Journal of Experimental Sciences Vol. 2, Issue 3, Pages 04-06 [2011]
www.jexpsciences.com

Regular Article

Antimicrobial and Phytochemical Screening of *Plumbago zeylanica* Linn. (Plumbaginaceae) Leaf

D.A. Dhale[1*] and S.K. Markandeya[2]

[1]Post-Graduate Department of Botany, SSVP Santha's L.K. Dr. P. R. Ghogrey Science College, Dhule-424005 (India); [2]Government Institute of Science, Caves Road, Aurangabad-431004 (India)

ABSTRACT: The antimicrobial effect of *Plumbago zeylanica* Linn. (Plumbaginaceae) leaf extract was evaluated on microbial strains like gram positive species *Staphylococcus aureus*, and *Bacillus subtilis* and gram negative species *Escherichia coli* and *Pseudomonas aeruginosa*. The solvent used for extraction of plant were petroleum ether, chloroform and alcohol. The alcoholic extract of leaves of *Plumbago zeylanica* shows maximum antimicrobial activity. The *in vitro* antimicrobial evaluation was carried out by agar disc diffusion method. The significant antibacterial activity of active extract was compared with standard antibiotic Amphicillin. The samples of leaves were further used for the phytochemical studies. Results of the phytochemical analysis indicated the presence of alkaloids, glycoside, reducing sugars, simple phenolics, tannins, Lignin, saponins and flavonoids. The antibacterial activities of the leaves were due to the presence of various secondary metabolites.

Key words: Antimicrobial, Physico-chemical, Phytochemical, *Plumbago zeylanica*

Introduction

In fact, plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982). Natural products play on important role in drug development programmes in the pharmaceutical industry (Baker et al., 1995). There are a few reports on the use of plants in traditional healing by either tribal people or indigenous community (Sandhy et al., 2006; Ayyanar and Ignacimuthu, 2005; Rajan et al., 2002; Natarajan et al., 1999 and Ignacimuthu et al., 1998). The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem (Austin et al., 1999). Natural products of higher plants may give a new source of antimicrobial agents. There are many research groups that are now engaged in medicinal plants research (Samy et al., 1998; Hamil et al., 2003; Motsei et al., 2003). Silver and Bostian (1993) have documented the use of natural products as new antibacterial drugs. There is an urgent need to identify novel substances active towards highly resistant pathogens (Recio, 1989; Cragg et al., 1997). In an effort to discover new compounds, many research groups screen plant extracts to detect secondary metabolites with the relevant biological activities. In this regard, several simple bioassays have been developed for screening purposes (Hostettmann, 1991).

The present study was carried out on the phytochemical and antibacterial activity of leaf of *Plumbago zeylanica* Linn. (Plumbaginaceae) is a tropical shrub. It grows wild as a garden plant in eastern, northern and southern India and Ceylon. The leaves of *P. zeylanica* are widely used medicinally in India and China. Traditionally, *P. zeylanica* is believed to kill intestinal parasites, and it is used clinically to treat rheumatism, intestinal parasites, anemia due to "stagnant blood", external and internal trauma, toxic swelling and malignant furunculous scabies (Jiangsu, 1979). In India it is usually used to treat fever or malaria. Pharmacological studies have indicated that *P. zeylanica* extract has antiplasmodial (Simonsen et al., 2001), antimicrobial (Ahmad et al., 2000), antifungal (Mehmood *et al.*, 1999), anti-inflammatory (Oyedapo, 1996), antihyperglycemic (Olagunju *et al.*, 1999), hypolipidaemic and antiatherosclerotic activities (Sharma et al., 1991).

Materials and Methods

Sample collection and Authentication

The fresh, mature healthy leaves of *Plumbago zeylanica Linn* (Plumbaginaceae) were collected from were collected from the Government institute of Science, Aurangabad (M.S.) campus. The plant materials were identified using the Flora of Marathwada (Naik, 1998a) at Post graduate Department of Botany, Government institute of Science, Caves Road, Aurangabad (M.S.) India.

Sample preparation

Fully grown leaves of *P. zeylanica* were weighed (1kg). The plant samples were shade dried ground and sieved with 2mm copper sieve to form uniform powder and stored in airtight bottles.

Preparation of extract

The dried plant material was pulverized into fine powder using a grinder (mixer). About 50 g of powdered material was extracted in soxhlet extraction apparatus with 250 ml of each of the following solvents; petroleum ether, chloroform, and alcohol (Vogel, 1988). The extracts obtained with each solvent were filtered through Whatman filter paper No. 1 and the respected solvents were evaporated (at 40ºC) with the help of heating mantle. The sticky greenish-brown substances were obtained and stored in refrigerator for prior to use (Beyer and Walter, 1997). Some of the extracts of each solvent were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods (Harborne, 1984; Trease and Evans, 1987; Ajaiyeoba, 2000; Edeoga et al., 2005). The positive tests were noted as present (+) and absent (-).

