Antimicrobial, Cytotoxic and Antioxidant Activities of *Desmodium heterocarpon*

Abdullah Al Hasan, Choudhury Mahmood Hasan and A.T.M. Zafrul Azam*

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Abstract

Methanolic extract (ME) of powdered whole plant of *Desmodium heterocarpon* and its six vacuum liquid chromatographic (VLC) fractions (F_{a-f}) were investigated for antimicrobial, cytotoxic and antioxidant activities. Only fractions F_c and F_d showed mild antimicrobial activity. Significant free radical (DPPH) scavenging activity was found in ME (IC_{50} value is 19.14±0.14 µg/ml) and F_f (IC_{50} value is 29.71±0.44 µg/ml). The total phenolic content was measured involving Folin-Ciocalteu reagent and it was the highest in fraction F_f (179.08±1.23 mg of GAE/gm of sample). Fractions F_b and F_d showed strong cytotoxicity in brine shrimp lethality bioassay having LC_{50} values 8.19±0.08 µg/ml and 6.46±0.09 µg/ml, respectively.

Keywords: *Desmodium*, antimicrobial, brine shrimp lethality bioassay, total phenolic content, free radical scavenging.

Introduction

Desmodium heterocarpon (L.) DC., is a perennial non-climbing shrub of the Fabaceae family. This family encompasses plants whose characteristics are of high industrial, pharmaceutical, scientific, and cultural importance. The plant is found in India, Myanmar, Bhutan, Thailand, Vietnam, Bangladesh and some other Asian countries. Traditionally many *Desmodium* species are used in typhoid, asthma, bronchitis, piles, cough, dysentery, diarrhoea, haemorrhage, biliousness, convulsions etc. and some of them can induce hypotension (Ghani, 1998).

Desmodium exhibit a wide spectrum of pharmacological activities. *D. gangeticum* protects heart against myocardial ischemia reperfusion injury (Kurian *et al.*, 2010). Analgesic, anti-inflammatory, and antipyretic activities were observed in *D. caudatum* (Ma *et al.*, 2011) and *D. podocarpum* (Zhu *et al.*, 2011). *D. adscendens* possesses anti-anaphylactic property (Addy and Awumey, 1984), analgesic and hypothermic actions as well as inhibitory influence on the propagation of clonic-tonic PTZ (pentylenetetrazole) seizure (N'gouemo *et al.*, 1996). Recently, Tsai *et al.* (2011) studied 10 *Desmodium* species from Taiwan and found that *D. sequax* is a potent antioxidant medicinal plant, and chlorogenic acid may be an important factor in the antioxidant activity of this plant. On the other hand, chemical investigations of *Desmodium* species have revealed the presence of isoflavones, C-glucosyl flavonoids, coumarono-chromones, and pterocarpans (Zhao *et al.*, 2007; Botta *et al.*, 2003). Triterpenoid saponins, tetrahydroiso-quinolones, phenylethylamines and indole-3-alkyl amines have been isolated from the leaves of *Desmodium adscendens* (Addy, 1989). Several flavonoid glycosides, pterocarpanoids, lipids, glycolipids, and alkaloids were isolated and identified from *D. gangeticum* (Mishra *et al.*, 2005). *D. canum* is known to contain isoflavan, isoflavanones (Zappia *et al.*, 2009). However, no phytochemical studies of *Desmodium heterocarpon* have been found in literature to date.

The present work was an endeavor to screen the methanolic extract (ME) of *D. heterocarpon* and its chromatographic fractions for probable antibacterial, cytotoxic and antioxidant activities.

Materials and Methods

Plant material: Whole plant of *Desmodium heterocarpon* (L.) DC., was collected from Rajendrapur, Gazipur, Bangladesh in the month of August, 2009, and was identified in Bangladesh National Herbarium, Dhaka, Bangladesh. A voucher specimen (ACC. No. 34392) of the plant has been deposited in Herbarium.

*Corresponding author: A.T.M. Zafrul Azam, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh. E-mail: zafrulazam@yahoo.com

Preparation of extracts: The powdered whole plants (1000 g) of *D. heterocarpon* were soaked in methanol (2.5 L) for 15 days. Part of the residue (10 g) obtained from methanol extract was subjected to vacuum liquid chromatography using *n*-hexane, *n*-hexane–EtOAc, EtOAc, EtOAc-MeOH, MeOH, MeOH-H_2O and H_2O in order of increasing polarities. As a result, 15 fractions were obtained and on the basis of TLC behavior same were combined to yield test samples F_a (1-3), F_b (4-5), F_c (6-7), F_d (8-10), F_e (11-12) and F_f (13-15).

