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CHEMICAL AND BIOLOGICAL PROFILING OF *DERRIS SCANDENS* (ROXB.) BENTH.

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Abstract

Plants have been a valuable source of bioactive phytochemicals since ancient times, providing mankind with important compounds that can be utilized in the development of innovative therapeutics for various diseases. Chemical investigation of *Derris scandens* (Roxb.) Benth., a plant with a long history of traditional use, led to the identification of two flavonoids such as Scandenone and Lupalbigenin. The ^{1}H NMR spectroscopic analysis along with comparison to similar compounds in the literature, helped to elucidate the structures of these compounds. The crude methanol extract was fractionated, and different fractions were evaluated for their antioxidant, cytotoxic, and thrombolytic activities. Among the fractions, the ethyl acetate and chloroform soluble fractions showed the highest DPPH free radical scavenging activity, while the chloroform soluble fraction demonstrated significant lethality in the brine shrimp lethality bioassay. The petroleum ether soluble fraction exhibited substantial thrombolytic activity. The extraction of these important phytochemicals from the plant has brought new insights into the field of drug discovery, potentially facilitating the development and exploration of novel therapeutics. However, additional investigations are advised to gain a comprehensive understanding of their precise molecular mechanisms and potential toxicological effects.

Introduction

Derris scandens (Roxb.) Benth., locally called Amkurchi or Kali lota, belonging to Fabaceae, is an evergreen climbing shrub with twisted stems that can grow up to 20 meters long emerging from the taproot (Ito *et al.* 2020). It is widely distributed throughout Asia and has a rich history in traditional medicine. In Ayurvedic, Thai, and Chinese remedies, it has been utilized for its therapeutic properties in relieving pain associated with muscle aches, joint pain, arthritis, and headaches (Mohotti *et al.* 2020). Traditionally, the vine portion of the plant was used for various purposes, including as a diuretic, expectorant, anti-tussive, antidysentery agent, and muscle pain reliever. Moreover, it has been advocated as an herb for cardiovascular patients and postmenopausal women, believed to have cancer-preventive qualities and promote overall well-being (Hussain *et al.* 2015, Puttarak *et al.* 2016, Ito *et al.* 2020). In Thailand, *D. scandens* dry powder (0.5-1 g immediately following meal, three times daily) and 50% hydroethanolic extract (400 mg immediately following meal, twice daily) are included in the National List of Essential Medicines as a herbal product for treating musculoskeletal pain (Puttarak *et al.* 2016). It is used to treat inflammation and osteoarthritis (Laupattarakasem *et al.* 2004, Kuljittichanok *et al.* 2018, Ito *et al.* 2020). Notably, the native Sri Lankans use this plant to treat wounds and immobilize fish during traditional fishing practices (Chandraratne 2016). In cholangiocarcinoma and hepatoma cell lines, the plant's ethanol extract has demonstrated anti-metastatic efficacy (Kuljittichanok *et al.* 2018). Due to the anticancer properties exhibited by the extract of *D. scandens* (Roxb.) Benth and its utilization in conventional treatments, there has been a research interest in exploring its phytochemical composition. Previous studies have identified benzyl derivatives, isoflavones, coumarins, and terpenoids as some of the phytochemical constituents present in the plant (Ito *et al.* 2020).

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In the present investigation, two isoflavones were isolated and subjected to structural characterization from the leaves of *D. scandens* (Roxb.) Benth, collected in Bangladesh. Different fractions of the plant's crude extract were subjected to antioxidant, cytotoxicity and anticoagulant assay and exhibited remarkable result.

Materials and Methods

In March 2021, *Derris scandens* stems were collected from the Sundarbans in Bangladesh. They were identified by an expert taxonomist at the Jahangirnagar University Herbarium (JUH) (accession no: JUH 10164).

The collected sample was cleaned, dried, and processed into a 2.2 kg coarse powder for analysis. Powdered *D. scandens* stems (2.2 kg) were soaked in 7.5 liters of ethyl acetate, split into two 5-liter amber containers, and left undisturbed for two weeks with periodic shaking. After filtering, the filtrate was reduced in volume using a Buchi Rotavapor under low pressure and temperature (30°C). The resulting crude dry extract weighed 34.7 g, with a yield of 1.58%.

