

Free Radical Scavenging, Membrane Stabilizing and Thrombolytic Potentials of the Leaves of *Quisqualis indica* (L.)

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Abstract

Bangladesh is a land of thousands of plants which might provide a scope to obtain therapeutically important natural drugs. Systematic screening of plants is very vital to fulfill this goal. *Quisqualis indica* (L.) is a vine and it belongs to the Combretaceae family. It grows all over the Bangladesh. It has some local uses to treat headache, skin disease, diarrhea, fever, cough, etc. In order to assess its biological potential, the leaves of the plant were collected and subjected to methanol extraction followed by fractionating into petroleum ether, carbon tetrachloride, chloroform and aqueous soluble materials. Primary free radical scavenging power of the extractives was evaluated using the radicals generated by DPPH and H₂O₂. Methanol crude extract as well as aqueous and chloroform fractions showed noticeable radical scavenging potential. Besides, these extracts had higher level of phenolics. The membrane stabilizing potential of the extractives was estimated based on the prevention of hemolysis of RBC prompted by hypotonic solution as well as heat. The aqueous fraction showed the highest level of membrane stabilizing potential indicating its primary anti-inflammatory ability. The extractives were also subjected to thrombolytic study. Mild thrombolytic ability was displayed by them compared to the standard. Further studies are required to explore bioactive compounds.

Key words: *Quisqualis indica*, Free radical scavenging, DPPH, Hydrogen peroxide, Phenolic, Membrane stabilizing, Thrombolytic.

Introduction

Medicinal plants serve as vital sources of lead molecules. A significant portion of the existing drugs are the natural products or derivatives of natural products. In this aspect, screening of the bioactivity and phytochemical of the plants is essential to proceed for drug discovery from natural sources. Bangladesh is a land of varieties of plants which provide an opportunity to obtain pharmacologically important extracts leading to drug discovery (Rahman *et al.*, 2008).

Free radicals generated in cell drastically damage nucleic acids, carbohydrates, protein, fats, etc. It might also trigger multiple complexities like inflammation, mutation, cancer, etc. Antioxidant capable of scavenging free radicals might be useful to

counteract these disorders. Inflammation is always a concern during many pathological states (Clement and Luo, 2020). To find better anti-inflammatory drugs, numerous candidates always remain in the process of screening (Colebatch *et al.*, 2012). Thrombosis is a very critical issue for patients with vascular diseases as it can lead to death gradually. Drugs, diets, life style changes, etc. are always tried to counteract thrombolytic disorders (Hilleman and Campbell, 2011). Regarding all of the above-mentioned issues, natural products are getting priority to discover new lead molecules (Abu Bakar *et al.*, 2018; Pisoschi *et al.*, 2016; Shilpi *et al.*, 2012; Yan *et al.*, 2015). In this context, plants of Bangladesh should be screened in a methodical way to explore their biological potentials.

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Quisqualis indica L. (family- Combretaceae; English name- Rangoon-creeper, Chinese honey-suckle; Bangla name- Basantilata, Madhabilata, Modhumalati, Modhumanjuri) is a shrubby vine having light pink to crimson star shaped flowers (Sahu et al., 2012). Leaves of *Q. indica* reported to have quisqualic acid, trigonelline, L-proline, rutin, L-asparagine and cysteine synthase. Pelargonidin-3-glucoside and rutin were isolated from flowers. Seeds contain linoleic acid, palmitic acid, oleic acid, stearic acid, arachidic acids, quisqualic acid and so on (Ghani, 2003; Rastogi and Mehrotra, 1990 and 1993). Analgesic, antimicrobial, immunomodulatory, anti-diarrheal, anti-staphylococcal and hypolipidemic effects, etc. have been reported earlier (Jahan et al., 2008; Jahan et al., 2009; Kulshreshtha et al., 2018; Sahu et al., 2012). The plant is traditionally used for treating headache, skin disease, diarrhea, fever, cough, flatulent distention of the abdomen, etc. (Mahajan and Aher, 2017; Sahu et al., 2012). Earlier, a study on the stem bark of *Q. indica* showed some potentials as antioxidant (Kaisar et al., 2009). Besides, a free radical scavenging assay was performed with the polar parts of the leaves *Q. indica* but low polar fraction was not prepared and evaluated (Bose et al., 2009). The current investigation was designed to assess comprehensively the free radical scavenging, membrane stabilizing and thrombolytic potentials of leaves of *Q. indica* using various fractions of different polarities.

