ORIGINAL ARTICLE

Evaluation of antioxidant and phytochemical activity in solvent extracts from *Delonix regia* flowers

Baskar Ramakrishnan, S. B. Akshaya, R. Akshitha, G. Dhilip Kumar, G. Poorani

Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu, India

Abstract

Background: Antioxidants are biological molecules that prevent oxidation, and with their enormous potency, they are broadly used in food and pharmaceutical industry. Free radicals possess an odd number of electrons that is in turn the primary cause for a variety of human disorders and diseases. Antioxidants react to the oxygen molecules, thereby preventing oxidative damages to the cell and its environment. Many plants and plant parts act as a good source of antioxidants. **Materials and Methods:** The present study aims to evaluate the antioxidant and phytochemical potential of flowers of *Delonix regia.* The dried flower was subjected to solvent extraction with methanol, ethyl acetate, acetone, and chloroform. A broad range of *in vitro* free radical scavenging and phytochemical assays was performed with the obtained extract. The structural features of the compounds in the flowers were studied through high-performance pre-parative thin-layer chromatography (TLC), TLC, and Fourier transform infrared (FTIR) analysis. **Results and Discussion:** The *in vitro* free radical scavenging assays shows that the ethyl acetate hot extract of *D. regia* flowers is very potential even at low concentrations. As a result of phytochemical estimation, it is evident that the extract contains anthocyanin in higher quantity than tannins, flavonoids, alkaloids, and total phenols. Through FTIR analysis, the presence of conjugated ketone, amide, and phenolic C-O stretch is confirmed. **Conclusion:** The results suggest that the ethyl acetate extract possesses a significant antioxidant activity and the compounds from *D. regia* are found to be ideal candidates for antioxidant therapy.

Key words: Antioxidants, *Delonix regia,* Fourier transform infrared, free radicals, high-performance thin-layer chromatography, phytochemical

INTRODUCTION

Biological antioxidants are compounds that save the biological systems from the harmful effects of oxidation. The antioxidants are present in three different forms: Phytochemicals, vitamins, and enzymes.[1] A lot of intracellular and extracellular mechanisms are essential to neutralize the hazards of reactive oxygen species (ROS). The human system is strong enough to produce its own antioxidant defense to curtail free radical chain reaction and thereby protect cells and tissues from oxidative damage.[2] The dysfunctionality of endogenous antioxidants leads to increase in number of free radicals, that might further cause various health disorders. Under such circumstances, one has to depend on exogenous antioxidants to suppress the roughness of the singlet oxygen on healthy cells.

Antioxidant-based drugs are gaining wide attention worldwide, because of its excellent capacity in scavenging free radicals. For the past three decades, antioxidants are used to treat complex disorders such as cancer and Alzheimer's disease.[3] Plants are extensively studied by the researchers nowadays to extract biologicals to treat human diseases. Plant-derived products such as polyphenols, terpene, and other phytochemicals possess antioxidants and exhibit its action even at lowest concentrations. The antioxidants from plant sources are also used in other applications such as an

Address for correspondence:
Dr. R. Baskar Ramakrishnan, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore - 641 049, Tamil Nadu, India.
Email: baskar.r.bt@kct.ac.in

Received: 07-08-2018
Revised: 19-09-2018
Accepted: 26-09-2018

ingredient in food industry, as a bioactive nutraceuticals and as an active component in biopharmaceuticals.[4]

A broad spectrum of plant species has been screened so far by many researchers to analyze and to extract the antioxidant compounds. In general, the colored fruits, vegetables, and flowers are highly rich in antioxidants. *Delonix regia* is a deciduous tree with fern-like leaves. Previous reports state that *D. regia* contains alkaloids, saponins, and secondary metabolites such as tannins as a potential component.[5] *D. regia* has been widely exploited for its health and economic benefits. Other than antioxidant potential, *D. regia* possesses antidiabetic, anti-inflammatory, antidiarrheal, hepatoprotective, antihelminthic, and wound healing activity.[6]

Despite the extensive uses, there have been only limited attempts to explore the biological properties of the *D. regia* in relation to their medicinal properties. The present study aims to extract antioxidant compounds from *D. regia* using organic solvents of increasing polarity, namely methanol, acetone, ethyl acetate, and chloroform, by exhaustive Soxhlet extraction and evaluate antioxidant levels by an array of *in vitro* antioxidant capacity assays. This study also aims to quantify phytochemicals and elucidate functional groups present in it through Fourier transform infrared (FTIR) and high-performance thin-layer chromatography (HPTLC) analysis.

MATERIALS AND METHODS

Plant Material and Chemicals

D.regia flowers were collected from in and around Coimbatore district, and it was authenticated by scientist in-charge, the Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore. The voucher specimen of the plant sample has been deposited in the herbarium of the department (Voucher No. BSI/SRC/5/23/2015/Tech/723). The plant samples were dried under shade and ground to coarse powder. Hot extraction of the flowers was performed by Soxhlet apparatus using different solvents. 150 ml of four different solvents of varying polarity index, namely methanol, ethyl acetate, acetone, and chloroform, were used along with 10 g of each powdered sample. The extracts were collected after 20 cycles of extraction and dried to obtain a dry residue which was stored for further analysis. All essential chemicals were purchased from HiMedia and of analytical grade.