Vacuum Liquid Chromatography (VLC) was used to separate a fraction of the 34.7 g crude extract, employing hexane, ethyl acetate (EtOAc), and methanol (MeOH) as solvents with increasing polarity. This process resulted in 27 VLC fractions. Each of these VLC fractions was then divided into smaller subfractions using $CHCl_3$ as the eluting solvent on a Sephadex LH-20 column. Five compounds were isolated from the various VLC fractions of the crude ethyl acetate extract of the stem of *D. scandens*. Among them two compounds (Compound 1 and Compound 2) were characterized as flavonoids i.e. Compound 1 as Scandenone and Compound 2 as Lupalbigenin. Structure elucidation of these two compounds was carried out with the help of ^1H NMR spectrum data which were provided by the INARS (Institute of National Analytical Research and Service) of BCSIR (Bangladesh Council of Scientific and Industrial Research), Dhaka, Bangladesh.

The solvent-solvent partitioning protocol, initially proposed by Kupchan and later improved by Van Wagenen and colleagues (VanWagenen *et al.* 1993), was employed to separate the components of a crude extract weighing 5 g. The extract was dissolved in a 10% aqueous methanol solution and then sequentially fractionated using pet-ether, chloroform, and ethyl acetate. Each fraction was evaporated separately using a Rotary evaporator, resulting in the isolation of the following fractions: petroleum ether soluble fraction (DSP, 1.4 g), chloroform soluble fraction (DSC, 1.9 g), ethyl acetate soluble fraction (DSE, 1.6 g), and aqueous soluble fraction (DSA, 0.7 g). These fractions were used for different biological investigations.

The free radical scavenging properties of different fractions of plant extracts were assessed using DPPH. Different concentrations of the extract (ranging from 200 g/ml to 0.78125 g/ml) were mixed with DPPH solution, and antioxidant activity was evaluated by comparing the decolorization with Ascorbic Acid (ASA) as a reference (Ashrafi *et al.* 2022, Ashrafi *et al.* 2022, Singh and Singh 2018, Süzen 2007).

$$\text{Inhibition of free radical DPPH (\%)} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control reaction}}\right) \text{x } 100$$

In the brine shrimp lethality bioassay, the plant extracts and Vincristine Sulfate (VS) were tested at concentrations ranging from 400 g/ml to 0.78125 g/ml. Dimethylsulphoxide (DMSO) was used as the negative control at the same concentration range. Using a micropipette, samples at various concentrations were added to pre-marked vials. Surviving nauplii were counted after 24 hrs (Jasiewicz *et al.* 2021).

$$\text{Mortality}(\%) = \frac{\text{Number of nauplii death}}{\text{Number of nauplii taken}} \text{x}100$$

Venous blood (5 ml each) from healthy individuals was collected in preweighed, sterile Eppendorf tubes, with 0.5 ml of blood in each tube. After incubating at 37 °C for 45 min, the serum was carefully removed, leaving clots behind. The clots were weighed, and 100 µl of extract solutions were added, along with positive and blank controls. After another 90-min incubation at 37 °C, the fluid generated from the clot was removed, and the tubes were weighed again (Sultana *et al.* 2022).

$$\text{Clot lysis}(\%) = \frac{\text{Weight of the clot after lysis}}{\text{Weight of clot before lysis}} \text{x}100$$

Results and Discussion

Two compounds were isolated from the crude methanol extract of the stems of *D. scandens* (Roxb.) Benth. by following repeated chromatographic separations (Fig. 1). The structures of the isolated compounds were elucidated as Scandenone (1) (Rahman *et al.* 2010), and Lupalbigenin (2) (Singhal *et al.* 1980) by analyzing the NMR spectral data and comparing those data with published values.

