Biolife 5(2):284-294
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

Bio-activity guided fractions, isolation and antimicrobial evaluation of active constituents of *Plumbago zeylanica Linn*.

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ABSTRACT

Plumbago zeylanica belonging to the family *plumbaginaceae* , proved to be a richer source of components with possible pharmacological values. Isolated from the root of this plant crude extracts were exhibited antimicrobial, antidermatophyte property, the active extracts were subjected for isolation of active constituent by using different techniques. On the basis of antimicrobial activity *P. zeylanica* have been chosen for the present study. The Petroleum ether, chloroform, ethanol and distilled water extracts of roots of *P. zeylanica* were prepared and these crude extracts were screened for their antimicrobial activity by agar dilution method. All the crude extracts has shown promising antimicrobial activity and the results were recorded in minimum inhibition concentration (MIC) values. The crude cold methanol extract showed inhibition at a concentration of 35 µg/ml for *S. aureus*. The results of antimicrobial activity are recorded as minimum inhibition concentration (MIC) value in µg/ml at the concentration where the growth of the microorganism is inhibited. Thus the plant *Plumbago zeylanica* proved to be active among the other plants chosen under bioactivity selection of plant. Root is considered more potent than others.

Key words: *Plumbago zeylanica L*, Antimicrobial activity, Minimum Inhibitory Concentration (MIC), Crude extracts.

INTRODUCTION

There is a worldwide belief that herbal remedies are safer and less damaging to the human body than the synthetic drugs. Recent surveys have shown that percentage of natural products in the modern drug armamentarium is considerable. This estimate is varying from 35% to 50%, most of them are plant derived. They are pilocarpine, vincristine, emetine, physostigmine, digitoxin, quinine, atropine, reserpine, are few well-known examples (Farnsworth and Bringel, 1986). The traditional role of developing countries in plant drug production has immense importance (Farnsworth,

1998). Indian traditional medicine is based on various systems including Ayurveda, Siddha and Unani. Materia medica of India provides lot of information on the folklore practices and traditional aspects of therapeutically important natural products (Narayana *et al.*, 1998; Mukherjee *et al.*, 1998). A large number of medicinal plants are claimed to be useful in the treatment of skin diseases. The following eighteen plants were selected on the basis of literature survey (Anonymous, 1959; Kirtikar and Basu, 1987; Chopra *et al.*, 1999) of traditional medicine and local folk medicine for dermatological infections.

Plumbago zeylanica Linn. (Fig-1) (Family: Plumbaginaceae) commonly found throughout India (Behl *et al.*, 1993). Known as The Wealth of India, 1948; Nadkarni) known as 'White Leadwort', Sanskrit-Chitraka; Hindi-Chita, Chitrak, Chitawar, Chiti, Chitra; Telugu-Chitramulamu.

How to Site This Article:
Sudhakara Reddy M, Sivasankar R, Udaykiran V, Muniya Naik M, Subahan M (2017). Bio-activity guided fractions, isolation and antimicrobial evaluation of active constituents of *Plumbago zeylanica Linn*.. Biolife. 5(2), pp 284-294.

DOI: 10.5281/zenodo.7364672
Received: 8 April 2017;
Accepted; 30 May 2017;
Available online : 5 June 2017

Definitely, the plant kingdom still holds many species of plants containing constituents of medicinal value, which have yet to be discovered. Large numbers of plants are constantly being screened for their possible medicinal value. One of these plants i.e., *Plumbago zeylanica* belonging to the family plumbaginaceae proved to be a richer source of components with possible pharmacological values (Anonymous, 1948). But still more pharmacological investigations are needed.

Figure-1. *Plumbago zeylanica Linn*. Roots.

Medicinal properties of *Plumbago zeylanica* :

Plant:

The plant is used as vulnerary in New Caledonia (Kirtikar and Basu, 2000).

Leaves:

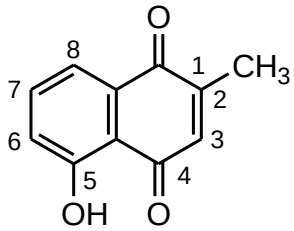
Leaves are caustic, vesicant, aphrodisiac and good for scabies (Kirtikar and Basu, 2000).

Root:

Root and root bark are bitter, hot, dry, stomachic, carminative, astringent to the bowel, antihelmintic, alterative, cure intestinal troubles, dysentery, leucoderma, inflammation, piles, bronchitis, itching, diseases of the liver, treatment of anaemia. The root has a bitter sharp taste, laxative, expectorant, stomachic, tonic, abortifacient, alexipharmic, good appetiser, useful in laryngitis, rheumatism, diseases of the spleen, leucoderma, ringworm and scabies. For external administration it is made into a paste with milk, vinegar or salt and water. Such a paste may be applied externally in leprosy and other skin diseases of an obstinate character and be allowed to remain until a blister has formed (Kirtikar and Basu, 2000).

