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Research paper

In vitro antioxidant, antimicrobial, membrane stabilization and thrombolytic activities of *Dioscorea hispida* Dennst.

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ABSTRACT

Introduction: *Dioscorea hispida* Dennst. is a perennial herb, found in Bangladesh, and is traditionally used for the treatment of arthritis, ulcer and skin infections. In this study, the antioxidant, anti-microbial, thrombolytic and membrane stabilization activities of methanol extract of the plant and its aqueous (AQSF), petroleum ether (PETSF), dichloromethane (DCMSF) and carbon tetrachloride (CTCSF) soluble fractions were investigated with respect to its medicinal implications in traditional systems.

Methods: Antioxidant activity of the plant fractions was investigated in the terms of their total phenolic content and free radical scavenging effect. Their anti-microbial assay was carried out against a total of twelve bacterial and three fungal pathogens. Thrombolytic activity was verified by the means of human blood clot lysis and membrane stabilization effect was determined against heat- and hypotonic solution-induced erythrocyte membrane lysis.

Results: The CTCSF of the plant was found to have a substantial phenolic content, strong free radical scavenging and moderate antimicrobial effects which were at their maximum in the methanol extract and its aqueous, dichloromethane and petroleum ether fractions. The PETSF and CTCSF significantly ($p < 0.05$) inhibited heat- and hypotonic solution-induced erythrocyte membrane lysis, compared to crude extract and the standard drug, acetylsalicylic acid, respectively. The AQSF demonstrated significant ($p < 0.05$) thrombolytic activity compared with organic fractions of methanol extract of the plant.

Conclusion: The investigation revealed bioactive extracts of *D. hispida* by the means of their antioxidant, anti-microbial, thrombolytic and membrane stabilization activities, which may significantly aid in the isolation of bioactive metabolites from the plant.

1. Introduction

The extensive application of antibiotics in human, agriculture and animal care is of concern given their increased and disorganised use and this has contributed to the development of microbial resistance. There are a limited number of antibiotic therapies in the current world and now there is great demand for the development of new anti-microbial agents [1,2]. The synthetic antibiotics have been found to produce potential side effects in the body. This, in turn, has led to the search for new antimicrobials from natural products [3]. Inflammation is characterized by a biological response of tissues in the body which may involve a complex array of activation of cellular enzymes followed by labialization as well as disruption of cell membranes. Therefore, stabilization of cell membrane could be a significant target for anti-inflammatory action [4–6]. Corticosteroid, steroid and non-steroid drugs induce anti-inflammatory effects by membrane stabilization [7]. However, their action is not devoid of noticeable side effects [8,9]. In this context, natural products with membrane stabilizing property could be a greater choice for the search of anti-inflammatory therapies. It has been found that the rate of mortality is increasing gradually due to the thrombotic disorders such as cerebral and myocardial infarction [10]. Arthritis may also increase the risk of thrombosis [11]. Significant efforts have also been made to develop antithrombotic agents from natural products [12]. Free radicals are the toxic products which may induce harmful diseases including oxidative stress, thrombotic disorders, arthritis and inflammation. Antioxidants play a major role in scavenging the free radicals and against these pathogenic conditions [13,14]. Therapeutic application of synthetic antioxidants became hindered by their significant side effects. Therefore, natural antioxidants such as phenolics have gained the interest of current

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researchers as they may be helpful in aiding the development of therapeutic remedies without causing side effects [13,15].

Natural products derived from the medicinal plants provide significant opportunities for the development of new therapeutically active agents [16]. Evaluation of biological activities of medicinal plants which are used in the traditional systems of medicine may led to the discovery of antimicrobial, antioxidant, analgesic, anti-inflammatory and cardioprotective agents [16,17].

Dioscorea hispida Dennst. is a perennial herb of the family Dioscoreaceae. It is commonly known as Suaralu in Bangladesh and distributed in the forests of Chittagong and Chittagong hill tracts of Bangladesh. The herb is used in the treatment of swelling, rheumatism, dropsy in traditional systems of medicines [18]. Besides, the plant is also used in the treatment of ulcer, boils, sores, tumors, fish poisoning, streptococcal infections of skin and deworming of wounds in ethnomedicine [18–20]. The tuber of the plant is used in the treatment of arthritic pain, indigestion, vomiting and possesses, contraceptive, purgative and narcotic properties [21,22]. The plant leaves are used for treating hernia, bloating and asthma [22]. Preliminary phytochemical screening has revealed that the plant contains alkaloid, saponin, carbohydrates, proteins, tannins, glycosides and phenolics [22]. Diosgenin (steroid), prazerigenin A (saponin glycoside), dioscorine (alkaloid) and ascorbic acid have been isolated from the plant. Furthermore, phenolic acids including chlorogenic acid, caffeic acid, methyl ester of protocatechuic acid and p-hydroxybenzaldehyde have been isolated from the tuber of the plant. Pharmacological investigations have revealed that the leaves of *D. hispida* exert significant analgesic as well as anti-inflammatory effects. Tuber extracts of the plant have been found to exhibit potent antioxidant, anticancer and hypoglycemic activities [23]. Biological studies have demonstrated that *Dioscorea* species may exert promising antioxidant, antimicrobial, anti-inflammatory, thrombolytic activities [24–26]. On this basis, the present study was carried out to justify the antioxidant, antimicrobial, membrane stabilization and thrombolytic activities of methanol extract of aerial parts of *D. hispida* and its organic fractions.

