Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 3 (4): 134-140, 2009
ISSN 1819-3455

Biological Activity of *Merremia emarginata* Crude Extracts in Different Solvents

A.V. Babu, R.S.C. Rao, K.G. Kumar, B.H. Babu and P.V.V. Satyanarayana
Department of Chemistry, Acharya Nagarjuna University,
Nagarjuna Nagar-522 510, A.P., India

Abstract: The plant *Merremia emarginata* (Burm. f.) Hallier f., belongs to Convolvulaceae family. In traditional medicinal system, different parts of *M. emarginata* have been mentioned to be therapeutically used as deobstruent, diuretic, for cough, headache, neuralgia and rheumatism. In the present study, biological activities of different solvent extracts isolated from *M. emarginata* were tested. Hexane (IA), ethyl acetate (IB), methanol (IC) and aqueous methanol (25%) (ID) extracts of *M. emarginata* were examined. Antioxidant properties of the extracts were studied by DPPH (1,1-Diphenyl-2-Picrylhydrozyl) radical scavenging activity method and superoxide radical scavenging activity method. Methanol extract exhibited better antioxidant activity than other extracts with IC_{50} of 8.59 μg mL^{-1} in DPPH radical scavenging method. Methanol and hexane extracts exhibited α-amylase inhibitory activity with IC_{50} of 104.5 and 133.4 μg mL^{-1}, respectively. Ethyl acetate extract showed cytotoxicity with ED_{50} of 34.29 μg mL^{-1} in brine shrimp lethality assay. The present study revealed that the extracts IB and IC of *M. emarginata* were found to be showed promising biological activities. Methanol extract of this plant might be use full for antioxidant and antiobesity activities with minimal toxicity.

Key words: *Merremia emarginata*, antioxidant, α-amylase inhibitory activity

INTRODUCTION

Natural products produced by plants, microorganisms, insects and animals have been isolated as biologically active pharmacophores (Gordon and David, 2005; Cragg and Newman, 2007; Wang *et al*., 2006). Approximately one-third of the top-selling drugs in the world are natural products or their derivatives often with ethno pharmacological background (Kingston, 2009). The advantage of natural products for random screening is the structural diversity provided by natural products, which is greater than provided by most available combinatorial approaches based on heterocyclic compounds (Harvey, 1999). Cox (1994) suggested that the ethno-directed sampling is most likely to succeed in identifying drugs used in the treatment of cancer, inflammatory and dermatological complaints. According to Chatterjee *et al*. (2006) tribal healers in most of the countries, the ethnomedical treatment is frequently used to treat cut wounds, skin infection, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snakebite and gastric ulcer, provide instructions to local people as how to prepare medicine from herbs. India possesses rich floristic wealth and diversified genetic resources of medicinal plants (Arora *et al*., 2003).

Corresponding Author: B. Hari Babu, Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar-522 510, A.P., India

The use of the plant extracts and pure compounds isolated from natural sources provided the foundation to modern pharmaceutical compounds (Alluri *et al*., 2006).

Antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and propagation of oxidative stress related diseases such as autoimmune (Willet, 1994) cardiovascular (Vinson *et al*., 1995) and neurovascular diseases (Aggarwal and Harikumar, 2008). Recently, more attention has been paid to the role of natural antioxidants, mainly phenolic compounds, which may have higher antioxidant activities than those of conventional vitamins C, E and β-carotene (Hafidh *et al*., 2009). Cytotoxicity screening models like brine shrimp lethality assay are the preliminary methods for selection of active plant extracts against cancer (Al-Fatimi *et al*., 2007). The management of diabetes by chemical drugs without any side effects is still a challenge to the medical system. Many efforts have been made to identify new antidiabetic agents from different sources, especially medicinal plants because of their effectiveness, fewer side effects and relatively low cost (Bhandari *et al*., 2008). The stable blood glucose level is important for diabetic patients, because it prevents the hyperglycaemia and the complications associated with diabetes. Carbohydrate hydrolyzing enzyme like α-amylase inhibitors are one of the essential drugs for managing type II diabetes (Nickavar *et al*., 2008).

