

Antimicrobial and Antioxidant activity of leaf and flower extract of *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum*

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ABSTRACT

The present study was undertaken to determine antimicrobial and antioxidant activities of leaf and flower extracts of *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum*. Antimicrobial activity was tested against *Staphylococcus aureus*, *Salmonella typhi*, *Candida albicans* and *Cryptococcus neoformans* by Agar well diffusion assay. Antioxidant activity was determined by DPPH free radical scavenging assay, ABTS free radical scavenging assay, Ferric reducing assay and Total antioxidant capacity determination. Total phenolic content of extracts was estimated by Folin-Ciocalteu Reagent method. *S. typhi* and *C. neoformans* were susceptible to extracts to greater extent than *S. aureus* and *C. albicans* among bacteria and fungi respectively. Except *C. pulcherrima* extract, the leaf extracts were more effective in inhibiting bacteria than flower extracts. Leaf extracts have shown high antifungal activity than flower extracts. The extracts have shown dose dependent scavenging of DPPH and ABTS radicals. Scavenging of ABTS radicals was more efficient than that of DPPH radicals as revealed by low IC50 values. All leaf extracts except *C. pulcherrima* displayed stronger scavenging activities than flower extracts. Similar results were observed in ferric reducing assay and total antioxidant capacity determination. Total phenolic content was found to be higher in leaf extracts (except *C. pulcherrima*) than flower extracts. A correlation has been observed between phenolic content of leaf and flower extracts and the antioxidant activity. A marked antimicrobial and antioxidant activity of leaf and flower extracts was observed which may be attributed to the presence of phenolic compounds and other phytochemicals. The plants can be used to control infectious diseases and oxidative damage.

INTRODUCTION

Infectious diseases are caused due to a complex interaction between the pathogen, host and the environment. The discovery of antibiotics and their subsequent use had eradicated the infections that once challenged mankind. However, therapy using antibiotics is going through a crisis due to development of resistance by pathogens. *Staphylococcus aureus* is one of the most important pathogens that has become resistant to almost all known antibiotics. Other examples for antibiotic resistant bacteria are vancomycin resistant enterococci, multidrug resistant tuberculosis and others. Moreover, these pathogens have the ability to transmit the resistance gene and thereby create a serious issue in the field of

medicine (Ojala *et al.*, 2000; Hemaiswarya *et al.*, 2008; Davies and Davies, 2010). Plants have been used long before the discovery of antibiotics as remedies for a number of human diseases. They contain a great array of secondary metabolites having therapeutic value. Traditional healers, often referred as herbal healers, from various parts of the world use plants as anti-infective agents. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Al-Bakri and Afifi, 2007; Cowan, 1999). Reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, singlet oxygen, and hydrogen peroxide are produced in the body during normal metabolism or on exposure to exogenous factors. These reactive species can initiate deterioration of biomolecules such as proteins, lipids, carbohydrates and nucleic acids and are implicated in several diseases such as ageing, atherosclerosis, inflammatory injury, cancer, cardiovascular

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disease, neurological disorders etc. The oxidative stress results when the balance between the generation of ROS and antioxidant defense system of the body is disturbed. Cells have innate defense system which protects against the adverse effects caused by these ROS and includes enzymatic and non-enzymatic defense. However, during pathophysiological conditions, there is an extra need for antioxidants from exogenous sources. Synthetic antioxidants such as BHA, BHT, PG, TBHQ etc have been suspected to cause or promote negative health effects. Hence, there is a need for development of safer antioxidants particularly from natural sources. Many studies have demonstrated the efficacy of plant derived products as antioxidants against various diseases induced by these free radicals. It has been shown that the antioxidant nature of plants is mainly attributed to phenolic compounds, such as flavonoids, phenolic acids etc (Kulisic *et al.*, 2004; Katalinic *et al.*, 2006; Letelier *et al.*, 2008; Ho *et al.*, 2010; Junaid *et al.*, 2013). The aim of the present study was to determine and compare the antimicrobial and antioxidant activity of leaf and flower extracts of *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum*.

MATERIALS AND METHODS

Collection and identification of plant material

Leaves and flowers of selected plants were collected at college campus during May 2013 and authenticated by Mr. Gopal T.D, Assistant Professor, Department of Botany, Sahyadri Science College (A), Shivamogga. The leaves and flowers were shade dried and powdered in a blender. The powdered plant materials were stored in air-tight container. Table 1 represents the biological activities reported on the selected plants.

Table. 1: Plants selected in the study.

Name of the plant	Family	Part used	Reported biological activities of the plant
<i>C. pulcherrima</i>	Fabaceae	Leaf and flower	Antioxidant (Chakraborty and Badujar, 2009; Kumbhare <i>et al.</i> , 2012; Chew <i>et al.</i> , 2011), Antifertility (Kumar <i>et al.</i> , 2013), Antimicrobial (Prakash <i>et al.</i> , 2009; Pulipathi <i>et al.</i> , 2012; Chew <i>et al.</i> , 2011; Parekh <i>et al.</i> , 2005; De Britto <i>et al.</i> , 2011), Cytotoxic (Islam <i>et al.</i> , 2004), Analgesic (Patel <i>et al.</i> , 2010; Afroz <i>et al.</i> , 2013; Kumbhare and Sivakumar, 2011), Antiinflammatory (Patel <i>et al.</i> , 2010; Kumbhare and Sivakumar, 2011), Larvicidal (Govindarajan <i>et al.</i> , 2013), Anti-diarrhoeal (Afroz <i>et al.</i> , 2013), Anthelmintic (Dhaked <i>et al.</i> , 2011; Satwadhar <i>et al.</i> , 2012), Anti-plasmodial (Ogu <i>et al.</i> , 2012), Immunomodulatory (Madagundi <i>et al.</i> , 2012), Antidiabetic (Balasubramanian <i>et al.</i> , 2012), Antiulcer (Ali <i>et al.</i> , 2013), Weight lowering (Chichioco-Hernandez and Leonido, 2011), Antiviral (Chiang <i>et al.</i> , 2003)