In Vitro Antioxidant Assays

Total antioxidant capacity assay

Samples were prepared by dissolving in their respective solvents used for extraction at different concentrations (200–1000 µg/ml). 1.0 ml of the reagent (disodium hydrogen phosphate + ammonium molybdate + sulfuric acid) was mixed with 0.1 ml of the extracts. The tubes were capped and incubated at 95°C for 90 min in boiling water bath. After the incubation, the tubes were cooled to room temperature (RT) and the absorbance was measured at 675 nm. The standard used for this assay is ascorbic acid, and the results were expressed as ascorbic acid equivalents/g extract.[7]

2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay

The sample extracts were prepared by dissolving it in methanol at different concentrations. 2.0 ml of varying concentrations of sample (200–1000 µg/ml) in triplicates were prepared. 0.5 ml of DPPH reagent was added to all test tubes and mixed well. The test tubes were incubated in the dark for 30 min and then measured in spectrophotometer at 517 nm. 2.0 ml of methanol and 0.5 ml of DPPH reagent were taken as control. Methanol served as blank and Trolox (synthetic antioxidant) was used as standard. The percentage inhibition of DPPH radical by the antioxidants in the extracts was calculated by the following formula: Absorbance of control - absorbance of test/absorbance of control×100.

2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assay

Equal volumes of ABTS solution (7 mM) and ammonium per sulfate (2.45 mM) were mixed well and were kept in the dark for 16 h at RT to form a dark color solution. The initial absorbance of the solution was measured at 745 nm and was found to be exceeding the visible range (2.5+). Final absorbance of 0.700(±0.02) was obtained by diluting the solution with methanol at RT. Different extracts were dissolved in their respective solvents (1 mg/ml), and varying concentrations (200–1000 µg) of the sample in triplicates were prepared. About 1.0 ml of ABTS standard solution was mixed with 30 µl of each sample mixture. After 6 min, the decrease in absorbance of the mixture was read at 745 nm. 30 µl of ethanol and 1.0 ml of ABTS solution were taken as control, and ethanol was set as blank. The percentage inhibition of the ABTS radical by the antioxidants in the sample was calculated as before.[8]

Nitric oxide (NO^-) radical scavenging assay

About 1.0 ml of varying concentrations of the extract (200–1000 µg/ml) was added to 1.0 ml of sodium nitroprusside solution. The contents were mixed well and incubated at RT for 30 min. To this, 2.0 ml of Griess reagent was added. The colored complex formed was read at 546 nm. The percentage inhibition of the NO^- radical by the antioxidants in the sample was calculated.[9]

Reducing power assay

Samples of varying concentrations (200–1000 µg/ml) were taken and made up to 2.5 ml with methanol. To all the reaction mixture, 2.5 ml of 1% potassium ferricyanide and 2.5 ml of

0.2 M phosphate buffer were added and incubated at 50°C for 20 min. Excluding the sample, the rest of the reaction mixture was taken as control. After the incubation time, the reaction was terminated by the addition of 2.5 ml of 10% trichloroacetic acid (TCA) followed by centrifugation at 3000 rpm for 10 min. Nearly 2.5 ml of the supernatant was taken and 2.5 ml of deionized water and 0.4 ml of 0.1% ferric chloride solution were added. The color complex formed was read at 700 nm.[10]

Ferrous ion chelation assay

Different extracts were prepared in their respective solvents (1 mg/ml), and varying concentrations of the sample in triplicates (200–1000 µg/ml) were prepared. 1 ml of each extract was mixed with 0.1 ml of 2 mM FeCl2 and 3.7 ml of methanol. Then, it was mixed with 0.2 ml of 5 mM ferrozine and incubated at RT for 10 min. The decrease in absorbance was measured at 562 nm.[10]

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method using an easily reduced oxidant, Fe (III). Reduction of a ferric tripyridyltriazine complex to ferrous-(2,4,6-tripyridyl-s-triazine)2 that is ferric (III) [colorless] to ferrous (II) [blue] can be monitored by measuring absorbance at 593 nm. To 0.09 ml of extract, 2.7 ml of FRAP reagent and 270 µl of water were added. The mixture was incubated at 37°C for 30 min. It was cooled to RT, and an increase in absorbance was measured at 595 nm.

Lipid peroxidation inhibition assay

Goat liver was washed thoroughly in cold phosphate-buffered saline (pH 7.4) and minced with mortar and pestle with a measured volume of buffer in ice. The mined meat was subjected to homogenization in a homogenizer to give a 10% homogenate. The homogenate was filtered using a cheese cloth to remove unwanted residue. The filtrate was centrifuged at 10,000 rpm for 10 min under refrigerated condition. The supernatant obtained was used for the assay. About 0.5 ml of this 10% homogenate was taken. To this, 0.5 ml of the extracts (50–250 µg/ml) in water was added. The total volume of the reaction mixture should be 1 ml and made up with distilled water. 0.05 ml of 0.07 mM ferrous sulfate was added to the mixture. The solution was incubated at RT for 30 min. To the incubated solution, 1.5 ml of 20% acetic acid, 1.5 ml of 0.8% thiobarbituric acid (TBA) (in 1.1% sodium dodecyl sulfate), and 0.05 ml of 20% TCA were added. The tubes were vortexed to ensure proper mixing. The tubes were then incubated at 100°C for 30 min. The pink color formed was read at 530 nm. The reaction mixture without sample serves as control.

Phytochemical Analysis

Estimation of flavonoids

Samples were dissolved in their respective solvents (1 mg/ml) to serve as stock. The volume of sample used for the assay is 25 µl, and the total volume was made up to 2.5 ml with distilled water. To the sample, 75 µl of 5% sodium nitrite solution was added and incubated at RT for 5 min. After incubation, 150 µl of 10% aluminum chloride was added and incubated at RT for 6 min. About 0.5 ml of 1 M sodium hydroxide was added and the color complex formed was read at 510 nm. Quercetin was used as a standard to prepare a calibration curve. The results were expressed as mg quercetin equivalents/g extract.

Estimation of total phenols

Sample was prepared by dissolving the extracts in their respective solvents (1 mg/ml). Nearly 0.1 ml of the sample was taken and made up to 2 ml with distilled water. 0.5 ml of Folin–Ciocalteu reagent was added and the tubes were incubated for 3 min. After the incubation period, 2 ml of sodium carbonate was added and the tubes were kept in water bath for 1 min at 50°C. Then, the absorbance of the color complex formed was read at 650 nm. Catechol was used as standard to draw the calibration curve. The results were expressed as catechol equivalents/g extract.

