

## Screening of Stem Extracts of *Plumbago Zeylanica* for Phytochemical, Antimicrobial and Anti-Arthritis Activity

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The plant *Plumbago Zeylanica* is known for its traditional medicinal use because of its wide phytochemical constituency. On same basis its screening for anti-microbial and anti-arthritis activity was carried out. The results showed that the stem of *Plumbago Zeylanica* could be used as best remedy against bacterial and fungal originated diseases. Among plant extracts, chloroform extract showed highest rate of inhibition against all the concerned bacterial strains followed by petroleum ether extract. The water extract did not show much response as an antimicrobial agent. Also the antifungal activity was observed for all the extracts, but ethanolic extract showed more inhibition potential followed by petroleum ether extract. The results of anti-arthritis activity shows, lowest IC<sub>50</sub> (46.55) value for water extract, hence high inhibition potential, followed by ethanolic extract (IC<sub>50</sub>) 48.50. Over all the plant showed good response as biomedicine against microbial originated diseases and against arthritis.

**Keywords:** Anti-arthritis, Anti-microbial, IC<sub>50</sub>, *Plumbago Zeylanica*, Phytochemistry.

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Almost, 70% modern medicines in India are derived from natural products. Medicinal plants play a central role not only as traditional medicines but also as trade commodities, meeting the demand of distant markets (Verma Sheetal, Singh SP, 2012). Moreover, in developing countries, synthetic drugs are presently too expensive and also are often adulterated (Jeyachandran R, *et al* 2009). The Rig Veda, dating 3500 B.C. to 1800 B.C., seems to be the earliest record available on medicinal plants.

Herbs seem to be very important component of medicine in other cultures too. Greek, African and Chinese medicines, to mention a few (Sandhya B, *et al* 2006).

The German market corresponds to about 50% of the European market, about \$ 3.5 billion. This market is followed by France, \$ 1.8 billion; Italy, \$ 700 million; the United Kingdom, \$ 400 million; Spain, \$300 million; the Netherlands, about \$ 100 million (Alok Sharma, *et al* 2004).

Herbal drugs are prescribed widely even when their biologically active components are

unknown because of their effectiveness, fewer side effects and relatively low cost. The aim of the present study "Screening of Stem Extracts of *Plumbago Zeylanica* L. for Antimicrobial and anti-arthritis Activity" is an endeavor to find a good natural drug for treatment of manifestations caused by microorganisms and of protein disinfections. Different extracts of the plant *Plumbago Zeylanica* Linn of family plumbaginaceae were tested for their antimicrobial potential against various pathogenic microbes.

Medicinal plant *Plumbago Zeylanica* L. is the member of family plumbaginaceae. The family plumbaginaceae consists of 10 genera and 280 species and is classified into two subfamilies, plumbaginoideae and staticoideae, well differentiated by morphological, chemical, and molecular characters. Plumbaginoideae comprise four genera, of which *Plumbago*, with approximately 20 species, is the largest. Distributed throughout the world, mostly in dry areas with saline soils, such as salt flats and sea coasts, especially in the Mediterranean and Western Asia (Perveen Anjum Qaiser, 2004, Kantha Devi Arunachalam, 2010, Dolores Lledo M, 2005).

*Plumbago zeylanica* L. commonly known as white chitrak is a rambling, subscandent, much branched perennial tropical or subtropical herb or undershrub of family plumbaginaceae. It grows wild up to 1400 meter above sea level and as garden plant in eastern, northern, and southern India. *Plumbago zeylanica* L. is an important medicinal plant traditionally used in India and China for the treatment of various diseases (Samant, SS, 1998, Chetty K, 2008). *Plumbago zeylanica* is distributed as a perennial herb in throughout the tropical and subtropical countries of the world. It is wide spread and is indigenous to south East Asia. It grows wild in forest of Uttarakhand and found in peninsular India, grows up to 1500 meter above sea level and is cultivated in gardens through India and is propagated. Leaves and stem of the plant contain volatile oil (Farroq S, 2005). The aerial parts contain naphthoquinones, sitosterol, lupeol, lupenylacetate, hentriacontane, and amino acids (Jeyachandran R, *et al* 2009). Earlier chemical examination of the plant revealed that the root contains- plumbagin (2- methoxy-5- hydroxyl- 1, 4-naphthoquinone); 3-chloroplumbagin; 3, 3-biplumbagin; chitranone (b-2Dihydro-1(s) 5, 5-trihydroxy-2, 3. Dihydroxy