Root is used as laxative and expectorant. Root paste is used in curing leucoderma (Behl *et al.*, 1993), ringworm, scabies, piles, leprosy and other skin diseases (Lin *et al.*, 2003). The root extracts possesses cytotoxic (Ahmed *et al.*, 1998), antimicrobial, anti-fungal, anti-fertility, larval mortality activity and itching. Naphthoquinones and phenolics are the main constituents isolated from this plant.

Plumbago zeylanica was purified by RP / HPLC method to afford plumbagin, a naphthoquinone (Fig-1). It showed significant activity against *Staphylococcus aureus*.



5-Hydroxy-2-methyl- 1 ,4-naphthoquinone (Plumbagin)

The principal action of plumbagin is on the muscular tissue. It stimulates the contraction of the muscular tissue of the heart, intestines, and uterus. The action is deep-seated. It stimulates the secretion of sweat, urine and bile. It has a stimulant action on the nervous system (Kirtikar and Basu, 2000).

Root is said to increase the digestive power and promote appetite. Plumbagin stimulates the central nervous system in small doses, while with larger doses paralysis sets in leading ultimately to death. The blood pressure shows a slight fall. Plumbagin is a powerful irritant and has well marked anti-septic properties. In small doses, the drug is sudorific, large doses cause death from respiratory failure.

MATERIALS AND METHODS

Collection of plant material:

The plant *Plumbago zeylanica* as whole / aerial / leaves / seeds / flower and Roots of selected plant was collected from Tirumala hills, and authenticated at the Herbarium (SVUB-617) of the Department of Botany, S.V. University, Tirupati. Andhra Pradesh. The different plants or parts collected were immediately sprayed with alcohol to cease the enzymatic degradation of

secondary metabolites. After shade drying the material was powdered. The plant is collected during flowering season. Part of the plant used is root, bark and milky juice. Flowering and fruiting between August and November. (Kini *et al.*, 1997).

Extraction of plant:

The shade-dried material was powdered and extracted by soaking in solvent (cold extraction) or in soxhlet extractor successively with solvents (hot extraction) from non-polar to polar The extracts were concentrated to dryness in a flash evaporator (Buchii) under reduced pressure and controlled temperature (50-60°C). On concentration, yielded respective solvent extracts. They were preserved in a refrigerator and used for phytochemical and microbial studies. The extracts found to be active were further used to isolate the active constituents present in it. Plant, its part used for extraction, method of extraction, solvent used for extraction and extract nature are tabulated.

Bioactivity Guided Selection of the Plant:

On the basis of antimicrobial activity in present study, *Plumbago zeylanica* have been chosen for the present study. The detailed study for the isolation of active principles from various extracts of *P. zeylanica* were studied.

Biological activities reported in literature:

Earlier reports on some biological activities are cytotoxicity (Lin *et al.*, 2003), antimicrobial activity (Ahmed *et al.*, 1998; Krishnaswamy and Purushottaman, 1980; Mukharya and Dahia, 1977; and Jeevan Ram *et al.*, 2004), anticancer (Krishnaswamy and Purushottaman, 1980; Shen *et al.*, 2003), antifungal (Krishnaswamy and Purushottaman, 1980), platelet aggregation (Simonson *et al.*, 2001), antiplasmodial activity, antihelicobacter pylori activity (Veluri and Diwan, 1999), hypolipidemic (Hazra *et al.*, 2002), antifertility (Hazra *et al.*, 2002), anti-feedant activity (Dinda *et al.*, 1997), pesticidal activity (Dinda *et al.*, 1997), larval mortality activity (Dinda *et al.*, 1997), macrophage bactericidal activity (Abdul *et al.*, 1995), tissue differentiation (Helbe *et al.*, 1974), hyperglycemia (Olanguju *et al.*, 1999), treatment of acne vulgaris (Olanguju *et al.*, 1999), breast cancer (Mahishi *et al.*, 2005), itching (Mahishi *et al.*, 2005), anticoagulant (Gupta *et al.*, 1993), rubifacient, leprosy, scabies and unhealthy ulcers (Behl *et al.*, 1993), wound healing (Sharma *et al.*, 1991), inhibition of allergic reactions (Singh *et al.*, 1997), anti-candidal (Masataka *et al.*, 1991) and antioxidant (Mehmood *et al.*, 1999).

Dosage:

Paste, powder, pills, tincture. (Behl *et al.*, 1993): Taken in large doses, this herb can cause paralysis leading ultimately to death. It can also cause death from respiratory failure. It is also a powerful poison. This herb is very dangerous and should not be taken except under the close supervision of a professional.