Antimicrobial activity: The samples were tested for antimicrobial activity by the standard disc diffusion method (Bauer *et al.*, 1966). The screening was done against 13 strains of bacteria. The results thus obtained were compared with standard antibiotic, kanamycin (30 µg/disc).

Cytotoxicity by brine shrimp lethality bioassay: In brine shrimp lethality bioassay (Meyer *et al.*, 1982) dimethyl sulfoxide (DMSO) was used as a solvent and negative control while vincristine sulfate (VS) served as the positive control. For cytotoxicity screening, DMSO solutions of the test samples were applied against *Artemia salina* in a 1-day *in vivo* assay. For the experiment, 4 mg of each of the test samples was dissolved in DMSO and solutions of varying concentrations (400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/ ml) were obtained by serial dilution technique.

Total phenolics analysis: Total phenolics of the samples were measured by Folin-Ciocalteu reagent (Skerget *et al.*, 2005). To 0.5 ml of sample solution (0.25 mg/ml) in water, 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of sodium carbonate (7.5 % w/v) solution were added. After 20 minutes of incubation at room temperature the absorbance was measured at 760 nm using UV-visible spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the known concentrations of standard gallic acid (0-100 µg/ml). The phenolic contents of the sample were expressed as mg of GAE (gallic acid equivalent)/gm of the sample.

Free radical scavenging activity: The free radical scavenging activity (antioxidant capacity) of the test samples were measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Brand-Williams *et al.*, 1995). Here 2.0 ml of a methanol solution of the sample (test sample/ standard) at different concentration (500 µg/ml to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min of reaction at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer. Inhibition of free radical DPPH in percent (I %) was calculated as follows: $(I\ \%) = (1 - A_{sample}/A_{blank}) \times 100$

where A_{sample} is the absorbance of the sample and A_{blank} is the absorbance of the control (containing all reagents except the test material). Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted with inhibition percentage against sample/standard concentration.

Statistical analysis: Three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD (n=3). Probability (P) value of 0.05 or less ($P < 0.05$) was considered significant.

Results and Discussion

In vitro antimicrobial activity: In vitro antibacterial activity of all test samples were investigated against five gram positive bacteria namely, *Bacillus cereus, B. megaterium, B. subtilis, Sarcina lutea,* and *Staphylococcus aureus* and eight gram negative bacteria namely, *Escherichia coli, Pseudomonas aeroginosa, Salmonella typhi, S. paratyphi, Shigella boydii, S. dysenteriae, Vibrio mimicus,* and *V. parahaemolyticus.* Test samples F_c and F_d showed weak antimicrobial activity but other samples were found inactive (data not shown). The zones of inhibition of F_c and F_c were found to be 8-9 mm using 400 µg/disc against gram positive bacteria. On the other hand, 7-9 mm of zone of inhibition were observed against gram negative bacteria for the same test samples (400 µg/disc). The zone of inhibition of samples were compared with the zone of inhibition of kanamycin (30 µg/disc) which showed 30-32 mm of zone of inhibition against all test organisms.

Cytotoxicity by brine shrimp lethality bioassay: The results of the brine shrimp lethality bioassay after 24 hours of exposure to the samples and the positive control, vincristine sulphate (VS) were investigated. The LC_{50} obtained from the best-fit line slope were found to be significant (in comparison with VS, 0.423 µg/ml) for ME (12.96±0.05 µg/ml), F_b (8.19±0.08 µg/ml), F_c (11.21 ± 0.06 µg/ml) and F_d (6.46 ± 0.09 µg/ml). The LC_{50} values

for the other samples were found to be insignificant (15.42 - 25.73 µg/ml) in comparison with positive control.

Antioxidant activity: Total phenolic content (Table 1) of samples was found to be the highest in F_f (179.08±1.23 mg of GAE/gm of sample) and the lowest in F_c (17.58 ± 0.31 mg of GAE/gm of sample). Free radical scavenging activities (Table 1) were found to be significant in ME (IC_{50} value is 19.14 ± 0.14 µg/ml) and F_f (IC_{50} value is 29.71±0.44 µg/ml).

Table 1. Total phenolic content and free radical scavenging activity of the samples.