Estimation of anthocyanin

About 0.5 ml of the sample was taken and 4 ml of formic acid was added, and the absorbance was read at 530 nm. The anthocyanin content was calculated on the basis of the following equation and determined as cyanidin-3-glucoside equivalent. Anthocyanin content (mg/100 g of dry matter) = A × MW × DF × (100/ε) × W, where A = absorbance, MW = molecular weight of cyaniding-3-glucoside chloride, ε = molar absorptivity, and W = weight of the sample.

Estimation of tannins

About 0.2 ml of the samples dissolved in their corresponding solvents were taken (1 mg/ml). 0.25 ml of sodium carbonate solution was added to all the samples and 0.2 ml of distilled water was taken as blank. All the tubes were made up to 5 ml with distilled water and incubated at RT for 90 min. The absorbance was read at 260 nm. Tannic acid was used as a standard and the results were expressed as mg tannic acid/g extract.

Estimation of alkaloids

Nearly 1.5 ml of each sample was taken in boiling tubes and 1 ml of 0.01 M SPI solution and 0.5 ml of 0.1 M acetic acid solution were added. 10 ml of distilled water was added to all the boiling tubes and was kept in the boiling water bath for 10 min. After incubation, 2 ml of the 0.01M 3-methyl-2-benzo thiazolinone hydrazone hydrochloride (MBTH) solution was added to all the tubes and kept in the boiling water bath for 2 min. The tubes were cooled and made up to 25 ml with distilled water. The complex formed was read at 630 nm. Theophylline was used as a standard which was prepared by dissolving 10 mg of theophylline in 20 ml of methanol, and then, it was made up to 100 ml with distilled

water. The results were expressed as mg theophylline equivalents/g extract.

HPTLC

The given plant sample 100 mg was weighed in an electronic balance (Afcoset) dissolved in 1 ml ethyl acetate and centrifuged at 3000 rpm for 5 min. This solution was used as test solution for HPTLC analysis. 2 µl of test solution and 2 µl of standard solution were loaded as 5 mm band length in 3 × 10 silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The sample loaded plate was kept in TLC Twin Trough Developing Chamber (after saturated with solvent vapor) with respective mobile phase (flavonoid) and the plate was developed up to 90 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photodocumentation chamber (CAMAG REPROSTAR 3) and captured the images at visible light, UV 254 nm, and UV 366 nm. The developed plate was sprayed with respective spray reagent (flavonoid) and dried at 100°C in hot air oven. The plate was photodocumented in visible light and UV 366 nm mode using photodocumentation (CAMAG REPROSTAR 3) chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 366 nm. The peak table, peak display, and peak densitogram were noted. The software used was winCATS 1.3.4 version.

FTIR Spectroscopy

FTIR spectra of the *D. regia* flower solvent extracts were obtained using Toshvin Shimadzu FTIR spectrometer 8000 series, with absorbance range of 340–4700 cm^{-1} with a resolution of 2870 cm^{-1}.

Statistical Analysis

The results obtained from experiments were expressed as mean ± standard deviation of the triplicates, and two-way ANOVA analysis by SPSS software was performed for the data obtained. The significant difference obtained between the treatments groups was calculated by student *t*-test using Microsoft Excel. $P < 0.05$ was considered as statistically significant.

RESULTS

Free radicals, also called as ROS, have become a major threat to human health in recent times by paving way for many medical conditions. These free radicals are potent enough to damage the lipid bilayer membrane and act as a hindrance for various biochemical pathways. It also results in severe nerve disorders such as Alzheimer's disease and Parkinson's disease. Moreover, it acts as a key factor in inducing mutation, which ultimately results in cancer. Hence, to protect the human organ systems that are prone to damage by ROS, antioxidants are quiet essential.

Antioxidants neutralize the free radicals and impede the chain reaction initiation and propagation. *D. regia* was found to contain various classes of phytoconstituents such as phenolics, terpenoids, flavonoids, phytosterols, and glycosides. Being a rich source of phytochemicals, it plays a crucial role as an antioxidant and thus prevents various medical complications that leave human lives sabotaged.[11]

In Vitro Antioxidant Potential of *D. regia* Flowers

Total antioxidant capacity activity

The total antioxidant assay gives an estimate of overall antioxidant potential of the different solvent extracts of *D. regia* flowers. The assay is based on the formation of the green phosphate - molybdenum complex by the process known as reduction, where molybdenum (VI) is converted to molybdenum (V) if there is the presence of antioxidant in the extracts at acidic pH which is measured at 695 nm. The result obtained was in accordance with that obtained from the total antioxidant capacity for the extracts of sapota peel by Gomathy *et al.*,[12] where methanol extracts (METE) showed maximal antioxidant activity. From the results [Figure 1], it was observed that the antioxidant activity was high in extracts that were prepared using solvents with high polarity index. Thus, METE and acetone extract (ACE) whose polarity is high showed maximal antioxidant activity when compared to ethyl acetate extract (EAE) (moderately polar) and chloroform extract (CHE) (less-polar). The order of the effectiveness of antioxidant activity was ACE > METE > EAE > CHE.

