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Antibacterial, *In vitro* Lipid per Oxidation and Phytochemical Observation on Achyranthes Bidentata Blume

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Abstract: The aim of this study is to evaluate the antibacterial efficacy of Achyranthes bidentata against seven different microorganisms such as *Escherichia coli*, Bacillus subtilin, Proteus vulgaris, Salmonella typhi, Staphylococcus aureus, Pseudomonas species and Klebsiella pneumoniae. The values of zone of inhibition were measured and compared with standard values. All the extracts of root, stem, leaves and flowers showed high sensitive to Proteus, *Escherichia coli*, Bacillus subtilin, Salmonella typhi, Klebsiella pneumoniae, moderate and less sensitivity to Staphylococcus aureus and Pseudomonas species. The antiperoxidative effect of various parts of Achyranthes bidentata was also done. Goat liver was used as the lipid source. This *in vitro* evaluation was done by the measurement of thiobarbituric acid reactive substances (TBARS) in the experimental mixtures of tissue homogenates. The results suggest that the ethanolic extracts of Achyranthes bidentata root and flowers possessed significant *in vitro* lipid peroxidation inhibiting activities, which is possibly attributed to its free radical scavenging properties. All the parts of plant extracts were subjected to qualitative analysis to find out phytoconstituents present in them. Results showed the presence of alkaloids, flavonoids, phenols, and steroids and the absence of saponins, glycosides and tannins.

Key words: Achyranthes bidentata, antibacterial activity; antiperoxidative effect, phytochemical activity, ethanolic extract

Introduction

Plants have been an essential part of human society since the civilization started. Medicinal plants are boon of nature to cure a number of ailments of human beings. Practitioners of ayurveda and Unani system of Medicine regularly employ a large number of Indian medicinal plants as antibiotic agents. In many parts of the world medicinal plants were used against bacterial, virus and fungal infections. Our country represents a storehouse of genetic diversity of plants (Perumal *et al*., 2004).

Over the last 40 years, intensive efforts have been made to discover clinically used antibacterial and antifungal drugs (Sofowora, 1984; Valsaraj *et al*., 1996; Ahmed *et al*., 1998; sardari *et al*., 1998; werner *et al*., 1999; Kudi *et al*., 1999; Perumalsamy *et al*., 1999). In the present study, a plant named Achyranthes bidentata was selected.

Achyranthes bidentata, a member of Amaranthaceae (Tamil name: Sigappu Nayurivi) is an erect, annual herb distributed and grown in hilly districts of India, China, Japan and Java. It is in flower from August to September and the seeds ripen from September to October. It grows in moist soils. The plant is used in indigenous system of medicine as emmenagogue, antiarthritic, antifertility, laxative, ecbolic, abortifacient, antiviral, antispasmodic, antihypertensive, anthelmintic, anticoagulant, diuretic and antitumor (Ratra and Misra, 1970; Anonymous, 1985). Also it is useful to treat cough, renal dropsy, fistula, scrofula, skin rash, nasal infection, chronic malaria, impotence, fever, asthma, amenorrhoea, piles, abdominal cramps and snakebite (Anonymous, 1985; Selvanayagam *et al*., 1994). The roots, leaves and stem are widely used in Chinese medicine (Bown, 1995). The root contains triterpenoids, saponins, sitosterol and sigma sterol (Nguyen Van Dan and Doan Thi Nhu, 1989). The root juice is used in Nepal in the treatment of toothache. This juice is also used in the treatment of indigestion and is considered to be a good treatment for asthma (Manandhar, 2002). The stem of the plant is used as a toothbrush that is said to be good for the health and is also a treatment for pyorrhoea (Manandhar, 2002). The leaves and stems are harvested in the summer and are usually crushed for their juice or used in tinctures (Bown, 1995).

A perusal of literature revealed that the various parts of Achyranthes bidentata had not been subjected to screening for antibacterial and antiperoxidative properties so far.

