Research Article

Antioxidant and antibacterial activity of *Hygrophila spinosa* T Anders root extracts

P. Venugopalan*, C. N. Nimisha

Department of Chemistry,

Sree Neelakanta Government Sanskrit College Pattambi, (Affiliated to the University of Calicut) Kerala- 679306, India

Received: 15 March 2019 Revised: 30 April 2019 Accepted: 6 May 2019

Abstract

Objective: In recent years, medicinal plants used in folk medicine represent a suitable approach for the development of new drugs. In this context, herein we report the antioxidant and antibacterial activity of the extracts of the roots of medicinal plant *Hygrophila spinosa* T Anders **Materials and methods:** The roots of *Hygrophila spinosa* were extracted with n-hexane, dichloromethane and ethyl acetate. A few common compounds in the extracts were identified by GC MS analysis. Antioxidant activity of the crude extracts was compared by measuring DPPH radical scavenging activity, super oxide radical scavenging activity and lipid peroxidation inhibitory activity. The antibacterial properties of the extracts were tested against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) by disc diffusion method. **Results:** The antioxidant activity of *Hygrophila spinosa* root extracts were established by different methods. The extracts showed moderate to good antibacterial activity against both Gram-positive and Gram-negative tested organisms. **Conclusions:** The studies presented here reveal the antioxidant and antibacterial activity of *Hygrophila spinosa* root extracts.

Keywords: *Hygrophila spinosa* T Anders, antioxidants, antioxidant activity, DPPH, medicinal plants, antibacterial agents

Introduction

Antioxidants and antibacterial agents have considerable importance in modern medicine. During the cellular respiration, oxygen generates energy in the form of ATP and some free radicals called Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) as the by-products. These reactive species play a dual role in human as both toxic and beneficial compounds. At low or moderate levels, reactive species exert beneficial effects on cellular redox signaling and immune function, but at high concentrations, they produce oxidative stress, a harmful process that can damage cell function and structures (Valko et al., 2007; Saiket and Chakraborty, 2011). The delicate balance between their two opposite effects is undoubtedly a key aspect of life. Free radicals induced oxidative stress is now believed to be a fundamental mechanism underlying a number of human cardiovascular, neurologic and other disorders. It has been estimated that approximately 5% of inhaled oxygen is converted into several damaging ROS (Zhu et al., 2002). These ROS may oxidize proteins, lipids or DNA and can initiate degenerative diseases. Antioxidants that can trap free radicals are our crucial defense against free radical induced damage, and they can reduce the risk for many chronic diseases. Owing to various side effects of synthetic antioxidants, naturally occurring antioxidants have considerable importance in medicine and in food processing. Determination of antimicrobial effectiveness against specific organism is essential in clinical therapy and naturally occurring antibacterial agents recently gained considerable attraction as they are harmless and safer than synthetic drugs (Chen et al., 2007).

Hygrophila spinosa T Anders, belonging to the family *Acanthaceae*, widely distributed on marshy low lands throughout India is a promising medicinal plant (Figure 1) and its medicinal values are well documented in *Ayurveda* (Sharma et al., 2002; Kirtikar and Basu, 2005). The different parts of this plant are used for various disorders, which include anasaraca, diseases of the urinogenital tract, dropsy

Address for Corresponding Author:

P. Venugopalan

Department of Chemistry

Sree Neelakanta Government Sanskrit College Pattambi

(Affiliated to the University of Calicut) Kerala- 679306. India

E-mail: venugpamrita@gmail.com

DOI: https://doi.org/10.31024/ajpp.2019.5.5.15

Figure 1. *Hygrophila spinosa*

from chronic Bright's disease, hyperdipsia, vesical calculi, flatulence, diarrhea, dysentery, leucorrhea, gonorrhea, asthma, blood diseases, inflammation, painful micturition, menorrhagea (Patra, 2009). The plant contains various groups of phytoconstituents, namely, phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, alkaloids, amino acids, carbohydrates, hydrocarbons, flavonoids, terpenoids, vitamins, and glycosides (Kshirsagaret al., 2010). In view of high degree of bioactivity shown by *Hygrophila spinosa* , in this communication we report the antioxidant activity and antibacterial activity of *Hygrophila spinosa* root extracts.

Materials and Methods

Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH), nicotinamide adenine dinucleotide (NADH), butylated hydroxyl toluene (BHT), trichloroacetic acid (TCA), nitro blue tetrazolium (NBT), phenazinemethosulphate (PMS) and thiobarbituric acid (TBA) were procured from Sigma Aldrich, USA. Solvents and other chemicals used were of analytical reagent grade.