The plant *Merremia emarginata* (Burm. f.) Hallier f., belongs to Convolvulaceae family. In Sanskrit, it is called as Mooshikakarnee (Satyavati *et al*., 1987). In India, it is mainly found in Chennai and in some places of Andhra Pradesh (Pullaiah *et al*., 2000). The plant was therapeutically used as deobstruent, diuretic, for cough, headache, neuralgia and rheumatism (Chatterjee and Prakashi, 1995). The importance of *M. emarginata* as a biologically potent plant species was proposed the study *in vitro* antioxidant, α-amylase inhibition and cytotoxicity activities of different solvent extracts of *M. emarginata* is reported.

MATERIALS AND METHODS

Plant Material

The plant material (whole plant except flowers) was collected from the Botanical Garden of Acharya Nagarjuna University, Nagarjuna Nagar and authenticated by the Department of Botany.

Preparation of Extracts

The plant material was collected and air dried. Finely powdered plant material (1 kg) was extracted twice with hexane (5 L) and then the spent was continuously extracted with ethyl acetate, methanol and aqueous methanol (accordingly). The solvent extracts were collected and dried by using rotary vacuum evaporator. The dried weights of the crude extracts are as follows hexane (IA):12.3 g, ethyl acetate (IB):16.65 g, methanol (IC): 30 g and aqueous methanol (ID): 15 g.

Antioxidant Activity

DPPH Radical Scavenging Activity Method

The DPPH (1,1-Diphenyl-2-Picrylhydrozyl) radical scavenging activity was determined according to the method of Szabo *et al*. (2007), the Optical Density (OD) of colored methanolic solution of the DPPH free radicals was measured at 517 nm. Percent of inhibition was calculated by comparing absorbance of crude extract with that of control. The radical scavenging activity of the crude extracts was expressed as the percent of inhibition and the IC_{50} values were obtained from the plot drawn concentration (µg) verses % of inhibition.

Superoxide Radical Scavenging Activity Method

Superoxide radicals were generated *in vitro* by non enzymatic system and determined spectrophotometrically (560 nm) by Nitro Blue Tetrazolium (NBT) photo reduction method (McCord and Fridovich, 1969). The assay mixture consist of 6.6 mM EDTA containing 3 µg of NaCN, 2 µM of riboflavin, 50 µM of NBT, crude extract and 67 mM of phosphate buffer (pH 7.8) in a final volume of 3 mL. The optical density at 560 nm was measured before and after 15 min illumination. The superoxide radical scavenging activity of the crude extracts was expressed in IC_{50} values.

Alpha-Amylase Inhibitory Activity

The α-amylase activity was measured using the dinitrosalicylic acid (DNS) method developed by Bernfeld (1955), improved by Jamieson *et al*. (1969) and adopted for testing α-amylase inhibitory potential (Da silva *et al*., 2004) using 1% soluble starch as substrate. The test substance was pre-incubated with amylase (100 µL) at room temperature for 20 min prior to the addition of 100 µL of the substrate solution followed by incubation at 37°C for 10 min. The reactions were stopped by the addition of 200 µL of DNS reagent followed by color development by placing the tubes in boiling water for 5 min and then added 3.6 mL of distilled water. Acarbose was used as positive control. The absorbance was read at 470 nm and experiments were carried out in duplicates.

Brine Shrimp Lethality Bioassay

Brine shrimp (*Artemia salina*) nauplii were hatched using brine shrimp eggs in a conical shaped vessel (1 L), filled with sterile artificial sea water of 38 g L^{-1} of sea salt and adjusted to pH 8.5 using 1 N of NaOH and kept under constant aeration for 48 h. After hatching, 10 nauplii were drawn through a pipette and placed in each vial containing 4.5 mL brine solution and added various concentrations of crude extracts (0-300 µg mL^{-1}) and the final volume was made up to 5 mL using brine solution and maintained 37°C for 24 h under the light of incandescent lamps (McLaughlin *et al*., 1993, 1998). Assays were carried out in duplicates. The percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. Podophyllotoxin was used as a positive control.