Materials and Methods

Chemicals: acetylsalicylic acid, gallic acid, hydrogen peroxide, 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) and quercetin were procured from Sigma-Aldrich Co., St. Louis, USA. Folin-Ciocalteu's reagent and ascorbic acid were collected from Loba Chemie Pvt. Ltd., Mumbai, India.

Plant collection and extraction: Leaves of *Quisqualis indica* (L.) was collected from Curzon hall, Dhaka in June, 2014. A voucher specimen for *Q. indica* (L.) with DACB Accession No: 40167 have been deposited at Bangladesh National Herbarium (BNH), Mirpur Road-1, Dhaka for future references.

500 g of leaf was powdered and macerated by 2.5 L of methanol for 2 weeks at RT (room temperature) followed by filtering using Whatman filter paper number 1 and concentrating to obtain methanol crude extracts (MCE). 5 g of this was fractionated into petroleum ether (PE, 2.8 g), carbon tetrachloride (CTC, 1.8 g), chloroform (CF, 0.15 g) and aqueous (AQ, 0.25 g) soluble materials as per modified Kupchan method (VanWagenen et al., 1993).

DPPH free radical scavenging assay: Extracts (500-0.977 µg/ml methanol) were mixed separately with DPPH (20 µg/ml methanol) at 2:3 (v/v), incubated for 20 minutes (at dark, RT). The absorbance was measured at 517 nm. The concentration required to inhibit 50% of radical (IC₅₀) by plotting the % inhibition of radical against extract concentrations (Chaity et al., 2016).

H₂O₂ free radical scavenging assay: 3.4 ml of extracts (500-0.977 µg/ml) was taken to 0.6 ml of 40 mM H₂O₂ separately, incubated for 10 min at RT and absorbance was taken at 230 nm to calculate IC₅₀ (Ahmed and Rahman, 2016).

Phenolic content determination: It was estimated by using Folin-Ciocalteu reagent. Total phenolics were stated as mg of gallic acid equivalent (GAE) / g of the extract (Ahmed and Rahman, 2016).

Determination of membrane stabilizing activity: It was evaluated by utilizing hypotonic solution- and heat- induced RBC hemolysis (Shinde et al., 1999). In case of hypotonic solution- induced hemolysis, 0.5 ml of RBC suspension, 5 ml of 50 mM NaCl as hypotonic solution and plant sample (1 mg/ml) or acetylsalicylic acid (standard) (0.1 mg/ml) were put together and kept at RT (10 min), centrifuged (3000 g for 10 min) and the optical density (OD) of the supernatants were estimated at 540 nm. % inhibition of RBC hemolysis was calculated as $\{(OD_{\text{control}} - OD_{\text{test}}) / OD_{\text{control}}\} \times 100$. In case of heat- induced RBC hemolysis, 5 ml of isotonic buffer, extractive (1 mg/ml) or acetylsalicylic acid (0.1 mg/ml) and RBC suspension (30 µl) was put into a tube. One group of the tubes along with control samples were kept at 54 °C (20 min), while another group of samples were kept at 0-5 °C. It was then centrifuged (1300g, 3 min)

and OD of the supernatants were estimated at 540 nm. % Inhibition of RBC hemolysis was $\{1 - (\text{OD}_{\text{heated test}} - \text{OD}_{\text{unheated test}}) / (\text{OD}_{\text{heated control}} - \text{OD}_{\text{heated test}})\} \times 100$.

Determination of thrombolytic activity: The required clot was produced from human venous blood. Extractive (1 mg/100 μl water) was included to clot (37°C, 90 minutes). Streptokinase (100 μl , equivalent to 30,000 IU) (Beacon pharmaceuticals Limited, Bangladesh) was taken as positive control (Prasad *et al.*, 2006).

Statistical analysis: Values are presented as mean \pm standard deviation (n=3).