DPPH radical scavenging activity

The DPPH radical scavenging assay is based on the ability of the antioxidants present in the various extracts of *D. regia* to decolorize the DPPH radical. The principle behind the assay is the changing of color from purple to yellow color which shows the reduction of DPPH radical forming reduced DPPH by the scavenging activity of the antioxidants present in the extracts by the donation of protons. The color intensity is based on the number of electrons taken up for pairing which is measured by decrease in absorbance at 517 nm.[13] Free radical

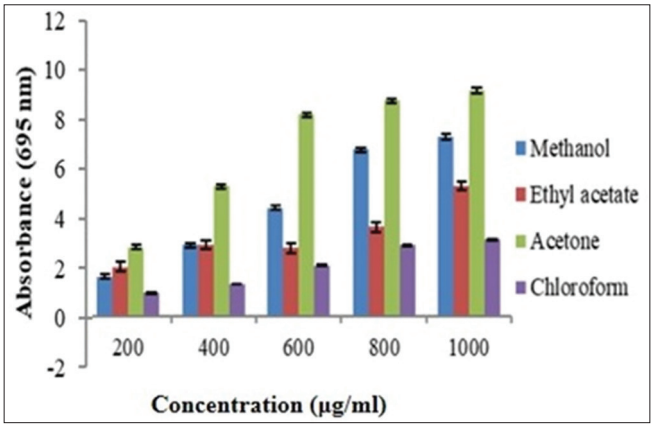

Figure 1: Total antioxidant capacity of solvent extracts of *Delonix regia* flowers

scavenging potential (DPPH) of different solvent extracts of *D. regia* flowers under extraction conditions is represented in Figure 2. The radical scavenging activity increases with an increase in the concentration of the sample which was reflected at the decrease in the absorbance. Trolox was used as standard. From the figure, it was inferred that, at 100 µg/ml, hot extracts of methanol and ethyl acetate exhibited maximal radical scavenging activity. Statistically, the scavenging activity of antioxidants was effective in the order of EAE > METE > ACE > CHE. Our results are in concordance with the reports made by Shabbir *et al.*, Apak *et al.* and Patil *et al.*[14-16]

ABTS radical scavenging activity

In ABTS assay, 2,2-azobis-3-ethylbenzothiazoline-6-sulfonic acid diammonium salt is converted to its radical cation by the addition of ammonium per sulfate, which results in the reduction of blue color read at 745 nm. The absorbance of the sample decreases with increase in the concentration of the sample and thus increases the percentage inhibition. Trolox was used as standard. Free radical scavenging potential (ABTS) of different solvent extracts of *D. regia* flowers under extraction conditions is represented in Figure 3. From the results, it was concluded that, at 100 µg/ml, hot extracts of ethyl acetate possess high antioxidant activity, which is evident from the maximal scavenging activity, followed by METE. Similar results were obtained for crude METE, which was inferred from the work done by Gomathy *et al*.[12] Statistically, the scavenging activity of antioxidants was effective in the order of EAE > METE > ACE > CHE.

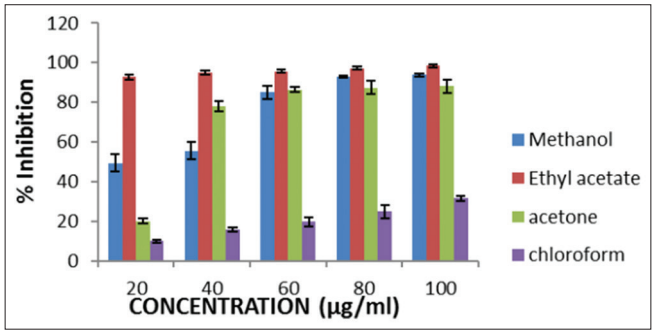

Figure 2: Scavenging effect on 2,2-diphenyl-1-picryl hydrazyl radical by solvent extracts of *Delonix regia* flowers

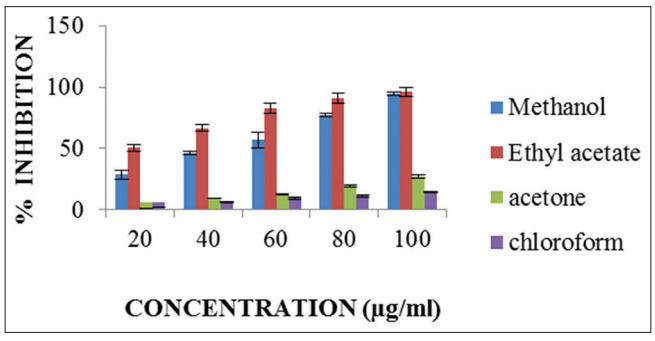

Figure 3: Scavenging effect on 2,2'-azino-bis-3-ethylben zothiazoline-6-sulfonic acid radical by solvent extracts of *Delonix regia* flowers

NO· radical scavenging activity

NO· scavenging effect is a reduction of NO·. NO· is generated in biological tissues by specific NO· synthases, which metabolizes arginine to citrulline with the formation of NO· through a five electron oxidative reactions. The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO·. Under aerobic conditions, NO· reacts with oxygen to produce stable products (nitrate and nitrite), the quantities of which can be determined using Griess reagent. NO· scavenging effect on the different solvent extracts of *D. regia* under hot extraction conditions is depicted in Figure 4. It is inferred from the figure that, at 100 µg/ml, hot extracts of methanol showed maximal NO· radical scavenging activity. However, no significant difference was observed between METE and EAE, while a significant difference was observed between METE and other two extracts (ACE and HE). Statistically, the scavenging activity of antioxidants was effective in the order of METE > EAE > ACE > CHE.

Reducing power capacity

Reducing power assay is based on the reduction by direct electron transfer. In this method, antioxidant compounds form a colored complex with potassium ferricyanide, TCA, and ferric chloride that was measured at 700 nm. Increase in absorbance of the reaction mixture indicates the increase in the reducing power of the sample. Reducing power of different solvent extracts of *D. regia* under hot extraction conditions increases with increase in concentration. From the results [Figure 5], it was shown that the hot extracts of ethyl acetate possess high antioxidant activity which is evident

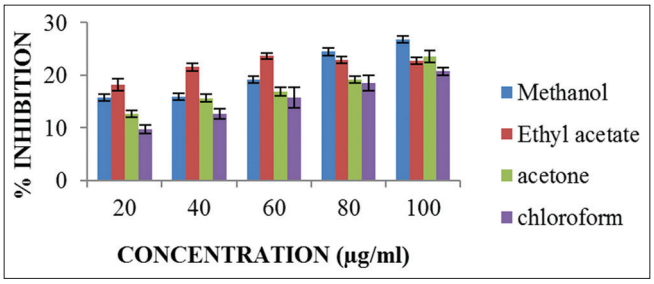

Figure 4: Nitric oxide scavenging effect by solvent extracts of *Delonix regia* flowers