benzoyl) butyric acid); enzymes as invertase and protease (Kumar Rajesh, 2009). Five coumarins – seselin; 5-methoxyseselin; suberosin; xanthyletin and xanthoxyletin have been isolated from the roots of *Plumbago zeylanica* (Jeyachandran R, *et al* 2009). The plant also contains lineleic acid; palmitic acid; nonylnonanoate; stigmasterolacetate; lupeol acetate; friedelinol; lupeol; lupanone; sitosterone and stigmasterone (Gopinath S, 2009), vanillic acid; 2,5-dimethyl-7-hydroxychromone; indole-3-carboxaldehyde (Nile Shivraj H, 2010), Plumbagin (2-methoxy-5-hydroxy-1,4-naphthoquinone) being the most active constitute possesses various pharmacological activities viz. antimalarial, antimicrobial, anticancer, cardiogenic and antifertility action. It stimulates muscular tissue at smaller doses but paralyzes at larger one. It stimulates muscular tissue of heart, secretion of bile and urine it also stimulates the CNS (Joshi Shankar Gopal, 2007).

*Plumbago zeylanica* has numerous therapeutic uses different parts of the plant are used in various ailments in different medicinal systems and different folks. Though the root, root barks, and seeds of *Plumbago zeylanica* are used medicinally, the root is the chief source of an acrid crystalline principle called plumbagin, a yellow naphthoquinone pigment, and also characteristic of plants in the tribe Plumbaginaceae, and is most used part for the medicinal uses (Sonibare Oluwadayo O, 2009). Leaves of the plant are used as aphrodisiac and in skin diseases. The aerial parts of the plant has hepatoprotective activity (Joshi Shankar Gopal, 2007). Flower of the plant show digestive stimulus activity. The plant possesses anti-inflammation, abortifacient, antitumor, antitussive, antiseptic, antifungal, anti-inflammatory, antihyperglycemic and antiatherosclerotic activity (Sonibare Oluwadayo O, 2009). The root of the plant is used as antidespeptic, antiepileptic, antiulcer. The plant is also used in dysentery, rubefaciency, leucoderma, files, bronchitis, liver diseases, and anemia. The roots used in vitiligo, in hysteria, prurigo, ophthalmia, paralytic affection and secondary syphilis. The roots are also used as laxative, expectorant, appetizer, carminative, and diuretic, abortifacient, astringent, in diarrhea, skin diseases, leprosy, and sudorific (Abera Balcha, 2008).

## MATERIALS AND METHODS

The fresh aerial parts of the plant *Plumbago zeylanica* L. were collected from a local herbal garden in Dehradun on September 21<sup>st</sup> 2019. The plant sample was authenticated by Botanical Survey of India, Northern Regional Centre, Dehradun. The voucher specimen was submitted at B.S.I herbarium with the accession no. BSD-112741. The plant stem was separated from leaves and dried in shed at room temperature in the Laboratory and then crushed to coarse powder.

### Extraction of Drug

#### Extraction

About 60 gm of accurately weighed dry stem powder was taken in a thimble and about 300 ml of solvent (Petroleum ether, DCM, Ethanol and Water successively as per their polarity index) was taken in an RBF and fitted condenser on a heating mental and extracted for 36 hours.

On completion of extraction with petroleum ether, the extract was collected and dried in an oven. Then the residue was extracted with other solvents (DCM and Ethanol) successively in the same manner. The same residue after ethanol extraction was then extracted by water by decoction method, in which the residue was dissolved in

500ml of water. The material was heated till the whole water gets absorbed, then finally more 500ml of water was added heated till half of the water gets evaporated. The mixture was finally filtered and the filtrate was collected and evaporated to dry powder. All the extracts after completely air dried were kept in air tight bottles for further use.