Preparation of microorganism

Isolation of bacterial species of Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Echerichia coli* and *Pseudomonas aeruginosa*) takes place. The cultures of these bacteria were checked for purity by doing gram staining and biochemical test and they were grown in nutrient broth at 37°C and maintained in nutrient agar slants at 2-8°C. Nutrient agar medium was used as bacterial culture medium in the antibacterial assays.

Selection of Reference antibiotic

Reference antibiotic Amphicillin was obtained from authorized medical shop Aurangabad (M.S.). The purity of the antibiotic is 99.8%

Dilutions and Inoculum preparations

The dried plant extracts of *P. zeylanica* and antibiotic Amphicillin were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentration of 50, 100mg/ml. The inoculums of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were prepared in nutrient broth medium and kept incubation at 37°C for 8 hours. After growth was observed, the cultures are stored in the refrigerator at 2-8°C for analysis.

** Corresponding Author, Email: datta.dhale@yahoomail.com*

Procedure for performing the Disc Diffusion test (Bayer et al., 1997)

The required amount of Petri plates is prepared and autoclaved at 121˚C for 15 minutes. And they were allowed to cool under Laminar air flow. Aseptically transfer about 20 ml of media into each sterile Petri dishes and allowed to solidify. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded with different concentrations of about 50, 100mg/ml of plant extract of *P. zeylanica* and antibiotic amphicillin into each separate disc of about 100µl. The paper diffuse discs were placed on the medium suitably apart and the plate were incubated at 5˚C for 1 hour to permit good diffusion and then transferred to an incubator at 37˚C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm).

Results and Discussion

Indian systems of medicine such as Ayurveda and Siddha uses majority of the crude drugs that are of plant origin. It is necessary that standards have to be laid down to control and check the identity of the plant an ascertain its quality before use. A detailed pharmacognostic evaluation therefore is highly essential prerequisite (Ramana, 2007). Methanol, chloroform and alcoholic extracts of *P. zeylanica* Linn. leaves were tested against various Gram-negative and Gram-positive bacteria (Table.1). Among the extracts assayed, the alcohol leaf extracts of *P. zeylanica* exhibited good activity against *P. aeruginosa* at 100mg/ml for example, 17 mm was recorded as diameter zone of inhibition. This was followed by 16 mm *E. coli*, 11 mm *B. subtilis* and *Staphylococcus aureus* 10 mm respectively. The least activity 3 mm against *E. coli,* 4 mm and *B.subtilis* at 50mg/ml was recorded by petroleum ether extracts. Activities of the various extracts were comparable to those of standard antibacterial agent Ampicillin.

In the present antimicrobial activity of both plant extract towards drug resistant or clinically significant microbes are reported and it was observed that active constituent of plant material seep out in organic solvent to display biological activity. Further phytochemical studies for identification and elucidation of active constituent in plant material tested in expected to serve as lead in the development of novel bioactive antimicrobial compound.

Phytochemical evaluation

The results of qualitative screening of phytochemical components in leaves of *P. zeylanica* revealed the presence of alkaloids, glycoside, reducing sugars, simple phenolics, tannins, Lignin, saponins and flavonoids presented in Table 2.

Table 1. Antibacterial efficacy of different solvent extracts of *Plumbago zeylanica* leaves

Sr. No.	Microorganism	Strain + / -	Concentration (mg / ml)	Zone of inhibition (mm)			
				Petroleum ether	Chloroform	Alcohol	Amphicillin (40 µg / ml)
1.	*Escherichia coli*	-ve	50	03	06	08	19
			100	05	09	16	
2.	*Pseudomonas aeruginosa*	-ve	50	06	07	12	23
			100	12	06	17	
3.	*Staphylococcus aureus*	+ve	50	05	08	08	21
			100	06	12	10	
4.	*Bacillus subtilis*	+ve	50	04	09	12	20
			100	06	14	11	

Table 2: Phytochemical components of different solvent leaves extracts of *Plumbago zeylanica* leaves

Phytochemical constituents	Petroleum ether	Chloroform	Alcohol
Alkaloids	-	-	+
Coumarins	-	+	-
Glycoside	-	-	+
Reducing sugars	+	-	+
Simple phenolics	-	-	+
Tannins	-	-	+
Lignin	-	-	+
Saponins	-	-	+
Flavonoids	-	-	+
Terpenoid	-	-	-

Acknowledgement

The authors wish to thank Head of Botany Department, Government Institute of Science, Aurangabad for providing the necessary laboratory facilities.

References

Ahmad I., Mehmood Z., Mohammad F. and Ahmad S. 2000. Antimicrobial potency and synergistic activity of five traditionally used Indian medicinal plants. *Journal of Medicinal and Aromatic Plant Sciences* 23: 173–176.

Ajaiyeoba E.O. 2000. Phytochemical and antimicrobial studies of *Gynandropeis gynandra and Buchholzia coriaceae* extracts. Afr. J. Biomed. Res. 3(3): 161-165.

Austin. D.J., Kristinsson. K.G. and Anderson.R.M. 1999. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc Natl Acad Sci USA., 96:1152-6.

