



Antioxidant and antibacterial activity of *Berberis tinctoria* root

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Abstract

Herbs have always been the principle form of medicine in developing nations and presently they are becoming popular throughout the developed world as people strive to stay healthy in the face of chronic stress and to treat illness with medicines that work in concert with body's own defences. The aim of the present study was to evaluate the antioxidant and antibacterial potential of *Berberis tinctoria* root. Plant material collected and extracted with various solvents. Different concentrations of extracts were used to evaluate the potential. *Berberis tinctoria* root at a concentration of 1000µg/ml shows high antioxidant activity and relatively all extracts possessing strong to moderate antibacterial activity. In addition, during phytochemical screening, we got saponins and sterols from its root, when extracting with organic solvents. Thus, root extract of *Berberis tinctoria* might be good candidate for the synthesis of antibacterial drugs in the future.

Keywords: *Catharanthus roseus*, Antimicrobial activity and Phytochemical analysis.

Introduction

Plants have been part and parcel of human life since time immemorial and the history of drugs is intricately interwoven with plants from the ancient times. Herbs have always been the principle form of medicine in developing nations and presently they are becoming popular throughout the developed world as people strive to stay healthy in the face of chronic stress and to treat illness with medicines that work in concert with body's own defences. The earliest mention of the medicinal use of plants has been found in 'Rig Veda', which was written between 4000 and 1600 B.C. The all five traditional systems of medicine viz, Ayurveda, Siddha, Unani, Tibetan and Homeopathy (Baruah et al, 1984) mentions about - 2000 plant species of medicinal value.

Medicinal plants have curative properties due to the presence of various complex chemical substances of

different composition, which are found as secondary plant metabolites in one or more parts of these plants. These plant metabolites according to their composition are grouped as alkaloids, glycosides, corticosteroids, essential oils etc. The alkaloids form the largest group, which includes morphine and codein, strychnine and brucine, cocaine etc. Glycosides form another important group represented by igoxin, stropanthin, barbolin, sannocides etc. Corticosteroids have come into prominence recently (Prajapathi et al, 2003). Approximately 119 pure natural compounds extracted from higher plants are used in medicine throughout the world (Farnsworth et al, 1985). Thus the phytochemical screening and extraction of bioactive compounds of therapeutic significance from plants is one of the most intensive areas of medicinal plant research today.

Living organisms have evolved with antioxidant defence system with enzymes to protect against active oxygen and free radicals. Their function are, (a) suppression of generation of ROS, (b) scavenging of ROS, (c) repairing and reconstitution of cell damage and (d) induction of antioxidant proteins and enzymes (Noguchi et al, 2000)

However, imbalance occurs between oxidants and antioxidants when the free radicals are produced excessively from endogenous or exogenous sources. This is known as oxidative stress that leads to damage in biomolecules resulting in various disease. Increased oxidative stress, in the absence of an appropriate response from indigenous antioxidant network, leads to the expression of gene products that cellular damage that are ultimately responsible for late diabetic complications (Tiwari, 2001), and also root cause for atherosclerosis and cancer. Malondialdehyde (MDA), a byproduct of lipid peroxidation (LPO), is said to be involved in DNA adduct formation, which contribute to the carcinogenicity and mutagenesis in mammalian cells (Marnet and Tuttle, 1980).

In recent years, several synthetic antibiotics are employed in the treatment of various infectious diseases but researchers are working hard to find a substitute for them as they cause many side effects. The medicinal plants have been proved to be an excellent substitute as they exhibit immense antimicrobial activity due to the presence of biologically active chemical constituents. Over the last 40 years intensive efforts have been made to discover

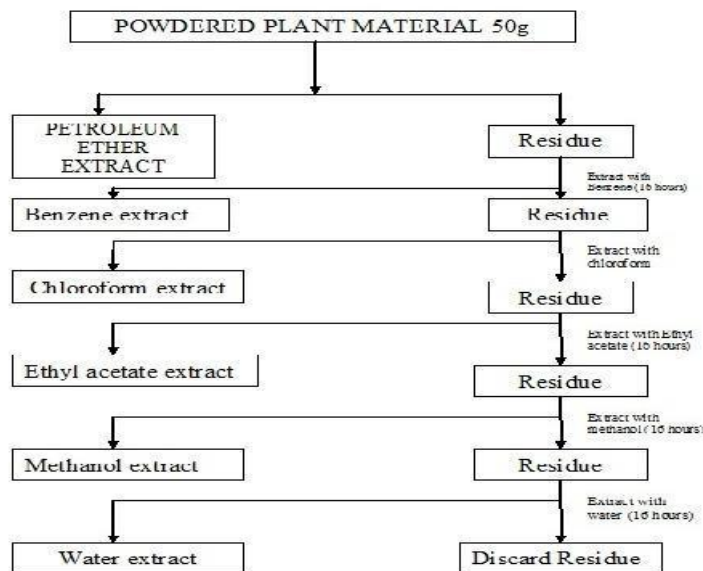
clinically useful antibacterial/antifungal drugs (Odebiyi and Sofawora, 1979; Valsaraj et al., 1996; Kudi 1999; Perumalswamy and Ignacimuthu, 2000).