Hence with respect to above stated information about the Achyranthes bidentata, a present study was carried out The main objective of the present study is to 1) test the antimicrobial activity of various parts of Achyranthes bidentata on different pathogenic micro organisms 2) determine the *in vitro* inhibition of lipid per oxidation in

Table 1: Antibacterial activity (zone of inhibition mm diameter) of various parts of Achyranthes bidentata

Organisms	Control Tetra-cycline	Ethanolic extract of Root		Ethanolic extract of Stem		Ethanolic extract of Leaf		Ethanolic extract of Flower	
		100µg	200µg	100µg	200µg	100µg	200µg	100µg	200µg
Proteus Vulgaris,	21	14	20	15	22	14	20	15	22
Escherichia coli	19	13	18	14	20	13	18	14	21
Bacillus subtilin	25	12	18	13	20	15	20	13	21
Salmonella typhi	21	10	15	11	18	10	16	12	19
Klebsiella pneumoniae	19	08	14	10	15	08	14	10	16
Staphylococcus Aureus	17	08	12	09	12	09	12	10	15
Pseudomonas species	20	07	10	07	10	07	10	08	12

liver homogenate by root, stem, leaves and flower extracts of Achyranthes bidentata and 3) phytochemical observation using the ethanolic extract of various parts of Achyranthes bidentata.

Materials and Methods

Collection of Plant material: The medicinal plant Achyranthes bidentata was selected and the different plant parts like root, stem, leaves and flowers of Achyranthes bidentata was collected from the hills of Ponnuthu village at coimbatore district, Tamilnadu, India and authenticated by the botanist Dr. Aurumugaswamy, Department of Botany, Kongunadu college of Arts and Science, Coimbatore and the voucher specimens of Achyranthes bidentata is preserved in the Department of Biochemistry, Dr. N.G.P Arts and Science college, Coimbatore, India.

Selection of Bacterial strains: The test organisms used were *Escherichia coli*, Bacillus subtilin, Proteus vulgaris, Salmonella typhi, Staphylococcus aureus, Pseudomonas species and Klebsiella pneumoniae. The bacterial strains were obtained from the postgraduate department of Microbiology, Dr. N.G.P Arts and Science College, Coimbatore.

Preparation of plant extract: The collected parts of medicinal plant (root, stem, leaves and flowers) were cleaned and dried under shade for 7 days and crushed into coarse powder. The powder thus obtained (30g) was extracted with ethanol (60-80°C) using soxhlet extractor. The extraction was continued for 10 hours. These extracts are then filtered and kept in oven at 40°C for 24 hours to evaporate the alcohol from it. Dark brown and greenish black residues were obtained. Dissolved 0.2g of these residues in 1ml of ethanol and this solution is used as the extract.

Preparation of Medium

Nutrient agar: Pentone-5g; sodium chloride-5g; Beef extract-3g; Yeast extract-2g; agar-2%; Distilled water-1000ml. Here 7g of nutrient agar was used directly and dissolved in 250 ml of sterile distilled water pH adjusted to 7.2 and the solution was autoclaved at 121°C for 20 minutes.

Antibiotic sensitivity assay (Disc diffusion method): A 100ml of 5-20 hour culture was poured into the nutrient agar media by micropipette. This nutrient agar media is poured into petridish and allowed to solidify. Antibiotic discs were placed on the medium with the help of sterile forceps. These plates were incubated at 37°C for a period of 24 hours. These sensitivity zones around the disc of each organism plate were measured using zone diameter scale. Filter paper discs with normal saline served as the control. Standard antidiabetic disc containing 30 µg of Tetracycline served as the reference (Bauer *et al.*, 1966).