<i>P. ferrugineum</i>	Fabaceae	Leaf and flower	Antibacterial (Dandapat <i>et al.</i> , 2012), Antimutagenic (Dandapat <i>et al.</i> , 2012), Antioxidant (Pavagadhi <i>et al.</i> , 2012)
<i>D. regia</i>	Fabaceae	Leaf and flower	Antimicrobial (Radhaiah <i>et al.</i> , 2012; Sharma <i>et al.</i> , 2010; Jahan <i>et al.</i> , 2010; Salem, 2013), Antioxidant (Salem, 2013; Chitra <i>et al.</i> , 2010; Aqil <i>et al.</i> , 2006), Hypoglycemic (Rahman <i>et al.</i> , 2011), Antiarthritic (Chitra <i>et al.</i> , 2010), Antiinflammatory (Shewale <i>et al.</i> , 2012), Cytotoxic (Jahan <i>et al.</i> , 2010), Anthelmintic (Ahirrao <i>et al.</i> , 2011), Hepatoprotective (Ahmed <i>et al.</i> , 2011), Diuretic (Velan <i>et al.</i> , 2012), Termiticidal (Rupal <i>et al.</i> , 2011)

Extraction of powdered leaf and flower material

For extraction, about 25g of dried and powdered leaf and flower materials were extracted with methanol in Soxhlet apparatus. The extract was filtered through Whatman No. 1 filter paper, concentrated in vacuum under reduced pressure and dried in the desiccator (Pavithra *et al.*, 2013).

Antimicrobial activity of flower extract

In order to determine antimicrobial activity of leaf and flower extracts, Agar well diffusion assay was performed. Antibacterial activity was tested against *Staphylococcus aureus* NCIM-2079 and *Salmonella typhi* MTCC-734. Antifungal activity was determined against *Candida albicans* NCIM-3466 and *Cryptococcus neoformans* NCIM-3378. The test bacteria and fungi were grown in sterile Nutrient broth (HiMedia, Mumbai) and Sabouraud dextrose broth (HiMedia, Mumbai) tubes respectively overnight. The broth cultures of bacteria and fungi were then aseptically swabbed on sterile Nutrient agar (HiMedia, Mumbai) and Sabouraud dextrose agar (HiMedia, Mumbai) respectively using sterile cotton swabs. Wells of 6mm diameter were created in the inoculated plates using sterile cork borer. 100µl of leaf and flower extracts (20mg/ml of 25% Dimethyl sulfoxide [DMSO]), standard antibiotic (1mg/ml of sterile distilled water) and DMSO (25%, in sterile water) were filled in labeled wells. Streptomycin and Fluconazole were used as standard antibacterial, antifungal antibiotics. The plates were incubated at 37°C for 24 hours (for bacteria) and 48 hours (for fungi) and the zone of inhibition was recorded (Pavithra *et al.*, 2013).

Antioxidant activity of leaf and flower extracts

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay

The radical scavenging activity of leaf and flower extracts was determined on the basis of the radical scavenging effect on the DPPH free radical. 1ml of different concentrations of extracts was mixed with 3ml of DPPH solution (0.004% in methanol) in labeled tubes. The tubes were incubated in dark for 30 minutes at room temperature and the optical density was measured at 517nm using UV-Vis spectrophotometer. The

absorbance of the DPPH control (extract replaced by methanol) was also noted. Ascorbic acid was used as reference standard. The scavenging activity was calculated using the formula:

Scavenging activity (%) = $[(A_o - A_e) / A_o] \times 100$, where A_o is absorbance of DPPH control and A_e is absorbance of DPPH in the presence of extract/standard (Elmastas *et al.*, 2006). The IC50 value for each of the extracts was calculated. IC50 denotes the concentration of extract required to scavenge 50% of DPPH free radicals.

ABTS (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid]) radical scavenging activity

The efficacy of leaf and flower extracts to scavenge free radicals was determined using ABTS radical scavenging assay with minor modification (Li *et al.*, 2011). The ABTS radical was generated by mixing 7mM ABTS stock solution with 2.45mM potassium persulfate and the mixture was left in the dark for 12–16 hours at room temperature. The resulting solution was diluted with distilled water to an absorbance of 0.70 at 730nm. 1ml of different concentrations of leaf and flower extracts (5-100µg/ml) were added to 4ml of ABTS solution in labeled tubes and the tubes were incubated for 30 minutes followed by measuring the absorbance at 730nm. Ascorbic acid was used as reference standard. The radical-scavenging activity was calculated using the formula:

Scavenging activity (%) = $(A_{\text{control}} - A_{\text{test}} / A_{\text{control}}) \times 100$, where A_{control} is the absorbance of the ABTS solution without extract/standard and A_{test} is the absorbance of ABTS solution in the presence of extract/standard. The IC50 value for each of the extracts was calculated. IC50 denotes the concentration of extract required to scavenge 50% of ABTS free radicals.

