Bangladesh Pharmaceutical Journal **26**(2): 162-166, 2023 (July)

Evaluation of Antioxidant, Cytotoxic, Thrombolytic and Membrane Stabilizing Activities of *Canna indica* L. Leaves (Family: Cannaceae)

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(Received: April 7, 2023; Accepted: June 15, 2023; Published (web): July 25, 2023)

Abstract

Canna indica L., a perennial plant known as Indian shot and African arrowroot, is a member of the family Cannaceae. Historically, this plant was used to treat menstrual cramps, diarrhea and dysentery. The purpose of this investigation was to assess the antioxidant, cytotoxic, thrombolytic and membrane stabilizing activities of the methanol extract of *C. indica* leaf and its organic soluble fractions. The DPPH free radical scavenging method and the total phenolic content determination were utilized to evaluate the antioxidant capacity, whereas the brine shrimp lethality bioassay was implemented to study the cytotoxic potential. The ability of the extractives to prevent the hemolysis of red blood cells (RBC) in response to the application of heat and hypotonic solutions was used to estimate of their membrane stabilizing potential. In all of these experiments, the methanolic leaf extract (ME) displayed moderate activity, while the petroleum ether soluble fraction (PSF) and the dichloromethane soluble fraction (DSF) demonstrated the most promising antioxidant and cytotoxic activities among the extractives. However, the strongest thrombolytic and membrane stabilizing activities were observed by the chloroform soluble fraction (CSF). Additional research may be necessary to determine which phytoconstituents are most likely to be responsible for the aforementioned qualities.

Key words: *Canna indica*, antioxidant, cytotoxic, thrombolytic, membrane stabilizing.

Introduction

Cannaceae, also known as the canna family, is a monotypic family consisting of a single genus, *Canna* and about 60 species. Canna's native habitat is tropical and subtropical America. One species *Canna bidentata* is tropical African. *Canna indica* is a popular garden plant that is nearly universally cultivated (Christenhusz and Byng, 2016). The vegetation is dispersed from the southeastern region of North America to South America. Numerous cultivars have been developed in order to cultivate these plants as ornamentals due to their appealing blossoms and foliage (Khoshoo and Guha, 1976). Common names for this plant include Indian shoot, African arrowroot, purple arrowroot and Sierra Leone arrowroot (Al-Snafi, 2015). The plants are tropical perennials with rhizomes (underground stalks) and erect, three-meter-tall (10-foot-tall) stems. The foliage, which may be towering or diminutive, features spirally arranged, green or bronze leaves (Nagati *et al.,* 2014).

This plant has traditionally been reported as a treatment for menstrual cramps. Both gonorrhea and amenorrhea can be treated with the root. For the

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DOI: https://doi.org/10.3329/bpj.v26i2.67806

treatment of diarrhea and dysentery, the root is ground into a powder and consumed in Nigeria. Additionally, the blossoms are used as a malaria treatment (Ghani, 1998; Sultana *et al.,* 2022). The phytochemical analysis of *C. indica* revealed that it contained numerous phytochemicals, such as alkaloids, cardiac glycosides, anthocyanin pigments, flavonoids, steroids, terpenoids, tannins, saponins, carbohydrates, proteins, fats and numerous other chemical compounds (Al-Snafi, 2015, Sultana *et al.,* 2022). The complete pharmacological effects of *C. indica* are yet to be established. The present study aimed to assess the *in-vitro* antioxidant, cytotoxic, thrombolytic and membrane stabilizing properties of the methanol extracts of *C. indica* leaf and its fractionates.

Materials and Methods

Gathering of plant parts: Fresh leaves of *C. indica* were sourced from Bangladesh Forest Research Institute (BFRI) Herbarium, Dhaka, Bangladesh, in the month of June, 2022 and a sample was placed as a voucher. A professional at the Bangladesh National Herbarium (BNH) described and identified that plant and it was given an accession number (DACB 66976). The plant parts were thoroughly washed, treated to two weeks of shade drying with adequate ventilation and cleared of any visible dirt and contaminants. Finally, using a large capacity grinder, 1550 g of leaf powder was produced.

Preparation of plant extracts: The pulverized and ground materials were collected in 3-liter clean, amber-colored containers, where they were soaked in enough methanol for 20 days while being sometimes shaken and stirred. The crude methanolic extract was obtained by filtering the entire blend through a clean cotton plug before using a Whatman No. 1 filter paper and submitting it to Buchii rotary evaporator at a low temperature (not above 40°C) and pressure. The concentrated crude extract was carefully placed in storage and kept for further use.

