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IN VITRO ANTHELMINTIC EFFICACY OF FRACTIONS *FROM PLUMBAGO ZEYLANICA L* (FAMILY- PLUMBAGINNACEAE) ROOT EXTRACT

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ABSTRACT: *Unlike synthetic drugs plants have different phytoconstituents which can act collectively by which helminths cannot resist them or there could be active constituent(s) in the plant with superior potency. The aim of this study is to investigate the anthemelmintic activity of both crude and fractions of Plumbago zeylanica root extract. Crude extract was subjected to column chromatography from which nine pure compounds were isolated. Chloroform crude extracts recorded less paralysis and death time than ethanolic crude extracts. In addition, the isolated compounds were higher in their anthelmintic activity than crude extracts at almost all concentrations. Both crude and fractions paralyse and kill the worms with less time than that of the positive control and even less than 10 fold especially at low concentrations in case of chloroform extracts.*

KEYWORDS: Plumbago Zeylanica L, In vitro test, anthelmintic activity, extraction

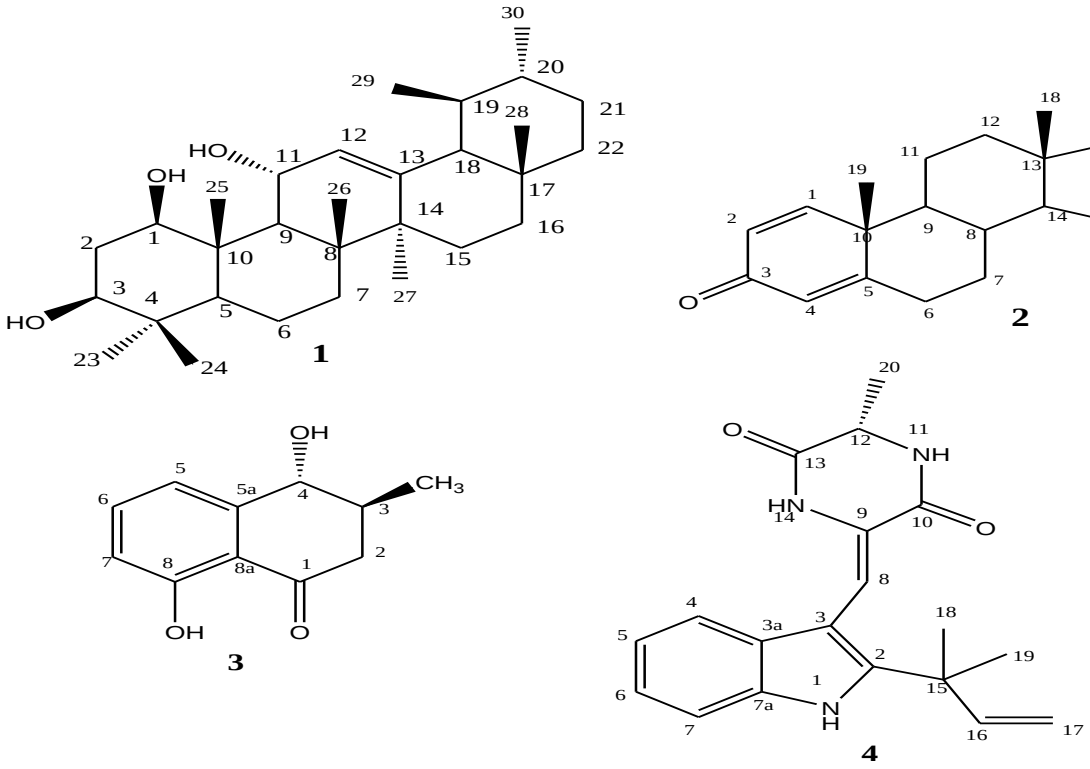
INTRODUCTION

In addition to infectious diseases parasitic worms are another alarm. They cause substantial privation and diminutive growth in animals and man. There are conditions that excides malaria and tuberculosis. Massive drug administration to control human helminthes can minimize but then it leads to emergence of anthelmintic resistance (Vidyadhar et al., 2010, Tiwari et al., 2011). When anthelmintic drug is administered sequentially, it eliminates susceptible helmenths without affecting for parasites that are resistant. The resistant parasites in turn pass their resistant genes on to the next generation of worms (Kumar et al., 2005). The majority of diseases caused by helmenths are persistent, weakening nature; and probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites (Suleiman et al., 2015). Therefore, unless drugs especially those synthetic origin are modified or substituted by plant origin drugs with the same or higher potency, drug resistance will be unmanageable. Especially in developing countries like Ethiopia the issue is even more critical.