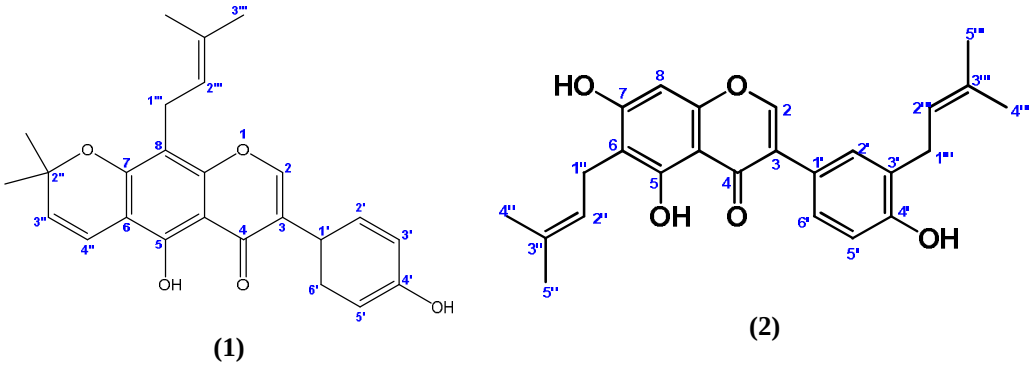

Fig. 1. Structures of isolated phytochemicals from *D. scandens* using NMR techniques:
(1) Scandenone and (2) Lupalbigenin.

Scandenone (1)*:* Yellow mass and soluble in ethyl acetate and chloroform; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (1H s, H-2), 7.35 (2H d, *J* = 8.7 Hz, H-2'/6'), 6.85 (2H d, *J* = 8.7 Hz, H-3'/5'), 5.62 (1H d, *J* =10 Hz, H- 3''), 6.73 (1H d, *J* = 10 Hz, H-4''), 1.46 (6H, s, 2 Me-2"), 3.43 (2H, d, J = 7.0 Hz, H-1‴), 5.10 (1H, t, J = 7.0 Hz, H-2‴), 1.73 (3H, s, H-4‴) and 1.53 (3H, s, H-5‴).

Lupalbigenin (2)*:* Yellow mass and soluble in ethyl acetate and chloroform; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (1H s, H-2), 6.37 (1H s, H-8), 7.24* (H-2'), 6.86 (1H d, *J* = 8.8 Hz, H-5'), 7.24* (H-6'), 13.25 (1H s, 5-OH), 3.39 (2H d, *J* = 7.2 Hz, H-1''), 5.28 (1H t, *J* = 7.2 Hz, H-2''), 1.77 (3H s, H-4''), 1.79 (3H s, H-5''), 3.46 (2H d, *J* = 7.2 Hz, H-1'''), 5.34 (1H t, *J* = 7.2 Hz, H-2'''), 1.77 (3H s, H-4'''), 1.84 (3H s, H-5'''). * = peak overlapped by the residual solvent peak

Compound 1 was obtained in the form of yellow solid. When the compound 1 was applied to a TLC plate and treated with a vanillin in sulfuric acid reagent, it produced a green-colored spot. The TLC plate was then heated for 5 min to enhance the visibility of the spot. The ^{1}H NMR

spectrum (400 MHz, $CDCl_3$) of compound 1 demonstrated H-2 of an isoflavone at δ 7.85 (1H, s, H-2) and AA′ BB′ type signals at δ 7.35 (2H, d, *J* = 8.7 Hz), 6.85 (2H, d, *J* = 8.7 Hz), assignable to H-2′, 6′ and H-3′, 5′, respectively of the B-ring together with typical signals due to 3,3-dimethylallyl group [δ 3.43 (2H, d, *J* = 7.0 Hz, H-1‴), 5.10 (1H, t, *J* = 7.0 Hz, H-2‴), 1.73 (3H, s, H − 4‴) and 1.53 (3H, s, H − 5‴)]. The chromene methyl groups appeared as a singlet integrating for six protons at δ 1.46 (6H, s, H-4″ and H-5″). By analyzing the spectral data and comparing them with the previously published report, compound 1 was identified as Scandenone (Rahman *et al.* 2010).

Compound 2 was obtained as yellow amorphous powder. The proton ^{1}H-NMR (400 MHz, $CDCl_3$) of compound 2 indicated two γ,γ- dimethylallyl groups and an 1,3,4-trisubstituted at δ 7.24*, 6.86 (1H, d, *J* = 8.8 Hz) and 7.24* assigned to H-2', 5', and 6', respectively. The first group was obtained at δ 1.77 and 1.79 (3H each, s), 3.39 (2H d, *J* = 7.2 Hz), 5.28 (1H t, *J* = 7.2 Hz). The remaining group was obtained at δ 1.77 and 1.84 (3H each, s), 3.46 (2H d, *J* = 7.2 Hz), 5.34 (1H t, *J* = 7.2 Hz). Also, the rest of peaks from the ^{1}H-NMR spectrum was in close agreement with those of published data. From the above analysis, compound 2 was characterized as Lupalbigenin (Singhal *et al.* 1980). * = peak overlapped by the residual solvent peak.