The Kupchan method described by Van Wagenen *et al.* (1993) was applied in order to achieve solvent-solvent partitioning. The resultant methanolic leaf extract (5 g) was dissolved in 10% aqueous methanol and was progressively extracted with petroleum ether, dichloromethane and chloroform. All of the fractions were dried by evaporation before being employed in further investigations.

Chemicals and drugs: Acetylsalicylic acid (Square Pharmaceuticals Limited, Bangladesh), streptokinase (SK) (Beacon Pharmaceutical Limited, Bangladesh), normal saline (Beximco Pharmaceuticals Limited, Bangladesh) and tween-20 (BDH Chemicals, UK) were used in this research work. All the other reagents that were used here were of analytical grade.

Determination of antioxidant activity by total phenolic content method and the DPPH free radical scavenging activity method: By using the Folin-Ciocalteu reagent as an oxidizing agent and gallic acid as the standard for equivalency, the total phenolic content in the plant samples was assessed spectrophotometrically (Islam *et al*., 2022). Additionally, the method described by Brand-Williams *et al.* (1995) regarding 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging experiment was used to assess the antioxidant capacity of the bulk methanolic extract and its various organic soluble fractions.

Cytotoxicity study: The approach described in the published literature (Jaman *et al*., 2023) was used to determine the general toxic characteristics of the plant extractives. This technique, also referred to as the brine shrimp lethality bioassay, is effective for detecting the presence of bioactive chemicals in dimethyl sulfoxide (DMSO) solutions containing plant extracts against *Artemia salina*. The drug vincristine sulphate was used as a positive control. The median lethal concentration (LC_{50}) was determined using the logarithm of the sample concentration.

Thrombolytic activity: The clot lysis method reported in previous studies (Meyer *et al.*, 1982, Alam *et al*., 2020, Emon *et al*., 2021) was implemented to estimate the thrombolytic activity of all the test samples. Streptokinase was used as standard.

Evaluation of membrane stabilizing activity: By preventing the release of inflammatory mediators, any substance that can both stabilize the cellular membrane and the lysosomal membrane can have anti-inflammatory effects. As a result, *in vitro* observations of the membrane stabilizing effects that various substances had on the erythrocyte membrane were made. To anticipate the potential impact of the samples on the lysosomal membrane, the data were extrapolated and compared with the data collected in case of standard acetylsalicylic acid (Shahriar *et al.*, 2023).

Statistical analysis: For every test, triplicates of each sample were employed. Mean (M) along with standard error of the mean (SEM) values were used to express the dataset. Dunnett's test was used to determine whether there was a significant difference between the blank or the negative control group and the standard and test groups, with p values below 0.05 being considered statistically significant.

Results and Discussion

Two tests - the total phenolic content test and the DPPH free radical scavenging activity test were used to assess the antioxidant capacity. Both testes produced promising results (Table 1). Total phenolic content fluctuated with extractives, extending from 11.185 ± 0.362 mg of GAE /gm of extractives to 53.531 ± 0.124 mg of GAE /gm of extractives of *C. indica*. Among all extractives of *C. indica,* the highest phenolic content was found in PSF (53.531 ± 0.124 mg of GAE /gm of extractives) followed by DSF (52.451 ± 0.212 mg of GAE /gm of extractives). The antioxidant activity indicated by the IC_{50} values in the DPPH method were found to be differed in different extractives and ranged from 21.824 ± 0.211 µg/mL to 107.14 ± 0.021 µg/ml. Among all extractives of the plant, the highest free radical scavenging activity was given by the petroleum ether soluble fraction (21.824 ± 0.211 µg/mc) followed by the dichloromethane soluble fraction (36.29 ± 0.112 µg/ml). The PSF and DSF showed the most significant activity among the fractionates. It might be inferred that the PSF and the DSF contained the most potent antioxidant compounds and that more research should be directed towards these fractions.

The results of the different samples from the brine shrimp lethality bioassay (Table 2) showed that the PSF had the most cytotoxic effect, with an LC_{50} value of 1.454 ± 0.114 µg/mL in comparison to the standard reference vincristine sulphate (0.904 ± 0.097 µg/ml). The DSF demonstrated similar cytotoxic activity (1.769 ± 0.321 µg/mL) to the PSF, suggesting that the cytotoxic substance is likely present in the non-polar fractions of the ME.

Table 1. DPPH free radical scavenging activity and total phenolic content level of *C. indica* leaf methanolic extract and their fractionates.

