Bangladesh J. Bot. **54**(3): 433-439, 2025 (September) DOI: https://doi.org/10.3329/bjb.v54i3.84427

ANTIOXIDANT, THROMBOLYTIC, CYTOTOXIC AND MEMBRANE STABILIZING ACTIVITIES AND FATTY ACID COMPOSITION OF *ACMELLA CALVA* (DC.) FLOWER

MD OMAR SHA RAFI*, NUSRAT TABASSUM SHRISTY[1], SAUDA SULTANA MIMI, SADIA AFRIN CHHANDA, MD HASANUR RAHMAN, MOHAMMAD SHARIFUR RAHMAN[2], MOHAMMAD A RASHID[2] AND TANVIR MUSLIM

Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh

Keywords: Acmella calva, Antioxidant, Thrombolytic activity, Cytotoxicity, Membrane, Stabilizing activity

Abstract

In the present study, crude methanolic extract (CRME) of *Acmella calva* flower along with its *n*-hexane (HSF), ethyl acetate (EASF), dichloromethane (DCMSF) and aqueous (AQSF) soluble fractions were subjected to antioxidant, thrombolytic, cytotoxic and membrane stabilizing activities. Fatty acid composition of the *n*-hexane extract was analysed by gas chromatography-flame ionization detector. None of the extracts showed any cytotoxicity against both carcinoma (HeLa) and normal cell lines (Vero and BHK-21). EASF scavenged the DPPH free radical most actively with IC_{50} value of 35.05 µg/ml. CRME and HSF showed profound membrane stabilizing activity by protecting human erythrocyte cell against hypotonic solution-induced and heat-induced lysis. CRME showed the highest (46%) clot lysis. Stearic acid (51.11%) and palmitoleic acid (39.38%) were found in the highest amount in both bound and free form of fatty acid, respectively. The observation from this study indicates the possibility of *A. calva* to be a good source of different bioactive phytochemicals.

Introduction

The importance of medicinal plants is multifaceted. They offer natural, accessible, and sustainable solutions to health problems, serve as sources of modern pharmaceuticals, and are deeply embedded in cultural practices. As modern medicine continues to evolve, medicinal plants remain a vital component of global health care, both as a tool for disease prevention and treatment and as a source of inspiration for new medical breakthroughs (Kayser 2018, Benjamin *et al.* 2024, El-Berawey 2024).

Acmella calva (DC.) R.K. Jansen grows mainly in tropics and subtropical regions of the world. It is a perennial herb, stoloniferous, glabrous to compactly hairy, 20-30 cm in length. This plant is popular for treating some dental and periodontal disorders such as toothaches, stomatitis, infections of throat and gums and psoriasis. It was also reported to possess local anaesthetic, diuretic, insecticidal, hypoglycemic and anti-diabetic activities (Sha Rafi *et al.* 2023). Alkaloids, glycosides, phenolics, flavonoids, steroids, tannins and so on were found in this plant. The pungent odour of its flowers has been found to be due to the presence of Spilanthol, which is an unsaturated alkyl amide present in its highest concentration (Nabi *et al.* 2016).

The flower of the plant has not yet been studied comprehensively. The aim of the present study was to assess the biological potentials of the flower of *A. calva*, i.e. antioxidant, thrombolytic, cytotoxic and membrane stabilizing activities as well as estimation of the fatty acid components of the flower.

*Author for correspondence: <osrafi@du.ac.bd>. [1]Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh. [2]Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka-1000, Bangladesh.

Materials and Methods

The fresh flower sample of *A. calva* was collected from Nandail, Mymensingh, Bangladesh in March 2022 and was taxonomically identified by the Dhaka University Salar Khan Herbarium (Voucher specimen number: DUSH-10813).