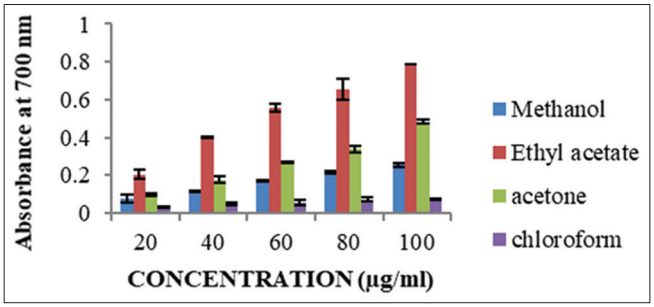

Figure 5: Reducing power capacity of solvent extracts of *Delonix regia* flowers

from the maximum reducing power capacity followed by other extracts. The present results are in close coincidence with the reports made by Jancyrani *et al.*[11] Statistically, the reducing power of antioxidants (hot extraction) was effective in the order of EAE > ACE > METE > CHE.

Ferrous ion chelation activity

The ferrous ion chelating assay works on the principle of reduction of the Fe^{3+} ions to Fe^{2+} ions, which then react with ferrozine to form a colored complex. The color intensity is directly proportional to the iron concentration and can be measured using a spectrophotometer. The ferrous ion chelating effect on the different solvent extracts of *D. regia* flowers extraction conditions is represented in Figure 6. The results suggest that, at 1000 µg/ml, the hot extract of ethyl acetate possesses high antioxidant activity, which is evident from the maximum chelating activity. Statistically, the chelating ability of antioxidants was effective in the order of EAE > METE > ACE > CHE.

FRAP activity

The FRAP assay uses antioxidants as reductant in a redox-linked colorimetric method using an easily reduced oxidant, and Fe (III) reduction of a ferric tripyridyltriazine complex to ferrous-(2,4,6-tripyridyl-s-triazine) that is ferric (III) [colorless] to ferrous (II) [blue] can be monitored by measuring absorbance at 593 nm. The absorption readings are related to the reducing power of the electron-donating antioxidants present in the test compound. The ferric reducing antioxidant potential of different solvent extracts of *D. regia* flowers under hot extraction conditions is depicted in Figure 7. It is evident that, at 1000 µg/ml, hot extracts of ethyl extract displayed significant ferric reducing antioxidant potential compared to other extracts. Statistically, the ferric reducing antioxidant potential of plant extracts was effective in the order of EAE > METE > ACE > CHE.

Lipid peroxidation inhibition activity

The product of lipid peroxidation, malondialdehyde, formed as a result of induction by Fe_2SO_4 (Fenton reaction) reacts with TBA reactive substances to form a pink-colored complex is measured at 530 nm. The extent of inhibition of lipid peroxidation is assessed by decrease in pink color with increasing concentration of the extracts.[17] A free radical prefers to steal electrons from the lipid membrane of a cell, initiating a free radical attack on the cell known as lipid peroxidation. ROS targets the carbon-carbon double bond of polyunsaturated fatty acids leading to oxidation of lipids in the membrane. The capacity of antioxidants to inhibit this peroxidation process by donating electrons to the free radical, thereby inhibiting chain reaction, is determined in this assay.

The lipid peroxidation inhibition effect on the different solvent extracts of *D. regia* flowers under hot extraction conditions is presented in Figure 8. From Figure 8, it is understood that, at 100 µg/ml, hot extracts of ethyl acetate exhibited significant lipid peroxidation inhibition ability, when compared to other extracts. In contrast, ACE showed least inhibition activity. Statistically, the lipid peroxidation inhibition potential of plant extracts was effective in the order of EAE > METE > CHE > ACE.

Phytochemical Analysis

Flavonoids, a group of polyphenol compounds, widely found in fruits and vegetables. Numerous positive health effects of flavonoids have been described. They have been reported to

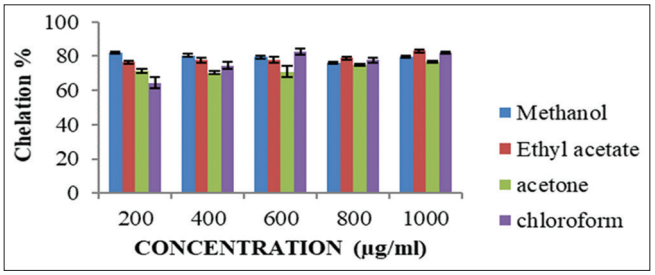

Figure 6: Ferrous ion chelating effect on the solvent extracts of *Delonix regia* flowers

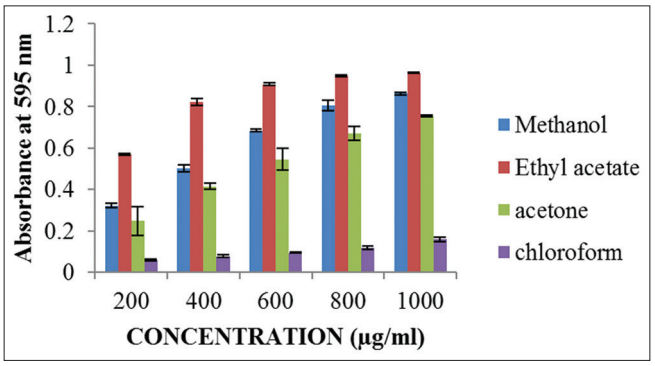

Figure 7: Ferric reducing antioxidant potential of solvent extracts of *Delonix regia* flowers