Chemistry of *Plumbago zeylanica L*

The plant has demonstrated promising bio activity for its wide range chemical constituents.

One investigation done on a real parts of *Plumbago zelanica L.* 95% ethanol extract confirmed the presence of seven compounds with the aid of various chromatographic and spectroscopic techniques. According to the study the one triterpenoid (compound **1**) was new while compounds **2**, **4**–**7** were obtained from this genus for the first time. Their structures together with their names are displayed (Fig. 1) below.



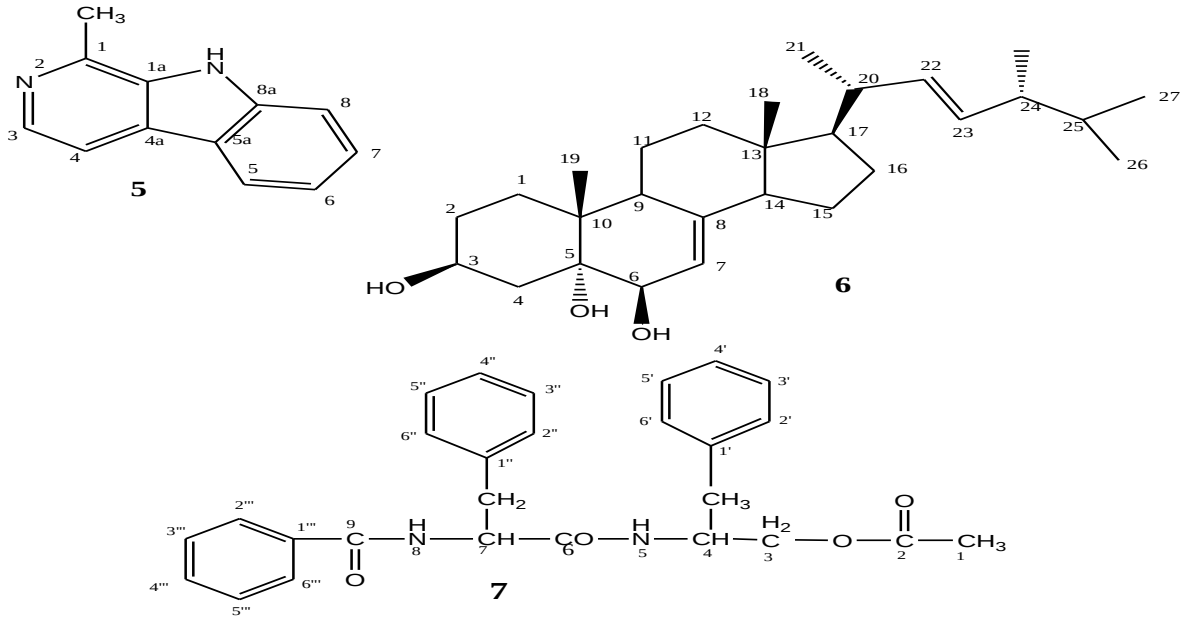


Fig 1. Structures of compounds 1-7 from *Plumbago zeylanica L.* (1), 1β,3β,11α-trihydroxy-urs-12-ene, (2), androsta-1,4-diene-3,17- dione, (3), isoshinznolone, (4), neoechinulin A, (5), harman, (6), ergostadiene-3β,5α,6β-triol and (7), *N*-(*N′*-benzoyl-*S*-phenylalaninyl)-*S*phenylalaninol (Huang et al., 2008).

Phytochemical analysis of crude extracts showed the presence of alkaloids, phenols and flavonoids (Dhal and Markandeya, 2011; Ahmad and Aquil, 2006). In addition the presence of tannins and saponins was detected from methanolic root extracts (Dhal and Markandeya, 2011). The root was found to contain the naphthoquinone plumbagin, composed naphthoquinones, like 3-biplumbagin, chloroplumbagin, chitranone and elliptone; the coumarins seselin, 5-methoxyseselin, suberosin and xanthyletin. Among all these compounds plumbagin (5-hydroxy-2-methyl-1,4- naphthoquinone, ($C_{11}H_8O_3$)) reported to be the major ingredient with 1% in the whole plant, but with higher percentages in the root. The stem brings only a trace and the leaves bring no plumbagin (Sharma et al.,1991). Plumbagin in general is found in different plant families including Plumbaginaceae, Droseraceae, Ancestrocladaceae and Dioncophyllaceae. Plumbagin is also present along with a series of other structurally related naphthoquinones (Aqil et al., 2008).