Reducing power assay (Ferric reducing activity)

The reducing power of leaf and flower extracts was determined by Ferric reducing assay. In brief, 1ml of different concentrations of extracts (10-100µg/ml) in 1ml of methanol were mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml of potassium ferricyanide ($[K_3Fe(CN)_6]$, 1%). The tubes were incubated at 50°C for 20 minutes in water bath, cooled and 2.5ml of trichloroacetic acid (10%) and 0.5ml of 0.1% ferric chloride ($FeCl_3$) were added to each tube. The absorbance of reaction mixtures was measured at 700nm. Increased absorbance of the reaction mixture on increasing the concentration of extracts indicated increased reducing power. Ascorbic acid was used as reference standard (El Hajaji *et al.*, 2010).

Total antioxidant capacity

0.3ml dilute concentration of leaf and flower extracts was mixed with 3ml of reagent solution (0.6M Sulfuric acid, 28mM Sodium phosphate and 4mM Ammonium molybdate) in labeled tubes. The tubes were capped and incubated in boiling water bath at 95°C for 90 min. The tubes were cooled and the absorbance of the solution was measured at 695nm using a spectrophotometer. In case of blank 0.3ml of methanol was used in place of extracts. Ascorbic acid was used as reference standard and the antioxidant

capacity of extracts was expressed as µg ascorbic acid equivalents (AAE)/mg of extract (El Hajaji *et al.*, 2010).

Total phenolic contents in leaf and flower extracts

The content of total phenolics in leaf and flower extracts was estimated by Folin-Ciocalteu reagent (FCR) method (Saeed *et al.*, 2012) with minor modifications. A dilute concentration of extract (0.5ml) was mixed with 0.5ml of FC reagent (1:1) and 2ml of sodium carbonate (7%). The reaction mixtures were allowed to stand for 30 minutes and the optical density was measured colorimetrically at 765nm. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000 µg/ml) and the TPC of extracts was expressed as µg Gallic acid equivalents (GAE) from the graph.

RESULTS

Antibacterial activity of leaf and flower extracts

Result of inhibitory efficacy of leaf and flower extracts against *S. aureus* and *S. typhi* is shown in Table 2. Among test bacteria, susceptibility was recorded higher in case of *S. typhi*. Highest and least inhibitory efficacy was shown by flower extract of *C. pulcherrima* and flower extract of *D. regia* respectively. Inhibition of test bacteria by Streptomycin was higher when compared to leaf and flower extracts. Reference antibiotic caused more inhibition of *S. aureus* than *S. typhi*. There was no inhibition in case of DMSO.

Table. 2: Antibacterial activity of leaf and flower extracts.

Extract	Zone of inhibition in cm	
	<i>S. aureus</i>	<i>S. typhi</i>
PLME	1.5	1.6
PFME	1.4	1.6
CLME	1.4	1.8
CFME	1.9	1.9
DLME	1.3	1.5
DFME	1.1	1.4
Streptomycin	3.9	3.6
DMSO	0.0	0.0

(ME- Methanol extract; PL- *Peltaphorum* leaf; PF- *Peltaphorum* flower; CL- *Caesalpinia* leaf; CF- *Caesalpinia* flower; DL- *Delonix* leaf; DF- *Delonix* flower)

Table. 3: Antifungal activity of leaf and flower extracts.

Extract	Zone of inhibition in cm	
	<i>C. albicans</i>	<i>C. neoformans</i>
PLME	1.4	1.5
PFME	1.2	1.2
CLME	1.6	1.6
CFME	1.3	1.2
DLME	1.6	1.6
DFME	0.8	1.2
Fluconazole	3.9	4.1
DMSO	0.0	0.0

Antifungal activity of leaf and flower extracts

Table 3 shows antifungal activity of leaf and flower extracts against two human pathogenic fungi *C. albicans* and *C. neoformans*. Test fungi showed varied susceptibility to extracts. *C. neoformans* was more susceptible than *C. albicans*. Leaf extracts of *C. pulcherrima* and *D. regia* have shown more or less similar

inhibitory activity. Least inhibition of test fungi was observed in case of flower extract of *D. regia*. Fluconazole exhibited higher inhibition of test fungi than leaf and flower extracts. DMSO showed no inhibition of test fungi.

DPPH free radical scavenging activity of leaf and flower extracts

The DPPH radical scavenging effect of leaf and flower extracts is shown in Figure 1. The extracts have shown dose dependent scavenging of DPPH radicals. The radical scavenging effect of leaf and flower extracts is in the order PLME > CFME > CLME > PFME > DLME > DFME. IC₅₀ (µg/ml) of PLME, CFME, CLME, PFME, DLME and DFME was found to be 26.69, 27.78, 31.08, 33.87, 35.97 and 41.19 respectively.

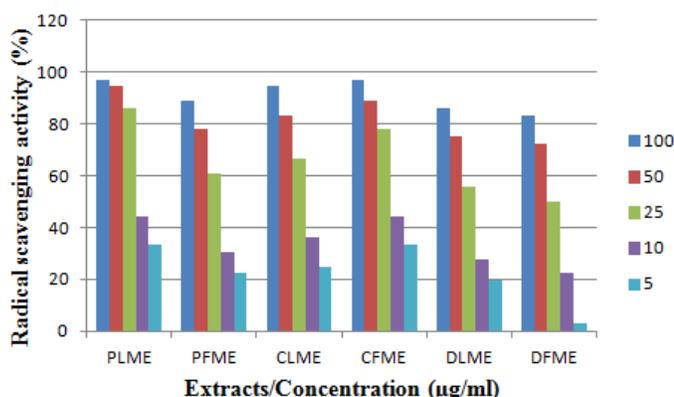


Fig. 1: DPPH radical scavenging activity of leaf and flower extracts .