Extraction of roots

The extracts of *Hygrophila spinosa* roots were prepared as per the general procedure given below. The roots were collected from matured plants, cleaned and dried under shade for two weeks. It is then well grounded using a domestic mixer grinder. 10g of the powdered roots was stirred with 50 mL of organic solvent for nearly 3 hours, using a magnetic stirrer, in an Erlenmeyer flask. The solution was decanted and the residue was again extracted twice with the same solvent. The extracts were combined together and concentrated in a rotary evaporator. The dried mass is collected and used as such for analysis. The solvents used are dichloromethane, ethyl acetate and n-hexane.

GC-MS analysis

The GC-MS analysis was carried out at SAIF IIT Madras, Chennai using a gas–chromatography (make–Agilent, 7890) coupled with mass spectrometer (Joel AccuTOFGCV4G). It is a combined analyser (GC HRMS) that has a superior ability in analyzing organic compounds both quantitatively and qualitatively. The components were separated on Rtx – 5MS quartz capillary column (60m x 0.25mm) with diphenyldimethylpolysiloxane stationary phase. The temperature increased to 180°C at a rate of 10°C/min and kept for 4 minutes, then with 15°C/min to 300°C and kept for 20 minutes. Sample injection volume was 0.3 ML with a split ratio 1:20, runtime 35 minutes and pressure at the column inlet 163kPa with Helium carrier gas at 1.21 ml/min flow rate. Compounds were identified by comparison of mass spectra with database of National Institute Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Determination of DPPH free radical scavenging activity

DPPH is a well known radical to monitor chemical reactions involving radicals and recently it is most widely used for antioxidant assay (Mensoret al., 2001) When a solution of DPPH (1) having a strong absorption at 517 nm is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form of DPPH (2) which can be monitored by measuring the absorbance at 517 nm. Lower absorbance at 517 nm represents higher DPPH scavenging activity.

In this method, 0.2 mL of extract solution in ethanol (95 %) at different concentrations (0.2, 0.5, 0.8 and 1.2 mg mL^{-1}) was added to 8 mL of 0.004 % (w/v) stock solution of DPPH in ethanol (95 %). The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm until the reaction reached the steady state, using a UV–VISIBLE spectrometer (UV-160A, Shimadzu Co., Japan). All determinations were performed in triplicate. The DPPH radical scavenging activity ($S\%$) was calculated as:

$$S\% = ((A_{control} - A_{sample})/A_{control}) \times 100$$

Where, $A_{control}$ is the absorbance of the blank control (containing all reagents except the extract solution) and A_{sample} is the absorbance of the test sample. Radical scavenging potential of the extracts were compared from EC_{50} value, which represents the sample concentration at which 50% of the DPPH radicals scavenged.

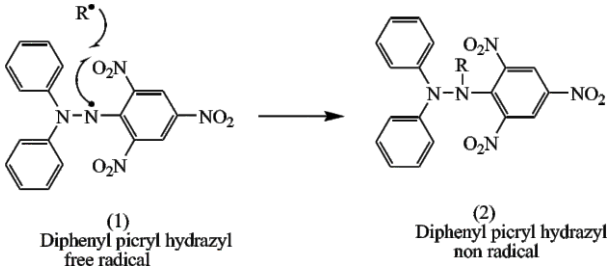

Figure 2. Radical and non radical forms of DPPH

Determination of Super oxide radical scavenging activity

The super oxide scavenging ability was assessed by reported method (Fontana et al., 2001).The reaction mixture contained NBT (0.1 mM) and NADH(0.1 mM) with or without sample to be assayed in a total volume of 1 ml of Tris–HCl buffer (0.02 M, pH 8.3). The reaction was started by adding PMS (10 µM) to the mixture, and change in the absorbance was recorded at 560 nm every 30 s for 3 min. The percent inhibition was calculated against a control without test sample. Radical scavenging potential of the extracts were compared from EC_{50} value, which represents the sample concentration at which 50% of the radicals scavenged.