From fractionation of areal parts of *Plumbago zeylanica L.*A in bioassay guided system ß-sitosterol, ß-sitosteryl-3ß-glucopyranoside-6`-O-palmitate, lupenone, lupeol acetate, plumbagin, and trilinolein was revealed to be isolated (Nguyen et al., 2004).
On the other hand phytochemical investigation on the leaf; alkaloids, glycoside, reducing sugars, simple phenolics, tannins, Lignin, saponins and flavonoids gave positive results (Tyagi and Menghani, 2004). In a search for larvacidal activity one study found β-Sitosterol (17-(5-Ethyl-6-

methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol) and plumbagin. The study had utilized column chromatography and1D, 2D NMR to find out these compounds (Maniafu et al., 2009). Also another journal intended at evaluating the anti-inflammatory and cytotoxic effects of extract from *Plumbago zeylanica* found out betasitosterol and gugultetrol-18-ferrulate with the help of silica gel column chromatography, high performance liquid chromatography (HPLC) and proton and carbon nuclear magnetic resonance spectroscopy analysis (1H and 13C NMR), Infra red and mass spectroscopy. In the same study prelimenary phytochemical screening of dichloromethane extract of *Plumbago zeylanica* root confirmed the presence of terpenoids, flavanoids and absence of steroids, carbohydrate, alkaloids and tannins (Arunachalam et al., 2010).

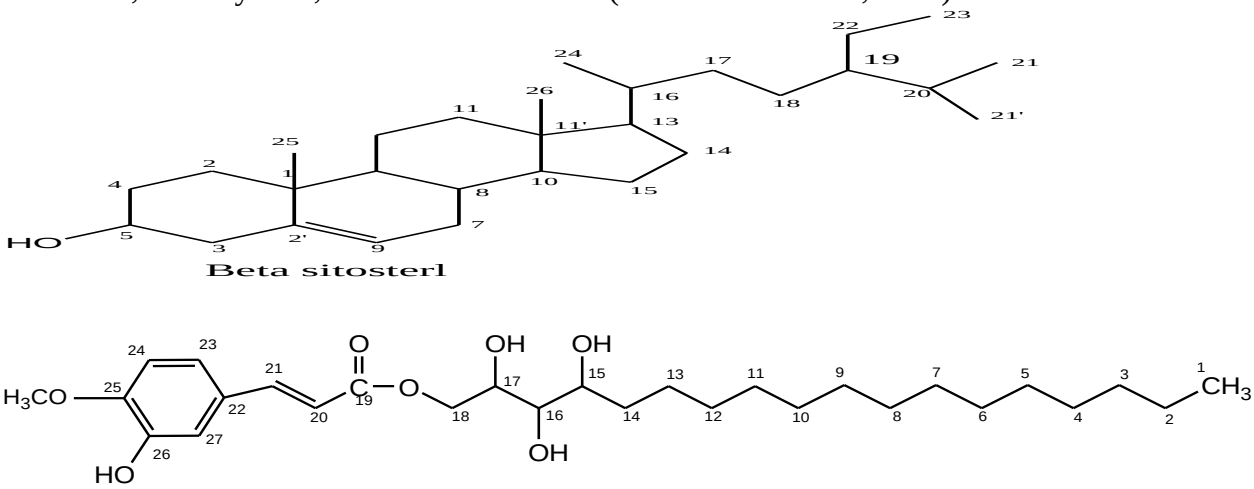


Fig 2. Beta sitosterl and guggltetrol-18-ferrulate

On one study, *Plumbago zeylanica L* extracts were run in TLC with chloroform/methanol solvent system (8:2) and yield four bands. Plumbagin alone was detected by spraying with 10% (w/v) ethanolic solution of KOH, followed by heating at 100°C until the red color appeared to the first band. Further it was confirmed by comparison of ^{1}H, ^{13}C NMR and GC-MS spectral data with values described in the literature for plumbagin (Jeyachandran et al., 2009).