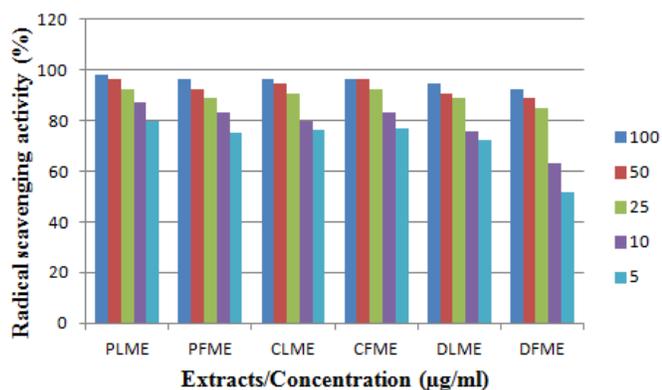


Fig. 2: ABTS radical scavenging activity of leaf and flower extracts .

ABTS radical scavenging activity of leaf and flower extracts

The ABTS radical scavenging effect of leaf and flower extracts is shown in Figure 2. The extracts have shown dose dependent scavenging of ABTS radicals. The radical scavenging effect of leaf and flower extracts is in the order PLME > CFME > CLME > PFME > DLME > DFME. IC₅₀ (µg/ml) of PLME, CFME, CLME, PFME, DLME and DFME was found to be 20.96, 21.30, 21.73, 21.78, 22.50 and 24.88 respectively.

Ferric reducing activity of leaf and flower extracts

In ferric reducing assay, the absorbance of reaction mixtures increased on increasing the concentration of extracts.

Ferric reducing activity of extracts is in the order PLME > CFME > PFME > CLME > DLME > DFME (Figure 3).

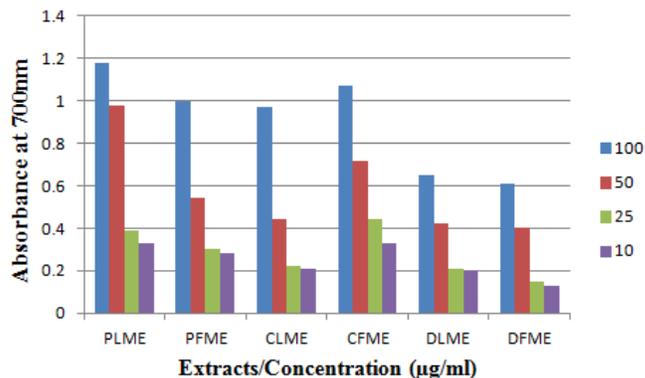


Fig. 3: Ferric reducing activity of leaf and flower extracts.

Total antioxidant capacity of leaf and flower extracts

Total antioxidant capacity of leaf and flower extracts was assessed by phosphomolybdenum blue method and the result is shown in Figure 4. The total antioxidant capacity, expressed in terms of µg AAE/mg extract, was high in PLME (88.60) followed by CFME (79.16), CLME (71.56) and others.

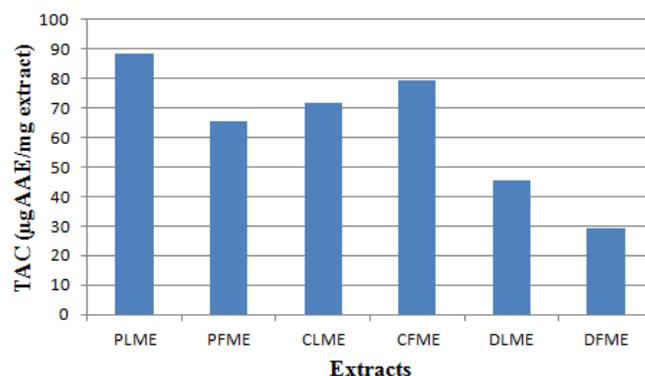


Fig. 4: Total antioxidant capacity of leaf and flower extracts of selected plants.

Total phenolic content of leaf and flower extracts

The content of total phenolics in the leaf and flower extracts was estimated by FCR method. Total phenolic content, as estimated in terms of µg GAE/mg extract, was high in PLME (385.6) followed by CFME (270.5), CLME (235.8) and others. DFME contained lowest phenolic content (Figure 5).

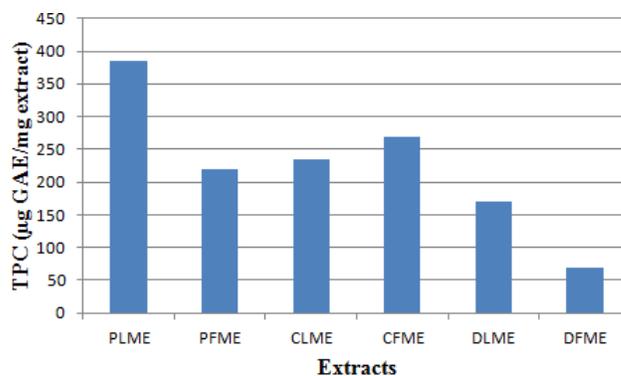


Fig. 5: Total phenolic content of leaf and flower extracts of selected plants.