Phytochemical studies on medicinal plants are required for the following reasons. (i) phytochemical information on a species of medicinal plant in essential basis for a fine chemical analysis to be followed by *invitro* and clinical studies. (ii) Almost every species of medicinal plants contains more than one active compound and it is necessary to know this composition before other studies were undertaken. (iii) A phytochemical survey would provide information on the distribution of certain chemical compounds in different species, to offer a wider choice of material for the work of other scientists (Rao et al., 1985). Therefore, the present study was aimed to detect the presence of active compounds and evaluation of antioxidant and antibacterial potential of *Berberis tinctoria*.

Materials and Methods

Preparation of different plant extracts

The powdered medicinal plant material was taken and subjected to successive solvent extraction. The extraction was carried out for 16 hours with the following solvents in the increasing order of polarity. The preparation of plant extract was detailed schematically in fig 1.



Antioxidant activity

The sample was subjected to antioxidant assays using a 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) model (Blois, 1958) and Reducing power model systems. (Oyaizu, 1986).

Test for DPPH Radical Scavenging Activity

Different concentrations (1000µg/ml, 500µg/ml, 250µg/ml, and 125µg/ml) of methanol extract of *B. tinctoria* sample was taken in test tubes and the volume was adjusted to 1ml. Then 5ml of a 0.1mM methanolic solution of DPPH was added, and the tubes were shaken vigorously. They were then allowed to stand at 35°C for 30 minutes. The control was prepared without any sample, and methanol was used for baseline corrections in absorbance (OD) of samples measured at 517nm.

Test for Reducing Power

Different concentration (1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml) of *B. tinctoria* sample was taken in test tubes and the volume was adjusted to 1ml. To that 2.5ml of phosphate buffer (0.2M, H 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was then kept in a 50°C water-bath for 20 minutes. The resulting solution was then cooled rapidly, spiked with 2.5ml of 10% trichloroacetic acid, and centrifuged at 3000rpm for 10 minutes. The supernatant (5ml) was then mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride. The absorbance at 700nm was then detected after reaction for 10 minutes. The higher the absorbance represents the stronger the reducing power.

Antimicrobial susceptibility test

Different concentrations (10mg , 5mg and 2.5mg) of the plant extract were made by diluting the extracts in Dimethyl Sulphoxide (DMSO). There are seven pathogenic bacteria was used in this study (i)

Escherichia coli (ii) *Staphylococcus aureus* (iii) *Streptococcus pyogenes** (iv) *Bacillus subtilis** (v) *Klebsiella pneumoniae** (vi) *Pseudomonas aeruginosa** (vii) *Salmonella typhi* MTCC 734. In the aforesaid microbes, (* Asterisk) marked pathogens were clinical isolates and procured from the Department of Microbiology, Karpagam Arts and Science College, Coimbatore. The microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi* were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. Antibacterial activity was done using the modification of the method originally described by Bauer et al (1996).

Phytochemical screening tests

Phytochemical screening is done for analysing secondary metabolites which are responsible for curing ailments. The phytochemical screening of the plant extract was carried out by the methods of Trease and Evans (1978) and Harborne (1984). We have analysed alkaloids, steroids and sterols, flavonoids, tannins and phenolic compounds, carotenoids, phlobatannins, cardioglycosides, saponins, fixed oils and fats and terpenoids for *Berberis tinctoria*.

Results and Discussion

The present investigation encompasses antioxidant and antibacterial activity of *Berberis tinctoria*.

Antioxidant assay

Reduction of the DPPH radicals can be observed by the decrease in absorbance at 517nm. The scavenging capacity of the extract was found to be 69.36 ± 0.309 with the IC₅₀ being 144.54 ± 0.023 (Table 1).

Table-1: Antioxidant activity of methanol extract of *Berberis tinctoria* (root) DPPH assay

Concentration (µg/ml)	Inhibition (%)	IC 50 (µg/ml)
1000	69.36 ± 0.309	144.54 ± 0.023
500	61.36 ± 0.601	
250	54.43 ± 0.917	
125	48.13 ± 0.873	

The reducing power of *Berberis tinctoria* sample is shown in table 2. At a concentration of 1000 µg/ml, relatively high reducing power was observed. The

reducing power was increased by increasing the sample concentration. The reducing power of the extract was found to be 0.567 ± 0.0348543 (Table 2).