Results and Discussion

Free radicals are perilous elements to cause biological havoc by damaging macromolecules. It can initiate the cascade of biochemical reactions to cause cancer, inflammation, oxidative stress and so on. Many natural products have been proved to

contain potent radical scavengers (Nimse and Pal, 2015). Free radical scavenging assay might be a useful tool to identify the plant extracts having potent radical scavenging power (Nigri *et al.*, 2004; Schetter *et al.*, 2010). In the present study, the free radicals were generated from DPPH and H_2O_2 to screen radical inhibitory potential of the plant materials. In the DPPH assay (Fig. 1A), different extractives of *Q. indica* also showed noteworthy free radical scavenging property. The methanol crude extract showed the maximum scavenging activity having IC_{50} = of 9.73 $\mu\text{g}/\text{ml}$. Among the fractions the aqueous fraction showed noticeable radical scavenging (IC_{50} = 11.73 $\mu\text{g}/\text{ml}$). The radical scavenging ability can be ordered as MCE > AQ > CF > CTC > PE. In the H_2O_2 assay (Fig. 1B), the methanol crude extract also showed highest free radical scavenging activity (IC_{50} = 201.85 $\mu\text{g}/\text{ml}$). The order of antioxidant activity displayed here can be presented as MCE > CF > AQ > CTC > PE.

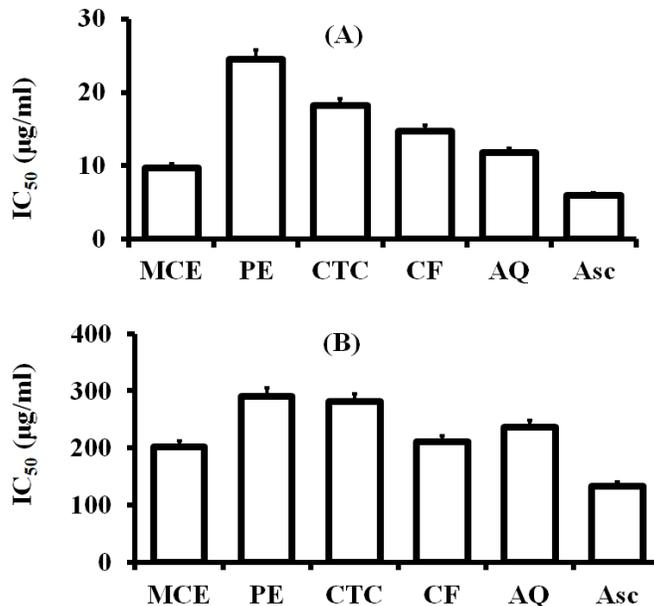


Figure 1. IC_{50} values of the extracts of *Q. indica* in (A) DPPH free radical scavenging and (B) H_2O_2 free radical scavenging assays. Asc, Ascorbic acid (standard).

Phenolic compounds are known as potent antioxidants. They are also known as free radical scavengers. The level of phenolic contents might act

as an important clue to predict the radical scavenging potential of any extract (Huyut *et al.*, 2017). In the current study, the upmost level of phenolics was

found in the MCE. The order of the level of phenolics in the extracts can be assigned as MCE > AQ > CF > CTC > PE (Fig. 2). The potent radical scavenging ability of MCE, AQ and CF might be linked with level of total phenolic contents to some extent (Baharfar *et al.*, 2015).

Many anti-inflammatory agents (extracts and compounds) have been marked from nature (Aswad *et al.*, 2018; Maroon *et al.*, 2010). Erythrocyte membrane stabilization assay is a tool to recognize the primary anti-inflammatory effect of plant extractives based on the prevention of hemolysis

mimicking the protection of the lysozyme membrane. If lysozyme is released, it might stimulate the inflammatory mediators like prostaglandins, free radicals, etc. (Chou, 1997). In the hypotonic solution induced assay, the highest level of inhibition of hemolysis was displayed by aqueous fraction (71.50 %). The order of the membrane protection potential can be ordered as AQ > MCE > CF > PE > CTC. In the heat induced hemolysis assay, the aqueous fraction also showed the highest level inhibition of hemolysis (39.46 %). The order of inhibition of hemolysis is AQ > CF > MCE > PE > CTC.