Ayyanar. M. and Iganacimuthu. S. 2005. Traditional knowledge of kani tribals in Kouthalai of Tirunelveli hills. Tamil Nadu, India. Journal of Enthopharmacology, 102:246-255.

Baker. J.T., Borris. R.P., Carte B. et al. 1995. Natural products drug discovery and development. New perspective on international collaborations. Nat prod., 58: 1325-1357.

Beyer H. and Walter W. 1997. Organic chemistry, 4th ed. Published by Jerry March, pp. 146-148.

Cragg G.M., Newman D.J. and Snader K.M. 1997. Natural products in drug discovery and development. *Journal of Natural Products* 60: 52–60.

Edeoga H.O., Okwu D.E., and Mbacbie B.O. 2005. Phytochemical constituents of some Nigerian medicinal plants. Afri J Biotech, *Vol.* 4(7): 685-688.

Farombi. E.O. 2003. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. African. J. Biotech., 2: 662-671.

Hamil. F.A., Apio. S., Mubiru. N.K., Bukenya-Ziruba.R., Mosanyo. M., Magangi O. W. 2003. Traditional herbal drugs of southern Uganda, II. Literature analysis and antimicrobial assays. *J Ethnopharmacol*. 84: 57-78.

Harborne J.B. 1984. Phytochemical methods, Chapman Hall, London, pp 100 - 101.

Hostettmann K. 1991. *Assays for bioactivity*. *Methods in Plant Biochemistry,* Academic Press, San Diego.6, 360.

Ignacimuthu. S., Sankarasivaraman.K. and Kesavan. 1998. Medico-ethno botanical survey among Kanikar tribals of mundentherai sanitary fitoterapia. 69:409-414.

Jiangsu. 1979. *New Medical College*, "*Zhongyao Dictionary* (*Encyclopedia of Chinese Materia Medica*), Scientific & Technological Press, Shanghai" 711–712.

Mehmood Z., Ahmad I., Mohammad F. and Ahmad S. 1999. Indian medicinal plants: A potential source of anticandidal drugs. *Pharmaceutical Biology* 37: 237–242.

Motsei. M.L., Lindsey. K.L., Vanstaden. J. and Jaeger. A.K. 2003. Screening of traditionally used South African pants for antifungal activity against Candida albicans. J Ethnopharmacol., 86:235-41.

Natarajan. B., Paulsen. B.S. and Pushpangadan. P. 1999. An Enthopharmacoloical study from the Coimbatore district, Tamil nadu, India. Traditional knowledge compared with modern biological science, pharmaceutical biology., 37: 378-390.

Olagunju J.A., Jobi A.A. and Oyedapo O.O. 1999. An investigation into the biochemical basis of the observed hyperglycaemia in rats treated with ethanol root.

Oyedapo O.O. 1996. Studies on the bioactivity of the extract of *Plumbago zeylanica*. *Phytotherapy Research* 13: 346–348.

Rajan. S., Sethuraman. M. and Mukherjee. P.K. 2002. Ethnobiology of the Niligries Hills, India, phytotherpy-Research., 16: 98-116.

Ramana, P. 2007. Quality control and standardization of herbal drugs- A tool between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc Natl Acad Sci USA., 96:1152-6.

Recio M.C. 1989. A review of some antimicrobial compounds isolated from medicinal plants reported in the literature 1978–1988. *Phytotherapy Research* 3: 117–125.

Samy. R.P., Ignacimuthu. S. and Sen. A. 1998. Screening of 34 Indian medicinal plants for antibacterial properties. J. Ethnopharmacol. 62:173-81.

Sandhy. B., Thomas. S., Isabel. W. and Shenbagavathai. R. 2006. Ethnomedicinal plants used by the valaiyan community of Piranmalai hills (Reserved forest), Tamil Nadu, India. A pilot study, African Journal of Traditional Complements and Alternative Medicines, 3: 101-114.

Sharma I., Gusain D. and Dixit V.P. 1991. Hypolipidaemic and antiatherosclerotic effects of plumbagin in rabbits. *Indian Journal of Physiology and Pharmacology* 35: 10–14.

Silver L.L., and Bostian K.A. 1993. Discovery and development of new antibiotics: the problem of antibiotic resistance. *Antimicrobial Agents and Chemotherapy* 37: 377–383.

Simonsen H.T., Nordskjold J.B., Smitt U.W., Nyman U., Palpu P., Joshi P. And Varughese G. 2001. *In vitro* screening of Indian medicinal plants for antiplasmodial activity. *Journal of Ethnopharmacology* 74: 195–204.

Stuffness. M., and Douros. J. 1982. Current status of the NLT plant and animal product program. J. Nat prod., 45: 1-14.

Trease E, and Evans W.C. 1987. Pharmacognosy, Billiare Tindall, London.

Vogel A.I. 1988. In: Elementary practical organic chemistry (Second edition), Orient Longman Limited, pp. 45-168.