Table-2 : Reducing power test of methanol extract of *B. tinctoria* (root)

Concentration (µg/ml)	O.D at 700 nm Mean ± SD
Control	0.0616 ± 0.002
125 µg	0.0873 ± 0.040
250 µg	0.1730 ± 0.024
500µg	0.4460 ± 0.046
1000µg	0.567 ± 0.0348

Antibacterial activity

The antibacterial activity of *Berberis tinctoria* sample is showed in Table 3. The sample showed significant antibacterial activity against *E.coli*, *B.subtilis*, *S.pyrogen*, *S.aureus* at all the three concentrations

(10mg/ml, 5mg/ml and 2.5mg/ml), while it showed moderate activity against *P. auroginosa*, *K. pneumoniae* at only one concentration (10mg/ml). Antibacterial activities are indicated by clear zone of inhibition.

Table -3: Antimicrobial activity of different extracts of *B.tinctoria* (root)

Microorganism	Zone of inhibition (mm)															
	Petroleum Ether			Chloroform			Ethyl Acetate			Methanol			Benzene			Chloram-phenicol
	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	
	mg/ml			mg/ml			mg/ml			mg/ml			mg/ml			30
<i>B.subtilis</i>	9	7	6	11	10	9	11	10	7	8	7	6	8	7	6	14
<i>S.aureus</i>	9	8	7	15	13	10	11	7	6	16	8	6	10	8	6	13
<i>S.pyogenes</i>	13	11	8	12	9	7	9	8	5	15	11	9	14	9	7	14
<i>K.pneumoniae</i>	10	8	9	11	10	9	10	9	8	12	9	8	12	8	7	34
<i>E.coli</i>	14	11	8	12	10	8	9	7	6	16	8	6	12	11	10	25
<i>S.typhi</i>	9	8	7	10	8	6	10	9	7	13	10	8	16	15	8	17
<i>P.aeruginosa</i>	12	9	7	9	8	6	10	9	7	9	7	6	12	11	10	33

Phytochemical screening

The petroleum ether extract of *B.tinctoria* root comprises steroids and sterols, fixed oils and fats, saponins. The test for alkaloids, tannins and phenolic compounds, carotenoids, amino acids and proteins, carbohydrates, flavanoids, anthracene glycosides showed negative results. Benzene extract of the root showed the presence of tannins and phenolic compounds, steroids and sterols, fixed oils and fats, saponins, amino acids and proteins, carbohydrates. The tests for alkaloids, carotenoids, flavanoids, and anthracene glycosides showed negative results. The chloroform extract of *B.tinctoria* root consist of

steroids and sterols, fixed oils and fats, saponins. The test for alkaloids, tannins and phenolic compounds, carotenoids, amino acids and proteins, carbohydrates, flavanoids, anthracene glycosides showed negative results. The Ethyl acetate extract of the root consist of showed the presence of gelatin, fixed oils and fats. The rest of the chemical components were found to be absent. The methanol extract of *B.tinctoria* root comprises tannins and phenolic compounds, steroids and sterols, fixed oils and fats, saponins, carbohydrates are presents. The test for alkaloids, carotenoids, amino acids and proteins, flavanoids, anthracene glycosides showed negative results.

Table-4: Results of phytochemical screening

Plant Name	Part Used	Extract Name	AL	TP		ST	OF	CA	SA	AA	CH	FA	AG
				GE	LE								
<i>Berberis tinctoria</i>	Root	PE	–	–	–	+	+	–	+	–	–	–	–
		BE	–	+	+	+	+	–	+	+	+	–	–
		CH	–	–	–	+	+	–	+	–	–	–	–
		EA	–	+	–	–	+	–	–	–	–	–	–
		ME	–	+	+	+	+	–	+	–	+	–	–

PE=Petroleum ether BE=Benzene CH=Chloroform EA=Ethyl acetate ME=Methanol +=Present -=Absent	Al=Alkaloids TP=Tannins and phenolic compounds GE=Gelatin LE=Lead acetate ST=Steroids and sterols	OF=Fixed oils and fact CA=Carotenoids SA=Saponins AA=Amino acids & Protein CH=Carbohydrate FA=Flavanoids AG=Anthraacene glycosides
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Conclusion

The pathogenic microorganisms play a vital role in human life. The microbes cause many infections and communicable diseases. Several synthetic antibiotics are employed in the treatment of disease caused by microbial pathogen. But these pathogens are trying to developed resistance to synthetic antibiotics. Hence the present study is an attempt to find out new antimicrobials. Antibacterial activities of *Berberis tinctoria* were determined by agar well diffusion method. Antibacterial activities are indicated by clear zone of inhibition. The *Berberis tinctoria* sample shows most significant activity against pathogenic bacteria. This study proves that *Berberis tinctoria* can be used in the treatment of various life threatening diseases.

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