Journal of Applied Pharmaceutical Science Vol. 3 (06), pp. 114-120, June, 2013 Available online at http://www.japsonline.com DOI: 10.7324/JAPS.2013.3619 ISSN 2231-3354 CC) BY-NC-5A

Antioxidant and antimicrobial activity of flowers of Wendlandia thyrsoidea, Olea dioica, Lagerstroemia speciosa and Bombax malabaricum

Pavithra G.M¹, Saba Siddiqua¹, Abhishiktha S. Naik¹, Prashith Kekuda T.R¹*, Vinayaka K.S² ¹Department of Microbiology, SRNMN College of Applied Sciences, NES Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, India. ²Department of Botany, Indira Gandhi Government College, Sagar-577401, Karnataka, India.

ARTICLE INFO

Article history: Received on: 13/04/2013 Revised on: 29/04/2013 Accepted on: 15/05/2013 Available online: 27/06/2013

Key words: Wendlandia thyrsoidea, Olea dioica, Lagerstroemia speciosa, Bombax malabaricum, DPPH, Ferric reducing, Agar well diffusion

ABSTRACT

Flowers of higher plants have been used for centuries for several purposes such as medicine, food and garnishing food in many parts of the world. In the present study, we have determined the antioxidant and antimicrobial activity of methanol extract of flowers of Wendlandia thyrsoidea (Roemer & Schultes) Steudel (Rubiaceae), Olea dioica Roxb. (Oleaceae), Lagerstroemia speciosa L. (Lythraceae) and Bombax malabaricum DC. (Bombacaceae). Antioxidant efficacy of various concentrations of flower extracts was evaluated by DPPH free radical scavenging assay and Ferric reducing assay. Antimicrobial activity was determined against four bacteria and two fungi by agar well diffusion method. Total phenolic and flavonoid contents were determined by Folin-Ciocalteau reagent and Aluminium chloride colorimetric estimation methods respectively. The DPPH free radical scavenging effect of flower extracts was concentration dependent and was higher in case of extract of L. speciosa followed by W. thyrsoidea, B. malabaricum and O. dioica. In ferric reducing assay, it was shown that the absorbance of reaction mixture at 700nm increased on increasing the concentrations of flower extracts indicating reducing power of extracts. The reducing ability was also highest in L. speciosa extract. Extract of L. speciosa displayed marked inhibitory activity against bacteria and fungi than other flower extracts. Gram positive bacteria have shown more susceptibility than Gram negative bacteria. Among fungi, C. neoformans was more inhibited than C. albicans. Extracts of B. malabaricum and O. dioica were not effective against C. albicans. The phenolic and flavonoid contents were higher in L. speciosa and O. dioica respectively. A positive correlation has been observed between total phenolic content of flower extracts and antioxidant and antimicrobial activity. The flowers can be employed as a remedy for treatment of infectious diseases and oxidative damage. Further, isolation of active components from flower extracts and their biological activity determinations are under progress.

INTRODUCTION

A free radical is any atom or molecule that has an unpaired electron in an outer shell. The production of ROS during metabolite processes is a normal and necessary process that provides important physiological functions. However, when an imbalance between ROS production and antioxidant defences occurs, it leads to oxidative stress which is implicated in over hundred human disease conditions, such as cancer, cardiovascular diseases, aging and neurological disorders. This free radical induced oxidative stress can be prevented by the intake of sufficient amount of antioxidants. It has been reported that antioxidant capacity of plants is because of phenolics, flavonoids and anthocyanins. These phytochemical act on ROS and prevent damage to DNA, proteins and membrane lipids and therefore are significant from the point of health (Elmastas *et al.*, 2006; Li *et al.*, 2009; Gulcin *et al.*, 2011; Rekha *et al.*, 2012; Junaid *et al.*, 2013a). Phenolic compounds are a large and diverse group of secondary metabolites of plants and are shown to possess a range of biological activities including antioxidant activity.

These compounds are widely distributed in fruits, vegetables and cereals. Phenolic compounds have strong antioxidant activity which is associated with their ability to scavenge free radicals, break radical chain reactions and chelate metal ions. Increased consumption of phenolic compounds is found to be associated with reduced risk of several diseases such as cardiovascular diseases and certain types of cancer (Tilak *et al.*, 2004; Dasgupta and De, 2004; Chung *et al.*, 2006; Kaviarasan *et al.*, 2007; Kaisoon *et al.*, 2011).

Department of Microbiology, SRNMN College of Applied Sciences, NES Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, India

^{© 2013} Pavithra G.M et al. This is an open access article distributed under the terms of the Creative Commons Attribution License -NonCommercial-ShareAlike Unported License (<u>http://creativecommons.org/licenses/by-nc-sa/3.0/</u>).

Flavonoids are a group of plant polyphenols. Flavonoids constitute a large group of naturally occurring plant phenolic compounds and include flavones, flavonols, isoflavones, flavonones and chalcones. They contain a characteristic C6-C3-C6 structure with free hydroxyl groups attached to aromatic rings. They are known to possess diverse biological activities including antioxidant activity. The antioxidant property of flavonoids is due to scavenging free radicals or by other mechanisms such as singlet oxygen quenching, metal chelation and lipoxygenase inhibition (Chung *et al.*, 2006; Kaisoon *et al.*, 2011; Izzreen and Fadzelly, 2013).