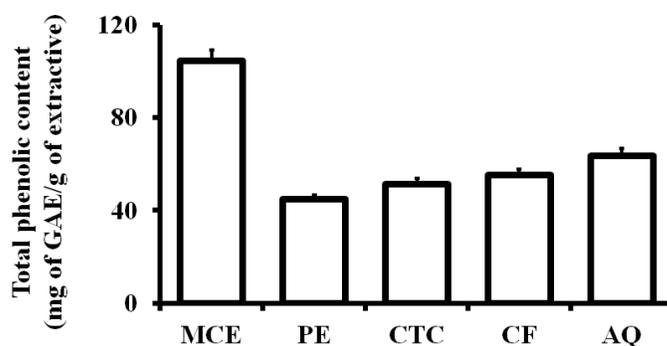


Figure 2. Total phenolic contents of different extractives of *Q. indica*.

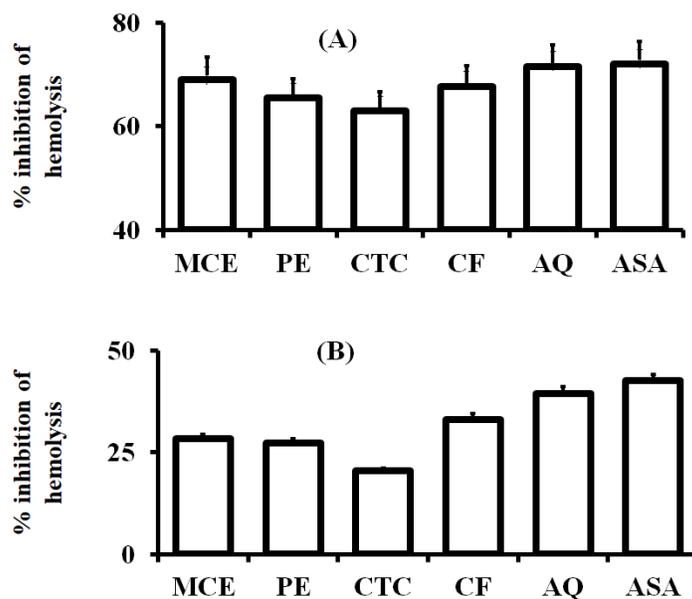


Figure 3. % inhibition of hemolysis of RBC by different extractives of *Q. indica* in hypotonic solution- (A) and heat- (B) induced conditions. ASA, Acetylsalicylic acid (standard).

Clot lytic potential of plants can be utilized for patients with thrombosis (Furie and Furie, 2008). Here, different extractives of *Q. indica* showed very weak clot lysis activity in compare to streptokinase

(standard). Among different fractions, the carbon tetrachloride soluble fraction showed the highest clot lysis activity of 25.92%.

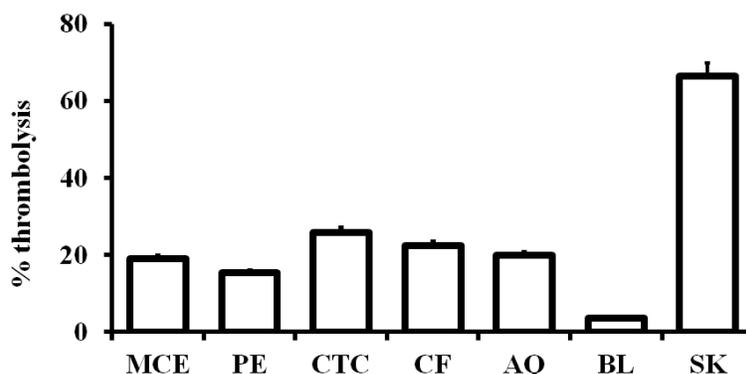


Figure 4. % clot lysis of different extractives of *Q. indica*. BK, Blank (water); SK, Streptokinase.

Conclusion

The present study comprehensively investigated the leaves of *Q. indica*. The methanol extract of the leaves and its different organic fractions exhibited noticeable free radical scavenging power as well as membrane stabilizing capacity. However, their thrombolytic actions were very weak. Further detail studies are required to identify the bioactive molecules.

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