Bangladesh Pharmaceutical Journal ***26****(1): 67-72, 2023 (January)*

Comparison of Antimicrobial and Membrane Stabilizing Properties of Leaf and Stem Extracts of *Derris scandens* (Roxb.) Benth. (Family: Fabaceae)

Saimon Shahriar[1], Asif Uj Jaman[1], Samir Saha[1], Md. Sabbarul Islam[1], Md. Rafiqul Islam[1], Biprojit Saha[1], Md. Al Amin Sikder[2] and Mohammad A. Rashid[2]

[1]Department of Pharmacy, School of Pharmacy and Health Sciences, State University of Bangladesh
77 Satmasjid Road, Dhanmandi, Dhaka-1205, Bangladesh
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka
Dhaka-1000, Bangladesh

(Received: July 10, 2022; Accepted: November 28, 2022; Published (web): January 31, 2023)

Abstract

Derris scandens (Roxb.) Benth. (Family: Fabaceae), a recognized medicinal plant colloquially known as 'Kalilata' in Bangladesh, has been traditionally used as analgesic in Thailand. This study was intended to evaluate and compare the antimicrobial and membrane stabilizing potentials among the methanol extracts of *D. scandens* leaf and stem, and their fractionates. The stem and leaves of the plant were extracted by methanol separately and fractioned into aqueous, dicholoromethane (DCM), ethyl acetate and *n*-hexane soluble fractions. They were tested for antimicrobial activity by using the agar-well diffusion method where ciprofloxacin and fluconazole were used as references. The highest antimicrobial activity was observed against a Gram-negative bacteria (*Escherichia coli*) by the DCM soluble fraction of the leaf and stem extracts with 20 mm and 15 mm zones of inhibition, respectively. The *n*-hexane soluble fraction of methanol extract of stem revealed weak to moderate antimicrobial activity against Gram-positive bacteria. The ethyl acetate soluble fractionates of methanol extracts of leaf and stem showed similar antifungal property against *Saccharomyces cerevisiae* with a zone of inhibition of 12 mm. 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 *n*-hexane and DCM soluble fractions of both extracts showed around 75% inhibition of hypotonic solution- and heat-induced hemolysis. All in all, the extracts and several fractionates showed moderate antimicrobial and membrane stabilizing properties and further investigations are warranted to find out active phytoconstituents responsible for these properties.

Key words: *Derris scandens*, antimicrobial, antifungal, membrane stabilizing.

Introduction

The Fabaceae family (also known as the Leguminosae family) consists of almost twenty thousand species in 772 accepted genera, which makes it the third-largest land plant family. These plants are of cosmopolitan distribution- widely represented in Africa, South America, North America and Australia (Ahmad *et al.*, 2016; Magallon and Sanderson, 2001; Pennington *et al.*, 2004).

Derris is a genus under the family Fabaceae. It is composed of 63 accepted species. One of the species, *D. scandens* is native to Eastern Australia and Tropical and Subtropical Asia. It is mostly found in Bangladesh, India (mainly in Assam), Myanmar, Sri Lanka, Nepal, Thailand and the Southwest Pacific Islands (Hussain *et al.*, 2015; Ito *et al.*, 2020; Rani *et al.*, 2013). Inside Bangladesh, Teknaf of Cox's Bazar district and Nijhum Dweep are its native range.

Corresponding author: Mohammad A. Rashid; Email: r.pchem@yahoo.com
DOI: https://doi.org/10.3329/bpj.v26i1.64220

This plant is locally known as Kalilata in this country (Bhuiyan *et al.*, 2019).

Previous studies have isolated a plethora of compounds from *D. scandens.* Isolated and characterized chemical constituents include coumarins, phenyl coumarins, triterpenes, isoflavones, flavones, isoflavone glycosides, and steroids (Bhuiyan *et al.*, 2019; Madhiri and Panda, 2018). Traditionally, *D. scandens* plant extract has mostly been used for its analgesic property. Since long ago, this plant has been used in the treatment of osteoarthritis, joint diseases, musculoskeletal diseases, rheumatic diseases and muscle tension (Ayameang *et al.*, 2020; Hematulin *et al.*, 2014; Ito *et al.*, 2020; Punjanon, 2018; Sookaromdee and Wiwanitkit, 2019). *D. scandens* plant extract was reported to possess for its antitussive, expectorant, anti-dysentery, antihypertensive, antibacterial, anti-HIV, immunomodulatory, abortifacient, antimicrobial and diuretic properties. It was also utilized in the treatment of cachexia, diarrhea, reptile-associated poisoning and stomach infections (Bhuiyan *et al.*, 2019; Hussain *et al.*, 2015; Jansakul *et al.*, 1997; Sittiwet and Puangpronpitag, 2009). This plant was also used by indigenous people of different regions as an insecticide and a fish poison. Its root was found to contain rotenone and lonchocarpic acid, which are potent insect repellents (Bhuiyan *et al.*, 2019; Hussain *et al.*, 2015). The present study aimed to compare the antimicrobial and membrane stabilizing properties of the methanol extracts of *D. scandens* leaf and stem, and their fractionates.