DISCUSSION

Traditional antimicrobial therapy with antibiotics suffers from the major drawback of rapid development of resistance to existing antimicrobial agents. Thus, it is necessary to develop new antimicrobial agents against drug resistant strains that continuously undergo genetic change (Ojala *et al.*, 2000). Recently WHO reported that >75% of world population, in particular developing and underdeveloped countries, rely chiefly on traditional medicines that are based on plants and their products (Bhattacharjee *et al.*, 2011). On screening plant extracts for antimicrobial activity, it has been shown that higher plants represent a promising source of new antimicrobial agents (Ojala *et al.*, 2000). Plants produce a number of substances and most are secondary metabolites which act as defense against predators, responsible for typical odors and characteristic pigmented nature of plants. Most of the phytochemicals are extensively used as medicinal compounds for treatment of various ailments all over the world (Cowan, 1999). Medicines derived from plants have no apparent side effects that are associated with modern drugs. In the present study, the leaf and flower extracts of selected plants exhibited antimicrobial activity against human pathogenic bacteria and yeasts. Overall, leaf extracts were more inhibitory against test bacteria than that of flower extract, except in case of *C. pulcherrima*. In antifungal activity also, leaf extracts were more inhibitory to test fungi than flower extracts. The medicinal, in particular antimicrobial, properties of plants are attributed to the presence of bioactive components such as polyphenols including flavonoids, alkaloids and other compounds (Cowan, 1999).

Polyphenols of plant kingdom are one of the most effective antioxidative constituents. It is important to estimate phenolic contents of plant extracts so as to justify their contribution to antioxidant activity (Choi *et al.*, 2007). In the present study, we estimated total phenolic content of leaf and flower extracts by FCR method. FCR method is one of the oldest and commonly used colorimetric techniques for estimating total phenolic contents of a range of substances including plant extracts. The phenolic compounds react with FCR only under basic conditions to form blue complex having maximum absorption near 750nm. Though the chemical nature of FCR is undefined, the total phenols assay by FCR is convenient, simple, and reproducible. 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.*, 2013). Phenolic contents ($\mu\text{g GAE/mg extract}$) was in the order PLME > CFME > CLME > PFME > DLME > DFME.

DPPH is a nitrogen centred stable free radical having maximum absorption at 517nm in alcoholic solution. It becomes a stable diamagnetic molecule on accepting an electron or hydrogen atom. In the presence of an extract capable of donating a hydrogen atom, the free radical nature of DPPH is lost and the purple color changes to yellow (diphenylpicrylhydrazine). The bleaching of

DPPH radical is one of the most widely used strategies to evaluate the antioxidant activity of herbal extracts. This method is simple, rapid and measures the capacity of herbal extract to bleach the DPPH radical. The method is sensitive and requires small amount of samples (Kulicic *et al.*, 2004; Kaviarasan *et al.*, 2007; Letelier *et al.*, 2008; Kekuda *et al.*, 2011; Junaid *et al.*, 2013; Pavithra *et al.*, 2013). In the present study, we monitored the decrease in DPPH absorption in the presence of varying concentrations of leaf flower extracts at 517nm. Leaf extracts of all plants except *C. pulcherrima* showed high scavenging potential when compared to flower extracts. Leaf extract of *P. ferrugineum* and *D. regia* and flower extract of *C. pulcherrima* and *D. regia* displayed highest and least scavenging of DPPH radicals. 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). A positive correlation was observed between phenolic contents of extracts and radical scavenging activity i.e., extracts with high phenolic contents displayed higher scavenging of DPPH radicals.

Like DPPH assay, ABTS radical scavenging is another popular antioxidant assay which measures the radical scavenging nature of several types of compounds including herbal extracts (Katalinic *et al.*, 2006; Pawlaci *et al.*, 2010; Li *et al.*, 2011; Huang *et al.*, 2012). On interaction with ABTS, antioxidants either transfer electrons or hydrogen atoms to ABTS and thereby neutralizing the free radical character (Huang *et al.*, 2012). In the present study, the leaf extracts of all plants except *C. pulcherrima* showed high scavenging potential when compared to flower extracts. Leaf extract of *P. ferrugineum* and *D. regia* and flower extract of *C. pulcherrima* and *D. regia* displayed highest and least scavenging of ABTS radicals. Extracts containing high phenolic contents showed marked scavenging of ABTS radicals indicating a direct correlation between phenolic content and scavenging of radicals.