A flavonoid compound (Fig. 3) known as 2-(2, 4-Dihydroxy-phenyl)-3, 6, 8-trihydroxy-chromen-4-one (yield: 0.082% on dry weight) was also detected in another study by spraying with ferric sulphate reagent. To confirm this elucidation was done by means of UV, IR ^{1}H- and ^{13}C-NMR spectroscopic methods (Nile and Khobragade, 2010).

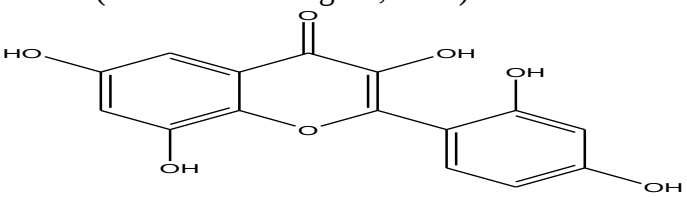


Fig 3. 2-(2, 4-Dihydroxy-phenyl)-3, 6, 8-trihydroxy-chromen-4-one

Biological activity of *Plumbago Zeylanica*

Antimicrobial activity:

A journal paper from Kollihills, south India revealed that *Plumbago zeylanica L.* extracts were active even than the standard streptomycin (10mg/disk). Chloroform extracts show highest activity. Moreover the methanolic extract exhibited moderate activity and the aqueous extract weak activity against the bacterial strains as assessed by disc diffusion assays. Bioassay guided isolation was done employing preparative thin layer chromatography and plumbagin alone was isolated and recorded highest activity than the crude extracts and than the standard drug against all the bacterial organisms utilized. The methanolic extract showed significant activity against these bacteria more at concentration of >11–18µg/disc (Jeyachandran et al., 2009). Plumbagin isolated from *Plumbago scandens* after soxhlet extraction with chloroform and fractionation with column chromatography (n-hexane, ethyl acetate 2%) was tested against one gram positive bacteria and one pathogen fungi. The data shows MIC to be 1.56µg/ml, 0.78µg/ml and MBC 25µg/ml, 1.56µg/ml for *Staphylococcus aureus* and *Candida albicans* respectively as determined by macro dilution technique (Paiva et al., 2003).

Ethanolic extract of *Plumbago zeylanica L* root was investigated for its antimicrobial activities against 11 human pathogenic bacteria and 6 phytopathogenic fungi using disc diffusion method and poisoned food technique respectively. The extract exhibited good antibacterial and antifungal activities against the test organisms. Among the test bacteria, *Vibrio cholerae* was found to be the most sensitive to the extract showing the highest diameter of zone of inhibition and lowest minimum inhibitory concentration (MIC) value (200mg/ml). Among the phytopathogenic fungi tested, *Curvularia lunata* exhibited the highest sensitivity to the extract with an MIC value of 150mg/ml (Rahman and Anwar, 2007).

A comparative study of the root versus callus of *plumbago zeylanica L.* in various test microorganisms revealed that the root and the callus as well have antimicrobial activity (in vitro). But the root has found to have highest activity. It was found that the root extract show zone of inhibition against all microorganism whereas callus extract show maximum zone of inhibition against the *S. aureus* and *M. luteus*. MIC of root extract against *S. aureus* and *M. luteus* was 1250 and 2500µg/ml respectively. Whereas the MIC of callus extract against these microorganisms was 5000 µg/ml as determined by turbidity method (Mittal and Sharma, 2010).
Plumbago zeylanica L. extracts (ethyl acetate fraction) also showed bactericidal activity against *Helicobacter pylori*. Four fold MIC concentrations of the extracts killed all the population with in the 4 hrs of incubation while the two fold concentration showed the similar effect in 8 hrs. *Plumbago zeylanica L.* demonstrated promising in vitro efficacy against multidrug resistant bacteria and it is ranked in a group of plants with over all broad spectrum of antimicrobial activity (Wang and Tung-Liang Huang, 2005).

Antioxidant activity:
Ethanolic root extracts *Plumbago zeylanica L* and isolated flavonoid (2-(2, 4-Dihydroxy-phenyl)-3, 6, 8- trihydroxy-chromen-4-one) were screened for antioxidant activity by free radical scavenging and superoxide radical scavenging assays. The plant root extracts showed significant antioxidant activity as compared to standard flavonoid (Quercetin). The antioxidant activity by DPPH was 96µg/ml and by NBT it was 4.6µg/ml which was greater than that of standard (Quercetin) 45µg/ml by DPPH and 10µg/ml by NBT assay (Nile and Khobragade, 2010).Including *Plumbago zeylanica L.* four Indian medicinal plants were assessed for their antioxidant capacity by ferric thiocyanate (FTC) assay and compared with thiobarbituric acid (TBA) method. *Plumbago zeylanica L.* showed highest antioxidant potential according to FTC assay. Further, the radical-scavenging activity of the extracts was measured as decolourizing activity followed by the trapping of the unpaired electron of DPPH. The percentage decrease of 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) standard solution was recorded significant for *Plumbago zeylanica L*. (73.41%). It was the second compared to the other plants.