Sample	Total Phenolic Content (mg of GAE/gm of sample)	Free radical scavenging activity (IC_{50} in µg/ml)
BHT	-	20.45 ± 0.17
ASA	-	2.9 ± 0.04
ME	30.75 ± 0.41	19.14 ± 0.14
F_a	34.08 ± 0.31	884.27 ± 21.52
F_b	21.67 ± 0.31	Value too high *i.e.* no activity
F_c	17.58 ± 0.31	53.68 ± 0.99
F_d	40.42 ± 0.51	34.48 ± 0.22
F_e	79.17 ± 0.62	46.16 ± 0.15
F_f	179.08 ±1.23	29.71 ± 0.44

The average values of three replicates are presented as mean ± S.D. (Standard Deviation).

Therefore, it can be concluded that, in the preliminary studies, some of the test samples obtained from *D. heterocarpon* revealed mild antibacterial activity, significant antioxidant activity as well as strong cytotoxic activity.

Acknowledgements

The authors would like to acknowledge the support received from Microbiology Research Laboratory and Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

References

Addy, M.E. 1989. Several chromatographically distinct components from *Desmodium adscendens* inhibit smooth muscle contractions. *Int. J. Crude Drug Res.* **27**, 81-91.

Addy, M.E., and Awumey, E.M. 1984. Effects of the extracts of *Desmodium adscendens* on anaphylaxis. *J. Ethnopharmacol.* **11**, 283-292.

Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* **45**, 493-496.

Botta, B., Gacs-Baitz, E., Vinciguerra, V. and Delle Monache, G. 2003. Three isoflavanones with cannabinoid-like moieties from *Desmodium canum*. *Phytochemistry* **64**, 599-602.

Brand-Williams, W., Cuvelier, M. E., and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.* **28**, 25-30.

Ghani, A. 1998. *Medicinal plants of Bangladesh: Chemical constituents and uses.* Asiatic Society of Bangladesh, Dhaka, Bangladesh, pp. 160-161.

Kurian, G. A., Suryanarayanan, S., Raman, A., and Padikkala, J. 2010. Antioxidant effects of ethyl acetate extract of *Desmodium gangeticum* root on myocardial ischemia reperfusion injury in rat hearts. *Chin. Med.* **5**, 3.

Ma, K.-J., Zhu, Z.-Z., Yu, C.-H., Zhang, H., Liu, J. and Qin, L.-P. 2011. Analgesic, anti-inflammatory, and antipyretic activities of the ethanol extract from *Desmodium caudatum*. *Pharm. Biol.* **49**, 403-407.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., and McLaughlin, J.L. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med.* **45**, 31-34.

Mishra, P.K., Singh, N., Ahmad, G., Dube, A. and Maurya, R. 2005. Glycolipids and other constituents from *Desmodium gangeticum* with antileishmanial and immunomodulatory activities. *Bioorg. Med. Chem. Lett.* **15**, 4543-4546.

N'gouemo, P., Baldy-Moulinier, M. and Nguemby-Bina, C. 1996. Effects of an ethanolic extract of *Desmodium adscendens* on central nervous system in rodents. *J. Ethnopharmacol.* **52**, 77-83.

Skerget, M., Kotnik, P., Hadolin, M., Hras, A. R., Simonic, M. and Knez, Z. 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem.* **89**, 191-198.

Tsai, J.-C, Huang, G.-J., Chiu, T.-H., Huang, S.-S., Huang, S.-C, Huang, T.-H., Lai, S.-C. and Lee, C.-Y. 2011. Antioxidant activities of phenolic components from various plants of *Desmodium* species. *Afr. J. Pharm. Pharmacol.* **5**, 468-476.

Zappia, G., Menendez, M. P., Lima, C. S. de A. and Botta, B. 2009. A p-quinol isoflavan and two new isoflavanones from *Desmodium canum*. *Nat. Prod. Res.* **23**, 665-671.

Zhao, M., Duan, J.-A. and Che, C.-T. 2007. Isoflavanones and their *O*-glycosides from *Desmodium styracifolium*. *Phytochemistry* **68**, 1471-1479.

Zhu, Z.-Z., Ma, K.-J., Ran, X., Zhang, H., Zheng, C.-J., Han, T., Zhang, Q.-Y. and Qin, L.-P. 2011. Analgesic, anti-inflammatory and antipyretic activities of the petroleum ether fraction from the ethanol extract of *Desmodium podocarpum*. *J. Ethnopharmacol.* **133**, 1126-1131.