The direct reduction of Fe^{+3} to Fe^{+2} was assessed in order to evaluate the reducing potential of leaf and flower extracts. It can be determined by measuring the absorbance resulting from the formation of Perl's Prussian blue complex on addition of excess of ferric ions. An increase in absorbance at 700nm on increasing concentrations of extracts indicated reducing capacity. The reducing properties of antioxidants are generally associated with the presence of reductones. The assay has been widely used by several researchers 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; Rekha *et al.*, 2012; Junaid *et al.*, 2013). 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 nature of a compound may serve as a significant indicator of its potent antioxidant activity (Hsu *et al.*, 2006). In the present study, it was observed that the reducing powers of all extracts increased with the increase of their concentrations. As in case of DPPH assay, the extracts containing high phenolic contents displayed greater reducing power. It is evident from the study that the

extracts possess reducing power and therefore, could serve as electron donors, terminating the radical chain reactions (Chung *et al.*, 2006). Total antioxidant capacity determination by Phosphomolybdenum blue method is one of the widely used antioxidant determination techniques which is used to estimate the antioxidant potential of various samples including plant extracts. In this method, the reduction of Mo (VI) to Mo (V) occurs by the antioxidants present in the samples and results in the formation of a green Mo (V) complex at acidic pH with a maximal absorption at 695nm (Jayaprakasha *et al.*, 2003; El Hajaji *et al.*, 2010; Silici *et al.*, 2010; Aliyu *et al.*, 2012). In our study, the total antioxidant capacity of leaf and flower extracts, expressed as equivalents of ascorbic acid, was in the order PLME > CFME > CLME > PFME > DLME > DFME. Among leaf extracts, the antioxidant capacity was highest in PLME followed by CLME and DLME. In case of flower extracts, the antioxidant capacity was highest in CFME followed by PFME and DFME. Overall, total antioxidant capacity of leaf extracts was higher than that of flower extracts. Here also, a direct correlation was obtained between the phenolic content of extracts and the total antioxidant capacity. In our study, the leaf and flower extracts of selected plants showed marked antioxidant activity. It has been observed that extracts containing high phenolic content exhibited stronger scavenging and reducing capacity. The results obtained are in justification with earlier studies which correlated the total phenolic content of plants with their antioxidant activity (Tilak *et al.*, 2004; Coruh *et al.*, 2007; Rekha *et al.*, 2012; Dileep *et al.*, 2012).

CONCLUSION

The present study revealed the inhibitory effect of leaf and flower extracts of selected plants against human pathogenic bacteria and yeasts. The extracts also exhibited marked antioxidant activity which may be attributed to the phenolic content of extracts. The plants can be used as potential source for the development of antimicrobial and antioxidant agents.

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REFERENCES

Afroz T, Ramproshad S, Mondal B, Haque A, Khan R. Antidiarrhoeal and analgesic activity of barks of medicinal plant *Caesalpinia pulcherrima*. International Journal of Pharmaceutical Sciences and Research. 2013; 4(5): 1946-1949.

Ahirrao RA, Patel MR, Hamid S, Patil JK. *In vitro* anthelmintic property of Gulmohar flowers against *Pheritima posthuma*. Pharmacologyonline. 2011; 1: 728-732.

Ahmed J, Nirmal S, Dhasade V, Patil A, Kadam S, Pal S, Mandal S, Pattan S. Hepatoprotective activity of methanol extract of aerial parts of *Delonix regia*. Phytopharmacology. 2011; 1(5): 118-122.

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

Ali SA, Mujahid S, Aatif SM, Khan MM. Anti-ulcer activity of ethanolic extract of *Caesalpinia pulcherrima* flowers on ethanol induced gastric ulcers in rats. Der Pharmacia Sinica. 2013; 4(2): 119-124.

Aliyu AB, Ibrahim MA, Ibrahim H, Musa AM, Lawal AY, Oshanimi JA, Usman M, Abdulkadir IE, Oyewale AO, Amupitan JO. Free radical scavenging and total antioxidant capacity of methanol extract of *Ethulia conyzoides* growing in Nigeria. Romanian Biotechnological Letters. 2012; 17(4): 7458-7465.

Aqil F, Ahmad I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turkish Journal of Biology. 2006; 30: 177-183.

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

Balasubramanian V, Seetaram P, Gayasuddin M, Venkataiah G. Demonstration of β -cell regeneration and anti-diabetic activity of *Caesalpinia pulcherrima* flower extract in alloxan induced diabetic rats. Der Pharmacia Lettre. 2012; 4(6): 1692-1697.

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

Bhattacharjee I, Chatterjee SK, Ghosh A, Chandra G. Antibacterial activities of some plant extracts used in Indian traditional folk medicine. Asian Pacific Journal of Tropical Biomedicine. 2011; 2(2): S165-S169.

Chakraborty GS, Badujar RS. Antioxidant activity of the successive extracts of *Caesalpinia pulcherrima* leaves. International Journal of Pharmaceutical and Clinical Research. 2009; 1(2): 75-76.

Chew YL, Chan EWL, Tan PL, Lim YY, Stanslas J, Goh JK. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. BMC Complementary and Alternative Medicine. 2011; 11: 12.

Chiang LC, Chiang W, Liu MC, Lin CC. *In vitro* antiviral activities of *Caesalpinia pulcherrima* and its related flavonoids. Journal of Antimicrobial Chemotherapy. 2003; 52: 194-198.

Chichioco-Hernandez CL, Leonido FMG. Weight-lowering effects of *Caesalpinia pulcherrima*, *Cassia fistula* and *Senna alata* leaf extracts. Journal of Medicinal Plants Research. 2011; 5(3): 452-455.

Chitra V, Ilango K, Rajanandh MG, Soni D. Evaluation of *Delonix regia* Linn. flowers for antiarthritic and antioxidant activity in female wistar rats. Annals of Biological Research. 2010; 1(2): 142-147.

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

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

Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999; 12(4): 564-582.

Dandapat R, Jena BS, Negi PS. Antimutagenic and antibacterial activities of *Peltophorum ferrugineum* flower extracts. Asian Pacific Journal of Tropical Disease. 2012; 2(S2): S778-S782.

