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In vitro Study of Antioxidant Potentials, and Thrombolytic Activity of the Leaves of *Delonix regia* (Family: Fabaceae)

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Abstract

This study was conducted to assess the antioxidant potential and thrombolytic activity of the methanolic extract of the *Delonix regia* leaves and its partitionates. The methanolic extract was partitioned into four fractions (pet ether, dichloromethane, ethyl acetate, and aqueous) according to polarity. These fractions were subjected to further investigation. The antioxidant potential was assessed by two methods one was by using the Folin-Ciocalteu reagent as oxidant and gallic acid as a standard; and another method was the DPPH assay. In Folin-Ciocalteu reagent using method, all fractions showed significant antioxidant potential and the ethyl acetate partitionate showed the highest antioxidant activity at 142.28 mg of GAE/gm of extractives among all. In the DPPH assay, the antioxidant potential was measured compared against BHT, all fractions showed better activity than the BHT and the highest IC_{50} value was 51.06 µg/mL of dichloromethane partitionate. In thrombolytic activity, streptokinase was the positive control, and water was the negative control. The highest thrombolytic activity was found in petroleum ether partition and methanolic extract and the IC_{50} value were 47% and 30.65%, respectively. Whereas the IC_{50} value of streptokinase and water was 64.22% and 15.48% respectively.

Key words: *Delonix regia*, antioxidant potential, Folin-Ciocalteu reagent, DPPH assay, thrombolytic activity, streptokinas.

Introduction

Plants are vital source of large variety of natural antioxidants and they have played a very crucial role in treating diseases throughout the world (Fallah Huseini *et al.,* 2006; Rafieian-Kopaei *et al.,* 2013). They have been used for anticancer antimicrobial, antidiabetic, anti-atherosclerosis, immune-modulatory and even reno-protection or hepatoprotective effects (Taji *et al.,* 2012; Chaleshtori *et al.,* 2011; Kazemi *et al.,* 2010; Khosravi-Boroujeni *et al.,* 2012; Shahrani and Rafieian-Kopaei, 2009; Baradaran and Rafieian-Kopaei, 2013; Rafieian-Kopaei and Baradaran, 2013). Moreover, in developing countries, numerous ailments have historically been treated mostly using natural remedies (Sivaraj *et al.,* 2014). Though 5000 species of angiosperms are reported to grow in Bangladesh, only 500 of them have been recognized as medicinal plants because of their biological activities (Harun-ur-Rashid *et al.,* 2014).

It has been estimated that 12500 tons of dried medicinal plant products are sold in Bangladesh which has a worth of Tk. 255 million to the rural economy. At the factory level, 5000 tones of medicinal plants are imported annually that cost around Tk. 480 million (Alam *et al.,* 1996).

A large portion of people in some Asian and African countries relies on complementary medicine for basic medical care. Whereas in affluent nations, 70% to 80% of the population utilize an alternative or

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supplementary form of medicine (e.g. acupuncture). The most often used traditional medicine is herbal therapy, which is also the most lucrative on the global market. In 2003–2004, Western Europe had annual revenues of $ 5 billion. Products were sold for a total of $ 14 billion in China in 2005. Brazil generated $ 160 million from the sale of herbal medicines in 2007 (Chaudhary *et al.,* 2010).

A sizable, deciduous tree with fern-like leaves is called a Krishnachura which is also familiar as the flame tree or royal poinciana or the peacock flower tree (*D. regia*) (Suhane *et al.,* 2016). Delonix is a genus of flowering plants that belongs to the Caesalpinioideae subfamily of the Fabaceae family. Many trees of that genus are native to Madagascar and East Africa (Singh and Kumar, 2014). These trees are also found in Brazil, Burkina Faso, Cyprus, Ethiopia, India, Bangladesh, Jamaica, Nigeria, Puerto Rico, Singapore, and South Africa (Suhane *et al.,* 2016).

It is a rich source of phytochemicals. The flowers were reported to contain alkaloids, cardiac glycosides, carbohydrates, flavonoids, and phenols (Hait *et al.,* 2018). The leaf contains alkaloids, glycosides, saponins, proteins, flavonoids, and diterpenes (Bhorga and Kamle *et al*, 2019). The root and the stem contain alkaloids, saponin, flavonoids, and steroids (Bhokare *et al.,* 2018).

The flowers, leaves, and bark of this plant have been investigated to contain most of the active constituents. The flowers possess antifertility, wound healing (De Groot, 1994), and antifeedant (Deepa and Remadevi, 2011) activities. The leaves of *D. regia* have been used in ulcers (Goel *et al.,* 1988), and fungal infections (Pandey *et al.,* 1982) and possess cytotoxic activities (Azab *et al.,* 2013). The bark has been used as an antiperiodic, febrifuge (Modi *et al.,* 2016). Leaves were used in bronchitis and pneumonia in infants (Jancy Rani *et al.,* 2011), as anti-diabetic medication (Rahman *et al.,* 2011), and also used in ulcerant problems, body aches, and rheumatic joints pain (Shewale *et al.,* 2011).