RESULTS AND DISCUSSION

Chemical constituents isolated from *plumbago zeylanica:*

P. zeylanica is greatly used for its chemical constituents that are present in various parts of the plants. (Richa Tyagi *et al*., 2014). To investigate the chemical constituents from the *Plumbago zeylanica L*. the chemical constituents were isolated from roots and aerial parts by various column chromatographic methods and their structures were elucidated as *plumbazeylanone* (Table-1).

In an effort for selection of plant with antimicrobial potential based on bioactivity guided selection, *Plumbago zeylanica* showed promising antibacterial, antidermatophytic activity and anti-candidal property. Naphthoquinones (Dinda *et al.*, 1997) isolated from the root of this plant exhibited antimicrobial, anti-dermatophyte. property (Dinda *et al.*, 1997). Although, *Plumbago zeylanica* has been studied for various biological activities (Dinda *et al.*, 1997), but no systematic approach has been made to identify the active fractions from the root of the plant. The active principles responsible for antidermatophytic property from the active fractions have not been studied. The systematic approach of bioactivity guided fractionation of the active extracts is helpful to understand and in search of the active principles responsible for the bioactivity.

The root part of *Plumbago zeylanica* on successive hot soxhlet extraction yielded pet-ether, chloroform and ethanol extracts which exhibited anti-dermatophytic, anticandidal, antibacterial activity in low concentration. Cold methanol extract of *P. zeylanica* have also exhibited antibacterial activity. In view of this, root part of the plant was also collected and subjected for extraction and fractionation in search of active part of plant with active principles in crude and fractionated extracts for antidermatophytic activity. These crude extracts were tested against bacteria and dermatophytes for their antimicrobial activity and then the active extracts were subjected for isolation of active constituent by using different chromatographic techniques.

Phytochemical screening of *Plumbago zeylanica:*

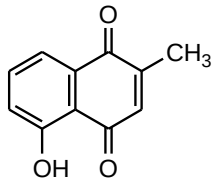
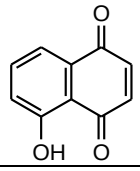
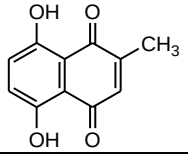
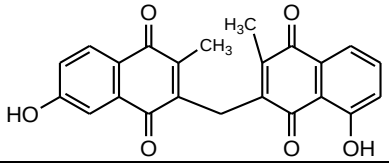
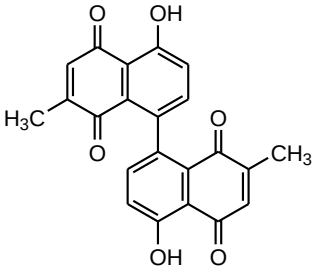
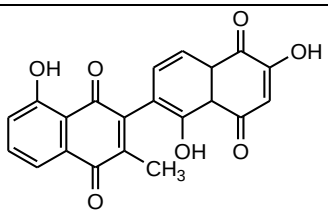
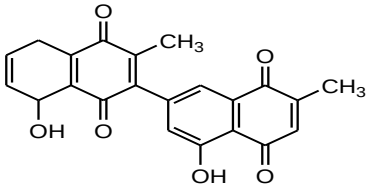
Phytochemical is a natural bioactive compound found in plant that is formed during plant's normal metabolic process. These chemicals are often referred as "Secondary metabolities".That Includes alkanoid, flavonoid, coumarins, gums, tannis, terpenes, phenols and so on. These phytochemicals are originated in plant food material that works through nutrients and dietary fibre to defend body against diseases. Research recommends phytochemicals as essential dietary content that works together with nutrients originate in fruits, vegetables and nuts and these nutrients might help to slow the aging process and diminish the hazard of several diseases, counting cancer, heart disease,