Infectious diseases, the world's leading cause of premature deaths are caused by bacteria, viruses, fungi and other parasites. These diseases are due to a complex interaction between the pathogen, host and the environment. The discovery of antibiotics and their subsequent use had eradicated these infections which once threatened mankind. However, the indiscriminate use of these commercial antimicrobial drugs led to the development of multidrug resistant pathogens that are serious challenge to the field of medicine. The ability of microbes to acquire and transmit the resistance genes made the situation ever worse as the resistance development in the susceptible strains has been noticed. Antibiotics such as Vancomycin, Methicillin, Penicillin etc., are no longer effective against many bacteria such as S. aureus, P. aeruginosa, Salmonella, Shigella, E. coli, Enterococci, M. tuberculosis and others. This situation is alarming in developing as well as developed countries. These drug resistant microbes have further complicated the treatment of infection diseases in immunocompromised patients, AIDS and cancer patients. This has necessitated a search for new antimicrobials from other sources including plants (Ahmad and Beg, 2001; Hemaiswarya et al., 2008; Mattana et al., 2010; Solorzano-Santos and Miranda-Novales, 2012).

Flowers are the important parts of the plants and many edible flowers have found their use in food, food garnishing etc., in various parts of the world for centuries. They have been consumed fresh or dried, in cocktails (in ice cubes), canned in sugar, preserved in distillates etc. Many natural antioxidants such as phenolic acids, flavonoids, anthocyanins and other phenolic compounds are found in flowers. Flowers are reported to possess several biological activities such as antimicrobial, acaricidal, antioxidant, anti-HIV, cytotoxic, antidepressant, anti-inflammatory etc. The high antioxidant activity of flowers may be attributed to the level of polyphenol compounds including flavonoid. They are also promising sources of various minerals which have key role in the normal health of an individual (Khodadad et al., 2007; Ho et al., 2010; Mert et al., 2010; Nabavi et al., 2011; Kaisoon et al., 2011; Raj et al., 2012; Rop et al., 2012; Ohran et al., 2012; Kietthanakorn et al., 2012; Gao et al., 2013). In the present study, we have investigated the antioxidant and antimicrobial efficacy of methanol extracts of flowers of Wendlandia thyrsoidea (Roemer & Schultes) Steudel (Rubiaceae), Olea dioica Roxb. (Oleaceae), Lagerstroemia speciosa L. (Lythraceae) and Bombax malabaricum DC. (Bombacaceae) and related the observed biological activities

with the phenolic and flavonoid content of the flowers.

MATERIALS AND METHODS

Collection and identification of plant material

The time and place of collection and the voucher numbers of the plants of this study is shown in Table 1. The plants were identified by Dr. Vinayaka K.S, Lecturer, Dept. of Botany, Indira Gandhi Government College, Sagara, Karnataka. The voucher specimens were deposited in the department herbaria, Dept. of Microbiology, SRNMN College of Applied Sciences, Shimoga, Karnataka.

Table. 1: Plants selected in this study.

Name of the plant	Place of collection	Month of collection	Voucher number
W. thyrsoidea (Rubiaceae)	Haniya, Hosanagara (Taluk), Shivamogga (District)	February 2013	SRNMN/PK/ PV-020
<i>B. malabaricum</i> (Bombacaceae)	Lakkinakoppa, Shivamogga (District)	February 2013	SRNMN/PK/ PV-021
<i>O. dioica</i> (Oleaceae)	Kanivebagilu, Hosanagara (Taluk), Shivamogga (District)	February 2013	SRNMN/MB/ PPV-03
L. speciosa (Lythraceae)	NES campus, Shivamogga (District)	March 2013	SRNMN/MB/ SRD-05

Extraction

Extraction of powdered flower materials was done in a Soxhlet apparatus. About 25g of each flower was extracted using methanol. The extract was filtered using Whatmann no. 1, concentrated in vacuum under reduced pressure and dried in the desiccator (Kekuda *et al.*, 2012).

Total phenolic content of flower extracts

Folin-Ciocalteau reagent (FCR) method was employed to estimate the total phenolic contents of flower extracts. A dilute concentration of each flower extract (0.5 ml) was mixed with 0.5 ml of FC reagent (1:1) and 4 ml of sodium carbonate (1M). The reaction mixtures were allowed to stand for 15 minutes and the optical density was measured colorimetrically at 765nm. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000 μ g/ml) and the total phenolic content of extracts was expressed as μ g Gallic acid equivalents (GAE) from the graph (Junaid *et al.*, 2013a).

Total flavonoid content of flower extracts

The content of total flavonoids present in flower extracts was estimated by Aluminium chloride colorimetric method. Here, a dilute concentration of each flower extract (0.5ml) was mixed with 0.5ml of methanol, 4ml of water, 0.3ml of NaNO₂ (5%) and incubated for 5 minutes at room temperature. Following incubation, 0.3ml of AlCl₃ (10%) was added and again incubated at room temperature for 6 minutes. Then, 2ml of NaOH (1M) 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 (standard, 0-120 μ g/ml) and the total flavonoid content of extracts was expressed as μ g Catechin equivalents (CE) from the graph (Zhishen *et al.*, 1999).

Antioxidant activity of flower extracts

Radical scavenging activity of flower extracts

The free radical scavenging ability of flower extracts was tested by employing DPPH radical scavenging assay. Briefly, 1 ml of different concentrations of flower extracts ($5-100\mu$ g/ml of methanol) was mixed with 3 ml of DPPH solution (0.004% in methanol) in separate tubes.