The flower samples were subjected to room temperature drying for several days followed by heating below 40°C. Then a grinder (Cyclotec 200 meshes) was used to grind the dried flowers into powder form followed by safe storage in an airtight container. This stored powdered sample was used for the extraction process. About 500 g of the sample powder was submerged in methanol in a flat-bottomed container and left for cold extraction for 06 days with intermittent stirring. The methanolic solution of the sample was then filtered, and evaporated by a rotary vacuum evaporator at below 40°C. The crude MeOH extract was solubilized in MeOH/H_2O and subjected to sequential extractions using *n*-hexane, dichloromethane (CH_2Cl_2) and ethyl acetate (EtOAc). Resultant solutions were evaporated, and used for further analysis.

Cytotoxic effects of different extracts of the sample were tested against HeLa (a human cervical carcinoma cell), Vero (a kidney epithelial cell extracted from African green monkey) and BHK-21 (a baby hamster kidney fibroblast cell) cell lines. The tests were carried out in Centre for Advanced Research in Sciences (CARS), University of Dhaka, Bangladesh. Samples were dissolved in 2.5% aqueous solution of DMSO, filtered and used for the determination of cytotoxicity using a Bio Safety Cabinet (Model: NU-400E, Nuaire, USA), CO_2 Incubator (Nuaire, USA), Trinocular microscope with camera (Optika, Italy), Hemocytometer, etc. Test cell lines were maintained in DMEM (Dulbecco's Modified Eagles' medium) containing 1% penicillin-streptomycin (1 : 1), 0.2% gentamycin and 10% fetal bovine serum (FBS). HeLa Cells (2×10^4 /100 µl), Vero cells (1.5×10^4/100 µl) and BHK-21 cells (1.5×10^4 /100 µl) were seeded onto 96-well plate followed by incubation at 37°C with 5% CO_2. 25 µl of each of the test extract solutions was added after 24 hrs. Inverted light microscope was used to examine the cytotoxicity after 48 hrs of incubation (Moniruzzaman *et al.* 2024).

The total phenolic content of the flower extracts was estimated by conducting an oxidation reaction using Folin-Ciocalteu reagent (Ahmed and Rahman 2016). Using the stable free radical of 1,1 diphenyl-2-picrylhydrazyl (DPPH), the free radical scavenging activity of the extract was measured (Brand-Williams *et al.* 1995).

Hemolysis of human erythrocytes by hypotonic solution- and heat- induced methods were used for membrane stabilization assay. In case of *in vitro* thrombolytic assay, venous blood was collected from healthy human contributors, clot was prepared and treated with extracts (Akter *et al.* 2022). 0.5 ml of venous blood was taken in ten pre-weighed Eppendorf tubes each, and incubated at 37°C for 45 min. After clot formation, the serum was completely removed, and the weight of the tube was again measured; the difference in weight of the tube gave the weight of the clot. 100 µl of the aqueous solution of the extracts was added to each tube. 100 µl of Streptokinase (SK) and distilled water served as positive and negative control, respectively. The tubes were then incubated at 37°C for 90 min and the lysis of clot was observed.

0.52 g of the *n*-hexane extract of *A. calva* flowers was dissolved in 50 ml of hexane and mixed with 5% sodium bicarbonate solution (50 ml). The solution was taken in a separatory funnel, shaken for half an hour and allowed to stand overnight. The resulting mixture was separated in two layers. The lower layer (aqueous) was separated for the analysis of free fatty acid (FFA). The upper layer (organic layer) was taken for analysis of bound fatty acid (BFA). Both the bound and free form of acids were converted to their corresponding methyl esters by acidic esterification using BF_3-MeOH. The methyl esters of both BFA and FFA were analysed by GC-

FID (Shimadzu 9A, column-BP-50, Detector-FID, temperature was programmed as: 170°C for 1 min at a rate of 4°C/min.; raised to 270°C; total program time was 30 min).