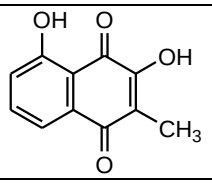
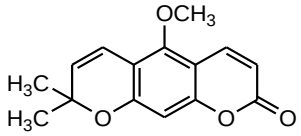
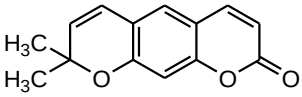
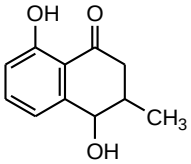
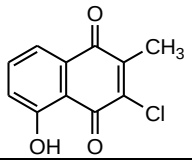
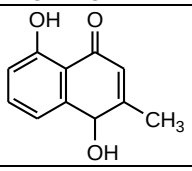
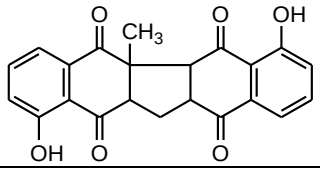
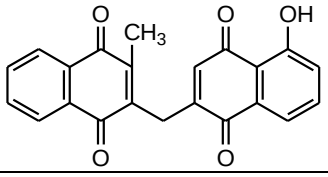
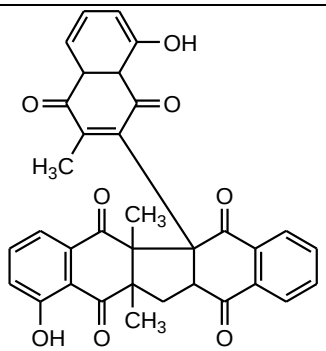
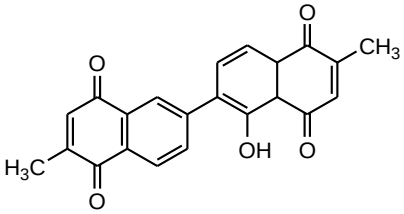
stroke, high blood pressure, cataracts, osteoporosis, and urinary tract infections.(Richa Tyagi *et al*.,2014)

Hot extraction:

The weighed amount (100 gm) of this material was successively extracted using soxhlet extractor with

Table-1. Earlier reports on chemical constituents isolated from this plant are tabulated below

Chemical constituents	Structure	Uses
	Chemical constituents isolated from roots and aerial parts	
Plumbagin		Anticancer (Lin *et al.*, 2003), Diuretic (Hazra *et al.*, 2002), Sudorific (Dinda *et al.*, 1997), Abortifacient (Ahmed *et al.*, 1998), Pesticidal (Dinda *et al.*, 1997), Larval mortality (Dinda *et al.*, 1997), Anticoagulant (Gupta *et al.*, 1993), Antifungal (Gupta *et al.*, 1993), Antimicrobial (Krishnaswamy and Purushottaman, 1980; Veluri and Diwan, 1999), Antifertility (Yaxian *et al.*, 2005; Lakshmi *et al.*, 1987; Hazra *et al.*, 2002; Selma *et al.*, 2003), CNS Stimulant in small doses (Hazra *et al.*, 2002; Bopaiah and Pradhan, 2001) Hypolipidemic (Hazra *et al.*, 2002) antiatherosclerotic (Suresh Reddy *et al.*, 2004), Antitumour (Sugie *et al.*, 1998), Cardiotonic (Devi *et al.*, 1999), Anticarcinogenic, Multidrug resistant plasmids from *E. coli* (Mehmood *et al.*, 1999) Antioxidant (Jai *et al.*, 2004)
	Chemical constituents isolated from roots	
Juglone		Antimicrobial (Didry *et al.*, 1994)
2-Methyl naphthazarin (Likhiwitayawuid *et al.*, 1998)		Antimalarial (Okamoto *et al.*, 2001)
Methylene 3,3′-diplumbagin (Kamal *et al.*, 1988)		Ichthyotoxic activity and germination inhibitory activity (Higa *et al.*, 2002)
Maritinone (Kamal et al., 1998; Okamoto et al., 2001 ; Sankaram et al., 1976 ; Sidhu and Sankaram, 1971)		Cytotoxic (Lin et al., 2003)
Chitranone (Hazra et al., 2002)		Cytotoxic (Hazra et al., 2002)
3,3′-Biplumbagin (Hazra et al., 2002 ; Sankaram et al., 1976 ; Sidhu and Sankaram, 1971)		Ichthyotoxic activity and germination inhibitory activity (Okamoto et al., 2001)

Chemical constituents	Structure	Uses
Droserone (Dinda et al., 1997 ; Kamal et al., 1983)		Antimalarial (Higa et al., 2002)
Xanthoxyletin (Lin et al., 2003)		Cytotoxic (Lin et al., 2003) and Antiplatelet (Tong et al., 1992)
Xanthyletin (Lin et al., 2003)		Cytotoxic (Lin et al., 2003) and Antiplatelet (Tong et al., 1992)
Isoshinanolone (Lin et al., 2003; Dinda et al., 1997 ; Kamal et al., 1983)		Cytotoxic (Lin et al., 2003) and Antiplatelet (Tong et al., 1992)
3-Chloroplumbagin (Dinda et al., 1997; Sidhu and Sankaram, 1971)		No biological reports
β-Dihydroplumbagin (Kamal et al., 1984)		No biological reports
Zeylanone (Kamal et al., 1988; Kamal et al., 1984 ; Peranjape and Kulkarni, 1995)		Cytotoxic and Antimicrobial (Sankaram et al., 1979)
Isozeylanone (Kamal et al., 1988; Kamal et al., 1984; Sankaram et al., 1979)		Ichthyotoxic activity and germination inhibitory activity (Okamoto et al., 2001)
Plumbazeylanone (Kamal et al., 1984)		No biological reports
Elliptinone (Veluir and Diwan, 1999; Sankaram et al., 1976)		Cytotoxic (Lin et al., 2003)