Induction of *In vitro* Lipid Per Oxidation: An *in vitro* model of goat liver homogenate was used for induction of lipid per oxidation, mediated by $FeSO_4$ as pro-oxidant. Application of the relevant Achyranthes bidentata plant root, stem, leaf and flower extract in the medium was tried with an objective of assessing the extent of inhibition of *in vitro* lipid per oxidation by the measurement of thiobarbituric acid reactive substances (TBARS) in the experimental mixtures. TBARS were measured spectrophotometrically at 535nm. The extent of inhibition of lipid peroxidation in the presence and absence of the various extract was determined in liver homogenate as proposed by Okhawa *et al.* (1979).

Phytochemical screening: In determining the presence of phytochemicals in various parts of Achyranthes bidentata, all the extracts was subjected to the tests by Kokate (1994); Trease and Evans (1994). It was found that the major chemical constituents of the extracts were polysaccharide, alkaloids and flavonoids.

Results and Discussion

In the present study, the ethanol extract of various parts of Achyranthes bidentata were selected for antibacterial activity on seven different microorganisms. The antibacterial efficacy of plant extract at 100µg to 200µg concentration is furnished in Table 1. All the extracts have exhibited different degrees of antibacterial activity which are compared with the reference standard Tetracycline.

Depending on the measured values of the complete inhibition diameter of the zone including the disc in

Table 2: Presence /absence of active phytochemical constituents in root, stem, leaves and flowers of Achyranthes bidentata

Ethanolic extracts of plant parts	Alkal-oids	Flavan-oids	Sapon-ins	Carbohy-drates	Protein	Phen-ols	Steroids	Glyco-sides	Resins	Tann-ins	Thiols
Root	+	+	-	+	+	+	+	-	-	+	+
Stem	+	+	-	+	+	+	+	-	-	-	+
Leaf	+	+	-	-	+	+	+	-	-	-	-
Flower	+	+	-	+	+	+	+	-	-	-	-

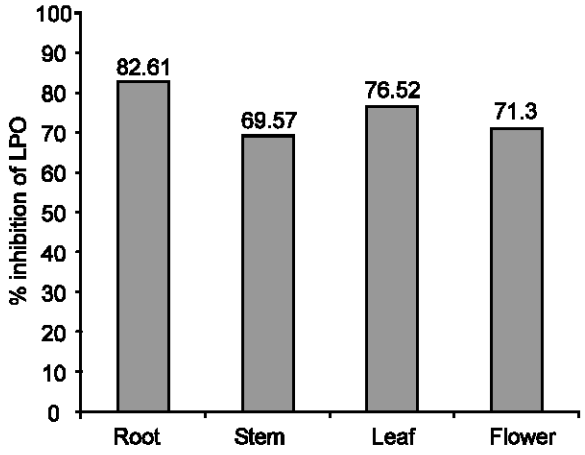

Fig. 1: Extent of inhibition of *in vitro* lipid per oxidation in Goat liver homogenate by the aqueous extracts of various parts of Achyranthes bidentata are statistically presented in Fig. 1

millimeter, the antibacterial activity can be classified into the following types, such as>12 mm zone of inhibition-high sensitive, 9-12 mm zone of inhibition-moderate sensitive, 6-9 mm zone of inhibition-less sensitive, and<6 mm zone of inhibition-resistant (Arora and Bhardwaj, 1997).

In spite of the inhibition of growth at 100µg for all the selected microbes, the inhibition is not significant and moderate sensitivity was found at 100µg. Among the seven micro organism tested, the ethanolic extract of stem and flower exhibited highest inhibition activity of 200µg concentration against Proteus vulgaris and the root showed least inhibition activity against pseudomonas species. These results suggest that the flavonoids found in the tested parts of plant may be responsible for their antibacterial activity. Several types of alkaloid, steroids and proteins have been reported to have antibacterial activity (Barnabas and Nagarajan, 1988; Bader *et al.*, 1987). The antibacterial activity shown by the extract might be also due to some antibacterial substance present in Achyranthes bidentata.