RESULTS

The crude extracts IA, IB, IC and ID were studied for antioxidant, α-amylase inhibitory and cytotoxic activities. The antioxidant activity was measured in two biological methods namely DPPH and superoxide radical scavenging activity method. The results of the two methods were incorporated in Table 1. In DPPH method, the IC crude extract showed better anti-oxidant activity (8.59 µg mL^{-1}) followed by IB (21.5 µg mL^{-1}), ID (31.2 µg mL^{-1}) and IA (56.2 µg mL^{-1}). In superoxide radical scavenging activity method, the extract IB exhibited

Table 1: Antioxidant activity of the crude extracts by DPPH and superoxide radical scavenging methods

	IC_{50} (µg mL^{-1})	
Test substrate	DPPH radical scavenging method	Superoxide radical scavenging method
IA	56.20	87.09
IB	21.50	45.10
IC	8.59	54.30
ID	31.20	89.40
Vitamin C	3.26	150.00

IA, IB, IC and ID are crude extracts of hexane, ethyl acetate, methanol and aqueous methanol, respectively

Table 2: Alpha amylase inhibitory activity of the crude extracts

Test substrate	IC_{50} (µg mL^{-1})
IA	133.4
IB	421.8
IC	104.5
ID	218.0
Acarbose	10.9

IA, IB, IC and ID are crude extracts of hexane, ethyl acetate, methanol and aqueous methanol, respectively

Table 3: Brine shrimp lethality test for the crude extracts

Test substrate	ED_{50} (µg mL^{-1})
IA	74.15
IB	34.29
IC	237.41
ID	145.40
Standard (Podophyllotoxin)	2.36

IA, IB, IC and ID are crude extracts of hexane, ethyl acetate, methanol and aqueous methanol, respectively

IC_{50} at 45.1 µg mL^{-1} followed by IC (54.3 µg mL^{-1}), IA (87.09 µg mL^{-1}) and ID (89.4 µg mL^{-1}). The *in vitro* α-amylase inhibitory activity (Table 2) results revealed that the extract IC showed IC_{50} at 104.5 µg mL^{-1} followed by IA. The brine shrimp results were incorporated in Table 3, the extract IB showed good cytotoxicity against brine shrimp larvae followed by IA. The ED_{50} value of the standard podophyllotoxin is 2.36 µg mL^{-1}.

DISCUSSION

We know that variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases (Fabricant and Farnsworth, 2001). Oxygen is easily activated by Ultra Violet (UV) radiation and heat from the sunlight to produce toxic Reactive Oxygen Species (ROS). These ROS are highly reactive because they can interact with a number of cellular molecules and metabolites there by leading to a number of destructive processes causing cellular damage (Hafidh *et al*., 2009). Researchers have been reported that convolvulaceae member like *Ipomoea aquatic* and *I. batatas* L. was showed potent antioxidant properties in their leaves and shoots. Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect including antioxidant , free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc. (Aher *et al*., 2009). The chloroform and ethyl acetate extracts of the aerial part of *Marsilea quadrifolia* have been reported profound antibacterial, cytotoxic and antioxidant effects (Ripa *et al*., 2009). The free radical scavenging activity of the 70% aqueous methanol and ethyl acetate extracts of *Annona senegalensis* leaves is due to their constituents of polyphenols (Potchoo *et al*., 2008). In the present study, methanol and ethyl acetate extracts from *M. emarginata* were found to be showed better antioxidant activities than other extracts.

Alpha-amylase inhibitors are drug-design targets for the development of compounds for treatment of diabetes, obesity and hyperlipaemia (Franco *et al*., 2000). Nickavar and Mosazadeh (2009) reported that three *Morus* species extracts have been exhibited *in vitro* α-amylase inhibitory activity with IC_{50} of 12 mg to 18 mg mL^{-1}. Methanol and hexane extracts of *M. emarginata* were found to be showed *in vitro* α-amylase inhibition with IC_{50} of 104.5 and 133.4 µg mL^{-1}, respectively. These results revealed that the IC extract has superior α-amylase inhibition activity than other substrates.

Brine shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity and it has been used for the detection plant extract toxicity (McLauglin *et al*., 1991). The use of the brine shrimp assay to screen plant extracts has been used successfully to biomonitor the isolation of cytotoxic, antimalarial, insecticidal and antifeedent compounds from plant extracts (Alluri *et al*., 2006). Moshi *et al*. (2009) reported that the extracts from different plants exhibited toxicity against brine shrimp larvae with LC_{50} values ranging from 15.35-374 μg mL^{-1}, ethyl acetate and dichloromethane extracts have been showed more cytotoxicity. In the present study, ethyl acetate extract from *M. emarginata* was showed better cytotoxicity effect than other extracts. Only few reports are available on biological activities of *Merremia* species. Austin (2007) reviewed the uses of *Merremia dissecta* (Convolvulaceae), the plant has been used for the treatment of inflammations, itching, snake bites and as intoxicant. The extracts of *M. emarginata*, belongs to the same family have not been reported previously for biological properties.