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

Davies J, Davies D. Origins and evolutions of antibiotic resistance. Microbiology and Molecular Biology Reviews. 2010; 74(3): 417-433.

De Britto AJ, Gracelin DHS, Sebastian SR. Antibacterial activity of a few medicinal plants against *Xanthomonas campestris* and *Aeromonas hydrophila*. Journal of Biopesticides. 2011; 4(1): 57-60.

- Dhaked PS, Panigrahy RN, Kshirsagar SN. *In vitro* evaluation of anthelmintic activity of *Caesalpinia pulcherrima* (Linn) flower extracts in Indian earthworm. International Journal of Pharmaceutical Sciences Review and Research. 2011; 7(1): 89-91.
- Dileep N, Rakesh KN, Junaid S, Poornima G, Swarnalatha SP, Kekuda PTR. *In vitro* Antioxidant Activity of Ripe Pericarp of *Polyalthia longifolia* Thw. Research Journal of Pharmacy and Technology. 2012; 5(10): 1312-1315.
- El Hajaji H, Lachkar N, Alaoui K, Cherrah Y, Farah A, Ennabili A, El Bali B, Lachkar M. Antioxidant properties and total phenolic content of three varieties of Carob tree leaves from Morocco. Records of Natural Products. 2010; 4(4): 193-204.
- Elmastas M, Gulcin I, Isildak O, Kufrevioglu OI, Ibaoglu K and Aboul-Enein HY. Radical scavenging activity and antioxidant capacity of Bay leaf extracts. Journal of Iranian Chemical Society. 2006; 3(3): 258-266.
- Govindarajan M, Rajeswary M, Amsath A. Larvicidal Properties Of *Caesalpinia pulcherrima* (Family: Fabaceae) against *Culex tritaeniorhynchus*, *Aedes albopictus* and *Anopheles subpictus* (Diptera: Culicidae). International Journal of Pure and Applied Zoology. 2013; 1(1): 15-23.
- Gulcin I, Topal F, Sarikaya SBO, Bursal E, Bilsel G, Goren AC. 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 AK and Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 2008; 15: 639-652.
- Hinneburg I, Dorman HJD, 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.
- Hsu B, Coupar IM, 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 RL. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry. 2005; 53: 1841-1856.
- Huang H, Hsieh W, Niu Y, Chang T. Inhibition of melanogenesis and antioxidant properties of *Magnolia grandiflora* L. flower extract. BMC Complementary and Alternative Medicine. 2012; 12: 72.
- Islam NAKM, Ali AM, Sayeed A, Islam A, Arefin KSM, Khatune NA, Khan AMGRM. Antimicrobial and cytotoxic effects of a glycoside from *Caesalpinia pulcherrima* Swartz. Journal of Medical Sciences. 2004; 4(1): 15-18.
- Jahan I, Rahman MS, Rahman MZ, Kaisar MA, Islam MS, Wahab A, Rashid MA. Chemical and biological investigations of *Delonix regia* (Bojer ex Hook.) Raf. Acta Pharmaceutica. 2010; 60: 207-215.
- Jayaprakasha GK, Selvi T, Sakariah KK. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. Food Research International. 2003; 36: 117-122.
- Junaid S, Rakesh KN, Dileep N, Poornima G, Kekuda TRP, Mukunda S. Total phenolic content and antioxidant activity of seed extract of *Lagerstroemia speciosa* L. Chemical Science Transactions. 2013a; 2(1): 75-80.
- Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chemistry. 2006; 94: 550-557.
- Kaviarasan S, Naik GH, Gangabagirathi R, Anuradha CV, Priyadarshini KI. *In vitro* studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum graecum*) seeds. Food Chemistry. 2007; 103: 31-37.
- Kekuda TRP, Vinayaka KS, Swathi D, Suchitha Y, Venugopal TM, 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.
- Kim S, Jeong S, Park W, Nam KC, Ahn DU, Lee S. Effect of heating conditions on grape seeds on the antioxidant activity of grape seed extracts. Food Chemistry. 2006; 97: 472-479.
- Kulisic T, Radonic A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chemistry. 2004; 85: 633-640.
- Kumar S, Singh J, Baghotia A, Mehta V, Thakur V, Choudhary M, Verma S, Kumar D. Antifertility potential of the ethanolic extract of *Caesalpinia pulcherrima* Linn. leaves. Asian Pacific Journal of Reproduction. 2013; 2(2): 90-92.
- Kumbhare M, Sivakumar T. Anti-inflammatory and antinociceptive activity of pods of *Caesalpinia pulcherrima*. Journal of Applied Pharmaceutical Science. 2011; 1(7): 180-184.
- Kumbhare MR, Sivakumar T, Udavant PB, Dhake AS, Surana AR. *In vitro* antioxidant activity, phytochemical screening, cytotoxicity and total phenolic content in extracts of *Caesalpinia pulcherrima* (Caesalpiniaaceae) pods. Pakistan Journal of Biological Sciences. 2012; 15(7): 325-332.
- Letelier ME, Molina-Berrios A, Cortes-Troncoso J, Jara-Sandoval J, Holst M, Palma K, Montoya M, Miranda D, Gonzalez-Lira V. DPPH and oxygen free radicals as pro-oxidant of biomolecules. Toxicology *In vitro*. 2008; 22: 279-286.
- Li P, Huo L, Su W, Lu R, Deng C, Liu L, Deng Y, Guo N, Lu C, He C. Free radical-scavenging capacity, antioxidant activity and phenolic content of *Pouzolzia zeylanica*. Journal of Serbian Chemical Society. 2011; 76 (5): 709-717.
- Madagundi SD, Pawadshetter MK, Sholapur HP, Habbu P, Biradar SM. A comparative study of isolated flavonoid and different extracts of *Caesalpinia pulcherrima* (L) Sw. for *In vitro* immunomodulatory effects on human neutrophils. Asian Journal of Traditional Medicines. 2012; 7(4): 159-167.
- Ogu GI, Aisuodionoe ME, Nwachukwu PU. Anti-plasmodial activity of *Caesalpinia pulcherrima* (Swartz) stem bark extract against *Plasmodium berghei* in albino mice. International Journal of Biology, Pharmacy and Allied Sciences. 2012; 1(2): 168-178.
- Ojala T, Remes S, Haansuu P, Vuorela H, Hiltunen R, Haahtela K, Vuorela P. Antimicrobial activity of some coumarin containing herbal plants growing in Finland. Journal of Ethnopharmacology. 2000; 73: 299-305.
- Parekh J, Jadeja D, Chanda S. Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. Turkish Journal of Biology. 2005; 29: 203-210.
- Patel SS, Verma NK, Chatterjee C, Gauthaman K. Screening of *Caesalpinia pulcherrima* Linn Flowers for Analgesic and Anti-inflammatory Activities. International Journal of Applied Research in Natural Products. 2010; 3(3): 1-5.
- Pavagadhi S, Joseph GS, Jena BS. Antioxidant principles in *Peltaphorum ferruginum* flower extracts. International Journal of Food Properties. 2012; 15(3): 549-557.
- Pavithra GM, Siddiqua S, Naik AS, Kekuda PTR, Vinayaka KS. Antioxidant and antimicrobial activity of flowers of *Wendlandia thyrsoidea*, *Olea dioica*, *Lagerstroemia speciosa* and *Bombax malabaricum*. Journal of Applied Pharmaceutical Science. 2013; 3(6): 114-120.
- Pawlaki K, Bylka W, Jazurek B, Matlawska I, Sikorska M, Manikowski H, Bialek-Bylka G. Antioxidant activity of flavonoids of different polarity, assayed by modified ABTS cation radical decolorization and EPR technique. Acta Biologica Cracoviensia Series Botanica. 2010; 52(1): 97-104.
- Prakash BS, Sharmistha P, Kumar RA. Antibacterial activity of methanolic extract of roots of *Caesalpinia pulcherrima*. International Journal of Chemical Sciences. 2009; 7(1): 16-18.
- Pulipathi S, Pallavi G, Sujan B, Babu AK, Babu SP. Evaluation of antibacterial activity of fresh and dry flower extracts of *Caesalpinia pulcherrima* L. International Journal of Biological and Pharmaceutical Research, 2012; 3(3): 360-365.

