American-Eurasian J. Agric. & Environ. Sci., 4 (5): 550-553, 2008
ISSN 1818-6769

Antioxidant and Antibacterial Activities of *Calotropis procera* Linn.

[1]Mst Nazma Yesmin, [2,3]Sarder Nasir Uddin, [4]Sanzida Mubassara and [4]Muhammad Ali Akond

[1]Department of Botany, Govt. B.L. College, National University, Bangladesh
[2]Biotechnology and Genetic Engineering Discipline, Khulna University, Bangladesh
[3]Department of Biology and Chemistry, Graduate School of Medicine,
Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8512, Japan
[4]Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh

Abstract: Methanol and aqueous extract of leaves of *Calotropis procera* Linn. were subjected to the potential antioxidant and antibacterial activities. The antioxidant potential of the methanolic extract was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. IC_{50} of the methanol extract of *Calotropis procera* Linn. was 110.25 µg/ml which indicated the strong antioxidant activity of the plant. However the aqueous extract showed mild antioxidant activity. For antibacterial activities test, the extract was subjected to its effectiveness against both Gram-positive and Gram-negative bacteria in agar diffusion method. The zones of inhibition produced by the crude methanol and aqueous extract against few sensitive strains were measured and compared with those of standard antibiotic Gentamycin. It is evident that both extracts are active against the bacteria at low concentrations. The obtained results provide a support for the use of this plant in traditional medicine and suggest its further advance investigation.

Key words: Antioxidant · Antibacterial · *Calotropis procera* Linn. · Activities · Extract

INTRODUCTION

A proper health care system can be established with supplying low cost medicine to population by using various medicinal plants. Medicinal plants are usually used for Ayuerbedic, Unani and other treatments in rural areas. Recent discovery shows that these plants have fewer side effects than the Allopathic medicine. So, the herbal medicine becoming popular for medication among whole over the world.

The number of plants with medicinal properties included in the Materia Medica of traditional medicine in this subcontinentat present stands at about 2000 [1]. More than 500 of such medicinal plants have so far been enlisted as growing in Bangladesh [2]. Thus the human race started using plants as a means of treatment of diseases and injuries from the early days of civilization on earth and its long journey from ancient time to modern age the human race has successfully used plants and plant products as effective therapeutic tools for fitting against diseases and various other health hazards [3]. *Calotropis procera* Linn. (Called Akond in Bengali; Swallow wort in English ; Akundia in Hindi), a wild growing plant of family Asclepiadaecae is known to possess multifarious medicinal properties. Different parts of the plant have been used in Indian traditional system of medicine for the treatment of leprosy, ulcers, tumors, piles and diseases of spleen, liver and abdomen [4]. Chemical investigations on *Calotropis procera* Linn. resulted in the isolation of octacosanoic acid, semiarenone and trematol, a triterpenoid alcohol and Column chromatography of the n-hexane extract of Calotropis procera followed by recrystallization afforded white needle shaped crystals. The structure of the compound was concluded to be b-sitosterol on the basis of its 1HNMR and IR spectral data by comparison to the data with those published in the literature [5]. The hypoglycaemic property of *Calotropis procera* Linn was also assesse by an oral glucose tolerance test(OGTT) in STZ-diabetic ratsAntibiotic susceptibility testing by a standardized single disc method [6].

The root of the *Calotropis procera* Linn is used as a carminative in the treatment of dyspepsia [7]. Further, the root bark and leaves of *Calotropis procera* are used

Corresponding Author: Sarder Nasir Uddin, Department of Biology and Chemistry,
Graduate School of Medicine, Yamaguchi University,
1677-1 Yoshida, Yamaguchi-735-8512, Japan

by various tribes of central India as a curative agent for jaundice [8]. The aqueous extract of the latex has been shown to inhibit cellular infiltration and afford protection against development of neoplastic changes in the transgenic mouse model of hepatocellular carcinoma [9]. The chloroform extract of the root has been shown to exhibit protective activity against carbon tetrachloride induced liver damage [10]. Methanol extract possess antioxidant activity in *Trema orientalis* [11] and *Senna tora* [12]. So, the present work was designated to investigate the antioxidant and antibacterial activities of of *Calotropis procera* Linn to know the scientific basis of the traditional use of this plant.

MATERIALS AND METHODS

Plant Materials: Fresh leaves of *C. procera* were collected from Khulna University Campus in Bangladesh in the middle of 2007. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka and a voucher specimen was kept for future reference. The dried leaves of *C. procera* were ground into a fine powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). About 400 g of powered material was extracted by soxhlet apparatus with 90% methanol at 55°C temperature. The extract thus obtained was concentrated by using a rotary evaporator (Bibby RE200, Sterilin Ltd., UK) to get a viscous mass. The viscous mass was then kept at room temperature under a ceiling fan to get a dried extract (about 16% yield). The extract thus obtained was used for pharmacological screening.