The tubes were then incubated at room temperature in dark for 30 minutes and the optical density was measured at 517 nm using UV-Vis spectrophotometer. The absorbance of the DPPH control was also noted. Ascorbic acid was used as reference standard. The radical scavenging activity of flower extracts and ascorbic acid was calculated using the formula:

Scavenging activity (%) = $[(Ao - Ae) / Ao] \times 100$, where Ao is absorbance of DPPH control and Ae is absorbance of DPPH and extract/standard combination (Elmastas *et al.*, 2006).

Ferric reducing activity of flower extracts

In this assay, various concentrations of flower extracts $(5-100\mu g/ml)$ in 1 ml of methanol were mixed in separate tubes with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of Potassium ferricyanide (1%). The tubes were placed in water bath for 20 minutes at 50°C, cooled rapidly and mixed with 2.5 ml of Trichloroacetic acid (10%) and 0.5 ml of Ferric chloride (0.1%). The amount of iron (II)-ferricyanide complex formed was determined by measuring the formation of Perl's Prussian blue at 700 nm after 10 minutes. The increase in absorbance of the reaction mixtures indicates increased reducing power. Ascorbic acid was used as standard (Junaid *et al.*, 2013a).

Antimicrobial activity of flower extracts

Antimicrobial activity of flower extracts was determined against four bacteria viz., Staphylococcus aureus, Bacillus cereus, Vibrio cholerae and Escherichia coli and two fungi viz., Candida albicans and Cryptococcus neoformans by Agar well diffusion method. The test bacteria and fungi were inoculated into sterile Nutrient broth (HiMedia, Mumbai) and Sabouraud dextrose broth (HiMedia, Mumbai) respectively and incubated overnight at 37°C. The broth cultures of test bacteria and fungi were aseptically swabbed on sterile Nutrient agar (HiMedia, Mumbai) and Sabouraud dextrose agar (HiMedia, Mumbai) respectively using sterile cotton swabs followed by punching wells of 6mm diameter using sterile cork borer. 100µl of flower extracts (50mg/ml of 25% DMSO), standard (Streptomycin (antibacterial) and fluconazole (antifungal), 1mg/ml) and DMSO (25%, in sterile water) were transferred into respectively labeled wells. The plates were incubated at 37°C aerobically for 24 hours (for bacteria) and 48 hours (for fungi) and the zone of inhibition (cm) formed around the wells was measured. The experiment was repeated twice and average zone of inhibition was noted (Junaid et al., 2013b). the

RESULTS

The content of total phenolics and flavonoids is shown in Table 2. The phenolic content was highest in extract of *L. speciosa* (Ls) followed by *W. thyrsoidea* (Wt), *B. malabaricum* (Bm) and *O. dioica* (Od). However, flavonoid content was higher in *O. dioica*. *L. speciosa* extract, displaying high phenolic content, contained lowest flavonoid content among all flower extracts.

Flower	Total phenolic content (µg GAE/mg extract)	Total flavonoid content (µg GAE/mg extract)		
W. thyrsoidea	200.63	30.00		
B. malabaricum	93.75	35.00		
O. dioica	91.25	46.25		
L. speciosa	260.68	22.65		

The efficacy of methanol extracts of flowers to scavenge free radicals was assessed by DPPH radical scavenging model and the results are shown in Figure 1. Extent of bleaching of color of DPPH radicals in the presence of varying concentrations of flower extracts was monitored at 517nm and the scavenging activity of extracts was compared with reference standard ascorbic acid. The scavenging effect was marked in case of extract of *L. speciosa* followed by *W. thyrsoidea*, *B. malabaricum* and *O. dioica*. In case of *L. speciosa*, 90% and higher scavenging of radicals was observed at 25mg/ml and higher concentrations of extract. However, the scavenging effects of all flower extracts were lesser than that of ascorbic acid (AA).





Fig. 1: DPPH free radical scavenging activity of flower extracts and ascorbic acid.



The reducing nature of extract of selected flowers was determined by employing ferric reducing assay. It was shown that the absorbance of reaction mixture at 700nm increased on increasing the concentrations of flower extracts. Like DPPH assay results, the reducing ability was also highest in L. speciosa extract followed W. thyrsoidea and other two extracts. Overall, the reducing powers of flower extracts were lesser than that of ascorbic acid (Figure 2). Result of antimicrobial activity of flower extracts is shown in Table 3. All the test bacteria were found to be susceptible to flower extracts. Here also, extract of *L. speciosa* was more effective against bacteria viz., S. aureus and B. cereus. Extract of O. dioica exhibited least effectiveness against bacteria. Among fungi, C. neoformans was more susceptible than C. albicans. Extracts of B. malabaricum and O. dioica were not effective against C. albicans. Overall, Gram positive bacteria have shown higher susceptibility to flower extracts and standard than Gram negative bacteria. Inhibition of test microorganisms by Streptomycin (against bacteria) and fluconazole (against fungi) was higher than that of flower extracts. There was no inhibition observed in case of DMSO.

 Table. 3: Antimicrobial activity of flower extracts and standard antibiotics.