Derris scandens extracts displayed dose-dependent free radical scavenging activity compared to the standard ASA. At 500 µg/ml, DSP and DSC showed significant scavenging activity (90.46% and 89.85%, respectively) with IC_{50} values of 122.19 and 119.58 µg/ml, while ASA had an IC_{50} value of 94.76 µg/ml. (Fig. 2). Different extracts of *D. scandens* showed dose-dependent mortality when compared to standard VS in the brine shrimp lethality test. DSC demonstrated strong cytotoxicity with an LC_{50} value of 1.09 µg/ml compared to VS (0.27 µg/ml) (Fig. 3). In the present study, different extract of *D. scandens* exhibited varying degrees of thrombolytic activity ranging from 11.67 to 27.94%. Among them, ethyl acetate fraction (DSP) demonstrated the highest anticoagulant effect (27.94%) compared to the standard Streptokinase (49.35%) (Table 1).

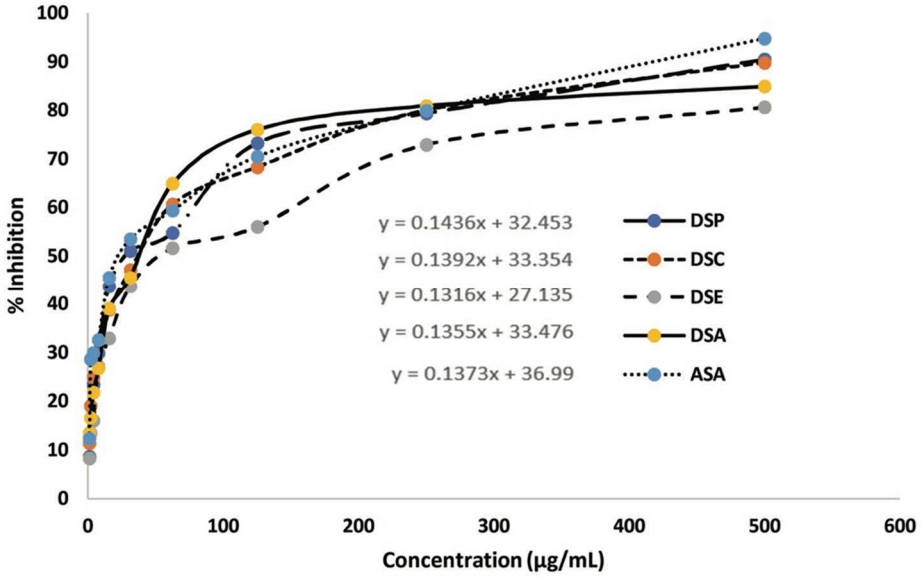

Fig. 2. Linear regression equations (IC_{50}) of Ascorbic Acid (ASA) and different extracts of *Derris scandens.*

