RESEARCH PAPER

Asian Journal of Bio Science (April, 2010) Vol. 5 Issue 1 : 6-9

Studies on antioxidant and anthelmintic activity of *Gnidia glauca* (Fresen) Gilg.

H.R. NETHRAVATHI[1], **T.R. PRASHITH KEKUDA**[2], K.S. VINAYAKA[3], N.B. THIPPESWAMY[1], S.J. SUDHARSHAN[2] **AND** S.V. PRAVEEN KUMAR[4]

[1]Department of Studies and Research in Microbiology, P.G. Institute of Studies and Research in Microbiology, Kuvempu University, Jnanasahyadri, SHANKARAGHATTA (KARNATAKA) INDIA

[2]Department of Microbiology, S.R.N.M.N. College of Applied Sciences, NES Campus, SHIVAMOGGA (KARNATAKA) INDIA

[3] Department of Studies and Research in Applied Botany, Kuvempu University, Jnanasahyadri, SHANKARAGHATTA (KARNATAKA) INDIA

[4]Department of Studies and Research in Microbiology, Kuvempu University, Shivagangothri, Tholahunase, DAVANGERE (KARNATAKA) INDIA

(Accepted : November, 2009)

The present study was carried to evaluate antioxidant and anthelmintic activity of methanol, chloroform, ethyl acetate, acetone and petroleum ether extracts of *Gnidia glauca* (Fresen) Gilg. The preliminary phytochemical tests showed the presence of tannins, terpenoids, steroids, saponins and flavonoids. A dose dependent antioxidant activity was observed in case of extracts. Methanol extract was found to possess greater radical scavenging potential than other extracts. All extracts were found to cause paralysis and death of worms but not to the extent of standard drug. Among extracts tested, the acetone extract took less time to cause paralysis and death of the worms when compared to other extracts. The antioxidant and anthelmintic activity of the solvent extracts could be mainly due to the presence of various phytoconstituents. Further studies are to be conducted to isolate active constituents and to find out the in vivo efficacy of the plant extracts tested.

Key words : *Gnidia glauca* (Fresen) Glig., Antioxidant activity, DPPH assay, Anthelmintic activity, Pheretima pasthuma

INTRODUCTION

In developing countries like India where poverty and malnutrition is rampant, knowledge of plant derived metabolites could reduce the cost of health care. India has a rich history of using various herbs and herbal components for treating various diseases (Ali *et al.,* 2008). Infectious diseases caused by bacteria, fungi, viruses, and parasites remain a major threat to public health, despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance (Okeke *et al*., 2005). Helminthes are recognized as a major problem to livestock production throughout the tropics. Parasitic helminthes affect human being and animals by causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic and debilitating in nature (Dewanjee *et al*., 2007). The origin of many effective drugs is found in the traditional medicine practices and in view of this several workers have undertaken studies pertaining to testing of folklore medicinal plants for their proclaimed anthelmintic activity (Temjenmongla and Yadav, 2005). Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman, 1996). The synthetic antioxidants like BHA, BHT, gallic acid esters etc., have been suspected to cause or prompt negative health effects. Strong restrictions have been placed on their application (Barlow, 1990; Branen, 1975). In recent years much attention has been devoted to natural antioxidant and their association with health benefits (Ali *et al*., 2008). *Gnidia glauca* (Fresen) Gilg belongs to the family Thymeliaceae and locally known as Mukkadakana gida. It is a large shrub, leaves alternate, linear oblong, head inflorescence flower and fruit in January and February. Fruit is indehiscent. It is traditionally used as pesticide in the paddy fields to control insects and to treat skin diseases (Gowda, 2004). The present study was conducted to evaluate antioxidant and anthelmintic potential of methanol, chloroform, ethyl acetate, acetone

and petroleum ether extracts of *G. glauca*.

MATERIALS AND METHODS

Collection and identification of plant :

Gnidia glauca (Fresen) Gilg was collected in the Sharavathi river basin of Central Western Ghats of Karnataka. The plant was authenticated in Department of Studies and Research in Applied Botany, Jnanasahyadri, Shankaraghatta and voucher specimens (KU/AB/KSV/237) were deposited in the department for future reference.

Extraction of plant material using solvents :

The leaves of *G. glauca* were washed thoroughly 2-3 times with running tap water and once with sterile water, shade dried, powdered and used for extraction. The powdered plant material (200g) was extracted with solvents namely methanol, chloroform, petroleum ether, ethyl acetate and acetone by soxhlet extraction and exhaustively extracted for about 48 hours. The extracts were filtered through Whatman filter paper No. 1 and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the desiccator (Manjunatha *et al*., 2006). All the extracts were subjected to preliminary phytochemical screening to screen the presence of various secondary metabolites (Parekh and Chanda, 2007).