Zone of inhibition in cm						
Ls	Wt	Bm	Od	Str	Flu	DMSO
2.2	1.7	1.5	1.4	3.8	NA	0.0
1.9	1.6	1.4	1.3	3.6	NA	0.0
1.7	1.4	1.2	1.1	2.9	NA	0.0
1.7	1.5	1.3	1.2	3.1	NA	0.0
1.5	1.2	0.0	0.0	NA	3.9	0.0
1.7	1.4	1.2	1.2	NA	4.1	0.0
	2.2 1.9 1.7 1.7 1.5	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Ls Wt Bm 2.2 1.7 1.5 1.9 1.6 1.4 1.7 1.4 1.2 1.7 1.5 1.3 1.5 1.2 0.0 1.7 1.4 1.2	Ls Wt Bm Od 2.2 1.7 1.5 1.4 1.9 1.6 1.4 1.3 1.7 1.4 1.2 1.1 1.7 1.5 1.3 1.2 1.5 1.2 0.0 0.0 1.7 1.4 1.2 1.2	Ls Wt Bm Od Str 2.2 1.7 1.5 1.4 3.8 1.9 1.6 1.4 1.3 3.6 1.7 1.4 1.2 1.1 2.9 1.7 1.5 1.3 1.2 3.1 1.5 1.2 0.0 0.0 NA 1.7 1.4 1.2 1.2 NA	Ls Wt Bm Od Str Flu 2.2 1.7 1.5 1.4 3.8 NA 1.9 1.6 1.4 1.3 3.6 NA 1.7 1.4 1.2 1.1 2.9 NA 1.7 1.4 1.2 3.1 NA 1.5 1.2 0.0 0.0 NA 3.9 1.7 1.4 1.2 1.2 NA 4.1

NA- Not applicable; Str- Streptomycin; Flu- Fluconazole

DISCUSSION

Evidences gathered in recent years suggest the involvement of free radicals and other oxidants as the major cause of oxidative stress that leads to a variety of diseases and disorders. Strong restrictions have been placed on the use of synthetic antioxidants such as BHT, BHA, gallates due to their carcinogenic potential (Da Silva and Paiva, 2012). This led to an increasing interest in natural products having antioxidant properties. Plants have been considered as richer sources of antioxidants. Polyphenolic compounds including flavonoids have shown to be excellent antioxidants due to their proton donating ability. This property of polyphenols is attributed to interruption of oxidation mechanisms and there by oxidative damage is prevented (Da Silva and Paiva 2012; Gulcin *et al.*, 2011; Ebrahimzedeh *et al.*, 2008).

It has been shown that polyphenolic compounds of plant kingdom are one of the most effective antioxidative constituents and hence it is important to estimate phenolic contents of extracts in order to assess their contribution to antioxidant activity (Choi *et al.*, 2007). In the present study, we have estimated total phenolic content of flower extracts by FCR method and expressed the phenolic content as μ g GAE/mg of dry extract. FCR method is one of the oldest and commonly used colorimetric techniques for the estimation of total phenolic content of a variety of substances including plant extracts. Phenolic compounds reacts with FCR only under basic conditions (adjusted by sodium carbonate solution to pH 10) to form blue complex which has maximum absorption near 750nm. Despite the undefined chemical nature of FCR, the total phenols assay by FCR is convenient, simple, and reproducible. As a result, a large data has been accumulated, and it has become a routine assay in studying the phenolic antioxidants (Dasgupta and De, 2004; Huang *et al.*, 2005; Chung *et al.*, 2006; Harish and Shivanandappa, 2006; Coruh *et al.*, 2007; Ardestani and Yazdanparast, 2007; Kekuda *et al.*, 2011; Rekha *et al.*, 2012; Junaid *et al.*, 2013a). The total phenolic content, as estimated in this study was higher in *L. speciosa* extract followed by *W. thrysoidea* and others.

Flavonoids are polyphenolic compounds representing the majority of plant secondary metabolites and have shown to possess remarkable health promoting effects including antioxidant activity (Chua *et al.*, 2011). Aluminium chloride colorimetric estimation is commonly used to quantify flavonoid content of plant extracts (Zhishen *et al.*, 1999; Penarrieta *et al.*, 2007; Rohman *et al.*, 2010). Total flavonoid contents can be determined by reaction with sodium nitrite, followed by the development of coloured flavonoid-aluminium complex formation using aluminium chloride in alkaline condition which can be monitored spectrophotometrically at maximum wavelength of 510 nm (Rohman *et al.*, 2010). In the present study, the flavonoid contents were higher in methanol extract of *O. dioica* followed by *B. malabaricum* and others.

DPPH is a stable nitrogen centred free radical having maximum absorption at 517nm. On accepting an electron or hydrogen atom, it becomes a stable diamagnetic molecule (Kaviarasan *et al.*, 2007). We have used DPPH free radical scavenging assay in order to determine the radical scavenging ability of flower extracts. This assay is one of the most widely used in vitro assays for determining the radical scavenging effect of extracts. In the presence of an extract capable of donating an hydrogen atom, the free radical nature of DPPH is lost and the color (purple) changes to yellow (diphenylpicrylhydrazine) (Dasgupta and De, 2004; Dixit *et al.*, 2005; Elmastas *et al.*, 2006; Coruh *et al.*, 2007; Kaviarasan *et al.*, 2007; Choi *et al.*, 2007; Ebrahimzadeh *et al.*, 2011; Rekha *et al.*, 2012; Poornima *et al.*, 2012; Junaid *et al.*, 2013a).