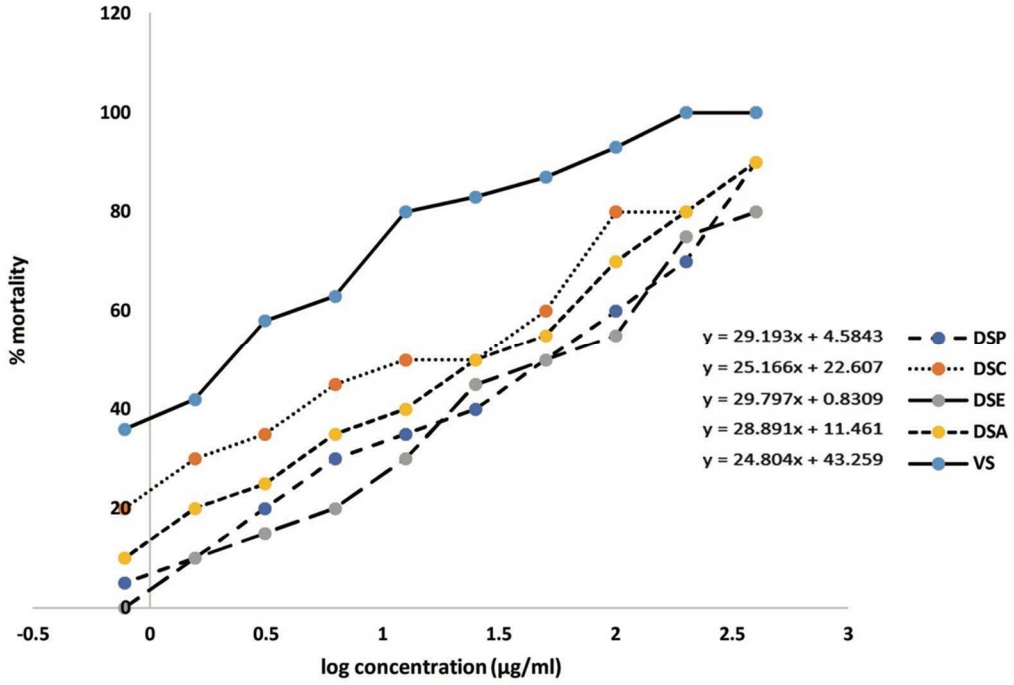

Fig. 3. Linear regression equations (LC_{50}) of Vincristine sulphate (VS) and different extracts of *Derris scandens.*

Table 1. Thrombolytic activity (in terms of % of clot lysis) of the extractives of *Derris scandens.*

Fractions	Weight of empty tube W_1 g	Weight of tube with clot before disruption W_2 g	Weight of tube with clot after disruption W_3 g	Weight of clot before lysis $W_4 = (W_2-W_1)$ g	Weight of lysed clot $W_5 = (W_2-W_3)$ g	% of lysis $(W_5 / W_4) \times 100\%$
DSP	0.9754	1.5388	1.3814	0.5634	0.1574	27.94
DSC	0.9761	1.5779	1.5077	0.6018	0.0702	11.67
DSE	0.9612	1.6822	1.5732	0.7210	0.1090	15.12
DSA	0.9287	1.5571	1.4817	0.6284	0.0754	12.01
Blank	0.9346	1.6826	1.5670	0.7480	0.1156	15.46
SK	0.9798	1.6367	1.3126	0.6569	0.3241	49.35

Traditional medicine, which includes various cultural and socioreligious practices, is vital in global healthcare, with approximately 80% of the World's population relying on traditional herbal remedies according to the World Health Organization (WHO). Worldwide, around 40,000 to 70,000 species of medicinal plants are used in traditional medicine (Anand *et al.* 2022, Ashrafi *et al.* 2022). Plant phenolics, including flavonoids and tannins, are prominent secondary metabolites known for their antioxidant properties and free radical scavenging abilities (Ahmed *et al.* 2014). The presence of flavonoids in *D. scandens* contributes to its ability to scavenge free

radicals. In the DPPH scavenging test, DSP and DSC exhibited remarkable antioxidant activity, with an IC_{50} values of 122.19 and 119.58 µg/ml. In comparison, the standard antioxidant ASA displayed an IC_{50} value of 94.76 µg/ml. The presence of flavonoids in a plant has been identified as a contributing factor to their potential antitumorigenic activity, which can impact various stages of cancer growth and development. This suggests that flavonoids may hold promise in the field of cancer research as potential therapeutic agents (Han *et al.* 2007). The cytotoxic activities observed in DSC (LC_{50} = 1.09 µg/ml) in comparison to Vincristine Sulfate (VS) (LC_{50} = 0.27 µg/ml) could be attributed to the presence of flavonoids in the plant. Further research is necessary to elucidate the specific mechanisms by which these compounds exert their cytotoxic effects and to explore their potential as anticancer agents. Thrombolytic drugs are employed to dissolve blood clots, a process known as thrombolysis (Emon *et al.* 2020). The exploration of natural sources for thrombolytic agents is crucial as it presents an opportunity to develop safer and more affordable alternatives to synthetic drugs. Flavonoids may inhibit platelet aggregation, which can help in preventing the formation of blood clots. Additionally, some flavonoids have been shown to inhibit the activity of enzymes involved in the coagulation cascade, such as thrombin and factor Xa (Guglielmone *et al.* 2002, Faggio *et al.* 2017). It is recommended to carry out further research to investigate the precise biological activities of the isolated compounds and explore their potential in diverse contexts. This will contribute to a broader understanding of the comprehensive pharmacological properties of flavonoids and their potential applications in various fields.

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