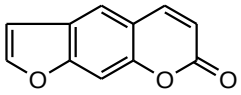
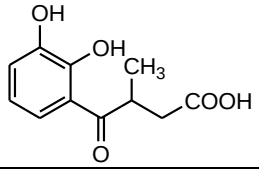
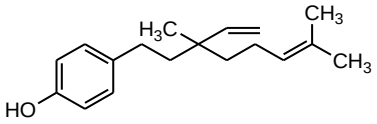
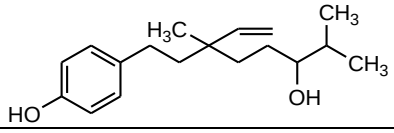
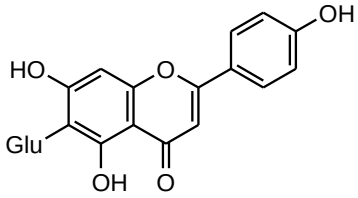
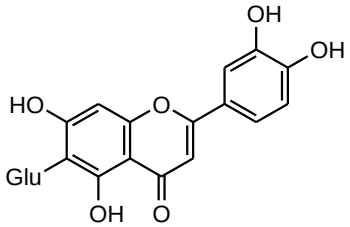
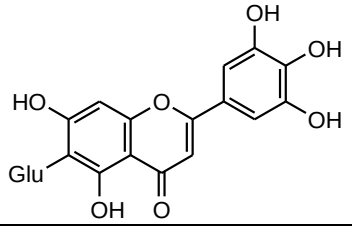
Chemical constituents	Structure	Uses
	Chemical constituents isolated from aerial parts	
Psoralen		Treatment of psoriasis and cutaneous T-cell lymphoma among other skin and blood disease and cytotoxic (Kawaii et al., 2001), antifungal (Lette et al., 2004), reproductive toxicity (Mourad et al., 2004)
Plumbagic acid (Diawara and Kulkosky, 2003)		Cytotoxic (The Wealth of India, 1948)
Bakuchiol (Linn and Chou, 2003)		Antioxidant (Jianging et al., 2005; Adhikari et al., 2003), Antitumour (Sarma et al., 2005), Cytotoxic (Sarma et al., 2005 ; Zasshi, 1989), Antimicrobial (Sarma et al., 2005), Anti-inflammatory (Sarma et al., 2005), Hepatoprotective(Chou et al., 2001)
12-Hydroxy isobakuchiol (Linn and Chou, 2003)		No reports
Saponaretin (Linn and Chou, 2003)		No reports
Isoorientin (Linn and Chou, 2003)		Inhibition of lipid peroxidation (Kupeli et al., 2004), Antinociceptive, Antiinflammatory, Gastroprotective and Antioxidant (Cheel et al., 2005)
Isoaffinetin (Linn and Chou, 2003)		Aldose reductase inhibitor (Haraguchi et al., 2003)

Table-2. *Plumbago zeylanica* part used, method of extraction, extract nature, colour of extract, amount obtained

Part of *P.zeylanica*	Method of extraction	Solvent for extraction	Nature of extract	Colour of extract	Amount of extract obtained for 100 gm
Roots	Hot	Pet-ether	Gummy Pale mass	Pale yellow	500 mg
		Chloroform	Semi solid	Pale yellowish brown	220 mg
		Ethanol	Semi solid	Dark brown	200 mg
		Water	Semi solid	Dark Brown	150 mg

solvents of varying polarity. Pet-ether (60-80° C, 500 ml), chloroform (500 ml) and ethanol (500 ml). The extracts were concentrated to dryness in a flash evaporator (Buchii) under reduced pressure and controlled temperature (50-60° C). On concentration, it yielded respective solvent extracts. The extracts so obtained were air-dried, weighed, packed and stored in a refrigerator. The preserved extracts were used for phytochemical and antimicrobial studies. The extracts found to be active were further used to isolate the active constituents present in it. Plant, its parts used for extraction, method of extraction, solvent used for extraction, extract nature, approximate amount of extract obtained were tabulated in (Table-2 and Table-3). The phytochemical tests of various extracts of *P. zeylanica* roots were studied. The results are shown in (Table-3).

Cold extraction:

The weighed amount of powdered roots of *P. zeylanica* was immersed in methanol with occasional shaking for two days. The extract was then filtered and concentrated extract was obtained after evaporating the solvent using Buchii evaporator, under reduced pressure and controlled temperature. The extract (brownish black semisolid) so obtained was air dried, weighed, packed and stored in a refrigerator. (Table-2 and Table-3).