Determination of Antioxidant Activities: The anti-oxidant potential of the methanolic extract was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH method is most widely used and easiest method to determine antioxidant activity [12]. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The aliquots of the different concentrations (1-500 µg/ml) of the extract was added to 3 ml of a 0.004% w/v solution of DPPH. Absorbance at 517 nm was determined after 30 min, and IC_{50} (Inhibitory concentration 50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals [13].

At first 6 test tubes were taken to make aliquots of 6 conc.(1, 5, 10, 50, 100 and 500 µg/ml). Plant extract and ascorbic acid were weighed 3 times and dissolved in ethanol to make the required concentration by dilution technique. Here ascorbic acid was taken as standard. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogeneously magnetic stirrer was used. After making the desired concentrations 3 ml of 0.004% DPPH solution was applied on each test tube by pipette. The room temperature was recorded and kept the test tubes for 30 mins in light to complete the reactions. DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank. After 30 mins, absorbance of each test tubes were determined by UV spectrophotometer. IC_{50} was determined from % inhibition *vs* concentration graph.

Determination Antibacterial Activities: Nutrient agar media was prepared by adding water to a dehydrated product that contains all the ingredients. Practically all media are available commercially in powdered form [14].

Three types of discs were prepared for antibacterial screening: One gram sample extracts was dissolved in 10 ml of ethanol to prepare sample solution, 0.03 gm/10ml gentamicin standard disc used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by known antibacterial agent with that produced by test samples, and third one was a blank sample (only ethanol) which was used as negative control to ensure that the residual solvents was not active. Specific organisms were inoculated into previously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile petri dish in an aseptic condition. It was stored in an incubator for about 24 hours to allow the proper growth of microbes. Prepared sample solutions were applied to the corresponding cups or holes with the help of a micropipette. The plates were then allowed to stand to diffuse the sample solution into the antibiotic medium at room temperature for 2 hours. The plates were then incubated at 37°C for overnight. After proper incubation, clear zones of inhibition around the point of application of sample solution were formed. These inhibition zones were measured by slide calipers and expressed in millimeter [6].

RESULTS

For Antioxidant Activities Test: In the present study, methanol extracts of the leaves of *C. procera* showed potential free-radical scavenging activity but aqueous extract showed very little free-radical scavenging activity.

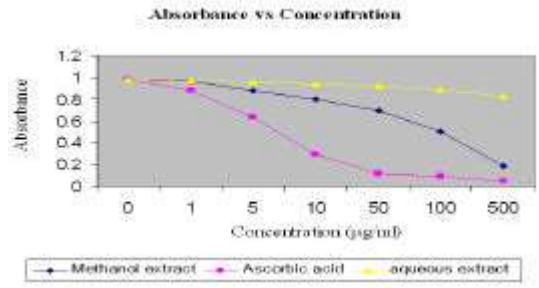

Fig. 1: DPPH Scavenging Assay of *Trema orientalis* compared with Standard ascorbic acid (absorbance vs concentration)

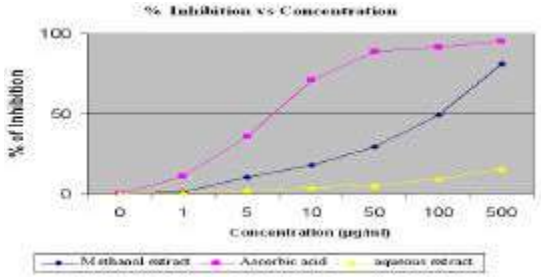

Fig. 2: DPPH Scavenging Assay of *Trema orientalis* compared with standard ascorbic acid (% of inhibition vs concentration)

IC_{50} of the methanol extract of *C. procera* was 121.25 µg/ml which indicated the strong antioxidant activity of the plant extract. However the aqueous extract showed mild antioxidant activity. DPPH Scavenging Assay of *C. procera* compared with Standard ascorbic acid absorbance vs concentrations are shown in Fig. 1 & % of inhibition vs concentration are shown in Fig. 2.

For Antibacterial Activities Test: The result of the antibacterial activity measured in term of diameter of zone of inhibition in mm. Standard antibiotic discs of Gentamycin was used as standard comparison purpose. Both extract showed antibacterial activity against both gram positive and gram negative bacteria. Aqueous extract showed higher anti microbial activities than methanol extract. Inference can be drawn that the antibacterial constituents are present in the extract in moderate concentration. Antibacterial activity of methanol and aqueous extract of leaves of *C. procera* are shown in Table 1.