Each extract was taken in three replicates (n=3) for total phenolic content, DPPH radical scavenging, and thrombolytic assay; the results are expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's HSD post-hoc statistical test was performed using SPSS statistical software (Version 20, IBM Corporation, USA). Values with $p < 0.05$ were considered significantly different.

Results and Discussion

Dried powdered sample was subjected to cold extraction using methanol, which was fractionated into *n*-hexane (HSF), dichloromethane (DCMSF), ethyl acetate (EASF), and aqueous (AQSF) soluble fractions. The extractive values of different fractions are shown in Fig. 1. Extractive percentage was found highest for the aqueous medium.

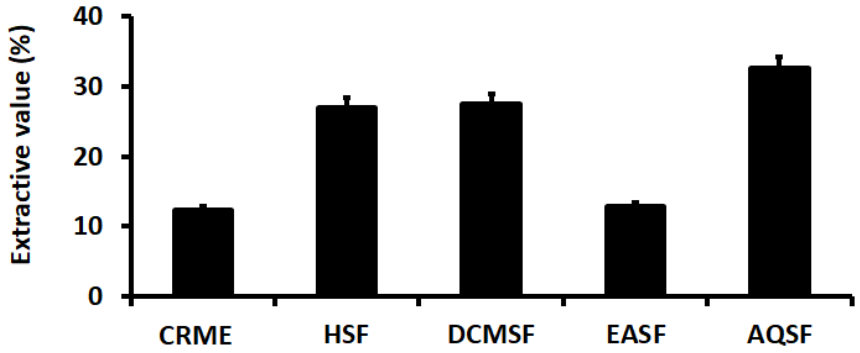

Fig. 1. Extractive value of different solvent extracts of *A. calva* flower. CRME: crude methanol extract, HSF: n-hexane soluble fraction of methanol extract, DCMSF: dichloromethane soluble fraction of methanol extract, EASF: ethyl acetate soluble fraction of methanol extract, AQSF: aqueous fraction.

Cytotoxicity for different extracts of the flowers of *A. calva* was tested against HeLa, BHK-21 and Vero cell lines. The results are mentioned in Table 1. The solvent used in this procedure, dimethyl sulfoxide (DMSO), exhibited a slight cytotoxic effect, killing about 5% of the test cells. The effect of the sample extracts (dissolved in DMSO) was found to be same as that of the solvent alone (95% cell survival) which indicate the non-toxicity of the sample extracts. The sample was not toxic to HeLa cancer cells as well as normal cell lines (BHK-21 and Vero). The non-toxic behaviour of the plant indicated its safety for traditional medicinal uses.

Table 1. Cytotoxicity for different extracts of *A. calva* flower.

Cell lines	Survival Percentages						
	Solvent (-)	Solvent (+)	Methanol fraction	Hexane fraction	Dichloromethane fraction	Ethyl acetate fraction	Aqueous fraction
Hela Vero BHK-21	100%	>95%	>95%	>95%	>95%	>95%	>95%
No cytotoxicity was observed for any of the extracts in all three cell lines							

The amount of total phenolics in the sample extracts are given in Fig. 2(A). Among all extractives of the *A. calva* flower, the highest phenolic contents were found in EASF (38.25±0.59) mg of GAE/g of extractives). Statistical analysis depicted similar variations in activity between CRME and EASF. The sample extracts were tested for their activity in scavenging DPPH free radical and the corresponding inhibitory concentrations of each extract are shown in Fig. 2(B). Ethyl acetate fraction (EASF) showed highest activity in scavenging DPPH radicals with an IC_{50} value of 35.05±0.32 µg/ml. For free radical scavenging assay, ANOVA followed by Tukey's HSD showed no significant similarity in activity among the sample extracts. The antioxidant effect of the samples might be due to phenolic components, such as flavonoids, phenolic acids and phenolic diterpenes. Phenolic compounds can absorb and neutralize the free radicals, thus playing a significant role as antioxidants (Villaño *et al.* 2007, Erkan *et al.* 2008). EASF extract of the *A. calva* flower showed comparable scavenging activity with the standard antioxidant BHT suggesting the presence of promising antioxidant compounds in the sample.