In the present study, the decrease in DPPH absorption in the presence of varying concentrations of flower extracts has been monitored at 517nm. It can be noticed that the extracts at high concentrations showed significant decrease in the absorption of DPPH radicals. Highest scavenging of radical was observed in case of *L. speciosa* extract followed by *W. thyrsoidea* and others. Although the scavenging abilities of flower extracts were lesser than that of ascorbic acid, it was evident that the extracts showed hydrogen donating ability and therefore the extracts could serve as free radical scavengers, acting possibly as primary antioxidants (Chung *et al.*, 2006). The measurement of direct reduction of $Fe^{+3}(CN)_6$ to $Fe^{+2}(CN)_6$ was employed in order to evaluate the reducing power of flower extracts. It is determined by measuring the absorbance resulting from the formation of Perl's Prussian blue complex on addition of excess of ferric ions (Fe⁺³). An increase in absorbance at 700nm on increase in extract concentrations indicates reducing capacity.

The reducing properties of antioxidants are generally associated with the presence of reductones. Ferric reducing assay has been employed by several researchers in order to evaluate antioxidant activity of compounds (Yuan *et al.*, 2005; Hinneburg *et al.*, 2006; Kim *et al.*, 2006; Barros *et al.*, 2008; Gulcin *et al.*, 2011; Poornima *et al.*, 2012; Rekha *et al.*, 2012; Junaid *et al.*, 2013a). In this assay, the presence of reductants (antioxidants) in the samples would result in the reduction of Fe⁺³ to Fe⁺² by donating an electron (Chung *et al.*, 2006). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Hsu *et al.*, 2006).

In the present study, it was observed that the reducing powers of all flower extracts increased with the increase of their concentrations. Methanol extract of *L. speciosa* displayed greater reducing power than other flower extracts and the higher reducing potential could be due to high phenolic and flavonoid contents. It is evident from the study that the extracts possess reductive potential and could serve as electron donors, terminating the radical chain reactions (Chung *et al.*, 2006).

Flowers are known to possess excellent antioxidant properties and the presence of phenolic and flavonoid compounds in flowers might play an important role in prevention of oxidative damage. Flower extracts of *Hemerocallis fulva* Linn displayed marked in vitro and in vivo antioxidant activities and the antioxidant activity was related to the presence of rutin, catechin and gallic acid present in the extract (Que *et al.*, 2007). Yang et al. (2007) observed marked antioxidant activity of fractions of extracts of *Camellia sinensis* flowers. Antioxidant phytochemicals isolated from flowers of *Balanophora laxiflora* displayed marked DPPH radical scavenging activity (Ho *et al.*, 2010).

Zakizadeh et al. (2011) found marked DPPH radical scavenging and ferric reducing activity of flower of *Alcea hyrcana* Grossh. The flower extract of *Pyrostegia venusta* showed marked reducing ability, DPPH and ABTS radical scavenging activity (Roy *et al.*, 2011). The flower extract of *Juglans regia* showed DPPH radical scavenging and iron chelating activity (Nabavi *et al.*, 2011).

Usoh et al. (2005) showed the antioxidant efficacy of dried flower extracts of *Hibiscus sabdariffa* L. on Sodium arsenite - induced oxidative stress in rats. In the present investigation, we have assessed the antioxidant efficacy of the selected flowers by DPPH free radical scavenging and ferric reducing assay. Superior antioxidant activity was observed in case of *L. speciosa* extract. A positive correlation was observed between the total phenolic content of flower extracts and the radical scavenging activity. Flower extracts containing high phenolics displayed marked

scavenging and reducing potential and is in justification with earlier studies where extracts/plants containing high phenolics displayed high scavenging potential (Tilak *et al.*, 2004; Coruh *et al.*, 2007; Rekha *et al.*, 2012).

Medicinal plants have been used for centuries as remedies for diseases. Antimicrobials of plant origin have enormous therapeutic potential which are due to the presence of certain metabolites. During the 2^{nd} half of the 20^{th} century, the acceptance of traditional medicine as an alternate form of primary healthcare and the drug resistance problems faced by the therapy using classical antibiotics led the researchers to investigate antimicrobial efficacy of several plants.

Antimicrobial of plant origin are effective in treating diseases and they do not have side effects that are often associated with synthetic drugs. The plant components are also shown to be effective against drug resistant microbes (Nascimento *et al.*, 2000; Ahmad and Beg, 2001; Al-Bakri and Afifi, 2007; Mattana *et al.*, 2010).

Plant derived antimicrobials are always the source of novel therapeutics and a number of studies have been carried out on the efficacy of the plants and plant derived agents against infectious microorganisms (Nostro *et al.*, 2000; Ahmad and Beg, 2001; Rajakaruna *et al.*, 2002; Holetz *et al.*, 2003; Sartoratto *et al.*, 2004; Duraipandiyan *et al.*, 2006; Manjunatha *et al.*, 2006; Al-Bakri and Afifi, 2007; Mattana *et al.*, 2010; Tellez *et al.*, 2010; Pathak *et al.*, 2012).

In our study, the antimicrobial efficacy of *L. speciosa* and *W. thyrsoidea* extracts was marked than other extracts. Gram negative bacteria are generally more resistant than Gram positive bacteria due to the presence of outer membrane which will resist the entry of many substances including antibiotics (Raj *et al.*, 2012). In the present study, Gram positive bacteria have shown higher susceptibility to flower extracts. *S. aureus* was found to be highly susceptible to flower extracts. Similar results were observed in case of flower extract of *Hibiscus rosa-sinensis* by Uddin et al. (2010).

Solvent extracts and purified components of flowers are shown to possess antimicrobial activity. The essential oil of *Leea indica* flowers showed inhibitory activity against pathogenic bacteria and fungi (Srinivasan *et al.*, 2009). Methanolic extract of *Thespesia populnea* flower showed dose dependent inhibition of bacteria (Saravanakumar *et al.*, 2009). The ethanol extract of flower of *Crotalaria juncea* was found to possess antibacterial activity against a panel of Gram positive and Gram negative bacteria (Chouhan and Singh, 2010).