CONCLUSION

In traditional medicinal system, different parts of *M. emarginata* have been mentioned to be therapeutically used as deobstruent, diuretic, for cough, headache, neuralgia and rheumatism. The present study revealed that the ethyl acetate (IB) and methanol extracts (IC) of *M. emarginata* were found to be showed promising biological activities. Methanol extract of this plant might be use full for antioxidant and antidiabetic activities with minimal toxicity.

ACKNOWLEDGMENT

The authors wish to thank Dr. G. Trimurthulu, Vice-President, Laila Impex R and D Centre, Vijayawada, for his help in biological activity studies.

REFERENCES

Aggarwal, B.B. and K.B. Harikumar, 2008. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int. Biochem. Cell Biol., 76: 1590-1611.

Aher, A.N., S.C. Pal, S.K. Yadav, U.K. Patil and S. Bhattacharya, 2009. Antioxidant activity of isolated phytoconstituents from *Casuarina equisetifolia* frost (Casuarinaceae). J. Plant Sci., 4: 15-20.

Al-Fatimi, M., M. Wurster, G. Schroder and U. Lindequist, 2007. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J. Ethnopharmacol., 111: 657-666.

Alluri, V.K., T.V.N. Rao, D. Sundararaju, M. Vanisree, H.S. Tsay and G.V. Subbaraju, 2006. Biological screening of medicinal plants collected from eastern ghats of India using *Artemia salina* (Brine Shrimp Test). Int. J. Applied Sci. Eng., 4: 115-125.

Arora, S., K. Kaur and S. Kaur, 2003. Indian medicinal plants as a reservoir of protective phytochemicals. Teratogenesis Carcinogenesis Mutagenesis, 1: 295-300.

Austin, D.F, 2007. *Merremia dissecta*, (Convolvulaceae): Condiment, medicine, ornamental and weed: A review. Econ. Bot., 61: 109-120.

Bernfeld, P., 1955. Amylases Alpha and Beta. In: Methods in Enzymology, Colowick, S.B. and N.O. Kaplan (Eds.). Academic Press, New York, pp: 149-153.

Bhandari, M.R., N. Jong-Anurakkun, G. Hong and J. Kawabata, 2008. A-glucosidase and α-amylase inhibitory activities of nepalese medicinal herb. Food Chem., 106: 247-252.

Chatterjee, A. and S.C. Prakashi, 1995. The Treatise on Indian Medicinal Plants. Vol. 4, NISC/CSIR, Govt. of India, New Delhi, ISBN: 81-7236-115-7, pp: 148-149.

Chatterjee, I., A.K. Chakravarty and A. Gomesa, 2006. Daboia russellii and Naja kaouthia venom neutralization by lupeol acetate isolated from the root extract of Indian sarsaparilla Hemidesmus indicus R. Br. J. Ethnopharmacol., 106: 38-43.

Cox, P.A, 1994. The ethnobotanical approach to drug discovery: strengths and limitations. Ciba Found. Symp., 185: 25-36.

Cragg, M.G. and D.J.Newman, 2007. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod., 70: 461-477.

Da Silva, M.C.M., L.V. Mello, M.V. Coutinho, D.J. Rigden, G. Neshich. and M.F. Grossi-de-Sa *et al*., 2004. Mutants of common bean alpha-amylase inhibitor-2 as an approach to investigate binding specificity to alpha amylase pesq. Agropec. Bras. Brasilia., 39: 201-208.

Fabricant, D.S. and N.R. Farnsworth, 2001. The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect., 109: 69-75.

Franco, O.L., D.J. Riggen, F.R. Melo, C. Bloch, C. Silva and M.F. Grossi, 2000. Activity of wheat α-amylase inhibitors towards bruchid α-amylases and structural explanation of observed specificities. Eur. J. Biochem., 267: 2166-2173.