This work aimed to investigate antioxidant, free radical scavenging, thrombolytic activities of various extracts of *D. regia*.

Materials and Methods

Collection of plant materials: Plant leaves of *D. regia* were collected from Chandrima Udyan in Dhaka, Bangladesh. The leaves were dried under sunlight for 30 days. After being dried, the leaves were then pulverized to coarse powder using grinding machine which was of high-capacity.

Preparation of extract: 300 gm of powder was filled in a cleaned, amber color reagent bottle (5 L). The powder was then immersed in methanol (2.0 L). The reagent bottle was closed by a bottle cap and kept for one month in these period. The reagent bottle was shacked and stirred occasional in this time period. The whole mixtures were then filtered twice. At first it was filtered through the fresh cotton plug and it was subsequently filtered with the Whatman No. 1 filter paper. Rotary evaporator was the method to reduce the volume of the filtrate at ambient temperature and low pressure. Rotary evaporation was continued until 70% of the solvent was evaporated.

Solvent-solvent partitioning was completed by modified Kupchan partitioning protocol by Van Wagenen *et al.,* (1993). The crude extract (5 g) was taken and dissolved in 10% of aqueous methanol solution. Petroleum ether, dichloromethane, and finally ethyl acetate were used to extract the crude. Five fractions were dried by evaporation and were utilized for analysis.

Table 1. Partitionates obtained from leaves of *D. regia*.

Plant part	Sample code	Test sample
Leaves of *D. regia*	MESF	Methanol extract soluble fraction
	PESF	Petroleum ether soluble fraction
	DCMSF	Dichloro-methane soluble fraction
	EASF	Ethyl acetate soluble fraction
	AQSF	Aqueous soluble fraction

Evaluation of antioxidant potential

Determination of total phenolics: The total phenolic content of *D. regia* extractives was tested by using Skerget *et al*., 2005 methodology, in which the Folin-Ciocalteu reagent and gallic acid was used as the oxidant and the standard, respectively (Majhenic *et al.*, 2007). Extractives (2 mg) were taken and diluted in distilled water separately (conc. 2 mg/ml). 2.5 ml Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml Na_2CO_3 (7.5% w/v) solution were added to 0.5 ml extract solution (conc. 2 mg/ml).

The mixture was kept in room temperature and the incubation time was 20 minutes. After that the absorbance of the mixture was measured by UV spectrophotometer at 760 nm wavelength. A standard curve was prepared using the gallic acid which was used to measure the total phenolic content of sample.

DPPH assay: The antioxidant potential of methanolic extracts and the several partitionates of the extracts were assessed using DPPH (Choi *et al.*, 2000). DPPH samples and different partitionates of the plant extracts were dissolved in 2.0 ml of methanol solution (conc. 500 µg/ml to 0.977 µg/ml). The solutions were kept in a dark place at room temperature and this incubation condition was maintained for 20 minutes. The absorbance of all solutions was taken at 517 nm. The percent inhibition of free radical DPPH was calculated from the following equations.

$$(I\%) = (1 - A_{sample}/A_{blank}) * 100$$

Where,

A_{blank} = Absorbance of the control (containing all reagents except the test material).

A graph was plotted using inhibition percentage vs extract concentration. IC_{50} value was determined using the equation from the graph.

Thrombolytic activity: All extractives were examined for thrombolytic activity where streptokinase (SK) was the standard drug. Methanol extracts (10 mg) of *D. regia* leaves and their distinctive fractions were added to 1ml distilled water in discrete vials. 1 ml of blood was drawn and transferred to eppendorf tubes which had been previously weighed, where it was allowed to clot.

Healthy volunteers contributed 5 ml venous blood, which were partitioned into ten pre-weighed sterile eppendorf tubes (0.5 ml/tube) and kept at 37 °C for 45 minutes. After the clot had formed, the serum was withdrawn entirely without disturbing the clot, and calculates the clot weight using following equation-

Clot weight = weight of clot containing tube – weight of tube alone 100 µl

Aqueous solutions of partitionates and crude extracts (100 ml) were supplied sequentially to each eppendorf tube containing pre-weighed clot. 100 µl of streptokinase (SK) was added to the control eppendorf tubes to make it a positive non-thrombolytic control and 100 µl of distilled water was added to the control eppendorf tubes to make it a negative non-thrombolytic control. Eppendorf tubes were then maintained for 90 minutes at 37 °C and clot lysis was observed. After the incubation period the released fluid was removed and the weight of the eppendorf tubes were measured again to analyses the difference in weight after the clot was ruptured. Finally the % clot lysis was measured by the following equation.

% clot lysis = (Weight of the lysis clot / Weight of clot before lysis) * 100

Results and Discussion

Total antioxidant activity was determined using the methanol extract of D. regia leaves as well as several partitionates, like as pet-ether, dichloromethane, and ethylacetate soluble partitionates of the methanol extract.

Total phenolic content fluctuates with extractive, extending from 75.37 mg of GAE/gm of extractives to 142.28 mg of GAE/gm of extractives. Ethylacetate (142.28 mg of GAE/gm of extractives) seemed to have the maximum phenolic concentration of all extractives, followed by AQP (75.37 mg of GAE/gm of extractives) (Figure 1).