Bergenin isolated from the flowers of *Peltophorum pterocarpum* showed antifungal activity against a panel of fungi including human dermatophytic fungi (Raj *et al.*, 2012). Edziri et al. (2012) showed antimicrobial activity of two flavonoids isolated from flowers of *Retama raetam*. In our study, the higher inhibitory efficacy of extracts might be related to their phenolic and flavonoid content as *L. speciosa* displaying high phenolic content also showed marked antimicrobial activity.

CONCLUSIONS

The present study revealed a marked antioxidant and antimicrobial potential of flower extracts. These flowers can be used as a remedy for treatment of infectious diseases and for prevention and treatment of oxidative damage caused by free radicals.

The antimicrobial and antioxidant activity of flower extracts might be attributed to the presence of phenolic and flavonoids. Further, isolation of active phytochemicals from these flower extracts and determination of their bio-efficacies are under progress.

ACKNOWLEDGEMENTS

The authors express thanks to Head, Department of Microbiology, Principal, SRNMN College of Applied Sciences, Shivamogga for providing all the facilities to conduct work. Authors also express thanks to NES, Shivamogga for the moral support.

REFERENCES

Ahmad I., Beg A.Z. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology. 2001; 74: 113-123

Al-Bakri A.G., Afifi F.U. Evaluation of antimicrobial activity of selected plant extracts by rapid XTT colorimetry and bacterial enumeration. Journal of Microbiological Methods. 2007; 68: 19-25

Ardestani A., Yazdanparast R. Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. Food Chemistry. 2007; 104: 21–29.

Barros L., Falcao S., Baptista P., Freire C., Vilas-Boas M., Ferreira I.C.F.R. Antioxidant activity of *Agaricus* sp. Mushrooms by chemical, biochemical and electrochemical assays. Food Chemistry. 2008; 111: 61-66

Choi Y., Jeong H., Lee J. Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chemistry. 2007; 103: 130-138

Chouhan H.S., Singh S.K. Antibacterial activity of seed and flower parts of *Crotalaria juncea* Linn. American-Eurasian Journal of Scientific Research. 2010; 5(3): 212-215

Chua L.S., Latiff N.A., Lee S.Y., Lee C.T., Sarmidi M.R., Aziz R.A. Flavonoids and phenolic acids from *Labisia pumila* (Kacip Fatimah). Food Chemistry. 2011; 127: 1186-1192

Chung Y., Chien C., Teng K., Chou S. Antioxidative and mutagenic properties of *Zanthoxylum ailanthoides* Sieb & zucc. Food Chemistry. 2006; 97: 418-425

Coruh N., Celep A.G.S., Ozgokce F., Iscan M. Antioxidant capacities of *Gundelia tournefortii* L. extracts and inhibition on glutathione-S-transferase activity. Food Chemistry. 2007; 100: 1249-1253

Da Silva M.C., Paiva S.R. Antioxidant activity and flavonoid content of *Clusia fluminensis* Planch. & Triana. Annals of the Brazilian Academy of Sciences. 2012; 84(3): 609-616

Dasgupta N., De B. Antioxidant activity of *Piper betle* L. leaf extract *in vitro*. Food Chemistry. 2004; 88: 219-224

Dixit P., Ghaskadbi S., Mohan H., Devasagayam T.P.A. Antioxidant properties of germinated fenugreek seeds. Phytotherapy Research. 2005; 19: 977-983

Duraipandiyan V., Ayyanar M., Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complementary and Alternative Medicine. 2006; 6: 35

Ebrahimzadeh M.A., Pourmorad F., Hafezi S. Antioxidant activities of Iranian corn silk. Turkish Journal of Biology. 2008; 32: 43-49

Edziri H., Mastouri M., Mahjoub M.A., Mighri Z., Mahjoub A., Verschaeve L. Antibacterial, antifungal and cytotoxic activities of two flavonoids from *Retama raetam* flowers. Molecules. 2012; 17: 7284-7293

Elmastas M., Gulcin I., Isildak O., Kufrevioglu O.I., Ibaoglu K., Aboul-Enein H.Y. Radical scavenging activity and antioxidant capacity of Bay leaf extracts. Journal of Iranian Chemical Society. 2006; 3(3): 258-266

Gao X., Shu L., Yang L., Shen Y., Zhang Y., Hu Q. Phenylethanoids from the Flowers of *Rosa rugosa* and Their Biological Activities. Bulletin of the Korean Chemical Society. 2013; 34(1): 246-248

Gulcin I., Topal F., Sarikaya S.B.O., Bursal E., Bilsel G., Goren A.C. Polyphenol contents and antioxidant properties of Medlar (*Mespilus germanica* L.). Records of Natural Products. 2011; 5(3): 158-175

Harish R., Shivanandappa T. Antioxidant activity and hepatoprotective potential of *Phyllanthus niruri*. Food Chemistry. 2006; 95: 180-185

Hemaiswarya S., Kruthiventi A.K., Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 2008; 15: 639-652

Hinneburg I., Dorman H.J.D., Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chemistry. 2006; 97: 122-129

Ho S., Tung Y., Cheng K., Wu J. Screening, determination and quantification of major antioxidants from *Balanophora laxiflora* flowers. Food Chemistry. 2010; 122: 584–588