Materials and Methods

Collection of plant materials: D. scandens whole plants were collected from Dhaka, Bangladesh and a voucher specimen was deposited. The plant was characterized and identified by a skilled professional at the Bangladesh National Herbarium (BNH) and issued an accession number (DACB 63764). The leaves and stems were cut into smaller pieces separately and roots were separated from the aerial parts of the plants. To remove dirt and other visible impurities, the plant parts were cleaned properly and were subjected to shade drying with sufficient ventilation options for a period of two weeks. Finally, 900 g stem powder and 750 g leaf powder were obtained after grinding by using a high capacity grinder.

Preparation of plant extracts: Both samples of the ground and powdered material were taken in clean, amber-colored bottles (3 liters) and soaked in a sufficient amount of methanol for 30 days, with occasional shaking and stirring. In both cases, the crude methanolic extract was collected by filtering the whole mixture through a fresh cotton plug and finally with a Whatman No.1 filter paper and subjected to Buchii Rotavapour at low temperature (not more than 40°C) and pressure. The concentrated crude extract was weighed accurately.

Table 1. Weight of fractions obtained from methanolic extracts.

Extract	Sample code	Fractionate	Amount (g)
Leaf of *D. scandens* (ME-L)	AF-L	aqueous soluble fraction	2.74
	DF-L	dichloromethane soluble fraction	2.26
	EF-L	ethyl acetate soluble fraction	2.89
	HF-L	*n*-hexane soluble fraction	1.67
Stem of *D. scandens* (ME-S)	AF-S	aqueous soluble fraction	3.62
	DF-S	dichloromethane soluble fraction	3.13
	EF-S	ethyl acetate soluble fraction	3.28
	HF-S	*n*-hexane soluble fraction	1.89

Solvent-solvent partitioning was conducted using the process developed by S. Morris Kupchan (1970) and improved by Van Wagenen *et al.* (1993). 5 g of the crude methanol extracts of stem (ME-S) and leaf (ME-L) were dissolved in 10% aqueous methanol separately. Both were extracted sequentially with *n*-

hexane, dichloromethane, ethyl acetate and finally water to obtain four respective fractions. All the fractions were evaporated to dryness and were used for further analysis. Their code name and weights are displayed in Table 1.

Sample preparation and evaluation of antimicrobial activity: The agar-well diffusion method was used for the antimicrobial activity testing (Alam *et al*., 2020; Rios *et al.*, 1988). Methanol was also used as a negative control, and ciprofloxacin (30 µg/disc) and fluconazole (30 µg/disc) were used as reference standards in antibacterial and antifungal tests, respectively. Microbial growth was determined by measuring the diameter of zone of inhibition after 24-48 hours in millimeters (mm). A scale was used to quantify the diameter of the clear zones (if greater than 5 mm) around each well.

Test microorganisms: The microbes used for the antimicrobial testing are listed in Table 2. They were obtained from Department of Pharmacy, School, of Pharmacy and Health Sciences, State University of Bangladesh, Dhanmondi, Dhaka, Bangladesh.

Table 2. List of bacteria and fungi used in antimicrobial screening.

Gram-positive bacteria	Gram-negative bacteria	Fungi
Bacillus cereus	*Escherichia coli*	*Aspergillus niger*
Bacillus megaterium	*Pseudomonas aeruginosa*	*Candida albicans*
Bacillus subtilis	*Pseudomonas aureus*	*Saccharomyces cerevisiae*
Staphylococcus aureus	*Salmonella paratyphi*	
Sarcina lutea	*Salmonella typhi*	
	Shigella boydii	
	Shigella dysenteriae	
	Vibrio mimicus	

Evaluation of membrane stabilizing activity: Any agent that can stabilize the cell membrane as well as the lysosomal membrane may exert anti-inflammatory properties by inhibiting the release of inflammatory mediators. Thus, the membrane stabilizing properties of different samples exerted on the erythrocyte membrane were observed *in vitro*. The data was extrapolated and compared with the data obtained for standard acetyl salicylic acid to predict the possible effect of the samples on the lysosomal membrane (Shinde *et al.*, 1999).