Phytochemical Analysis (Kokate CK, et al, 2005, Mukharjee PK, 2002, Khandelwal KR, 1996). The term 'Phyto' means the plants, so as the name indicates phytochemicals are the chemicals which are derived from the plant sources. Alkaloids, Terpenoids, Coumarins, Tannins, Quinones, Flavonoids, Glycosides, Steroids, and Saponins etc. are some classes of phytochemicals. They play different role in plants but they are biologically active as well and are used to cure various ailments.

### Antimicrobial Assay (Panikar Ananthanarayan, 2007)

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, viruses and fungi. Antimicrobials drugs either kill microbes called microbicidal or prevent the growth of microbes called as microbistatic.

### Source of Microorganisms

The antimicrobial assay of different extracts was performed under two heads that is antibacterial and antifungal assays. All the microbial strains were procured from the Department of microbiology UCBMSH and the study also was performed at microbiology lab.

**Table 1.** Selected Bacterial Strains

S. No	Bacterial Strain	Family
B <sub>1</sub>	<i>Staphylococcus aureus</i>	Enterobacteriaceae
B <sub>2</sub>	<i>Listeria monocytogenes</i>	Bacillaceae
B <sub>3</sub>	<i>Bacillus cereus</i>	Enterobacteriaceae
B <sub>4</sub>	<i>Acinetobacter baumannii</i>	Bacillaceae
B <sub>5</sub>	<i>Salmonella typhi</i>	Bacillaceae
B <sub>6</sub>	<i>Pseudomonas aeruginosa</i>	Micrococcaceae
B <sub>7</sub>	<i>Serratia marsecens</i>	Streptococcaceae
B <sub>8</sub>	<i>Klebsiella pneumoniae</i>	Enterobacteriaceae

**Table 2.** Selected Fungal Strains

S. No.	Fungal strains
F <sub>1</sub>	<i>Aspergillus flavus</i>
F <sub>2</sub>	<i>Aspergillus parasiticus</i>
F <sub>3</sub>	<i>Trichoderma rubrum</i>
F <sub>4</sub>	<i>Microsporium gypseum</i>

**Table 3.** The Percentage Yield of the Various Plant Extracts

S. No.	Solvent Used	Quantity of Crude Drug	Weight of extract	% Yield
E <sub>1</sub>	Petroleum Ether	120 gm	0.75gm	00.63
E <sub>2</sub>	Chloroform	119 gm	1.0gm	00.84
E <sub>3</sub>	Ethanol	118 gm	14.64gm	13.0
E <sub>4</sub>	Water	100 gm	8.35gm	08.4

### Evaluation of the antimicrobial potential of plant extracts

Eight gram positive and gram negative bacteria viz. *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Acinetobacter baumannii*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Serratia marsecens*, *Klebsiella pneumoniae* were taken to evaluate antibacterial potential of the different extracts.

All the crude extracts were first screened for preliminary test with the concentration 500mg/ml to know whether they were active against the particular bacteria or not. The sensitivities against standard drug Ofloxacin at concentration 5µg/ml were also observed. Only extracts with good activity were then assayed further at different concentrations for MIC test. All the extracts were made to dissolve in DMSO, a highly polar, aprotic organic solvent miscible with water and majority of organic solvents and is nontoxic to the microbes.

### Evaluation of the antifungal potential of plant extracts (Pelzer Michael J, 2007, Aneja KR, 2009)

The crude extracts were also screened for their possible antifungal potential. Antifungal activity of the extracts was observed against four pathogenic fungi namely *Aspergillus flavus*, *Aspergillus parasiticus*, *Trichoderma rubrum*, *Microsporium gypseum*.

### Anti-arthritis Activity

#### Inhibition of protein denaturation method (Deshpande V, 2009, M. Amin Mir, 2017)

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Plumbago zeylanica* extracts (100 and 250 mcg/ml of final volume). pH was adjusted at 6.3 using a small amount of 1 N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 30 min. After cooling the samples, 2.5 ml phosphate buffer

**Table 4.** Qualitative phytochemical analysis results of various plant extracts

S. No.	Constituents	Petroleum Ether extract	Chloroform extract	Ethanol extract	Water extract
1	Carbohydrates	+	-	+	+
2	Triterpenes	+	+	+	+
3	Proteins	-	-	-	-
4	Flavonoids	-	-	+	-
5	Glycosides	+	-	-	-
6	Alkaloids	-	+	+	-
7	Sterols	-	+	-	-
8	Tannins	-	-	-	-