Radhaiah A, Suman B, Babu SC, Muniswamy D. Phytochemical screening and anticandid activity of selected plants of genus *Caesalpinia*. Indian Journal of Plant Sciences. 2012; 1(2&3): 239-243.

Rahman MM, Hasan MN, Das AK, Hossain MT, Jahan R, Khatun MA, Rahmatullah M. Effect of *Delonix regia* leaf extract on glucose tolerance in glucose induced hyperglycemic mice. African Journal of Traditional, Complementary, and Alternative Medicines. 2011; 8(1): 34-36 34.

Rekha C, Poornima G, Manasa M, Abhipsa V, Devi PJ, Kumar VHT, Kekuda PTR. 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.

Rupal AV, Savalia DM, Narasimhacharya AVRL. Plant extracts as biotermiticides. Electronic Journal of Environmental Sciences. 2011; 4: 73-77.

Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complementary and Alternative Medicine. 2012; 12: 221.

Salem MZM. Evaluation of the antibacterial and antioxidant activities of stem bark extracts of *Delonix regia* and *Erythrina humeana* grown in Egypt. Journal of Forest Products and Industries. 2013; 2(2): 48-52.

Satwadhar ND, Mehta PP, Patil SR, Mute VM. Evaluation of anthelmintic activity of *Caesalpinia pulcherrima* (L). bark against *Pheretima posthuma*. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4(1): 76-77.

Sharma RA, Chandrawat P, Sharma S, Sharma D, Sharma B, Singh D. Efficacy of *Delonix regia* Rafin (Syn. *Poinciana regia* Bojer Ex. Hook) for potential antifungal activity. The Bioscan. 2010; 5(3): 441-444.

Shewale VD, Deshmukh TA, Patil LS, Patil VR. Anti-Inflammatory Activity of *Delonix regia* (Boj. Ex. Hook). Advances in Pharmacological Sciences Volume 2012, Article ID 789713, 4 pages, doi:10.1155/2012/789713.

Silici S, Sagdic O, Ekici L. Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys. Food Chemistry. 2010; 121: 238-243.

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

Velan SS, Prakash JG, Sindhan V, Somasekhar E, Bharathi S, Rajani B. Evaluation of Diuretic activity of *Delonix regia* (Gul Mohr) flowers in Albino rats. International Journal of Research in Pharmaceutical Sciences. 2012; 3(3): 369-372.

Yuan YV, Bone DE, Carrington MF. Antioxidant activity of dulce (*Palmaria palmata*) extract evaluated *in vitro*. Food Chemistry. 2005; 91: 485-494.

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