Evaluation of extracts of *P. Zeylanica* for antimicrobial screening (Table-4 and 5):

The results of antimicrobial activity are recorded as minimum inhibition concentration value in µg/ml at the concentration where the growth of the microorganism is inhibited. The MIC values of root part of plant extract were obtained by agar dilution method, results are tabulated in Table-5 and shown by graphical representation also (Figure-2). The cut off value of MIC of crude and fractionated extracts was chosen at 125 µg/ml.

Negative control

The negative control, DMSO chosen for the present study for preparation of test stock solutions did not inhibit the microorganisms i.e., *S. aureus, S. pyogens, C. albicans, T. rubrum, T mentagrophytes* and *E. flocussum.*

Positive control

- The standard drugs used for the antibacterial study are ciprofloxacin and Gentamycin, which inhibited *S. aureus* and *S. pyogens* at MIC of < 3.91 µg/ml.
- The Griseofulvin and Ketaconazole inhibited the *C. albicans* at MIC of < 3.91 µg/ml.
- *T. rubrum* was inhibited by Ketaconazole at MIC of 0.04 µg/ml, Miconazole at MIC 0.08 µg/ml, Griseofulvin at MIC of 1.25 µg/ml, Amphotericin B at MIC of 2.5 µg/ml and Ciclopirox Olamine at MIC of 5 µg/ml.
- *T. mentagrophytes* was inhibited by Ketaconazole at MIC 5 µg/ml, Miconazole at MIC 0.63 µg/ml,
- Griseofulvin at MIC 20 µg/ml, Amphotericin B at MIC of 5 µg/ml and Ciclopiroxolamine at MIC of 10 µg/ml.
- *E. flocussum* was inhibited by Ketaconazole at MIC of 10 µg/ml, Miconazole at MIC of 1.25 µg/ml, Griseofulvin at MIC of 20 µg/ml, Amphotericin B at MIC of 5 µg/ml and Ciclopiroxolamine at MIC of 5 µg/ml.

i) Activity of plant crude extracts against *Candida albicans*

All extracts of *P. zeylanica*, i.e., pet-ether, chloroform, ethanol and water extracts are found to be inhibiting the growth of *Candida albicans* at MIC of < 62.5 µg/ml whereas cold methanol extract of *P. zeylanica* was inhibiting at MIC of 1000 µg/ml, which is above the cutoff point considered in present investigation.

ii) Activity of *P. zeylanica* roots extracts against *S. aureus*

All the extracts of *Plumbago zeylanica* i.e., pet-ether, chloroform, ethanol and cold methanol extracts were inhibiting the growth of *S.aureus* at MIC values < 7.81, 31.25, 31.25 and 35 µg/ml, respectively.

iii) Activity of *P. zeylanica* roots crude extracts against *S. pyogens*

All the extracts of *P. zeylanica,* i.e. pet-ether, chloroform, ethanol and cold methanol extracts were inhibiting the growth of *S. pyogens* at MIC of 250 µg/ml.

iv) Activity of *P. zeylanica* plant crude extracts against *T. rubrum*

P. zeylanica root crude pet-ether, chloroform, ethanol and cold methanol extracts were found to inhibit the growth of *T. rubrum* at MIC of < 3.90, <3.90, 125 and 15.63 µg/ml, respectively.

v) Activity of *P. zeylanica* roots crude extracts against *T. mentagrophytes*

P. zeylanica roots crude pet-ether, chloroform, ethanol and cold methanol extracts were found to inhibit the growth of *T. mentagrophytes* at MIC of < 3.90, < 3.90, 250 and 31.25 µg/ml, respectively.

vi) Activity of *P. zeylanica* roots crude extracts against *E. floccusum*

All the root extracts of *P. zeylanica,* i.e., pet-ether, chloroform, ethanol and cold methanol extracts were inhibiting the growth of *E. floccusum* at MIC of 7.82, 31.25, 500 and 250 µg/ml, respectively.

vii) Activity of *P. zeylanica* roots crude extracts against *Propionobacterium acne*

All the root extracts of *P. zeylanica,* i.e., pet-ether, chloroform, ethanol and cold methanol extracts were inhibiting the growth of *P. acne* at MIC of 1000 µg/ml

Table-3. Phytochemical tests of crude extracts from the roots of *P. zeylanica*

Plant Part	Method of extraction	Solvent of extraction	Secondary metabolites					
			S	A	F	P	G	Sa
Plumbago zeylanica Root	Hot	Pet-ether	+	-	-	+	-	-
		Chloroform	+	-	-	+	-	-
		Ethanol	-	-	+	+	+	-
		Water	-	-	-	-	+	-
	Cold	Methanol	+	+	+	+	+	-