Methanolic extract of leaves of *Plumbago zeylanica L*. was also checked for their total antioxidant activity. At all the studied concentrations, the plant extract showed slightly higher activity than α-tocopherol (Kataki et al., 2009).In one study the in vitro antioxidant activity of ethanolic extract of roots of *Plumbago zeylanica* was investigated by DPPH free radical scavenging, nitric oxide scavenging and superoxide scavenging methods at dose of 100–1000µg/mL. The ethanol extract showed good antioxidant activity in these methods. The maximum activity was found in DPPH free radical scavenging model. The antioxidant activity was dose dependent.

There are various in vitro antioxidant test methods like reducing power and nitric oxide scavenging activity and in vivo models like tissue GSH levels and lipid peroxidation. Ethanol extracts of leaves of *Plumbago zeylanica L* exhibited significant in vitro and in vivo antioxidant activity in those assays (Khan et al., 2006).

Anthelmintic activity:
Leaf extracts *P. zeylanica L*.were tested for anthelimntic activity against adult earth-worms (*Pheretima posthuma*) at 25, 50 and 100 mg/ml concentrations. All of the three concentrations of extracts of *Plumbago zeylanica L.* showed significant dose dependent anthelmintic property. Results clearly indicated that 100 mg/ml concentration of the extract has the highest potency as an anthelmintic (took least time to cause paralysis and death of worms) when compared to standard drug piperazine citrate and albendazole (Kataki et al., 2010).Anthelmintic activity of the root as confirmed in another study done at various concentrations (5, 10, 15, 20mg/ml) reveal that methanolic extract of *Plumbago zeylenica* showed higher activity as compared to water extract. Methanolic extracts kill the worms in 81 ±1.5min at 20mg/ml compared to standard piperazine citrate which kill the worms in 36±0.9 at same concentration. Anthelmintic activity was observed by gradually increasing the dose of extract (Desai et al., 2009).

Plants such as *plumbago zeylanica* with all this phyto constituents and bioactivity should be assessed for different assays In different methods and at different places. As drug resistance is really a matter finding long lasting plant derived drug will be the immediate measurement. The goal of this study was to test the anthelmintic activity of *Plumbago zeylanica* root and fraction extracts. There are few reports especially on the anthelmintic property of root extract of the plant and this paper will be the first to report on the anthelmintic activity of fractions and that of crude extract at low concentrations.

EXPERIMENTAL

Materials

Chemicals and solvents

Sodium chloride, siligcagel, sodium sulphate anhydrous (Na_2SO_4), distilled water, cyclo hexane, diethyl ether, chloroform, dichloromethane, carbontetrachloride, ethyl acetate, acetone,n-hexane, methanol, ethanol and Tween-80.

Instruments and equipments

Ultra violet – visible light, rotary evaporator (laborata 4000, Heithbad bath, 230,50/60 Hz), electrical shaker, soxhlet extractor set up, separatory funnel, Thinlayer chromatography plate(glass and aluminum support), chamber, glass column chromatography, vacuum pump, oven, fridge and desikator were the equipments utilized.

Test organisms

Earthworm: *Pheretima posthuma*

Methods

Material collection

Plumbago zeylanica L. roots fresh, were obtained in the month Aug - Sep/ 2013. Voucher specimen was deposited at the National Herbarium of Addis Ababa University with voucher specimen number B (003).

Extraction

Shade-dried roots of *Plumbago zeylanica L* were crushed in to powder using mortar and pestle. The dried and powdered root material (156g) was extracted in 800ml chloroform for 36h at once using soxhlet extraction method. Root powder of *Plumbago zeylanica* (238g) was also extracted by maceration in 1.5 liters of ethanol for three day on an electrical shaker (shake speed 220 at room temprature). Both the extracts were filtered using What man No1 filter paper and the filtrate was concentrated by rotary evaporator at room temperature and further with vacuum pump (Mittal and Sharma, 2010; Dzoyem et al., 2007, Aladesanmi et al., 2017).