Determination of Lipid peroxidation inhibitory activity

Lipid peroxidation inhibitory activity was assessed according to reported method (Ruberto et al., 2000). In this method, egg lecithin (3 mg/ml phosphate buffer, pH 7.4) was sonicated for 3 minutes .The test samples (10–100µg/ml) were added to 1ml of liposome mixture, control was without test sample. Lipid peroxidation was induced by adding 10µl $FeCl_3$ (400 mM) and 10µl-ascorbic acid (400 mM). After incubation for 1 h at 37^0C, the reaction was stopped by adding 2 ml of 0.25N HCl containing 15% TCA and 0.375%TBA and the reaction mixture was boiled for 15 min then cooled, centrifuged and absorbance of the supernatant was measured at 532 nm. Inhibitory activity of the extracts were compared from EC_{50} value, which is sample concentration inhibited 50% of lipid peroxidation.

Determination of antibacterial activity

The antibacterial activity of the extracts were analysed by the disc diffusion method and broth dilution methods (Cruickshank et al., 1975; Collins, 1976). Standard inoculums (1–2 x 10^7 CFU/ml 0.5 McFarland standards) were spread onto the surface of sterile agar plates. The discs measuring 6 mm in diameter were prepared using Whatman No.1 filter paper and were sterilized by dry heat at 140^0C for 1h. The sterile discs previously soaked in a known concentration of the test compounds were placed in the nutrient agar medium. Ciprofloxacin (30µg) was used as positive control, while the disc poured in DMSO was used as negative control. The plates were inverted and incubated for 24 h at 37^0C. The susceptibility was assessed on the basis of the diameter of the zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. Minimum inhibitory concentrations (MICs) were determined by the broth micro dilution method. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 5x 10^5 CFU/ml of actively dividing bacteria cells. The cultures of the bacterial strains were incubated for 24 h at 37^0C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). To obtain the minimum bacterial concentration (MBC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of CFU was counted after 24 h of incubation at 35^0C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculum were killed.

Statistical analysis

All antibacterial and DPPH scavenging assays were run in triplicate. The results obtained are expressed as mean value ± standard error of the mean (SEM) in tables 1 and 2. The Student's T-test was used to compare results between the two assays using T. TEST function available in Microsoft Excel by assuming two-sample equal variance (homoscedastic) with one-tailed distribution. Probability values lower than 0.05 ($p < 0.05$) were obtained in all cases.

Results and Discussion

The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The common components in the extracts identified are myristic acid, palmitic acid, stearic acid, linoleic acid, lupenone, lupeol, stigmasterol, betulin, oleic acid, hentriacontane, glucuronic acid, asteracanthine,

Table 1. Antioxidant activity of compounds

Compounds	EC_{50} value [a] ($\mu g ml^{-1}$)		
	DPPH radical scavenging activity	Super oxide radical scavenging activity	Lipid peroxidation inhibitory activity
BHT (1)	10 ± 0.3	190 ± 3.1	85 ± 2.3
n-Hexane extract (2)	72 ± 4.2	262 ± 2.2	110 ± 2.4
Dichloromethane extract (3)	33 ± 3.2	256 ± 2.3	92 ± 2.2
Ethyl acetate extract (4)	26 ± 3.2	226 ± 2.2	74 ± 2.2

[a]Each value represents mean of three different observations ± SEM.

Table 2. MIC and MBC results of extracts with positive control ciprofloxacin

Extracts	Gram positive bacteria *Staphylococcus aureus* (ATCC 25923)		Gram negative bacteria *Escherischchia coli* (ATCC 25922)	
	MIC	MBC	MIC	MBC
n-Hexane extract	25	45	27	25
Dichloromethane extract	40	50	42	50
Ethyl acetate extract	38	45	52	62
Standard ciprofloxacin	9	16	11	19

MIC (µg/ml) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of bacteria completely;
MBC (µg/ml) = minimum bacterial concentration, i.e., the lowest concentration of the compound for killing the bacteria completely.

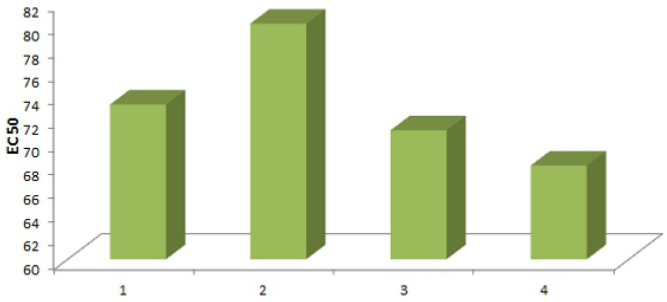

Figure 3. Comparison of EC_{50} value of extracts and BHT for DPPH radical scavenging. (1: Dichloromethane; 2: n-Hexane; 3: Ethyl acetate; 4: BHT)

asteracanthicine, phenyl alanine and lysine.