Biological membranes are a rich source of polyunsaturated fatty acids that are susceptible to lipid peroxidation, in the presence of metal ions and other prooxidants (Gutteridge and Halliwell, 1994). Lipid Per Oxidation (LPO) has been identified as one of the basic reactions involved in free radical induced cellular damages (Hallwell and Gutteridge, 1992). Free radicals are constantly being generated in the body through various mechanisms, and are also being removed by endogenous antioxidant defense mechanisms that act by scavenging free radicals, decomposing peroxides, and/or binding with pro-oxidant metal ions (Halliwell, 1990) Antioxidants are a broad group of compounds that destroy Reactive Oxygen Species and protect the body against oxidative damage to cells (Express Pharma Plus, 1999). Lipid peroxidation has been related to the pathophysiology of several diseases like joint injury in rheumatoid patients (Halliwell *et al.*, 1988). some pulmonary and hepatic diseases (Slater and Sawgar, 1971), cancer (Hallwell and Gutteridge, 1992) and toxicity of some drugs e.g. cardiotoxicity of doxorubicin (Luo *et al.*, 1997). So the balance between the pro-oxidant and antioxidant is very important for the survival. All antioxidants generally influence the redox status, thereby protecting cells against reactive oxygen species (ROS) under certain circumstances, while promoting ROS generation in others (Herbert, 1996). Anita and Jeyashree (1999) have reported that many green leafy plants of Indian region are rich sources of antioxidants. In our results the ethanolic extract of root of Achyranthes bidentata showed a significant level of inhibition of *in vitro* LPO by 82.61% inhibition, the extent of inhibition by root and flowers being higher for goat liver homogenate when compared with stem and leaves, proving that the root and flowers offer a good degree of protection against the biological end point of oxidative damage. Khajuria (1997) have reported that the mammalian cells have evolved myriad interrelated antioxidant defense mechanisms, which minimize the injurious events that result from toxic chemicals and normal oxidative products of cellular metabolism.

The result reveals that the presence of alkaloids and flavanoids in roots and leaves provides the maximum inhibition and are presented in Fig. 1. Flavanoid reduces lipid peroxidation by preventing or slowing the onset of cell necrosis and by improving vascularity (Tsuchiya *et al.*, 1996). The extent of inhibition differed with the type of plant part used, indicating that the antioxidants present in the roots, stems, leaves and flowers could react differently towards different lipid groups.

The present findings suggest that root and flowers of Achyranthes bidentata has the potential to reduce pro-oxidant-induced lipid-peroxidation, and thus to increase the therapeutic index of the Achyranthes bidentata by way of reducing toxicity, that may be mediated through free radical mechanisms. However a detailed study is required to conclude such hypothesis.

Phytochemical constituents of Achyranthes bidentata like alkaloids, flavanoids, carbohydrate, steroids, proteins, phenols, tannins, saponins, glycosides and thiols were analyzed by qualitatively and reported in Table 2. The phytochemical screening showed in all case the presence of alkaloids, flavanoids, proteins, phenols and steroids. Saponins, carbohydrates, glycosides, tannins, resins and thiols were absent. The present study confirmed that the root contained high content of phytochemicals compared with all other parts of Achyranthes bidentata. Thus with all the above results, the Achyranthes bidentata present investigation reveals the root exhibited maximum activity which is followed by leaves.

Conclusion: Achyranthes bidentata is used in the treatment of a wide range of disorders. The present study shows the antimicrobial activity of various parts of the plant is highly exhibited by stem and flower. The result of this study also reveals that the root of Achyranthes bidentata has the maximal antiperoxidative effect and contains flavanoids and has a well-defined expectorant, anti-inflammatory, antipyretic, antirheumatic and diuretic activity.

Hence the present study supports the view that this medicinal plant might be useful as antimicrobial agent in the development of novel drugs for many centuries to control diseases. Progression of studies in this medicinal plant will help its use to control diseases and infections.

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