Preparation of crude extracts and isolated fractions for bioactivity test

Exactly 0.2g of crude chloroform extract was dissolved in 2ml chloroform to get 100mg/ml concentration. This was then serially diluted to obtain 50mg/ml, 25mg/ml, 10 mg/ml and 5mg/ml concentrations as shown in (Fig. 4) below.

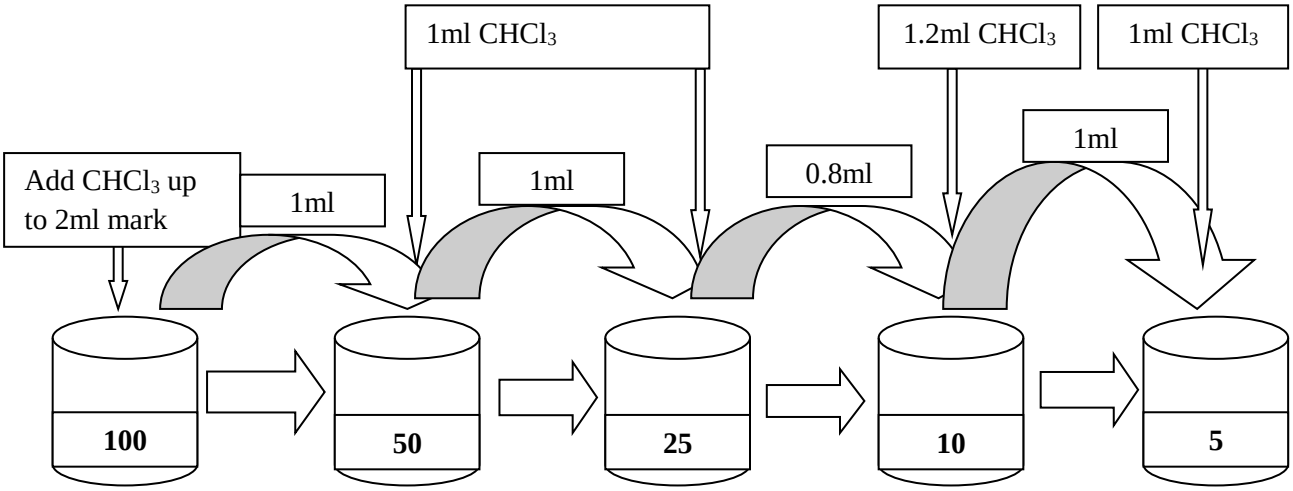


Fig. 4: Serial dilution procedure

This procedure was repeated for ethanol extracts using ethanol as a solvent for dilution. In preparation of samples for anthelmintic test 0.2g of both chloroform and ethanol extracts was taken separately and serialy diluted in the same procedure explained above, but the dilution was done with 2% Tween 20 suspended in normal saline solution (Shahverdi, et al., 2005). Similar dilution procedure was applied for the fractions corresponding to their yield. All of the fractions were prepared in two to three fold.

ANTHELMINTIC ACTIVITY

Earthworm's collection and authentication

Healthy adult earthworm (*Pheretima posthuma*) were collected from water logged area of the soil and identified in Department of Biology of Mekelle University. Earthworms in moist soil were washed with normal saline and used for the study. The earthworms of 4 -7 cm in length and 0.1- 0.2 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Because of easy availability, earthworms have been used extensively for the preliminary *in vitro* evaluation of anthelmintic compounds (Tiwari et al., 2011).

Anthelmintic investigation of the crude chloroform and ethanol extracts

The anthelmintic activity was done following the method described in (Lakshmanan, B. et al, 2011) with modest modification.

The worms were divided into three groups containing six-earth worms in each group. All the prototypes were dissolved in minimum quantity of 2%v/v Tween80 and the volume was adjusted to 10 ml with normal saline for making the concentration of 1, 2, 3, 4, 5, 10, 25, 50 and 100mg/ml for chloroform crude extracts and 5, 10, 25, 50 and 100mg/ml for ethanol extracts. All the prototypes and the standard drug were freshly prepared before commencement of the experiments. All the earthworms were washed in normal saline solution before they were released into 10ml of respective formulation as follows, vehicle (2% v/v Tween80 in normal saline), extracts and piperazine citrate at (1, 2, 3, 4, 5, 10, 25, 50 and 100mg/ml). The anthelmintic activity was determined in six observations. Six worms in about the same size per petridish were used. They were observed for their spontaneous motility and evoked responses. Observations were made for the time taken to paralysis and death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline with ice. Death was concluded when the worms lost their motility in cold water followed with fading away of their body color (Tiwari et al., 2011).