2. Materials and methods

2.1. Collection and preparation of the plant material

The plants of *D. hispida* were collected from Bandarban district, Chittagong, Bangladesh. The plant was identified by taxonomist of Bangladesh National Herbarium where a voucher specimen has been deposited for future reference (accession no. DACB 10782). The plants were dried in thermostatic oven at 40 °C. The dried plant materials were then ground to a coarse powder with a grinding machine. The powdered material (650 g) was soaked in 1.8 l of methanol for 15 days. The mixture was kept at room temperature and occasionally stirred. The content was then filtered through a fresh cotton plug followed by Whatman #1 filter paper. The solvent was evaporated by using a rotary evaporator (Heidolph Instruments GmbH & Co. KG, Walpersdorfer, Schwabach, Germany) to obtain crude methanol extract. An aliquot of concentrated (5 g) methanol extract was taken and fractionated following the modified Kupchan method developed by VanWagenen et al. [27]. The process of extraction of various fractions of methanol extract of *D. hispida* has been depicted in Fig. 1. The subsequent fractionating followed by drying allowed to obtain petroleum ether (PETSF), carbon tetrachloride (CTCSF), dichloromethane (DCMSF) and an aqueous soluble fraction (ASF) of the methanol extract of *D. hispida* (MEDH).

2.2. Chemicals and drugs

Methanol, carbon tetrachloride, dichloromethane, petroleum ether, sodium carbonate, Folin-Ciocalteu reagent, Tert- butyl-1-hydroxytoluene (BHT), gallic acid, ascorbic acid were purchased from Merck Co., Germany. Acetylsalicylic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich, USA. Standard discs of ciprofloxacin were purchased from Conda laboratories, Torrejón de Ardoz, Madrid, Spain. Streptokinase was obtained as a gift sample from Incepta Pharmaceuticals Ltd. Bangladesh.

2.3. Determination of total phenolic content

Total phenolic content of *D. hispida* fractions was estimated using Folin-Ciocalteu reagent as oxidizing agent following the method described by Skerget et al. [28]. Gallic acid was used to prepare standard solutions with their concentrations ranged from 0 to 100 µg/ml. A volume of 0.5 ml of gallic acid was mixed with 2.0 ml of Na_2CO_3 (7.5% w/v) and 2.5 ml Folin-Ciocalteu reagent. An amount of 10 mg of crude extract or its fraction was mixed with 5 ml of MeOH. Then, 0.5 ml of the plant fractions (2 mg/ml) was mixed with 2.5 ml of Folin-Ciocalteu reagent and 2.0 ml of Na_2CO_3 solution. The mixtures were incubated for 20 min at room temperature. After incubation, its absorbance was

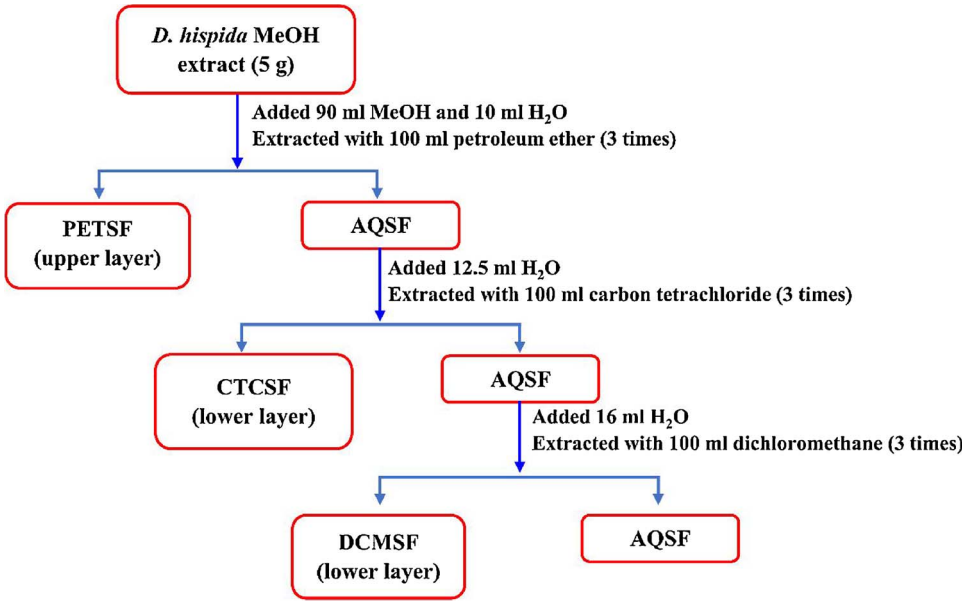

Fig. 1. Schematic diagram of modified Kupchan's method for the fractionation of methanol extract of *D. hispida*. PETSF = petroleum ether, CTCSF = carbon tetrachloride, DCMSF = dichloromethane, AQSF = aqueous soluble fraction of *D. hispida*.