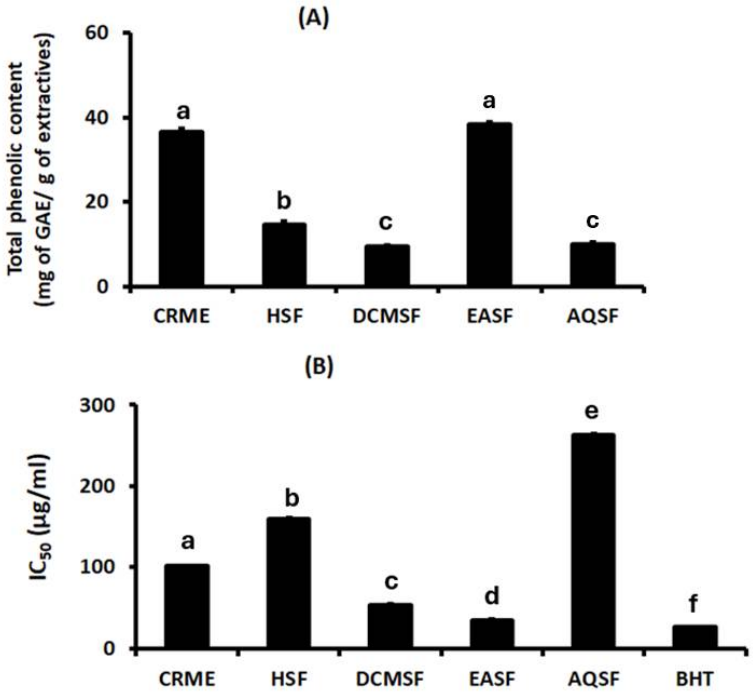

Fig. 2. Total phenolic contents A: and IC_{50} B: of the flower extracts of *A. calva*. BHT: butylated hydroxytoluene. Bars with different letters are significantly different ($p < 0.05$).

The activity of the crude extract of the plant part and its different fractions was tested for human blood clot lysis using Streptokinase as standard. The results are shown in Fig. 3. Cardiovascular diseases are one of the most severe disorders which are increasing at an alarming rate in the recent years. Blood clot or thrombus formation in the arteries is designated as one of the main reasons of these fatal diseases (Davies and Thomas 1984). Methanol extract of the sample (CRME) showed a remarkable potential for clot lysis (46%) compared to the standard Streptokinase (63.05%). Moreover, statistical analysis demonstrated significant variations in thrombolytic activity among the extracts of the sample.

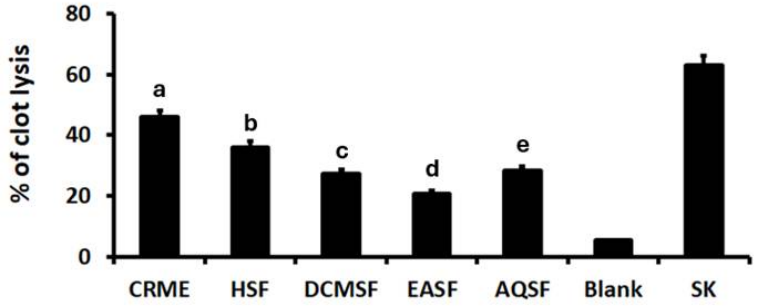

Fig. 3. Thrombolytic activity of the flower extracts of *A. calva*. SK: streptokinase, BL: blank. Bars with different letters are significantly different ($p < 0.05$).