Holetz F.B., Ueda-Nakamura T., Filho B.P.D., Cortez D.A.G., Morgado-Diaz J.A., Nakamura C.V. Effect of essential oil of *Ocimum* gratissimum on the trypanosomatid *Herpetomonas samuelpessoai*. Acta Protozoologica. 2003; 42: 269-276

Hsu B., Coupar I.M., Ng K. Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. Food Chemistry. 2006; 98: 317-328

Huang D., Ou B., Prior R.L. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry. 2005; 53: 1841-1856

Inglett G.E., Chen D. Contents of phenolics and flavonoids and antioxidant activities in skin, pulp, and seeds of Miracle fruit. Journal of Food Science. 2011; 76(3): 479-482

Izzreen M.N.N.Q., Fadzelly A.B.M. Phytochemicals and antioxidant properties of different parts of *Camellia sinensis* leaves from Sabah Tea Plantation in Sabah, Malaysia. International Food Research Journal. 2013; 20(1): 307-312

Junaid S., Rakesh K.N., Dileep N., Poornima G., Kekuda T.R.P., Mukunda S. Total phenolic content and antioxidant activity of seed extract of *Lagerstroemia speciosa* L. Chemical Science Transactions. 2013a; 2(1): 75-80

Junaid S., Dileep N., Rakesh K.N., Pavithra G.M., Vinayaka K.S., Kekuda T.R.P. Anticariogenic Activity of *Gnidia glauca* (Fresen.) Gilg, *Pothos scandens* L. and *Elaegnus kologa* Schlecht. Journal of Applied Pharmaceutical Science. 2013b; 3(03): 020-023

Kaisoon O., Siriamornpun S., Weerapreeyakul N., Meeso N. Phenolic compounds and antioxidant activities of edible flowers from Thailand. Journal of Functional Foods. 2011; 3: 88-99

Kaviarasan S., Naik G.H., Gangabhagirathi R., Anuradha C.V., Priyadarshini K.I. *In vitro* studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum graecum*) seeds. Food Chemistry. 2007; 103: 31-37

Kekuda T.R.P., Raghavendra H.L., Swathi D., Venugopal T.M., Vinayaka K.S. Antifungal and Cytotoxic Activity of *Everniastrum cirrhatum* (Fr.) Hale. Chiang Mai Journal of Science. 2012; 39 (1):76-83.

Kekuda T.R.P., Vinayaka K.S., Swathi D., Suchitha Y., Venugopal T.M., Mallikarjun N. Mineral Composition, Total Phenol Content and Antioxidant Activity of a Macrolichen *Everniastrum cirrhatum* (Fr.) Hale (Parmeliaceae). E-Journal of Chemistry. 2011; 8(4): 1886-1894.

Khodadad P., Mehdi R. Biological activities of chamomile (*Matricaria chamomile*) flowers' extract against the survival and egg laying of the cattle fever tick (Acari Ixodidae). Journal of Zhejiang University SCIENCE B. 2007; 8(9):693-696

Kietthanakorn B., Ruksiriwanich W., Manosroi W., Manosroi J., Manosroi A. Biological activities of supercritical Carbon dioxide fluid (scCO2) extracts from medicinal flowers. Chiang Mai Journal of Science. 2012; 39(1): 84-96

Kim S., Jeong S., Park W., Nam K.C., Ahn D.U., Lee S. Effect of heating conditions on grape seeds on the antioxidant activity of grape seed extracts. Food Chemistry. 2006; 97: 472-479

Li H., Wang X., Li Y., Li P., Wang H. Polyphenolic compounds and antioxidant properties of selected China wines. Food Chemistry. 2009; 112: 454-460

Manjunatha B.K., Patil H.S.R., Vidya S.M., Kekuda T.R.P., Mukunda S., Divakar R. Studies on the antibacterial activity of *Mucuna monosperma* DC. Indian Drugs. 2006; 43: 150-152.

Mattana C.M., Satorres S.E., Sosa A., Fusco M., Alcaraz L.E. Antibacterial activity of extracts of Acacia aroma against methicillinresistant and methicillin-sensitive *Staphylococcus*. Brazilian Journal of Microbiology. 2010; 41: 581-587

Mert T., Fafal T., Kivcak B., Ozturk H.T. Antimicrobial and cytotoxic activities of the extracts obtained from the flowers of *Alcea Rosea* L. Hacettepe University Journal of the Faculty of Pharmacy. 2010; 30(1): 17-24

Nabavi S.F., Ebrahimzadeh M.A., Nabavi S.M., Mahmoudi M., Rad S.K. Biological activities of *Juglans regia* flowers. Brazilian Journal of Pharmacognosy. 2011; 21(3): 465-470

Nascimento G.G.F., Locatelli J., Freitas P.C., Silva G.L. Antibacterial activity of plant extracts and phytochemicals on antibioticresistant bacteria. Brazilian Journal of Microbiology. 2000; 31: 247-256

Nostro A., Germano M.P., D'Angelo V., Marino A., Cannatelli M.A. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology. 2000; 30: 379-384

Orhan D.D., Ozcelik B., Hosbas S., Vural M. Assessment of antioxidant, antibacterial, antimycobacterial, and antifungal activities of some plants used as folk remedies in Turkey against dermatophytes and yeast-like fungi. Turkish Journal of Biology. 2012; 36: 672-686