(+) and (-) signs indicate presence an absence of the respective compound, repetitively

**Table 5.** The Antibacterial Activity of Various Plant Extracts

S. No.	Bacterial Strains	Zone of Inhibition in (mm)				Ofloxacin
		Pet. Ether extract	Chloroform extract	Ethanol Extract	Water extract	
B <sub>1</sub>	<i>Staphylococcus aureus</i>	8	12	9	-	20
B <sub>2</sub>	<i>Listeria monocytogenes</i>	10	12	-	-	22
B <sub>3</sub>	<i>Bacillus cereus</i>	-	4	8	-	20
B <sub>4</sub>	<i>Acinetobacter baumannii</i>	9	14	11	-	21
B <sub>5</sub>	<i>Salmonella typhi</i>	12	13	-	-	22
B <sub>6</sub>	<i>Pseudomonas aeruginosa</i>	14	14	-	-	23
B <sub>7</sub>	<i>Serratia marsecens</i>	11	16	10	-	23
B <sub>8</sub>	<i>Klebsiella pneumoniae</i>	-	8	10	-	20

(-) sign indicates no Zone of Inhibition

saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm for control test 0.05 ml distilled water was used instead of extracts while product control test lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows. Percent inhibition =  $100 - \frac{O.D. \text{ of test} - O.D. \text{ of product control}}{O.D. \text{ of control}} \times 100$

The control represents 100% protein denaturation. The results were compared with acetyl salicylic acid (250 mcg/ml) treated samples.

#### Observation and Results

The yields of various crude extracted fractions of powdered drug with different solvents were found to be as follows.

#### Phytochemical Screening

Following table represents the results of preliminary qualitative phytochemical screening of different extracts of *Plumbago zeylanica*.

#### Antimicrobial Analysis

Initially the petroleum ether, chloroform, ethanol and Aqueous extracts of stem of *Plumbago zeylanica* L. were tested at 500mg/ml against various gram positive and gram negative pathogenic bacteria. Among the extracts assayed chloroform extract exhibited good activity

against *Staphylococcus aureus* (12mm), *Listeria monocytogenes* (12mm), *Salmonella typhi* (13mm), *Pseudomonas auruginosa* (14mm), *Serratia marsecens* (16mm), *Klebsiella pneumoniae* (8mm) and moderate inhibition against *Acinetobacter baumanii*, (14mm) while there was no inhibition against *Bacillus cereus*.

#### Antimicrobial Analysis

On performing MIC analysis, the result showed that *Acinetobacter baumanii*, *Pseudomonas aeruginosa* and *Serratia marsecens* are sensitive against the least concentration the chloroform. MIC for *Acinetobacter baumanii*, *Pseudomonas, auruginosa* and *Serratia marsecens* were 25 mg/ml. the MIC of petroleum ether against *Klebsiella pneumonia* was also 25 mg/ml. The following table concludes the MIC analysis results:

The assay results indicate that the crude extracts of *Plumbago zeylanica* L. are active against various gram positive and gram negative pathogenic bacteria. The chloroform and petroleum ether extract were found to be active even in low concentration against bacteria for which the MIC test was performed.

#### Antifungal activity

The petroleum ether, chloroform, ethanol

**Table 6.** The Antimicrobial Activity of Petroleum ether and Chloroform extracts at various Concentrations

S. No.	Extract	Bacterial strain	Zone of Inhibition (mm)					
			400 mg/ml	300 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml
E <sub>1</sub> -B <sub>6</sub>	Petroleum ether	<i>PseudomonasAuruginosa</i>	8	8	7	5	5	3
E <sub>2</sub> -B <sub>4</sub>	Chloroform	<i>Acinetobacterbaumanii</i>	7	6	6	4	4	3
E <sub>2</sub> -B <sub>6</sub>	Chloroform	<i>Pseudomonasauruginosa</i>	8	7	6	5	5	3
E <sub>2</sub> -B <sub>7</sub>	Chloroform	<i>Serratiamarsecens</i>	9	9	8	5	3	2