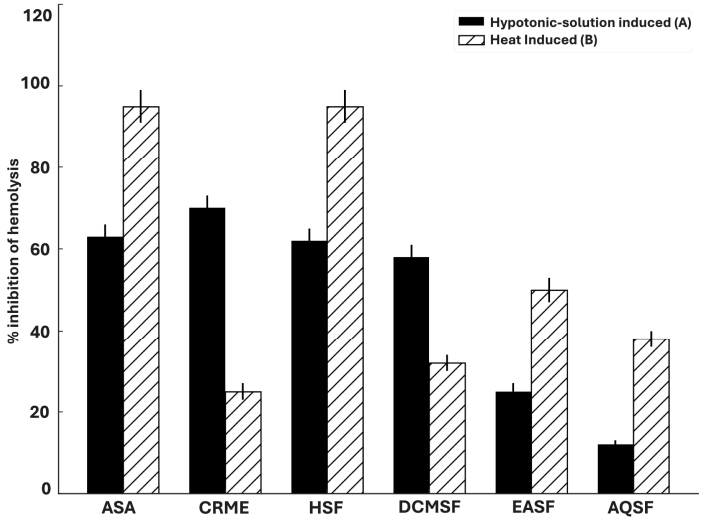

Fig. 4. (A) Hypotonic-solution induced and (B) Heat-induced hemolysis of the flower extracts of *A. calva*.

The sample extracts showed significant potential in stabilizing the human red blood cell membrane in both hypotonic and heat-induced media. The results are summarized in Fig. 4. When the living tissues face any injury, infection or irritation, they produce inflammatory responses. Inflamed tissues let out some lysosomal enzymes that induce variety of pathological disorders such as heart attacks, rheumatoid arthritis etc. Fixing the breakdown of lysosomal membrane can prevent the associated health conditions from inflammatory responses (Shinde *et al.* 1999, Chaity *et al.* 2016). The methanolic extract of the sample (CRME) was found to stabilize erythrocyte membrane prominently in the hypotonic solution induced haemolysis. Also, the *n*-hexane extract showed noticeable stabilization of the membrane against heat induced haemolysis.

The non-polar *n*-hexane extract of the sample was used for the determination of fatty acid composition. Both the bound fatty acid (BFA) and free fatty acid (FFA) were converted to their corresponding methyl esters and analyzed by gas chromatography-flame ionization detector (GC-FID). The resulting chromatogram gave the relative percentages of the acids present in the sample compared to the standard. Amounts of BFA and FFA obtained from n-hexane extract were 0.339

and 0.141g, respectively. A high percentage of fatty acid content obtained from the extract may be due to the incomplete esterification, weighing errors or possible contaminations during extraction. The results are summarized in Table 2. Amount of BFA in the crude methanol extract was higher (2.46%) than the FFA (1.02%), which indicated the trivial action of lipolytic enzymes on triglycerides and lower rancidity of the oil in the sample. The flower extract contains the highest percentage of Stearic acid (51.11%) as BFA. The percentage of palmitoleic acid was also found to be very high, 34.97% in BFA and 39.38% in FFA. The relative percentage of fatty acids exhibits a higher value for C18:0 acids rather than the common C16:0 acid, i.e., palmitic acid, which is the preliminary metabolic product of plant fats. This may be due to a rapid elongation of the acid chain in presence of particular enzymes, any genetic modification in the plant or due to environmental stress situations. The anomaly may also be a result of the rapid co-elution of the short-chain acids with the longer ones due to the selectivity of the analytical instrument. A higher proportion of saturated and unsaturated fatty acids were present in bound and free form, respectively.

Table 2. Relative percentages of bound fatty acid and free fatty acid in *A. calva* flower.

Types of fatty acids	Names	Bound fatty acids	Free fatty acids
Saturated	Caprylic acid	-	16.67
	Lauric acid	2.66	-
	Myristic acid	2.38	1.65
	Stearic acid	51.11	26.94
Unsaturated	Linoleic acid	6.81	8.02
	Palmitoleic acid	34.97	39.38
	Oleic acid	1.18	1.03

values are expressed as relative %.