Antioxidant activity of the crude extracts was compared by measuring DPPH radical scavenging activity, super oxide radical scavenging activity and lipid peroxidation inhibitory activity. The concentration of extract required to produce 50% scavenging activity (EC_{50} value) suggest that the ethyl acetate extract is having maximum radical scavenging activity and it is comparable with that of BHT. The n-hexane extract produced the same effect at higher concentrations. It is probably due to lower content of phenolic compounds in the extract. Comparisons of the results in DPPH radical scavenging activity are presented in figure 3.

The extracts were screened for their antibacterial activity against *Staphylococcus aureus* (ATCC 25923) and *Escherischchia coli* (ATCC 25922) bacterial strains by disc diffusion method. Ciprofloxacin (30 µg) was used as positive control, while the disc poured in DMSO was used as negative control. Inhibition zones were measured and compared with the controls. The MIC and MBC are given in table 2.The antibacterial screening data revealed that all tested extracts showed moderate to good antibacterial activity against both Gram-positive and Gram-negative tested organisms.

Conclusions

The potential antioxidant activity of *Hygrophila spinosa* root extracts was established by measuring DPPH radical scavenging, super oxide radical scavenging and lipid peroxidation inhibitory activities. Qualitative results reveal that ethyl acetate extract has the maximum antioxidant activity. It is probably due to the greater percentage of phenolic compounds in ethyl acetate extract which is more polar than the other solvents used in this study. Owing to the natural abundance of *Hygrophila spinosa* and its wide pharmacological activities, further studies are required to isolate various phytochemicals from different parts of the plant and screening of each component for different biological activities. The extracts were screened in vitro against *S. aureus* and *E. coli*. These studies presented here indicated antibacterial activity of the *Hygrophila spinosa* root extracts.

Acknowledgements

We thank SAIF IIT Madras for GC MS analysis.

Conflicts of interest

The authors declare no conflicts of interest.

References

Chen JC, Yeh JY, Chen PC, Hsu CK. 2007. Phenolic Content and DPPH Radical Scavenging Activity of Yam-Containing Surimi Gels Influenced by Salt and Heating. Asian Journal of Health and Information Sciences, 2:1-11.

Collins AH. 1976. Microbiological Methods, pp. 345-356, London, Butterworth.

Cruickshank R, Duguid JP, Marmion BP, Swain RHA. 1975. Medical Microbiology, Vol. 2, pp. 196–202, London. Churchill Livingstone.

Fontana M, Mosca L, Rosei MA. 2001. Interaction of enkephalines with oxyradicals. Biochemical Pharmacology, 61:1253-1257.

Kirtikar KR, Basu BD. 2005. Indian Medicinal plants, Vol III, pp. 667-700, Dehradun, International Book Distributors.

Kshirsagar AD, Ingale KG, Vyawahare NS, Thorve VS. 2010. *Hygrophila spinosa*: A comprehensive review. Pharmacognosy Review, 4(8):167-171.

Mensor LL, Menezes FS, Leitao GG, Reis AS, Santos TCD, Coube CS. 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research, 15(2):127-130.

Patra A, Jha SH, Murthy PN. 2009. Phytochemical and pharmacological potential of *Hygrophila spinosa* T. Anders. Pharmacognosy Review, 3:330-341.

Ruberto G, Baratta MT, Deans SG, Dorman HJ. 2000. Planta Medica, 66: 687-694.

Saiket S, Chakraborty R. 2011.The Role of Antioxidants in Human Life; In Oxidative Stress: Diagnostics, Prevention and Therapy. Andreescu S, et al. (Eds.), ACS Symposium series. Washington DC, American Chemical Society.

Sharma PC, Yelne MB, Dennis TJ. 2002. Database on Medicinal Plants Used in Ayurveda, Vol 4, pp753-765. New Delhi, Central Council for Research in Ayurveda and Siddha.

Valko M, Leibfrits D, Moncol J, Cronin MTD, Mazur M, Telser J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry & Cell Biology, 39:44-84.

Zhu QY, Hackman RM, Ensunsa JL, Holt RR, Keen CL. 2002. Antioxidant activities of Oolong Tea. Journal of Agricultural and Food Chemistry, 50:6929 – 6934.