Screening solvent extracts for antioxidant activity :

The antioxidant activity of solvent extracts of plants and the standard ascorbic acid was tested using DPPH free radical scavenging activity (Khalaf *et al*., 2008; Ravikumar *et al.*, 2008). Different concentrations of plant extracts and standard namely 0.125, 0.25, 0.5mg/ml and 1.0mg/ml were prepared in methanol. 0.002% of DPPH was prepared in methanol. In clean and labeled test tubes, 2ml of DPPH solution was mixed with 2ml of different concentrations of plant extract and standard separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517nm using UV-Vis Spectrophotometer. The degree of stable DPPH* decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. The scavenging activity of the extract against the stable DPPH* was calculated using the following equation.

$$\text{Scavenging activity in \%} = \frac{\mathbf{A - B}}{\mathbf{A}} \mathbf{X\ 100}$$

where, A was the absorbance of DPPH and B was the absorbance of solution of DPPH and extract.

Screening of solvent extracts for anthelmintic activity:

The assay was performed on adult Indian earthworm Pheretima pasthuma due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. The worms were washed with normal saline to remove all the extraneous matter. Standard drug (Piperazine citrate, 1%) and solvent extracts (10mg/ml) were prepared in normal saline (0.85%) and poured into respective labeled Petriplates (50 ml). Six worms of equal size (or nearly equal) were introduced into each of the plates. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors (Gireme *et al*., 2006). Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased (Temjenmongla and Yadav, 2005).

RESULTS AND DISCUSSION

Phytochemical screening of methanol extract revealed the presence of tannins, terpenoids, steroids, saponins and flavonoids (Table 1).

Table 1: Phytochemical constituents in the methanol extract of *G. glauca*

Phytochemical group	Methanol extract
Tannins	+
Terpenoid	+
Alkaloid	-
Steroid	+
Saponins	+
Flavonoids	+

The result of antioxidant activity of solvent extracts is shown in Table 2. The crude solvent extracts exhibited marked antioxidant activity by scavenging DPPH* (free radical) and converting into DPPHH. The extracts have exhibited concentration dependent radical scavenging activity *i.e.*, higher the concentration, more scavenging potential. Among the extracts, methanol extract exhibited high free radical scavenging activity followed by acetone, ethyl acetate, chloroform and petroleum ether extracts. The scavenging activity of standard (ascorbic acid) was greater than that of solvent extracts. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are

Table 2 : Antioxidant activity of solvent extracts of *G. glauca*

Solvent extract	Radical scavenging activity (%) of different concentrations of solvent extracts (mg/ml)			
	0.125	0.250	0.500	1.000
Methanol	75.16	83.53	89.72	92.63
Chloroform	56.67	62.83	68.13	75.68
Ethyl acetate	64.10	72.11	78.14	82.05
Acetone	63.82	75.42	83.78	88.22
Petroleum ether	56.31	61.09	66.04	75.30
Standard (Ascorbic acid)	88.69	92.52	95.12	97.33

Table 3 : Anthelmintic activity of solvent extracts of *G. glauca*

Treatment	Average time in minutes	
	Paralysis	Death
Methanol	95	151
Chloroform	85	149
Ethyl acetate	102	156
Acetone	78	146
Petroleum ether	112	159
Piperazine citrate (1%)	29	44
10% DMSO	-	-
Normal saline	-	-

through scavenging or chelating process (Kessler *et al*., 2003; Cook and Samman, 1996). Phenolic compounds are a class of antioxidant compounds which act as free radical terminators (Shahidi and Wanasundara, 1992). The compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for antioxidant effect in the plants (Das and Pereira, 1990; Younes, 1981). DPPH is relatively stable nitrogen centred free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Jayaprakasha *et al*., 2001). DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution, therefore, loses color stoichometrically depending on the number of electrons taken up (Blois, 1958). In this study, the scavenging activity was found to be dose dependent *i.e*., higher the concentration, more was the scavenging activity. Though the DPPH radical scavenging abilities of the extracts were less than that of ascorbic acid, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

The anthelmintic activity of solvent extracts of *G. glauca* is depicted in Table 3. It has been found that the acetone extract took less time to cause paralysis and death of the worms followed by chloroform, methanol and ethyl acetate extracts. Less anthelmintic efficacy was observed in case of petroleum ether extract. Standard drug exhibited more potent activity than the extracts. Paralysis and death of worms was not observed in case of control (DMSO) and normal saline (Table 2). Helminthes are recognized as a major problem to livestock production throughout the tropics. Parasitic helminthes affect human being and animals by causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic and debilitating in nature (Dewanjee *et al*., 2007). The origin of many effective drugs is found in the traditional medicine practices and in view of this several workers have undertaken studies pertaining to testing of folklore medicinal plants for their proclaimed anthelmintic activity (Temjenmongla and Yadav, 2005).

Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extracts for their biological activity may provide new anti-microbial substances. The extracts were found to be effective against worms. So the extracts could be used to treat intestinal helminthic worms. The plants could be used as a source of natural antioxidant as marked scavenging was observed in the study. Further experiments are to be conducted to isolate active principles from the extracts and to determine *in vivo* efficacy of phytoconstituents.

Acknowledgement :

The authors express their sincere thanks to HOD, Department of Microbiology and Principal, S.R.N.M.N. College of Applied Sciences, Shimoga. Authors also thank N.E.S for providing all facilities and moral support to conduct work.

REFERENCES

Ali, S.S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahu, A. and Bora, U. (2008). Indian medicinal herbs as sources of Antioxidants. *Food Res. Internat.*, **41**: 1–15.

Barlow, S.M. (1990). Toxicological aspects of antioxidants used as food additives. In : Food Antioxidants, Hudson BJF (ed.) Elsevier, London, pp. 253-307.

Blois, M.S. (1958). Antioxidant determinations by the use of stable free radical. *Nature*, **29**: 1199.

Branen, A.L. (1975). Toxicology and biochemistry of butylated hydroxyanisol and butylated hydroxytoluene. *J. American Oil Chemists Soc.*, **5**: 59-63.

Cook, N. and Samman, S. (1996). Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. *J. Nutr. Biochem.*, **7**(2): 66-76.

Das, N.P. and Pereira, T.A. (1990). Effects of flavonoids on thermal autooxidation of Palm oil: structure- activity relationship. *J. American Oil Chememist Soc.*, **67**: 255-258.

Dewanjee, S., Maiti, A., Kundu, M. and Mandal, S.C. (2007). Evaluation of anthelmintic activity of crude extracts of Diospyros peregrine, Coccinia grandis *and* Schima wallichii. *Dhaka Univ. J. Pharm. Sci.*, **6**(2): 121-123.

Gowda, B. (2004). *Vanaspathi Kosha, Plant Wealth of Sringeri*, Kalpatharu research Academy, Bangalore (Karnataka).

Grime, A.S., Bhalke, R.D., Ghogare, P.B., Tambe, V.D., Jadhav, R.S. and Nirmal S.A. (2006). Comparative *in vitro* anthelmintic activity of *Mentha piperita and Lantana camara* from Western India. *Dhaka Univ. J. Pharm. Sci.*, **5** (1-2): 5-7.

Jayaprakasha, G.K., Singh, R.P. and Sakariah, K.K. (2001). Antioxidant activity of grape seed extracts on peroxidation models *in vitro*. *J. agric. Food Chem.*, **55**: 1018.

Kessler, M., Ubeaud, G. and Jung, L. (2003). Anti- and pro-oxidant activity of rutin and quercetin derivatives. *J. Pharm. Pharmacol*., **55**: 131-142.

Khalaf, N.A., Shakya, A.K., Al-Othman, A., El-Agbar, Z. and Farah, H. (2008). Antioxidant activity of some common plants. *Turkish.J.Biol.*, **32**: 51-55

Kumpulainen, J.T. and Salonen, J.T. (1999). Natural antioxidants and anticarcinogens in nutrition, health and disease, *The Royal Society of Chemistry*, UK, pp 178-187.

Manjunatha, B.K., Patil, H.S.R., Vidya, S.M., Kekuda, T.R.P., Mukunda, S. and Divakar, R. (2006). Studies on the antibacterial activity of Mucuna monosperma DC. *Indian Drugs*, **43**: 150-152.

Okeke, I.N., Laxminarayan, R. and Bhutta, Z.A. (2005). Antimicrobial resistance in developing countries. Part 1: recent trends and current status. *Lancet Infect Disease*, **5**: 481-493.

Parekh, J. and Chanda, S.V. (2007). *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish J.Biol.*, **31**: 53-58.

Ravikumar, Y.S., Mahadevan, K.M., Kumaraswamy, M.N., Vaidya, V.P., Manjunatha, H., Kuma, V. and Satyanarayana, N.D. (2008). Antioxidant, cytotoxic and genotoxic evaluation of alcoholic extract of *Polyalthia cerasoides (roxb) Bedd. Environ. Toxicol. & Pharmacol.*, **26**: 142-146.

Shahidi, F. and Wanasundara, P.K.J.P.D. (1992). Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.*, **32**: 67-103.

Temjenmongla and Yadav, A.K. (2005). Anticestodal efficacy of folklore medicinal plants of Naga tribes in Northeast *India. African J. Trad. CAM*, **2**(2): 129-133.

Younes, M. (1981). Inhibitory action of some flavonoids on enhanced spontaneous lipid peroxidation following glutathione depletion. *Planta Med*., **43**: 240-245.