Hypotonic solution-induced hemolysis: The test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) with 4.5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffer saline (pH 7.4) containing either the different methanolic extract (2.0 mg/mL) or acetylsalicylic acid or ASA (2 mg/mL) as a reference standard. The mixtures were incubated for 10 minutes at room temperature, centrifuged for 10 min at 3000 rpm and the absorbance (OD) of the supernatant was measured at 540 nm.

$$\% \text{ inhibition of hemolysis} = (OD_1 - OD_2 / OD_1) \times 100$$

Where, OD_1 = Optical density of hypotonic-buffered saline solution alone (control) and

OD_2 = Optical density of test sample in a hypotonic solution

Heat-induced hemolysis: 5 ml of the isotonic buffer containing 1.0 mg/mL of different samples and erythrocyte suspension (30 µL) were put into two duplicate sets of centrifuge tubes followed by gentle mixing by inversion (Shinde *et al.*, 1999). One pair of the tubes was incubated at 54^{o}C for 20 min in a water bath. The other pair was maintained at 0-5^{o}C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 rpm and the absorbance of the supernatant was measured at 540 nm.

$$\% \text{ Inhibition of hemolysis} = 1 - (OD_2 - OD_1 / OD_3 - OD_1) \times 100$$

Here, OD_1= test sample unheated, OD_2= test sample heated, OD_3= control sample heated

Statistical analysis: Three replicates of each sample were used for each assay to facilitate statistical analysis and the values are reported as mean ± SD.

Results and Discussion

Bacterial resistance to antimicrobials has become a major issue in the field of clinical and public health which urges to discover new alternatives to solve the problems. In the current experiment, we have tried to compare the antimicrobial and membrane stabilizing potential among the methanol extracts of *D. scandens* leaf and stem, and their fractionates as scientific investigation of various plants still remains as an extensive area of research to find phytochemicals with such actions (Vineet *et al.*, 2015).

The results of the antibacterial activity of the methanolic extracts (*D. scandens* leaf and stem) and their fractionates demonstrated this plant to be more effective against Gram-negative bacteria than that of Gram-positive bacteria (Table 3). The dichloromethane soluble fraction of the extract of both leaf and stem showed the highest antimicrobial action against Gram-negative *E. coli*. Aqueous soluble fraction of methanol extract of stem provided no antibacterial action. The *n*-hexane soluble fraction of methanol extract of stem revealed broad spectrum antibiotic activity against Gram-positive bacteria compared to the *n*-hexane soluble fraction of methanol extract of leaf. In case of fungi, the ethyl acetate soluble fraction of methanol extract of leaf and stem showed similar results against *S. cerevisiae* with a zone of inhibition of 12 mm. The leaf extracts and fractionates showed antibacterial activity against *S. boydii*, whereas the stem extracts and fractionates could not. On the other hand, the stem extracts demonstrated antimicrobial activity against *P. aureus* and *C. albicans*, whereas the leaf extracts and fractionates did not show any activity against these microbes.

Table 3. Antimicrobial activity of *D. scandens* leaf and stem methanolic extracts and their fractionates.

