reductive ability (Chung *et al*., 2006; Meir *et al*., 1995; Kekuda *et al*., 2011). It was found that the reducing powers of LE and FE increased with increase in the concentration. FE showed more reducing potential than LE. The reductive abilities of extracts were slightly higher than that of standard (tannic acid).

Iron, a transition metal is essential for oxygen transport, respiration, and activity of enzymes, it is a reactive metal that is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human diseases such as cardiovascular diseases. Fe^{+2} also has shown to cause production of oxyradicals and lipid peroxidation, minimizing Fe^{+2} concentration in Fenton reactions afford protection against oxidative stress. The chelating effect of extracts

is the ability to reduce iron and then form Fe^{+2}-extract complexes that are inert. Binding of antioxidants to iron can suppress the accessibility of the iron to oxygen molecules by changing the redox potential, thus converting the ferrous ion to ferric and thereby inhibiting oxidative damage (Chung *et al*., 2006; Choi *et al*., 2007; Singh *et al*., 2007). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted and eventually that the red color of the complex fades. Measurement of colour reduction therefore allows estimation of the chelating activity of the co-existing chelator (Kekuda *et al*., 2011). The chelating effect of LE and FE was investigated by Ferrous chelating activity. It was found that Fe^{+2} chelating activity of LE and FE increased with increase in concentration but the chelating effect was considerably lesser when compared to standard.

Nowadays, there is growing interest in search of antioxidant chemicals of plants, because they inhibit the propagation of free radical reactions, protect the body from disease and retard oxidative rancidity in foods. The flavonoids and other plant phenolics are present in fruits, vegetables, leaves, nuts, seeds, barks, roots and in other parts. These substances have significance in the field of food chemistry, pharmacy and medicine due to a wide range of favorable biological effects including antioxidant properties. The antioxidant property of phenolics is mainly due to their redox property. Phenolic compounds act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators and thus are effective free radical scavengers and inhibitors of lipid peroxidation (Yen *et al*., 2005; Chung *et al*., 2006; Kaviarasan *et al*., 2007). Phenolic contents of plants have been extensively studied for their contribution to antioxidant activity of plants. There are many reports which correlate the total phenolic content of plants and their antioxidant activity (Tilak *et al*., 2004; Coruh *et al*., 2007; Kekuda *et al*., 2011; Rekha *et al*., 2012). In our study, the FE was found to contain high phenolic content and has shown high DPPH scavenging, ferric reducing and metal chelating activity. The antioxidant activity of extracts, as observed in this study could be directly related with the phenolic content of extracts.

CONCLUSION

The LE and FE of *A. lawii* exhibited marked antimicrobial and antioxidant activity *in vitro*. To the best of our knowledge, this is the first report on these bioactivities of the plant. The observed activities could be attributed to the presence of flavonoids and other phenolic contents of the extracts. Further, isolation of active principles present in the extracts and testing their bioactivities are to be carried out.

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