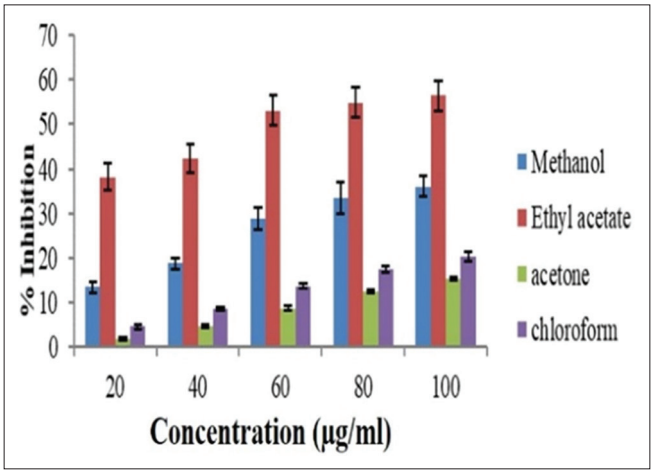

Figure 8: Lipid peroxidation inhibition effect (%) of solvents extracts of *Delonix regia* flowers

exhibit anticancer, antiviral, and anti-inflammatory effects and to reduce the risk of cardiovascular diseases. These activities are generally associated with antioxidant or free radical scavenging properties of flavonoids. The number of flavonoid derivatives is more than 4000, and their antioxidant properties are very different.[18]

The flavonoid content present in solvent extracts of *D. regia* flowers is represented in Table 1. In the present study, a significant elevation in flavonoid content is noticed in the ACE. It has been reported that flavonoids were also profoundly present in the bark extracts.

The total phenol assay relies on the transfer of reducing equivalents (electrons), in the alkaline medium, from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes, and manifested in the formation of blue color complexes that are determined in a spectrophotometer.[19] Total phenols are involved in the delaying the process of aging, and we have observed a profound elevation in total phenol content in the EAEs [Table 1].

Tannins known as the group of phenolic compounds are the significant plant secondary metabolites. These phenolic compounds may slow down the aging process as well as protect the human body against diseases such as atherosclerosis, coronary diseases, and cancer. The tannin content in the solvent extracts of *D. regia* flowers is depicted in Table 1. From Table 1, it is noted that EAE is highly rich in tannins.

Anthocyanin is representative of plant pigments widely distributed in color fruits and flowers. As anthocyanin is widely consumed, finding out additional biological activities related to these compounds would be of great interest. Anthocyanin is normally obtained by extraction from plants, and the extraction methods currently employed are with the use methanol, ethanol, acetone, water, or mixtures as solvents. In the present study, both anthocyanin and tannins were found to be significantly increased in EAE. It has been demonstrated that the floral parts of *D. regia* was found to contain two anthocyanin: Cyanidin-3-glucoside and cyanidin-3-gentiobioside. Table 1 represents the anthocyanin content in solvent extracts of *D. regia* flowers. A significant increase in alkaloid content was observed in the METE and is represented in Table 1. There is considerable anecdotal and epidemiological evidence that dietary anthocyanin and polyphenols confer preventive and therapeutic role in a number of diseases.

Alkaloids are a class of non-nutritive phytochemical compounds that are synthesized as secondary metabolites by the plant cells. They fight against free radicals and are capable of quenching their activity. The presence of these phytochemicals simply attributes to the antimicrobial and antioxidant activities of the flower extracts of *D. regia,* and it has also been reported that the wood and bark of this tree possess a high quantity of polyphenol.[20] Apart from the phytochemicals, other chemical constituents such as quercetin 3-rhamnoside, afzelin, astragalin, and isoquercetin were isolated from the leaf extracts.[21] Alkaloids are oxidized by sodium metaperiodate under mild acidic conditions to form an intermediate, N, N‟ dimethyl alloxan, which then react with MBTH to yield a blue color product and read in spectrophotometer at 630 nm.

HPTLC of Solvent Extracts of *D. regia* Flowers

The phytochemical evaluations of plants which have a suitable history of use in folklore have often resulted in the isolation of principles with remarkable bioactivities. Hence, methodologies that can generate a fingerprint of each extract in large collections would be useful to detect stability of the same extract over time. Preferably, the method should be based on electronic storage, retrieval, and analysis of the data. A lot of extraction methods and analytical methods as spectrophotometry, HPTLC, HPLC, gas chromatography, mass spectrometry, and FTIR spectroscopy are developed for the study about plant active compounds. HPTLC-based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase.

Table 1: Phytochemical content in different solvent extracts of *Delonix regia* flowers under hot extraction conditions

Solvents	Flavonoid	Total phenols	Tannins	Alkaloids	Anthocyanins
METE	2.05±0.52	2.03±0.18	3.79±0.04	0.09[a]±0.01	128.58±14.56
EAE	1.80±0.27	6.37±0.07	4.99±0.07	0.78[d]±0.02	43.97±1.93
ACE	4.99±0.86	1.99±0.15	3.28±0.02	0.18[b]±0.06	33.95±5.86
CHE	1.17±0.86	0.17±0.02	0.21±0.02	4.15[e]±0.01	20.59±0.96

Values represent mean±standard deviation of three replicates. Units: Flavonoids: mg quercetin equivalents/g extract; total phenols - mg catechol equivalents/g extract; tannins: mg tannic acid equivalents/g extract; alkaloids - mg theophylline equivalents/g extract; anthocyanins - mg cyanadin-3-glucoside equivalents/g extract. Comparison between groups; a - METE with EAE, ACE and CHE, b - EAE with ACE and CHE, d - ACE with CHE and METE, e – CHE with METE and EAE., METE: Methanol extracts, EAE: Ethyl acetate extract, ACE: Acetone extract, CHE: Chloroform extract

HPTLC fingerprinting is a highly reliable and advanced method of confirming the presence of phytoconstituents in the plant samples. The area under a peak and peak height from the densitogram [Figure 9] and the Rf values could be used to affirmatively declare the presence of a particular phytochemical. From the Rf values and heights of peaks mentioned in Table 2, it may be inferred that peaks 1–6 and 8 of EAEs of *D. regia* confirm the presence of flavonoids in the samples.