Test bacteria	Zone of inhibition (mm)										
	ME-L	HF-L	DF-L	EF-L	AF-L	ME-S	HF-S	DF-S	EF-S	AF-S	STD
Gram-positive bacteria											Ciprofloxacin
Bacillus cereus	7±0.68	-	8±0.91	-	-	6±0.74	-	8±0.69	-	-	36±0.27
Bacillus megaterium	-	-	-	-	-	-	-	-	-	-	25
Bacillus subtilis	8±1.08	-	8±0.72	-	-	8±0.14	9±2.07	-	-	-	25±1.03
Sarcina lutea	8±0.23	8±0.62	8±1.37	-	-	8±0.43	10±0.81	8±0.2.32	-	-	30±0.17
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	22±0.18
Gram-negative bacteria											Ciprofloxacin
Escherichia coli	7±1.34	8±0.92	20±0.14	-	7±0.85	8±1.23	9±0.77	15±0.33	-	-	30±1.07
Pseudomonas aeruginosa	8±0.32	8±0.51	-	-	-	7±0.28	9±1.02	-	-	-	30±0.52
Pseudomonas aureus	-	-	-	-	-	9±1.07	-	8±0.74	-	-	31±0.45
Salmonella paratyphi	8±0.71	-	8±0.62	-	-	7±1.37	-	8±0.77	-	-	25±0.56
Salmonella typhi	6±0.32	9±0.27	8±0.16	-	-	8±0.06	10±0.29	9±0.54	-	-	28±0.39
Shigella boydii	8±1.71	-	8±1.28	-	-	-	-	-	-	-	28±2.34
Shigella dysenteriae	9±0.49	8±0.39	-	13±0.21	-	8±0.55	10±0.84	8±0.64	12±0.17	-	33±0.52
Vibrio mimicus	8±0.84	8±0.45	-	-	-	8±0.27	9±0.31	-	-	-	35±1.04
Fungi											Fluconazole
Aspergillus niger	8±0.17	-	10±0.13	-	-	8±0.12	-	10±0.24	-	-	29±0.19
Candida albicans	-	-	-	-	-	8±0.96	8±0.87	8±1.02	-	-	28±0.51
Saccharomyces cerevisiae	7±0.32	-	-	12±0.15	-	7±0.24		-	12±0.13	-	29±0.35

- = no inhibition zone observed; STD = reference standard

Table 4. Membrane stabilizing activity of *D. scandens* leaf and stem methanolic extracts and their fractionates.

Sample	Hemolysis inhibition (%)	
	Hypotonic solution induced	Heat induced
Acetylsalicylic acid	99.91±0.21	97.84±0.09
ME-L	74.12±0.32	70.14±0.24
HF-L	77.41±0.29	72.11±0.28
DF-L	76.87±0.36	71.63±0.31
EF-L	24.69±0.89	19.47±0.51
AF-L	9.58±0.78	9.33±0.74
ME-S	70.14±0.30	67.48±0.36
HF-S	73.11±0.27	69.87±0.42
DF-S	71.87±0.33	68.44±0.29
EF-S	18.32±0.64	15.62±0.68
AF-S	8.12±0.81	7.41±0.93

The membrane stabilizing evaluation results (Table 4) were promising. Acetylsalicylic acid, as standard, showed almost 100% inhibition of hemolysis in both cases. The leaf and stem extracts also displayed close activity (around 70% inhibition). The fractions showed even better results as the *n*-hexane and dichloromethane soluble fractions of both extracts showed around 75% inhibition of hemolysis. It was clear that *D. scandens* leaf and stem extracts might possess anti-inflammatory properties and the constituents responsible for such properties were most likely to be present in the *n*-hexane and dichloromethane soluble fractions. The other two fractions did not display promising results.

Conclusion

The methanolic extracts of leaf and stem of *D. scandens* leaf and stem showed moderate membrane stabilizing and weak to moderate antibacterial and antifungal properties. Further investigations might be warranted to find out which phytoconstituents might be responsible for these properties.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgement

The authors would like to express appreciation to Mr. Akhteruzzaman Chowdhury for his assistance in collecting the plant sample. They would also like to express their gratitude towards Bangladesh National Herbarium, Ministry of Environment and Forest, Dhaka, Bangladesh for identifying the plants. They also recognize the supports of Department of Pharmacy, School of Pharmacy and Health Sciences, State University of Bangladesh for providing experimental facilities.

References

Ahmad, F., Anwar, F. and Hira, S. 2016. Review on medicinal importance of Fabaceae family. *Pharmacologyonline* **3**, 151-157.

Alam, S., Emon, N. U., Shahriar, S., Richi, F. T., Haque, M. R., Islam, M. N., Sakib, S. A. and Ganguly, A. 2020. Pharmacological and computer-aided studies provide new insights into *Millettia peguensis* Ali (Fabaceae). *Saudi Pharm. J.* **28**, 1777-1790.

Ayameang, O., Rattarom, R., Mekjaruskul, C. and Caichompoo, W. 2020. Anti-inflammatory activity and quantitative analysis of major compounds of the mixtures of *Derris scandens* (DZSS) formula. *Pharmacogn. J.* **12**, 828-834.