Anthelmintic investigation of fractions

Similar procedure was followed as for the crude extracts. The only differences were 2 -3 earth worms were included in a group and final dilutions were fixed to 2- 5 mililtres attributed to their yield.

Statistical analysis

Calculations were carried out in triplicate with their mean values and standard deviations by formula in the Microsoft excell.

RESULT AND DISCUSSION

Yield of plumbago zeylanica root powder

Soxhlet extaction of the root with chloroform and maceration with ethanol gave 0.82% w/w and 3.31% w/w of the powder extracted respectively.

Anthelmintic activity

Chloroform crude extracts

The anthelmintic activity of chloroform crude extracts was significant. They paralysed and killed the earthworms by less than half the time taken for piperazine citrate to paralyze and kill the worms (Table 1). At lower concentrations, the time taken to paralyse and kill the earthworm was less than 10 fold to that of the positive control. For example, the paralysis and death time for chloroform crude extracts was 540, 552 and 900, 960 seconds at 2 and 1mg/ml respectively. Whereas, for piperazine citrate they were 12000, 16200 and 30000, 35400 seconds at the same

concentrations. Even if chloroform is not as polar as ethanol, methanol or water some of the bioactive compounds such as alkaloids, flavonoids and quinones are extractable within it. The bioactivity of alkaloids on central nervous system also works for worms (Tiwari et al., 2011).

Table 1. Anthelimnti activity of crude chloroform extract of *Plumbago zeylanica L* against Adult earthworms *Pheretima posthuma*

Treatment group	Concentration mg/ml	Time taken (seconds)	
		Paralysis	Death
Chloroform extract	**100**	120±21	192±26
	50	150±22	240±33
	25	192±19	312±24
	10	282±20	378±59
	5	360±26	408±81
	4	468±33	480±90
	3	510±25	540±64
	2	540±16	552±82
	1	900±29	960±100
Piperazine citrate	**100**	300±27	1080±93
	50	540±35	1800±96
	25	960±28	3240±135
	10	1380±49	3740±125
	5	2700±56	4380±180
	4	3600±68	4680±200
	3	4920±45	5700±250
	2	12000±67	16200±320

	1	30000±36	35400±402

- Results on this biological study were reported as mean ± Standard deviation. n= 6 in each group.

Ethanolic crude extracts

Ethanolic extracts showed highest activity than the positive control but less than the chloroform extracts. Earth worms die at 600 and 960 seconds in ethanol extracts at 100 and 50mg/ml. While, the positive control killed the worms at 1080 and 1800 seconds at the same concentration. However, as the concentration decreased worms were paralyzed and killed by pirerazine citrate at relatively shorter time than the ethanolic extracts. Journal papers published in this assay suggested the reason for the potency of their plants is mainly relied to the presence of alkaloids, tannin, flavonoids etc (Vidyadhar et al., 2010; Sarojini et al., 2011; Parida et al., 2010; Mali and Mehta , 2008; Roy et al., 2010). The significant anthelmntic activity of ethanolic extracts in the present study can be arged in the same way.

Table 2. Anthelmintic activity of crude ethanolic extract of *Plumbago zeylanica L.* against adult earthworms *Pheretima posthuma*

Treatment group	**Concentration mg/ml**	**Time taken (seconds)**	
		Paralysis	**Death**
Ethanol extract	**100**	270±23	600±32
	50	300±30	960±23
	25	900±43	2580±95
	10	3000±51	4800±67
	5	3600±57	6300±26
Piperazine citrate	**100**	300±42	1080±67

	50	540±46	1800±55
	25	960±29	3240±65
	10	1380±68	3740±56
	5	2700±84	4380±92

- Results on this biological study were reported as mean ± Standard deviation. n= 6 in each group.