In DPPH assay, EASF showed the strongest antioxidant capability in this study, with an IC_{50} value of 10.49 µg/ml for leaves of *D. regia*. PESF showed antioxidant capability, with IC_{50} values of

21.38 µg/ml, whereas MESF and DCMSF partitionates had IC_{50} values of 39.24 µg/ml and 51.06 µg/ml, respectively (Figure 2). Therefore, DCMSF and EASF partitionates may be further investigated for antioxidant phytochemicals.

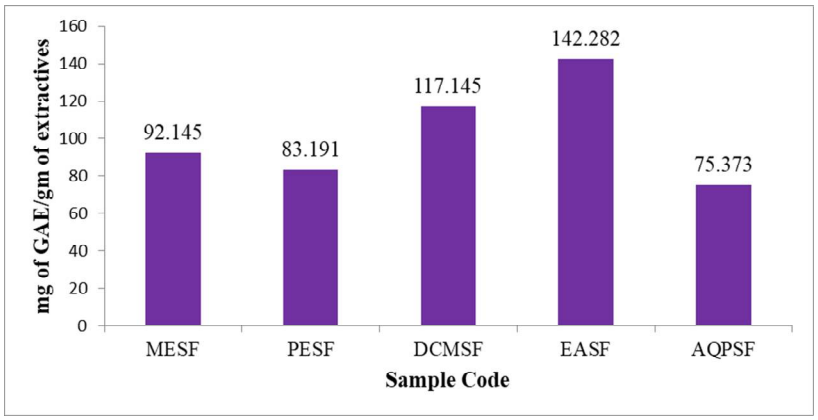

Figure 1. Total phenolic content of *D. regia leaves*.

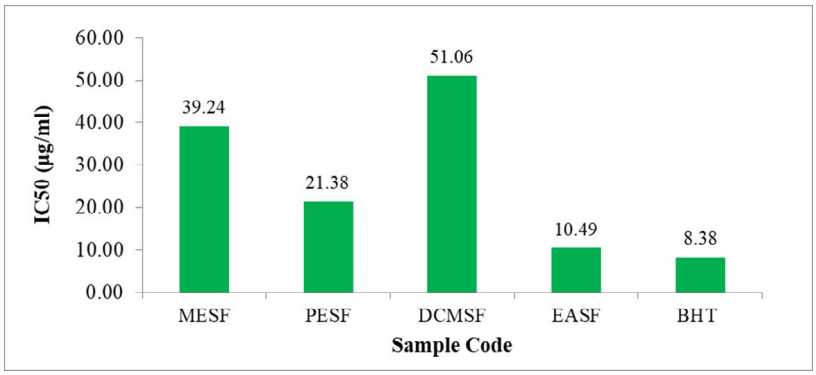

Figure 2. Antioxidant potential of *D. regia* leaves.

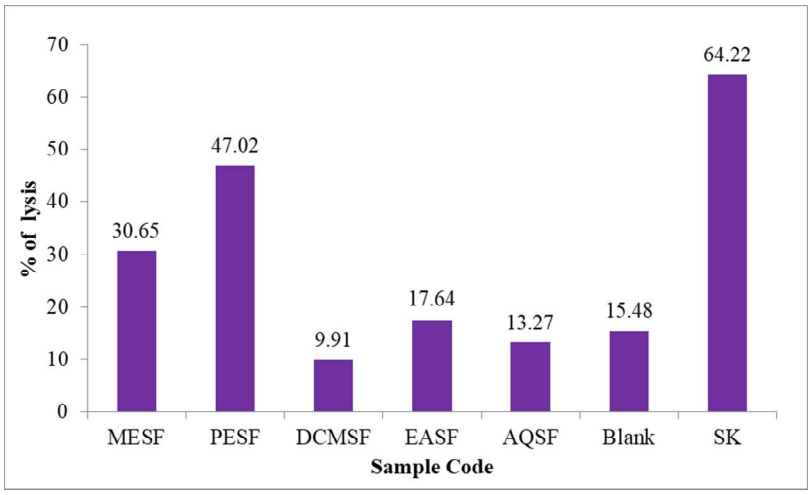

Figure 3. Thrombolytic activity of *D. regia* leaves extract.

The addition of 100 µl SK, a positive control (30,000 I.U.) to the clots, and subsequent 90-minute incubation at 37 ℃ resulted in 68.57% clot lysis. On contrary, distilled water was being used as a negative control, resulting in a small incidence of clot lysis (8.12%). The difference in clot lysis percentage between the positive and negative controls was found to be considered significant.

In this study, the methanolic extract of *D. regia* exhibited thrombolytic activity 30.65%, PESF exhibited 47.02%, DCMSF exhibited 9.91% and EASF exhibited 17.64% thrombolytic activity (Figure 3).

Conclusion

In vitro study effects of the leaves of *D. regia* were thoroughly investigated in the current study. The crude extract showed significant antioxidant and thrombolytic activity. The further chemical study may reveal the reason for these effects and help to understand the mechanism of these effects.

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