**Table 7.** The antifungal activity of various plant extracts

S. No.	Fungal Strain	Zone of Inhibition in (mm)				
		Pet. Ether Extract	Chloroform Extract	Ethanol Extract	Water Extract	Clotrimazole
F <sub>1</sub>	<i>Aspergillus flavus</i>	2	3	4	1	10
F <sub>2</sub>	<i>Aspergillus parasiticus</i>	1	5	7	-	10
F <sub>3</sub>	<i>Trichoderma rubrum</i>	3	5	3	2	10
F <sub>4</sub>	<i>Microsporium gypseum</i>	2	2	4	2	10

(-) sign indicates no zone of inhibition.

and water extracts of stem of *Plumbago zeylanica* L screened for possible antifungal activity. But the assay results unexpectedly were found too well.

#### Anti-arthritis activity of *Plumbago zeylanica*

The Anti-arthritis activity of various *Plumbago zeylanica* extracts Viz (petroleum ether, chloroform, ethanol and water) have been analyzed, and it was found that (water extract) (Fig 2) possess the highest anti-arthritis activity followed by ethanol extract (Fig 3). The less anti-arthritis activity was observed for chloroform and petroleum ether extracts (Figs, 4 & 5). The  $IC_{50}$  value was determined from the straight line graph.

The  $IC_{50}$  value of all the plant extracts was found lesser than the reference compound Acetyl Salicylic acid (Fig 1). As per the results of  $IC_{50}$  value the lesser value corresponds to more inhibition potential of an extract, and the concerned extracts of the plant shows  $IC_{50}$  values as (46.55, 48.50, 53.21 and 56.52).

#### DISCUSSION

The phytochemical analysis of different extract of *Plumbago zeylanica* shows the presence of Carbohydrate, Triterpenes and glycosides