The bioactivities found in *in-vitro* assays primarily predicted the potential of the flower extracts of *A. calva*. Further extensive studies on *in-vivo* activity and characterization of the phytochemicals of this plant can pave the way to understand the mechanism behind its therapeutic activities.

Acknowledgements

Authors are grateful to Center for Advanced Research in Sciences (CARS), University of Dhaka; Department of Pharmacy, State University of Bangladesh and Bose Centre for Advanced Study and Research in Natural Sciences, University of Dhaka for supporting the research work. The Author also gratefully acknowledges the Ministry of Science and Technology, Govt. of People's Republic of Bangladesh through R&D project for financial support to carry out the work.

References

Ahmed F and Rahman MS 2016. Preliminary assessment of free radical scavenging, thrombolytic and membrane stabilizing capabilities of organic fractions of *Callistemon citrinus* (Curtis.) skeels leaves. BMC Complement. Altern. Med. **16**: 247.

Akter M, Soma M, Rashid M and Rahman M 2022. Comparative assessment of free radical scavenging, membrane stabilizing and thrombolytic potentials of two Amaranthus species of Bangladesh. Bangladesh J. Bot. **51**: 139-145.

Benjamin MAZ, Mohd Mokhtar RA, Iqbal M, Abdullah A, Azizah R, Sulistyorini L, Mahfudh N and Zakaria ZA 2024. Medicinal plants of Southeast Asia with anti-alpha-glucosidase activity as potential source for type-2 diabetes mellitus treatment. J. Ethnopharmacol. **330**: 118239.

Brand-Williams W, Cuvelier ME and Berset CL 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Tech. **28**(1): 25-30.

Chaity FR, Khatun M and Rahman MS 2016. *In vitro* membrane stabilizing, thrombolytic and antioxidant potentials of *Drynaria quercifolia* L., a remedial plant of the Garo tribal people of Bangladesh. BMC Complement. Altern. Med. **16**: 184.

Davies MJ and Thomas A 1984. Thrombosis and acute coronary-artery lesions in sudden cardiac ischemic death. N. Engl. J. Med. **310**: 1137-1140.

El-Berawey DY 2024. Phytochemical and phylogenetic perspective of medicinal plants used for cancer treatment in Egypt. Nat. Prod. Res. **39**(10): 2944-2962

Erkan N, Ayranci G and Ayranci E 2008. Antioxidant activities of rosemary (*Rosmarinus Officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chem. **110**: 76-82.

Kayser O 2018. Ethnobotany and medicinal plant biotechnology: From tradition to modern aspects of drug development. Planta Med. **84**: 834-838.

Moniruzzaman M, Chowdhury AS, Ashrafi S, Hasan CM, Uddin MA, Rahman MS and Rashid MA 2024. Isomeric phenylpropanoid from *Stereospermum suaveolens* (Roxb.) DC growing in Bangladesh. Indian J. Chem. **63**: 928-930.

Nabi N, Wani T, Shrivastava M, Rashid A and Shah S 2016. *Spilanthes acmella* an endangered medicinal plant-its traditional, phytochemical and therapeutic properties-an overview. Int. J Adv. Res. **4**: 627-639.

Sha Rafi MO, Shristy NT, Chowdhury V, Rahman MH, Mimi SS, Afroz R and Chowdhury TA 2023. *Acmella calva* (DC.) R.K. Jansen flower: A comprehensive in vivo study of its central and peripheral analgesic, hypoglycemic, antidepressant, and anti-diarrheal properties. Dhaka Univ. J. Pharm. Sci. **22**: 65-71.

Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ and Saraf MN 1999. Membrane stabilizing activity-a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia **70**: 251-257.

Villaño D, Fernández-Pachón MS, Moyá ML, Troncoso AM and García-Parrilla MC 2007. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta **71**: 230-235.

(Manuscript received on 30 November, 2024; revised on 17 September, 2025)