In the literature it was discussed the anthlmintic activity of the methanolic extracts of *Plumbago zeylanica L*. leafs against adult earthworms *Pheretima posthuma*. Compared to present study anthlmintic activity of the leaf is much less. Leaf extracts paralysed and killed the worms at 26.833 and 33 minutes respectively (Kataki et al., 2010). Both chloroform and ethanolic extracts paralyse and kill worms in less than 11minutes at the same concentration (100 mg/ml). On another study, anthelimintic activity of metanolic extract of the root paralyse and kill the worms in 33 ± 1.6 and 81 ±1.5min at 20mg/ml while water extracts paralyse and kill the worms in 190 ±1.2 228± 1.2min at same concentrations. In comparison to this study, the present findings were even less than to that of approximately 4.7, 6.3 min paralysis and death time recorded by chloroform crude extracts (Desai et al., 2012).

Anthelmintic activity of fractions

Chloroform crude extract fractions

Isolated compounds of the chloroform crude extract were tested for anthelmintic activity at different concentrations. With respect to chloroform crude extract at parallel concentrations all the fractions show higher activity, but **Pure F_1** at 1mg/ml and **Pure F_5** at 34 and 15mg/ml. The time of paralysis and death of adult earth worms *Pheretima posthuma* decreased with increase in concentration (Table 3)

Table 3. Anthelimntic activity of nhexane – ethyl acetate (F_1-F_7) and chloroform (F_8) fractions of chloroform crud extracts of *Pumbago zeylanica L.* against adult earthworms *Pheretima posthuma*

Treatment groups	**Concentrations (mg/ml)**	**Time taken (seconds)**	
		Paralysis	**Death**
Pure F_1	1	4560±213	6060±184
	0.5	5040±301	7320±55
Mixture F_2	3	300±65	480±61
	1.5	600±23	840±34
Pure F_3	22.6	48±11	60±14
	10	72±9	120±17

Mixture F_4M	31.5	78±11	156±41
	15	162±12	240±58
Pure F_5P	34	180±19	540±97
	15	840±98	1020±123
Pure F_6P	25	72±10	120±17
	10	120±32	210±37
Pure F_7P	12	240±25	500±56
	5	300±24	550±43
Pure F_8P	4	90±16	120±21
	2	203±18	250±33

- Results on this biological study were reported as mean ± Standard deviation. n = 2-5 in each group.

Ethanolic crude extract fractions

The data from the Table 4. below shows that the isolated compounds have superior anthelmintic activity than analogous crude extracts. They take less time to paralyse and kill the worms compared to positive control. Bioactive plant chemo constituents are commonly extractable with ethanol. These phytochemicls are still the reason behind the significantly higher activity of these fractions too. In agreement to this study plants extracted with ethanol were found to be potent anthelminthes. Higher inhibition was recorded than the standard drug used in the assay. Ethanolic crude extracts of *Saraca indica* leaves for instance were superior in their anthelmntic activity than the methanol extracts and piperazine citrate a positive control in the assay within the same study (Sarojini et al., 2011). Extracts from *Symplocos racemosa* were also more active in those groups in which ethanol as extracting medium compare to pet ether extracts and similar with chloroform extracts (Rao et al., 2011). Whereas, crude ethanol extracts of *Pterospermum acerifolium Linn*. were found to exceed in their activity against *Pheretima posthuma* (worm) in contrast to pet ether, ethyl acetate, chloroform extracts of the bark(Parida et al., 2011).

Table 4. Athelmintic activity of n hexane – ethyl acetate (F_A-F_C) fractions of ethanolic crude extracts of *Plumbago zeylanica L.* against adult earthworms *Pheretima posthuma*

Treatment groups	**Concentrations(mg/ml)**	**Time taken (seconds)**	
		Paralysis	**Death**
Pure F_A P	8.7	300±29	420±45
	4	360±44	420±54
Mixture F_B	99.09	72±8	80±26
	50	78±19	96±13
Pure F_C P	11.75	420±21	1080±152
	5	1200±114	1560±196

- Results on this biological study were reported as mean ± standard deviation. n= 2-5 in each group.

CONCLUSION

Chloroform and ethanolic root extracts of have observed to be inhibiter to earth worms *Pheretima posthuma*. At all the concentrations used they paralysed and killed the worms by considerably shorter time than the standard piperazine citrate. Higher potency was recorded in chloroform extracts compared to ethanolic extracts. It could be concluded that *Plumbago zeylanica L.* root have anthelmintic efficacy. Extractable individual compounds which can be converted to antihelmintic drug can be obtained such as the nine pure compounds isolated here. The assays done here are in vitro which require further data from in vivo studies to be valuable. Though nine pure compounds are isolated here they lack spectroscopic analysis to identify the actual chemical constituents and to relate the data with their structures and functional groups.

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