The mobile phase used was toluene: ethyl acetate: formic acid (5:4:1). The spray reagent used was 1% ethanolic aluminum chloride reagent. For the spectral analysis, it was shown that yellow, yellowish blue-colored fluorescent zone at UV 366 nm mode was present in the tracks, and it was observed from the chromatogram [Figure 10a and b] after derivatization, which confirmed the presence of flavonoid/phenol carboxylic acid in the given standard and in the sample.

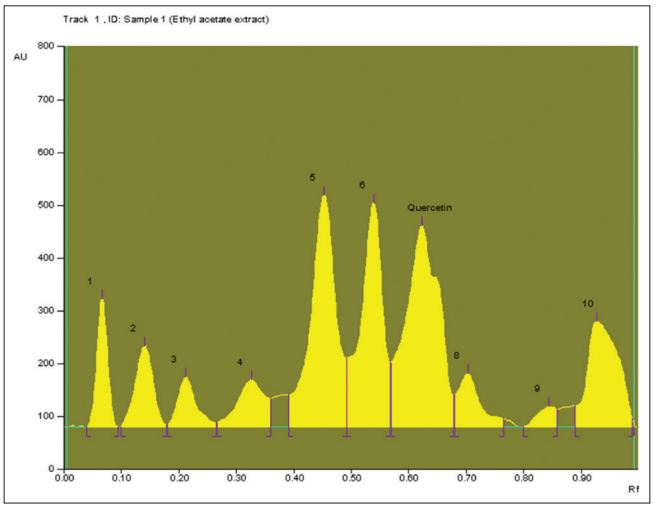

Figure 9: High-performance thin-layer chromatography densitogram of ethyl acetate extract of *Delonix regia* flower

FTIR of Solvent Extracts of *D. regia* Flowers

FTIR is an easy and commonly used method to elucidate the structural features of compounds present in plant samples. Each spectral range corresponds to unique features in a molecular structure, and hence, they can be called fingerprint regions. Similar studies on the FTIR analysis on *Bougainvillea glabra* flower extract[22] and *Aerva lanata* roots[23] were reported. Spectral analysis of EAE of *D. regia* flowers yielded the following spectral bands. Spectral analysis of EAEs of *D. regia* depicts the presence of amide, conjugated ketone, and phenolic C-O stretch (1660.71 + 1681.93 + 1056.07 cm^{-1}) in combination, which indicates a possible flavone backbone. Furthermore, a broad 90 spectrum of 3458.07 cm^{-1} indicates the presence of O-H bonds.[24] Similarly, the presence of carbonate (1519.91 cm^{-1}) in EAE indicates the possible existence of phenolic acids [Figure 11].

CONCLUSION

The EAE of the *D. regia* flowers can be concluded as the effective one among the all four extracts. The EAE shows the maximum scavenging, reducing, chelating, and inhibition of peroxidation activity in the following assays, namely DPPH assay, ABTS assay, reducing power assay, ferrous reducing power assay, ferrous ion chelation assay, and lipid peroxidation inhibition assay. The particular extract was further screened for the presence of flavonoid groups using HPTLC methods. The result of FTIR confirms the presence of phenolic groups and flavonoid groups. Since the total phenols are abundant than the flavonoids as per the phytochemical assays, the antioxidant potential of the EAE may be because of phenolic constituents present in it. Hence, *D. regia* is a potent source of antioxidants, which further elucidates that this plant, being of low economic importance, could pave the way for cheap future medicines.

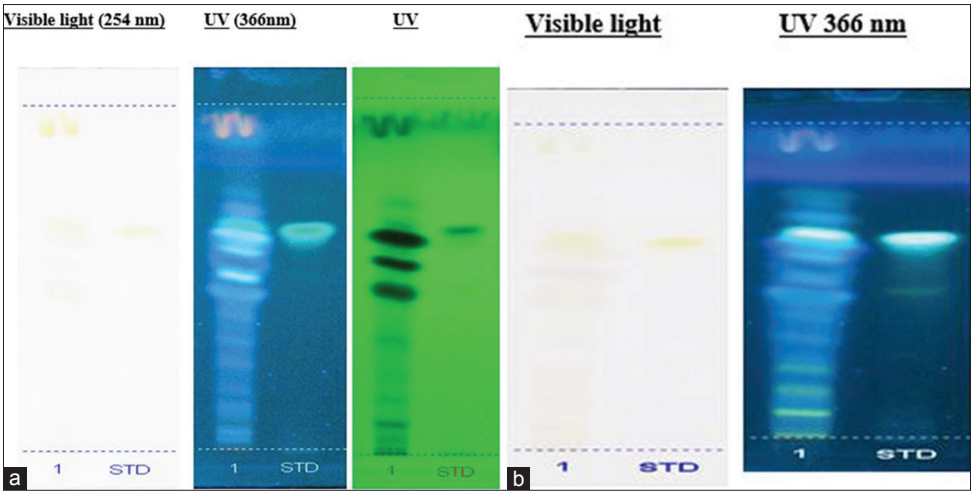

Figure 10: (a) Chromatogram before derivatization, (b) Chromatogram after derivatization

Table 2: HPTLC peak table

Track	Peak	Rf	Height	Area	Assigned substance
Sample 1	1	0.07	242.5	4087.3	Flavonoid 1
Sample 1	2	0.14	152.8	4369.3	Flavonoid 2
Sample 1	3	0.21	94.2	2776.6	Flavonoid 3
Sample 1	4	0.33	89.2	3921.1	Flavonoid 4
Sample 1	5	0.45	438.5	17569.7	Flavonoid 5
Sample 1	6	0.54	424.9	14680.0	Flavonoid 6
Sample 1	7	0.62	380.8	20335.7	Flavonoid 7 (quercetin)
Sample 1	8	0.70	100.7	3556.3	Flavonoid 8
Sample 1	9	0.84	38.5	1132.7	Unknown
Sample 1	10	0.93	199.9	9894.6	Unknown
STD	1	0.63	475.3	16933.2	Flavonoid standard (quercetin)