Bhuiyan, M.M.R., Nahar, U.J. and Begum, A. 2019. Evaluation of hypoglycemic effect of extracts from *Derris scandens* and *Thunbergia erecta* leaves in Swiss albino mice. *Res. J. Pharm. Technol*. **12**, 3467-3470.

Fernández-Agulló, A., Pereira, E., Freire, M.S., Valentao, P., Andrade, P.B., González-Álvarez, J. and Pereira, J.A. 2013. Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. *Ind. Crops Prod.* **42**, 126-132.

Hematulin, A., Ingkaninan, K., Limpeanchob, N. and Sagan, D. 2014. Ethanolic extract from *Derris scandens* Benth mediates radiosensitzation via two distinct modes of cell death in human colon cancer HT-29 cells. *Asian Pac. J. Cancer Prev.* **15**, 1871-1877.

Hussain, H., Al-Harrasi, A., Krohn, K., Kouam, S.F., Abbas, G., Shah, A., Raees, M.A., Ullah, R., Aziz, S. and Schulz, B. 2015. Phytochemical investigation and antimicrobial activity of *Derris scandens*. *J. King Saud Univ. Sci.* **27**, 375-378.

Ito, C., Matsui, T., Miyabe, K., Hasan, C.M., Rashid, M.A., Tokuda, H. and Itoigawa, M. 2020. Three isoflavones from *Derris scandens* (Roxb.) Benth and their cancer chemopreventive activity and *in vitro* antiproliferative effects. *Phytochemistry* **175**, 112376.

Jansakul, C., Srichanbarn, A. and Saelee, A. 1997. Some pharmacological studies of a hypotensive fraction from *Derris scandens*. *J. Sci. Soc. Thail.* **23**, 323-334.

Kavak, D.D., Altıok, E., Bayraktar, O. and Ülkü, S. 2010. *Pistacia terebinthus* extract: as a potential antioxidant, antimicrobial and possible β-glucuronidase inhibitor. *J. Mol. Catal. B Enzym.* **64**, 167-171.

Madhiri, R. and Panda, J. 2018. A review on phytochemistry and pharmacological aspects of *Derris scandens* (Roxb.) Benth. *Int. J. Sci.* **5**, 1-4.

Magallon, S. and Sanderson, M.J. 2001. Absolute diversification rates in angiosperm clades. *Evolution* **55**, 1762-1780.

Pennington, P.T., Cronk, Q.C.B., Richardson, J.A., Burnham, R.J. and Johnson, K.R. 2004. South American palaeobotany and the origins of neotropical rainforests. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **359**, 1595-1610.

Punjanon, T. 2018. The inhibitory effect of the *Derris scandens* extract on cytochrome P450 2E1 (CYP2E1)-associated aniline-4-hydroxylase *in vitro*. *Thai J. Toxicol*. **33**, 9-20.

Rani, P.U., Hymavathi, A., Babu, K.S. and Rao, A.S. 2013. Bioactivity evaluation of prenylated isoflavones derived from *Derris scandens* Benth against two stored pest larvae. *J. Biopestic.* **6**, 14-21.

Rios, J.-L., Recio, M.C. and Villar, A. 1988. Screening methods for natural products with antimicrobial activity: a review of the literature. *J. Ethnopharmacol.* **23**, 127-149.

Shinde, U., Phadke, A., Nair, A., Mungantiwar, A., Dikshit, V. and Saraf, M. 1999. Membrane stabilizing activity-a possible mechanism of action for the antiinflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia* **70**, 251-257.

Sittiwet, C. and Puangpronpitag, D. 2009. Antimicrobial properties of *Derris scandens* aqueous extract. *J. Biol. Sci.* **9**, 607-611.

Sookaromdee, P. and Wiwanitkit, V. 2019. Subjective complaint on joint pain symptoms of patients with osteoarthritis after switching therapy from NSAIDs to modern Thai traditional medicine drug, *Derris scandens* (Roxb.) Benth. *Acta Persica Pathophysiol*. **4**, 1-2.

VanWagenen, B.C., Larsen, R., Cardellina, J.H., Randazzo, D., Lidert, Z.C. and Swithenbank, C. 1993. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. *J. Org. Chem.* **58**, 335-337.

Vineet, K.M., Ajit, K.P., Nachimuthu, S.K. and Bhim, P.S. 2015. Antimicrobial and antioxidant activities of *Blumea lanceolaria* (Roxb.). *J. Med. Plants Res.* **9**, 84-90.