Table 1: Antibacterial activity of methanol and aqueous extract of leaves of *C. procera*

	Diameter zone of inhibition in mm		
Name of bacteria	Gentamycin (30µg/well)	Methanol extract (500 µg/well)	Aqueous extract (500 µg/well)
Gram positive bacteria			
Staphylococcus aureus	21	9	12
Staphylococcus epidermidis	18	9	10
Staphylococcus saprophyticus	32	-	22
Streptococcus pyogenes	21	-	10
Gram negative bacteria			
Plesiomonas shigelloides	14	6	7
Shigella dysenteriae	24	9	10
Vibrio cholerae	28	9	9
Salmonella typh	31	-	-
Shigella flexneri	21	-	10
Shigella boydii	23	-	-
Shigella sonnei	24	-	9
Pseudomonas aeruginosa	27	-	10

'-' No inhibition

DISCUSSIONS

DPPH is the best, easiest and widely used method for for testing preliminary free radical scavenging activity of a compound or a plant extract [12]. In present study, methanol extracts of the leaves of *C. procera* possess strong antioxidant activity. However the aqueous extract showed mild antioxidant activity. The free radical scavenging property may be one of the mechanisms by which this drug is effective as a traditional medicine. Most of the tannins and flavonoids are phenolic compounds and may be responsible for antioxidant properties of many plants [15]. So, this activity may be due to the presence of phenolic compounds (tannins and flavonoids) present in the extract [16].

Crude methanol extract of *C. procera* showed antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis, Plesiomonas shigelloides, Shigella dysenteriae, and Vibrio cholerae* on the other hand aqueous extract showed antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis, Staphylococcus saprophyticus*, *Streptococcus pyogenes Plesiomonas shigelloides, Shigella dysenteriae, Vibrio cholerae, Shigella Flexner, Shigella sonnei and Pseudomonas aeruginosa.* Both extracts did not show any activities against

Salmonella typhi and Shigella boydii. In fact, both methanol and aqueous extract of *C. procera* shown significant antibacterial activity against few gram positive and gram negative bacterial strains. The reputation of *C. procera* as a remedy for different microbial diseases traditionally including diarrhoea and dysentery was supported by the antibacterial screening tests.

Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi processed plant products to produce drugs and medicines. This huge foreign exchange can be saved if the indigenous medicinal plants or their semi-processed products are utilized by the manufacturers to satisfy their needs. So, further pharmacological and toxicological study is required to establish the therapeutic uses of the plant and particularly with its active principles.

REFERENCES

1. Chopra, J. and S. Rajasekharan, 1958. Indian Pharmacological Aspects, 55:87-95.
2. Yusuf, M., J.U. Chowdhury, M.A. Wahab and J. Begum, 1994. Medicinal Plant of Bangladesh. 3rd Edn., pp: 234-235.
3. Ghani, A., 2003. Medicinal Plants of Bangladesh. The Asiatic Society of Bangladesh, pp: 21-28.
4. Kirtikar, K.R. and B.D. Basu, 1935. Indian Medicinal Plants, Lolit Mohan Basu, Allahabad, India, pp: 1606.
5. MISSING
6. Bauer, A.W., W.M.M . Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol., 45: 493-496.
7. Kumar, V.L. and S. Arya, 2006. Medicinal uses and pharmacological properties of *Calotropis procera*. In: J.N. Govil, Editor, Recent Progress in Medicinal Plants 11, Studium Press, Houston, Texas, USA, pp: 373-388.
8. Samvatsar, S and V.B. Diwanji, 2000. Plant sources for the treatment of jaundice in the tribals of Western Madhya Pradesh of India. J. Ethnopharmacol., 73: 313-316.
9. Choedon, T., G. Mathan, S. Arya, V.L. Kumar and V. Kumar, 2006. Anticancer and cytotoxic properties of the latex of *Calotropis procera* in a transgenic mouse model of hepatocellular carcinoma. World J. Gastroenterol., 12: 2517-2522.
10. Basu, A., T. Sen, R.N. Ray and A.K. Nag Chaudhuri, 1992. Hepatoprotective effects of *Calotropis procera* root extract on experimental liver damage in animals, Fitoterapia, 63: 507-514.
11. Uddin, S.N., K.M.A.Uddin and F. Ahmed, 2008. Analgesic and antidiarrhoeal activities of Treama orientalis Linn. in mice, Oriental Pharmacy and Experimental Medicine, 8(2): 187-191.
12. Uddin, S.N., M.E. Ali and M.N. Yesmin, 2008a. Antioxidant and Antibacterial Activities of *Senna tora* Roxb, American J. Plant Physiol., 3(2): 096-100.
13. Gupta, M., U.K. Mazumdar, T. Sivakumar, M.L.M. Vamis, S. Karkis, R. Sambathkumar and L. Mainkandan, 2003. Antioxidant and Anti-inflammatory activities of *Acalypha fruticasa.* Nig. J. Nat. Prod. Med., pp: 25-29.
14. Pelczar, M.J., J.R. Chan, E.C.S. Noel and K.R. Krieg, 1993. Microbiology. Fifth edition. Tata McGraw-Hill Publishing Company Ltd., New Delhi, India, pp: 103-105, 510-539.
15. Larson, R.A., 1988. The antioxidants of higher plants. Phytochemistry, 27(4): 969-978.
16. Sadhu, S.K., E. Okuyama, H. Fujumoto and M. Ishibashi, 2003. Seperartion of leucas, aspara amedicinal plant of Bangladesh,guided by prostaglandin inhibitory and anti oxidant activities. Chemical and Pharmaceutical Bulletin, 51(5): 595-598.