S – Steroids; A – Alkaloids; F – Flavonoids; P – Phenolics; G – Glycosides; Sa – Saponins; '+' Positive '-' Negative

Table-4. Table showing the volume of solution used and concentration of extract in agar

Volume of solution (ml)	Diluent (ml)	Concentration in dilution (µg/ml)	Concentration of extract in agar (µg/ml)
200 mg of plant extract (stock)	100 ml of DMSO	20,000	2000
5 ml of stock	5 ml of PW	10,000	1000
5 ml of stock	5 ml of PW	5000	500
5 ml of stock	5 ml of PW	2500	250
5 ml of stock	5 ml of PW	1250	125
5 ml of stock	5 ml of PW	625	62.5
5 ml of stock	5 ml of PW	312	31.2
5 ml of stock	5 ml of PW	156	15.6
5 ml of stock	5 ml of PW	78	7.8
5 ml of stock	5 ml of PW	39	3.9
5 ml of stock	5 ml of PW	19.5	1.95

PW – Purified Water

Table-5. MIC values for crude extracts by agar dilution method

Plant part	Method of extraction	Solvent of extraction	MIC values in µg/ml						
			C. a	*S. a*	*S. p*	*T. r*	*T. m*	*E .f*	*P. a*
Plumbago zeylanica root	Hot	Pet-ether	<62.50	<7.81	250	<3.90	<3.90	7.81	1000
		Chloroform	<15	31.25	250	<3.90	<3.90	31.25	1000
		Ethanol	<62.50	31.25	250	125	250	500	1000
		Benzene	<62.50	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
		Water	<62.50	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
		Benzene+ water	1000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
		Benzene+ water insoluble	1000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
	Cold	Methanol	1000	35	250	15.63	31.25	250	>1000

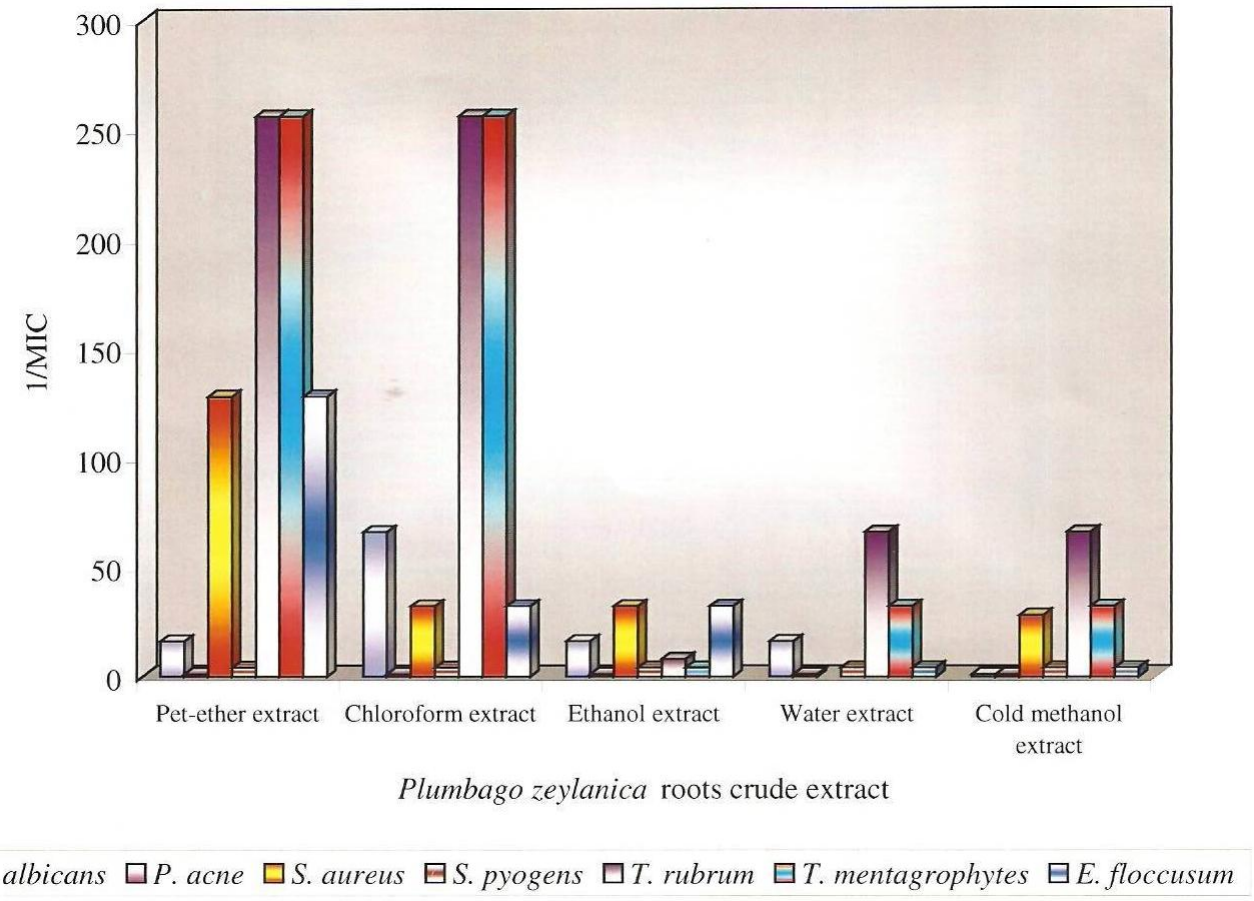
N.T. – Not Tested
C.a – Candida albicans *S.a – Staphylococcus aureus*
S.p. – Streptococcus pyogens *T.r. – Trichophyton rubrum*
T.m. – Trichophyton mentagrophytes *E.f. – Epidermatophyton flocussum*
P.a. – Propionobacterium acne