Sample	DPPH based free radical scavenging property (IC_{50} µg/ml)	Total phenolic content (mg of GAE/g of extracts)
ME	107.14 ± 0.021	11.185 ± 0.362
PSF	21.824 ± 0.211	53.531 ± 0.124
DSF	36.29 ± 0.112	52.451 ± 0.212
CSF	93.56 ± 0.362	22.728 ± 0.481
ASF	75.942 ± 0.238	34.272 ± 0.234
STD	17.715 ± 0.024	----

Values are mentioned as Mean ± SEM (n=4). ME=Methanol extract; PSF= Petroleum ether soluble fraction; DSF= Dichloromethane soluble fraction; CSF= Chloroform soluble fraction; ASF= Aqueous soluble fraction, STD= Positive control (butylated hydroxyl toluene)

Table 2. LC_{50} values of the *C. indica* leaf methanolic extract and their fractionates in brine shrimp lethality bioassay.

Sample	LC_{50} (µg/ml)
ME	3.412 ± 0.036
PSF	1.454 ± 0.114
DSF	1.769 ± 0.321
CSF	7.774 ± 0.234
ASF	12.405 ± 0.532
STD	0.904 ± 0.097

Values are mentioned as Mean ± SEM (n=4). ME=Methanol extract; PSF= Petroleum ether soluble fraction; DSF= Dichloromethane soluble fraction; CSF= Chloroform soluble fraction; ASF= Aqueous soluble fraction; STD= Positive control (vincristine sulfate)

The ability of each fraction to lyse blood clots was examined in order to assess the thrombolytic activity of the crude methanolic extract and its fractionates. The samples displayed thrombolytic activity ranging from 7.563 to 21.509% (Table 3). The clot lysis activity of *C. indica* extractives is less than that of the standard streptokinase. However, when compared to blank, the standard and the chloroform soluble fraction (CSF) demonstrated statistically significant thrombolytic activity (68.18 % and 21.509%, respectively).

Table 3. Thrombolytic property of the *C. indica* leaf methanolic extract and their fractionates.

Sample	% of clot lysis
Water	7.512 ± 0.352
STD	68.18 ± 0.054*
ME	14.491 ± 0.091
PSF	11.394 ± 0.115
DSF	5.722 ± 0.251
CSF	21.509 ± 0.211*
ASF	7.563 ± 0.157

Values are mentioned as Mean ± SEM (n=4). *$p < 0.05$ compared to water. ME=Methanol extract; PSF= Petroleum ether soluble fraction; DSF= Dichloromethane soluble fraction; CSF= Chloroform soluble fraction; ASF= Aqueous soluble fraction; STD= Positive control (streptokinase)

Table 4. Membrane stabilizing activity of the *C. indica* leaf methanolic extract and their fractionates.

Sample	Percent inhibition of the hemolysis	
	Induced by hypotonic solution	Induced by heat
STD	78.911 ± 0.212	87.34 ±0 .092
ME	40.641 ± 0.324	57.912 ± 0.245
PSF	17.959 ± 0.294	23.314 ± 0.284
DSF	28.258 ± 0.362	15.336 ± 0.315
CSF	51.004 ± 0.896	61.077 ± 0.511
ASF	49.458 ± 0.787	59.235 ± 0.745

Values are mentioned as Mean ± SEM (n=4). ME=Methanol extract; PSF= Petroleum ether soluble fraction; DSF= Dichloromethane soluble fraction; CSF= Chloroform soluble fraction; ASF= Aqueous soluble fraction; STD= Positive control (acetylsalicylic acid)

The evaluation results for membrane stabilising activity (Table 4) were also encouraging. In both hypotonic solution- and heat-induced hymolytic methods, the usual dose of acetylsalicylic acid demonstrated a nearly 80% suppression of hemolysis. The crude leaf extract showed moderate activity (an inhibition of about 50%). The chloroform and aqueous soluble fractions demonstrated around 55% and 53% suppression of hemolysis, respectively.

Conclusion

The methanolic extract of *C. indica* leaf demonstrated potential antioxidant, cytotoxic, thrombolytic and membrane stabilizing properties. It may be necessary to conduct additional research to determine which phytoconstituents are responsible for these activities.

Conflicts of interest

The authors have disclosed no conflicts of interest.

Acknowledgement

The authors would like to express gratitude towards Bangladesh National Herbarium, Dhaka, Bangladesh, for identifying the plants. Additionally, they acknowledge the Department of Pharmacy at the State University of Bangladesh for providing experimental facilities.

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