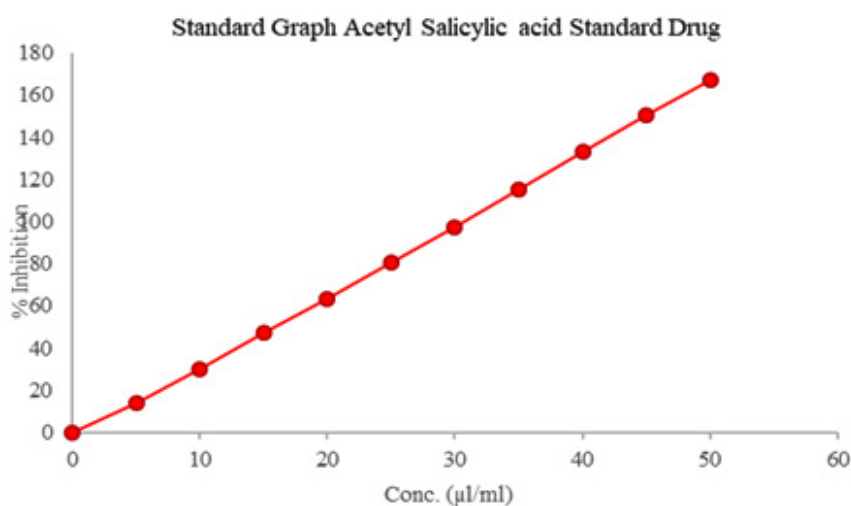


Fig. 1. Standard Graph of Acetyl Salicylic acid

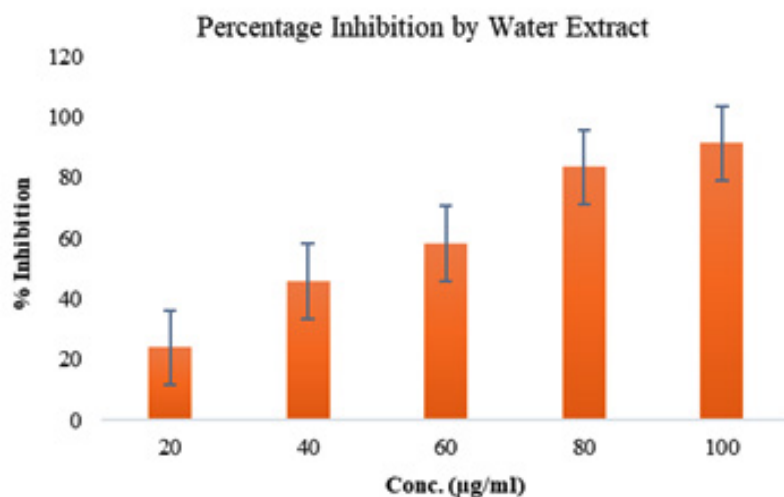


Fig. 2. Anti-arthritis activity by Water Extract

in the Petroleum ether extract. Carbohydrate, Triterpenes, flavonoids and alkaloids were found in Ethanol extract. The chloroform extract showed the presence of triterpenes, alkaloids and sterols, whereas water extract showed the presence of Carbohydrate and triterpenes.

The Extracts of *Plumbago zeylanica* were screened for anti-bacterial activity against various bacteria species, and the results indicate that the water extract showed no any activity against the concerned bacterial species. The petroleum ether extract inhibited the growth of all the selected bacteria excepted *Bacillus cereus* and *Klebsiella pneumoniae* and the maximum zone of inhibition was found against *Pseudomonas aeruginosa*. The chloroform extract inhibited the

growth of all the microbial species and maximum growth of inhibition was observed against *Serratia marsecens*. The ethanolic extract of the plant showed marginally less inhibition than the petroleum ether and chloroform extracts, although inhibited the growth of only *Staphylococcus aureus*, *Bacillus cereus*, *Acinetobacter baumanii*, *Serratia marsecens*, *Klebsiella pneumoniae* species. These results indicated that the plant *Plumbago zeylanica* have good antibacterial properties which could be because of the presence of many phytochemicals (Kirtikar KR, 1984, Thakur RS, 1989).

The antifungal Analysis of the plant extracts indicates that the ethanolic extract is most effective against all the selected fungus with maximum zone of in 7mm against the *Aspergillus*