which is above the cut-off value in present investigation. In the present investigation it is evident that there is no inhibition of microorganisms by DMSO which is used as negative control.

Different classes of standard drugs were used as positive control. The mode of action of the standard drugs depends upon the class and the structure of drug used. From the results obtained it is clear that different standard drugs are having different inhibition concentration against different kind of microorganisms, which range between 20 µg/ml to 0.04 µg/ml.

All the crude extracts of *P. zeylanica* roots found to have little comparable MIC values with standard drugs. The strength of the crude extracts of the plant was

Figure-2. MIC values for crude extracts of roots of *Plumbago zeylanica* by agar dilution method



determined by the inhibition of growth of *S. aureus, S. pyogens, C. albicans, T. rubrum, T. mentagrophytes* and *E.flocussum* which may be due to the phytoconstituents, mainly naphthoquinones present in these crude extracts, which is responsible for the antimicrobial property.

As in the screening approach, the cut-off value of MIC for these crude extract chosen was 125 μg/ml. Thus the other crude extracts of *P. zeylanica* i.e., the water, Benzene + water and Benzene + water insoluble extracts were not considered for further study since the MIC value is above the cut-off point.

The hot successive pet-ether extract is active at MIC < 3.90 μg/ml against dermatophytes, which was very much comparable with the standard drugs. But this extract also shown activity against *C. albicans* and *S. aureus,* at 62.5 and < 7.85, for *S. pyogens* at 250 μg/ml. This extract has shown potent antidermatophytic activity against the *T. rubrum, T. mentagrophytes* and *E. flocussum*.

The hot successive chloroform extract of *P. zeylanica* is active at < 3.90 μg/ml against *T. rubrum* and *T. mentagrophytes* whereas against *E. flocussum* at MIC of 31.25 μg/ml. At MIC of < 62.50 and 31.24 μg/ml for *C. albicans* and *S. aureus,* respectively. This extract had not shown activity against *P. acne*.

Cold methanol extract of *P. zeylanica* root has shown inhibition at a concentration of 35 μg/ml for *S. aureus*. This extract was used for further separation.

Thus the plant *Plumbago zeylanica* proved to be active among the 18 plants chosen under bioactivity selection of plant. Root is considered more potent than others.

CONCLUSION

The review clearly shows the importance of *Plumbago zeylanica,* is a perennial shrub used throughout the world for therapeutic purposes. It is the most important medicinal plant extensively used in herbal formulations. It is chemically rich with its diverse content of active compounds, such as plumbazin, and many useful naphthaquinone constituents as a multipurpose medicinal agent were present. It is used in the treatment of dyspepsia, piles, and diarrhea and skin diseases. The present study reveals that, The Petroleum ether, chloroform, ethanol and distilled water extracts of roots of *P. zeylanica* were prepared and these crude extracts were screened for their antimicrobial activity by agar dilution method. All the crude extracts has shown promising antimicrobial activity and the results were recorded in minimum inhibition concentration (MIC)

values. The review summarizes about the morphology of the plant along with its chemical composition, propagation, therapeutic use it can be said that plant act as a good antimicrobial drug.

Acknowledgement:

Authors are thankful to Ex-Principal, Prof. K.Jayantha Rao, (S.V.U.C.S) for their constant Encouragement**,** and also express deep sense of gratitude to Department of Zoology, Sri Venkateswara University. Tirupathi (A.P) for providing laboratory facilities to carry out this work and cooperation.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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