Gordon, M.C. and J.N. David, 2005. Plants as a source of anti-cancer agents. J. Ethnoharmacol., 100: 72-79.

Hafidh, R.R., A.S. Abdulamir, F. Abu Bakar, F. Abas, F. Jahanshiri and Z. Sekawi *et al*., 2009. Antioxidant research in Asia in the period from 2000-2008. Am. J. Pharmacol. Toxicol., 4: 48-66.

Harvey, A.L., 1999. Medicines from nature: Are natural products still relevant to drug discovery? Trends Pharmacol. Sci., 20: 196-198.

Jamieson, A.D., K.M. Pruitt and C.R.C. Caldwell, 1969. An improved amylase assay. J. Dental Res., 18: 483-483.

Kingston, D.G.I., 2009. Tubilin-interactive natural products as anticancer agents. J. Nat. Prod., 72: 507-515.

McCord, J.M. and I. Fridovich, 1969. Superoxide dismutase: An enzymic function for reythrocuprein (Hemocuprein). J. Biol. Chem., 244: 6049-6055.

McLaughlin, J.L., C.J. Chang and D.L. Smith, 1991. Bench Top Bioassay for the Discovery of Bioactive Natural Products: An Update. In: Studies in Natural Product Chemistry, Rahman, A.U. (Ed.). Elseiver, UK., pp: 383-409.

McLaughlin, J.L., C.J. Chang and D.L. Smith, 1993. Simple bench-top bioassay (brine shrimp and potato discs) for the discovery of plant antitumor compounds. Am. Chem. Soc. Sympos. Ser., 534: 112-134.

McLaughlin, J.L., L.L. Rogers and J.E. Anderson, 1998. The use of biological assays to evaluate botanicals. Drug Inform. J., 32: 513-524.

Moshi, M.J., E. Innocent, P.J. Masimba, P. Otieno, M.K. Lynes, Meachem, A. Hamilton and I. Urassa *et al*., 2009. Antimicrobial and brine shrimp toxicity of some plants used in traditional medicine in Bukoba District, north-western Tanzania. Tanzania J. Health Resh., 11: 23-29.

Nickavar, B., L. Abolhasani and H. Izadpanah, 2008. Alpha-Amylase inhibitory activities of six *Salvia species*., Iran. J. Pharma. Res., 7: 297-303.

Nickavar, B. and G. Mosazadeh, 2009. Influence of three *Morus*, species extracts on α-amylase activity. Iran. J. Pharma. Res., 8: 115-119.

Potchoo, Y., I.P. Guissou, M. Lompo, E. Sakie and B. Yaro, 2008. Antioxidant activity of aqueous methanol and ethyl acetate extract of leaves of *Annona senegalensis* pers from togo versus the one originates from burkina faso. Int. J. Pharmacol., 4: 120-124.

Pullaiah, T., V. Rama Krishna, S. Sandya Rani and P.N. Rao, 2000. Systematic Enumeration: Flora of Guntur District. Regency Publications, New Delhi, ISBN: 81-87498-16-1, pp: 243-244.

Ripa, F.A.L. Nahar, M.H. and M.M. Islam, 2009. Antibacterial, cytotoxic and antioxidant activity of crude extract of *Marsilea quadrifolia*. Eur. J. Sci. Res., 33: 123-129.

Satyavati, G.V., A.K. Gupta and T. Neeraj, 1987. Medicinal Plants of India. Vol. 2, India Council of Medical Research, New Delhi.

Szabo, M.R., C. Iditoiu, D. Chambre and A.X. Lupea, 2007. Improved DPPH determination for antioxidant activity spectrophotometric assay. Chem. Pap., 61: 214-216.

Vinson, J.A., Y.A. Dabbagh, M.M. Serry and J. Jang, 1995. Plant flavonoids, especially tea flavonoids are powerful antioxidants using an *in vitro* oxidation model for heart disease. J. Agric. Food Chem., 43: 2800-2802.

Willet, W., 1994. Diet and health-what should we eat. Science, 264: 532-537.

Wang, R., J.S. Yang. L.I. Chen and Y. Zheng, 2006. Anti-HIV-1 activities of extracts from the medicinal plant Rhus Chinensis. J. Ethnopharmacol., 105: 269-273.