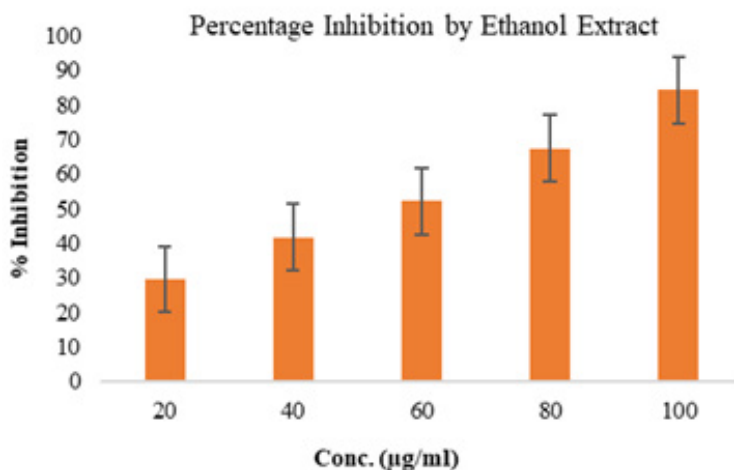


Fig. 3. Anti-arthritis activity by Ethanolic

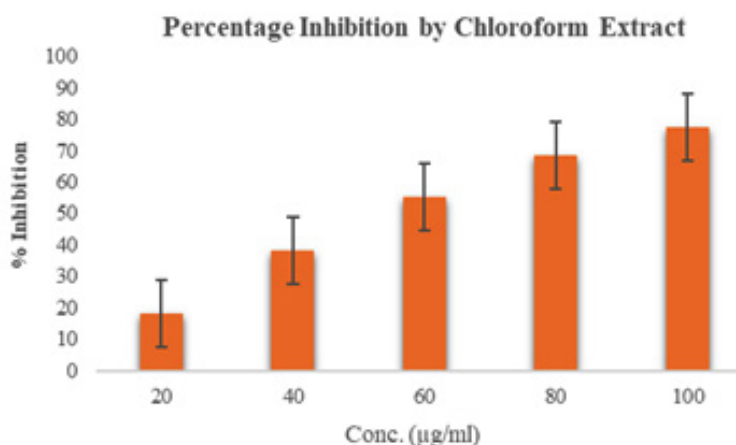


Fig. 4. Anti-arthritis activity by DCM Extract

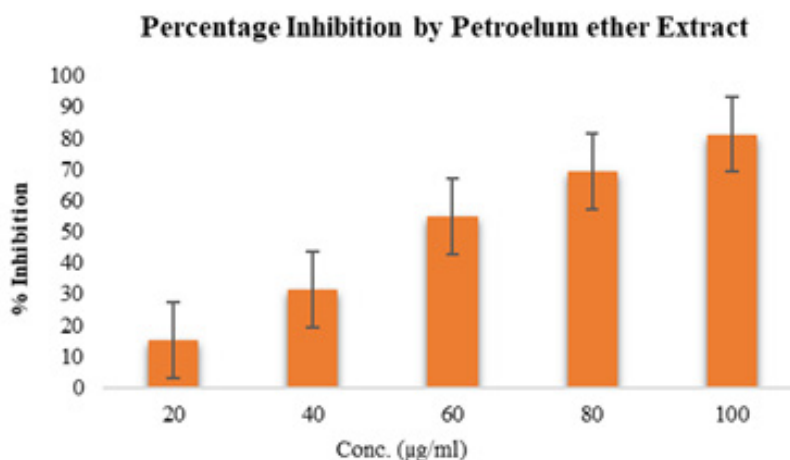
*parasiticus*. The Petroleum ether extract of the plant was effective against all the selected fungus with the max zone of inhibition of 3mm against *Trichoderma rubrum*. The chloroform extract shows the max zone of inhibition of 5mm against *Aspergillus parasiticus* and *Trichoderma rubrum*. The Aqueous extract shows the minimum zone of inhibition as compared with the other extract and was unveiled to inhibit the growth of *Aspergillus parasiticus*. The antifungal activities of *Plumbago zeylanica* could be mentioned due to presence of compounds (Tang Xiao-guang, 2002).

An arthritis or protein denaturation is a process in which proteins lose their tertiary and secondary structure due to external stress or compound, like strong acid or base, concentrated inorganic salt, an organic solvent or heat. All most all the animal proteins lose their biological function when denatured, like enzymes lose their activity, because the substrates can no longer bind to the active site. Protein denaturation is one of the cause of rheumatoid arthritis as elevated. Also in some cases the production of auto antigen may cause denaturation of protein in certain arthritic diseases. The Modulation of electrostatic, hydrogen, hydrophobic and disulphide bonding may cause the denaturation of proteins. This anti-denaturation effect further gets supported by the change in viscosities and it had been reported that the viscosities of protein solutions increase on denaturation. From the result of the present study, it can be stated that all the extracts of

*Plumbago Zeylanica* have the capacity to control the production of auto-antigens so inhibits the denaturation of proteins of both fresh egg albumin and bovine albumin in a dose dependent manner and there effect were compared with the standard drug Acetyl Salicylic acid. Water extract (Fig 2) of *Plumbago Zeylanica* have pronounced effect against arthritis, followed by ethanolic extract (Fig 3). The chloroform extract (Figure 4) showed less anti-arthritis activity as the  $IC_{50}$  value of about 53.21. Similarly the  $IC_{50}$  value 56.52 of petroleum ether extract showed less response against arthritis (Fig 5). All together the anti-arthritis activity of the concerned plant extracts could be due the presence of phyto-compounds with anti-arthritic activity (Tapiero H, 2002, Folts JD, 2002).

## CONCLUSION

The present study finds out a possible antimicrobial drug of natural source. Of the extracts screened for antibacterial activity mostly were active against most of the bacterial strains except aqueous extract that gave discouraging results and was not found to be active against any of the bacterial strains. So it could be concluded that the moderate antibacterial activity of the different extract have been analysed. The results of antifungal assay of the extracts unexpectedly were not found too well although the inhibition of fungi species was observed. Also the anti-arthritis activity shown by the plant extracts



**Fig. 5.** Anti-arthritis activity by Petroleum ether Extract



was found very encouraging as almost all types of extracts responded good for the production of auto-antigens so inhibits the denaturation of proteins. The antibacterial, antifungal and anti-arthritis activities can further be enhanced if the phyto-active components responsible for curing such diseases could be determined by GC-MS and NMR analysis, then isolated, purified and applied in proper dosage form.

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#### Conflict of interest

The authors declare no conflict of interest.

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