

# Total Phenolic Content, Membrane Stabilization and Thrombolytic Activities of *Corypha taliera* (Arecaceae) Flowers

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*Corypha taliera* Roxb. is a monocarpic plant species belonging to the Arecaceae (palm) family. The palm species is considered to be rare and critically endangered in nature.<sup>1</sup> It flowers once in nearly hundred years and the last flowering plant specimen was found in the campus of University of Dhaka.<sup>1-3</sup> Collection and characterization of different parts including flowers, fruits and seeds of the plant have been carried out and sustainable strategies towards its protection have been under taken.<sup>1,4</sup>  $\beta$ -amyrin, betulinic acid and  $\beta$ -sitosterol, have been isolated from the *n*-hexane soluble extract of flowers of *C. taliera*.<sup>5</sup> The unripe fruits of the plant have been reported to contain  $\beta$ -sitosterol, stigmasterol, lupeol,  $\beta$ -amyrin and betulinic acid.<sup>6</sup> GC-MS analysis of the seed pericarp revealed the presence of myristic, oleic, stearic, linoleic, lignoceric and arachidic acids where palmitic acid was predominant.<sup>3</sup>  $\beta$ -sitosterol-3-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranoside, a steroidal glycoside, have been isolated from the seed pericarp.<sup>6</sup> Recently, the palm species has drawn a significant attention of the scientific community for its potential medicinal values. Fruit extractives of the plant have been reported to exert promising antioxidant as well as cytotoxic activities. The fruit extractives also demonstrated mild to moderate antimicrobial effect against Gram-positive and Gram-negative pathogens.<sup>7</sup>

It is evident from our rigorous literature review that, biological investigation of *C. taliera* flower is still lacking. Therefore, as a part of continuation of our investigation on *C. taliera*<sup>2,5,7</sup> the methanol extract of *C. taliera* and its aqueous and organic soluble extractives have been primarily studied for their total phenolic content, membrane stabilization and thrombolytic activities.

Flowers of *C. taliera* have been collected from the plant available in the campus of University of Dhaka, during February-March, 2009. The collected plant flowers were identified by the authentic scientific officer of Bangladesh National Herbarium, where a voucher specimen (accession no: DACB-34180) has been kept for future reference. The collected flowers were subjected to air drying followed by cold extraction using methanol. The concentrated crude methanol extract of flowers of *C. taliera* (MECF) was further fractionated to obtain *n*-hexane (HECF) dichloromethane (DECF) and aqueous (AECF) soluble partitionates by Kupchan method described by Van Wagenen *et al.*<sup>8</sup> Quantification of total phenolic content in the extractives of flowers of *C. taliera* was carried out using Folin-Ciocalteu reagent according to the method described by Islam *et al.*<sup>9</sup>

Membrane stabilizing activity of the extractives (2 mg/ml) of flowers of *C. taliera* was determined by measuring the inhibition of human erythrocytes hemolysis on heat-and hypotonic-solution as described by Shinde *et al.*<sup>10</sup> Thrombolytic activity the extractives (2 mg/ml) was expressed by human blood clot lysis activity as previously described by Miah *et al.*<sup>11</sup> The experiments were conducted under the consent of the Institutional Ethical

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Review Committee, State University of Bangladesh and human volunteers who have donated bloods. The ethical guidelines described in the Declaration of Helsinki 2013 were followed for the experiments. The experiments were

carried out thrice for each sample. The data are presented as mean with standard error of mean (SEM) of three replicate experiments.

**Table 1. Total phenolic content, membrane stabilizing and thrombolytic activity of different extractives of *C. taliera* flowers.**

Test sample/ Standard drug	TPC in mg (GAE/gm extract)	Membrane stabilization (%)		Thrombolytic activity (% clot lysis)
		Heat-induced	Hypotonic solution-induced	
MECF	193.43 ± 3.27	56.86 ± 1.03	45.67 ± 2.22	31.59 ± 1.69
AECF	158.84 ± 1.72	52.75 ± 1.57	24.10 ± 1.92	27.16 ± 1.27
DECF	143.43 ± 3.27	44.34 ± 0.91	17.45 ± 2.57	23.57 ± 2.17
HECF	93.94 ± 5.64	18.69 ± 2.42	7.44 ± 0.62	18.17 ± 1.29
Acetyl salicylic acid	-	73.50 ± 1.60	61.95 ± 2.99	-
Streptokinase	-	-	-	63.09 ± 1.83

Data are presented as mean ± SEM (n = 3). TPC= total phenolic content, GAE= gallic acid equivalent. MECF, HECF, DECF, AECF represents methanol, *n*-hexane, dichloromethane, and aqueous soluble extract of flowers of *C. taliera*, respectively.

The results of the present investigation demonstrated that methanol extract and its different organic extractives of *C. taliera* possess potent membrane stabilizing and thrombolytic activities as well as substantial amount of phenolics (Table 1). The total phenolic content assay revealed that the crude methanol extract of the plant flowers (MECF) contain noticeable amount of phenolic compounds (193.43 ± 3.27 mg/gm equivalent to gallic acid), compared to its organic soluble partitionates.

The extract of *C. taliera* flowers showed prominent membrane stabilizing activity in human RBC (red blood cell) membrane stabilization assay. The extracts considerably inhibited the lysis of human RBC membrane induced by heat and hypotonic solution, as compared to the positive control acetyl salicylic acid. The MECF revealed highest inhibition of hemolysis of RBC membrane in hypotonic solution and heat-induced conditions by 56.86 ± 1.03 and 45.67 ± 2.22% as compared to other extractives. However, standard acetylsalicylic acid showed remarkable inhibition of the hypotonic and heat-induced RBC membrane lysis by 73.50 ± 1.60 and 61.95 ± 2.99%, respectively (Table 1). Human RBC membrane is analogous to lysosomal membrane and inhibition of both hypotonic solution and heat induced hemolysis of RBCs might be considered as a parameter of the plant's anti-inflammatory potential.<sup>12,13</sup> It has been reported that, tannins, phenolic compounds might act as promising

membrane stabilizer.<sup>14</sup> Therefore, phenolics present in *C. taliera* flowers extractives could be partly responsible for the membrane stabilizing activity.

The crude methanol extract of *C. taliera* flowers and its organic soluble materials were investigated to determine their thrombolytic activity by means of RBCs clot lysis activity. The result exhibited that, different extractives of *C. taliera* flowers showed considerable clot lysis of RBCs, ranging from 18.17 ± 1.29 to 31.59 ± 1.69 %. The highest activity was exhibited by the crude methanol extract of the plant flowers (MECF) compared to its other partitionates. However, the effect was more promising for the standard drug, streptokinase (63.09 ± 1.83%), when compared to the plant extractives (Table 1). Blood clot formation may cause clogging of blood vessels, with the help of deposition of fibrin, platelets and tissue factors and results in severe thromboembolic consequences such as stroke, arthritis, and myocardial ischemia.<sup>15,16</sup> The results suggest that the crude extract of the plant flowers might act prominently against blood clot formation as well thromboembolic disorders.

Finally, it can be deduced from the result of present investigation that the crude methanol extract of flowers of *C. taliera* and its aqueous and organic soluble partitionates possesses significant phenolic content. The methanol extract has considerable membrane stabilizing and thrombolytic potentials compared to its aqueous and organic soluble partitionates. The study primarily revealed

the potential bioactivities of the plant flowers extracts. However, further chemical and biological studies of the flowers extractives are required to investigate the substance(s) responsible for such bioactivities as well as isolation and characterization of the active molecules. Consideration of the outcomes of the present study might be worth for the future investigations.

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