HPTLC: High-performance thin-layer chromatography, STD: Standard

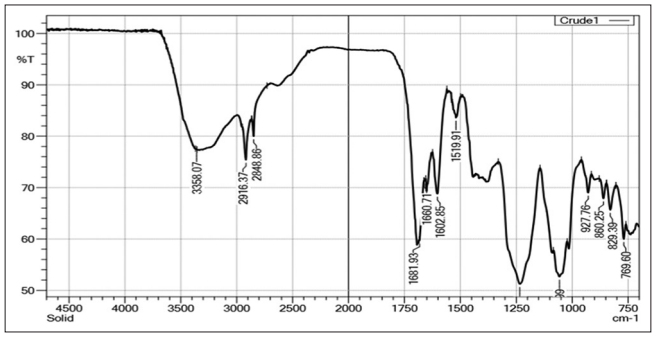

Figure 11: Fourier transform infrared analysis of ethyl acetate extract of *Delonix regia* flower

REFERENCES

1. Nabila Y, Mahmoud H, Amal SA. Mater nutritional and biological assessment of wheat biscuits supplemented by fenugreek plant to improve diet of anemic rats. Acad J Nutr 2012;1:1-9.
2. Sidhu K, Kaur J, Kaur G, Pannu K. Prevention and cure of digestive disorders through the use of medicinal plants. J Hum Ecol 2007;21:113-6.
3. Chirag JP, Tyagi S, Halligudi N, Yadav J, Pathak S, Singh SP, *et al*. Antioxidant activity of herbal plants: A recent review. J Drug Deliv Ther 2013;1:1-8.
4. Kusuma IW, Enos M, Arung T, Syafrizal, Kim YU. Antimicrobial and antioxidant properties of medicinal plants used by the bentian tribe from Indonesia. Food Sci Hum Wellness 2014;3:191-6.
5. Parekh J, Chanda SV. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol 2007;31:53-8.
6. Singh S, Singh S, Naresh S. A review : Introduction to genus delonix. World J Pharm Pharm Sci 2014;3:2042-55.
7. Islam F, Chowdhury SR, Quadery TM. Antioxidant, total phenolics, free radical scavenging and preliminary cytotoxicity studies of *Ophiorrhiza mungos*. Bangladesh Pharm J 2011;14:117-20.
8. Rakholiya K, Kaneria M, Chanda S. Vegetable and fruit peels as a novel source of antioxidants. J Med Plants Res 2011;5:63-71.
9. Saha K, Lajis NH, Israf DA, Hamzah AS, Khozirah S, Khamis S, *et al.* Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. J Ethnopharmacol 2004;92:263-7.
10. Selvakumar S, Sankar S. Phytochemical screening and antioxidant activity of combination of *Pleurotus florida* and *Agrocybe cylindracea*. J Pharm Res 2015;9:89-94.
11. Jancyrani MM, Kannan P, Kumaravel S. Screening of antioxidant activity, total phenolics and gas chromatograph and mass spectrometer (GC-MS) study of *Delonix regia*. Afr J Biochem Res 2011;2:341-7.
12. Gomathy K, Baskar R, Kumaresan K. Comparison of antioxidant potential in pulp and peel extracts of *Manilkara zapota* (L.) P. Royen. Afr J Biotechnol 2013;12:4936-43.
13. Alam MN, Bristi NJ, Rafiquzzaman M. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm J 2013;21:143-52.
14. Shabbir M, Khan MR, Saeed N. Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of *Maytenus royleanus* leaves. BMC Complement Altern Med 2013;13:143.
15. Apak R, Güçlü K, Demirata B, Ozyürek M, Celik SE, Bektaşoğlu B, *et al.* Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules 2007;12:1496-547.
16. Patil DD, Mhaske DK, Patare MA, Wadhawa GC. Antibacterial and antioxidant, anti-inflammatory study of leaves and root *Delonix regia*. Int J Pharm Chem Sci 2014;3:131-5.
17. Sangeetha R, Karthi N. Evaluation of antioxidant status and renal parameters in diabetic and non-diabetic users. Pharmacologyonline 2010;290:285-90.
18. Kumar TS, Baskar, R. Screening and quantification of phytochemicals in the leaves and flowers of *Tabernaemontana heyneana* Wall - Anear threatened plant. Ind J Nat Prod Resour 2014;5:237-43.
19. Karuppusamy S, Muthuraja G, Rajasekaran KM. Antioxidant activity of selected lesser known edible fruits from Western Ghats of India. Indian J Nat Prod Resour 2011;2:174-8.
20. Einbond LS, Reynertson KA, Luo XD, Basile MJ, Kennelly EJ. Anthocyanin antioxidants from edible fruits. Food Chem 2004;84:23-8.
21. Azab B, Shah N, Radbel J, Tan P, Bhatt V, Vonfrolio S, *et al.* Pretreatment neutrophil/lymphocyte ratio is superior to platelet/lymphocyte ratio as a predictor of long-term mortality in breast cancer patients. Med Oncol 2013;30:432.

22. Sahu R, Saxena J. Screening of total phenolic and flavonoid content in conventional and non-conventional species of *Curcuma*. J Pharm Phytochem 2013;2:24-6.
23. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: Properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem 2015;30:11-26.
24. Nirmaladevi D, Venkataramana M, Chandranayaka S, Ramesha A, Jameel NM, Srinivas C. Neuroprotective effects of bikaverin on H_2O_2-induced oxidative stress mediated neuronal damage in SH-SY5Y cell line. Cell Mol Neurobiol 2014;34:973-85.

Source of Support: Nil. **Conflict of Interest:** None declared.