Pathak A., Sardar A., Kadam V., Rekadwad B., Karuppayil S.M. Efficacy of some medicinal plants against human dental pathogens. Indian Journal of Natural Products and Resources. 2012; 3(1): 123-127

Penarrieta J.M., Alvarado J.A., Bergenstahl B., Akesson B. Spectrophotometric methods for the measurement of total phenolic compounds and total flavonoids in foods. Revista Boliviana De Quimica. 2007; 24(1): 5-9

Poornima G., Kekuda T.R.P., Vinayaka K.S. Antioxidant efficacy of *Olea dioica* Roxb (Oleaceae) leaves. Biomedicine. 2012; 32(4): 506-510

Que F., Mao L., Zheng X. *In vitro* and vivo antioxidant activities of daylily flowers and the involvement of phenolic compounds. Asia Pacific Journal of Clinical Nutrition. 2007; 16 (S1): 196-203

Raj M.K., Duraipandiyan V., Agustin P., Ignacimuthu S. Antimicrobial activity of bergenin isolated from *Peltophorum pterocarpum* DC. Flowers. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(2): S901-S904

Rajakaruna N., Harris C.S., Towers G.H.N. Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka. Pharmaceutical Biology. 2002; 40(3): 235-244

Rekha C., Poornima G., Manasa M., Abhipsa V., Devi P.J., Kumar V.H.T., Kekuda P.T.R. Ascorbic Acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe Citrus fruits. Chemical Science Transactions. 2012; 1(2): 303-310

Rohman A., Riyanto S., Yuniarti N., Saputra W.R., Utami R., Mulatsih W. Antioxidant activity, total phenolic, and total flavaonoid of extracts and fractions of red fruit (*Pandanus conoideus* Lam). International Food Research Journal. 2010; 17: 97-106 Rop O., Mlcek J., Jurikova T., Neugebauerova J., Vabkova J. Edible flowers- A new promising source of mineral elements in human nutrition. Molecules. 2012; 17: 6672-6683

Roy P., Ambedkar S., Kumar A., Singh V. Preliminary study of the antioxidant properties of flowers and roots of *Pyrostegia venusta* (Ker Gawl) Miers. BMC Complementary and Alternative Medicine. 2011, 11:69

Saravanakumar A., Venkateshwaran K., Vanitha J., Ganesh M., Vasudevan M., Sivakumar T. Evaluation of antibacterial activity, phenol and flavonoid contents of *Thespesia populnea* flower extracts. Pakistan Journal of Pharmaceutical Sciences 2009; 22(3): 282-286

Sartoratto A., Machado A.L.M., Delarmelina C., Figueira G.M., Duarte C.T., Rehder V.L.G. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Brazilian Journal of Microbiology. 2004; 35: 275-280

Solorzano-Santos F., Miranda-Novales MG. Essential oils from aromatic herbs as antimicrobial agents. Current Opinion in Biotechnology. 2012; 23: 136-141

Srinivasan G.V., Sharanappa P., Leela N.K., Sadashiva C.T., Vijayan K.K. Chemical composition and antimicrobial activity of the essential oil of *Leea indica* (Burm.f) Merr. flowers. Natural Product Radiance. 2009; 8(5): 488-493

Tellez N., Tellez M., Perdomo M., Alvarado A., Gamboa F. Anticariogenic activity of the active fraction from *Isertia laevis* against *S. mutans* and *S. sobrinus:* Comparison of two extraction methods. Acta Odontologica Latinoamericana. 2010; 23(3): 188-195

Tilak J.C., Adhikari S., Devasagayam T.P.A. Antioxidant properties of *Plumbago zeylanica*, and Indian medicinal plant and its active ingredient, plumbagin. Redox Report. 2004; 9(4): 220-227

Uddin B., Hossan T., Paul S., Ahmed T., Nahar T., Ahmed S. Antibacterial activity of the ethanol extracts of *Hibiscus rosa-sinensis* leaves and flowers against clinical isolates of bacteria. Bangladesh Journal of Life Science. 2010; 22(2): 65-73

Usoh I.F., Akpan E.J., Etim E.O., Farombi E.O. Antioxidant actions of dried flower extracts of *Hibiscus sabdariffa* L. on Sodium arsenite - induced oxidative stress in rats. Pakistan Journal of Nutrition. 2005; 4(3): 135-141

Yang Z., Xu Y., Jie G., He P., Tu Y. Study on the antioxidant activity of tea flowers (*Camellia sinensis*). Asia Pacific Journal of Clinical Nutrition. 2007; 16(S1): 148-152

Yuan Y.V., Bone D.E., Carrington M.F. Antioxidant activity of dulse (*Palmaria palmata*) extract evaluated *in vitro*. Food Chemistry. 2005; 91: 485-494

Zakizadeh M., Nabavi S.F., Nabavi S.M., Ebrahimzadeh M.A. *In vitro* antioxidant activity of flower, seed and leaves of *Alcea hyrcana* Grossh. European Review for Medical and Pharmacological Sciences. 2011; 15: 406-412

Zhishen J., Mengcheng T., Janming W. The determination of flavonoids contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999; 64: 555-559

How to cite this article:

Pavithra G.M, Saba Siddiqua, Abhishiktha S. Naik, Prashith Kekuda T.R, Vinayaka K.S., Antioxidant and antimicrobial activity of flowers of *Wendlandia thyrsoidea*, *Olea dioica*, *Lagerstroemia speciosa* and *Bombax malabaricum*. J App Pharm Sci. 2013; 3 (06): 114-120.