INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 9, No. 4, p. 81-91, 2016

RESEARCH PAPER OPEN ACCESS

Anthelminthic analysis of two local weeds of Punjab viz. *Trianthema portulacastrum* L. and *Zaleya pentandra* (L.) C. Jeffrey

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Key words: Anthelmintic, *Haemonchus contortus*, Piperazine citrate, Aizoaceae

http://dx.doi.org/10.12692/ijb/9.4.81-91 Article published on October 11, 2016

Abstract

The two plants of family Aizoaceae, *Trianthema portulacastrum* L. and *Zaleya pentandra* (L.) C. Jeffrey were compared from anthelmintic perspective. The process of steady-state maceration was used for the extract preparation and established that more phytochemical contents were macerated in fruit extract of *T. portulacastrum* L. and*Z. pentandra* (L.) C. Jeffrey. The dose-dependent anthelmintic appraisal carried out employing *Haemonchus contortus*at four concentrations (10, 20, 50 and 100mg/mL) render the stem macerates of *T. portulacastrum* L. and the fruit extracts of *Z. pentandra* (L.) C. Jeffrey most effective. Piperazine was used as a reference standard at a concentration of 10 mg/mL. Conclusively, the results of both plants under inquisition were relatable in some aspects but none of the test specimen outperformed other.

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Introduction

Bearing in mind the conventional consumption of plants in developing nations, the current study aims to evaluate the efficacy of the plant specimens as an anthelmintic substitute (Batista *et al*., 1999; Slomp *et al*., 2009). Even though sufficient number of plants bearing anti-parasitic attributes had been documented, still it deem necessary to scrutinize native flora in pursuit of more potential anthelmintics. Also, plants with average anti-parasitic features should also be notified as they might serve the purpose in an effective manner against any other nematode beside test organism (Saverino and Ambrosio, 2012).

Helmintics are not onlyamong one of the conspicuous determinants of the adverse influence on economy, instigating considerable losses by impacting agronomic production inducing diminutive growth (Geary, 2005) but are also accountable for numerous prolonged infirmities in livestock animals, domestic pets and human beings (Manoj and Pal, 2011). Approximately two billion folks harbour parasitic worm contagions rendering to the investigation completed by World Health Organization (Geary, 2005).

The medicaments engaged to cure contagions ensued considering the parasitic worms comprising either flat worms i.e., tapeworms and flukes or round worms i.e., nematodes, are designated as anthelmintics. Notwithstanding the pervasiveness of helmintics, the therapeutical markets had provided merely a slight fiscal and technical backing on medications having antihelmintic potency. One modest elucidation to this miserable circumstance is that these scrounging worms provoke ailments mainly in the tropical arena where nations are not economically sound and hence, could not afford the drug discovery. The anthelmintics produced primarily were merely to cure fauna and not the mankind. The ample range of response, extraordinary cure fraction with a single pharmaceutical dosage, free from noxiousness and cost efficiency are the few specifications that an ideal medicine has to accomplish. Unfortunately, neither of the anthelmintic medications agrees with these particularizations (Mali and Mehta, 2008).

With the progression in the resistance gradient of such pathogenic worms, the helmenthosis is disseminating at considerably rapid pace evoking apprehensions universally for the exploration of utmost effective herbal substitutions. One of the paramount worm ailment was induced by gastro-intestinal helminthes i.e., treatable earlier but the misuse of the chemically manufactured remedies had render it resilient to the corresponding treatments (Manoj and Pal, 2011).

Mother Nature had endowed mankind with diverse array of phytochemicals that can encounter the infirmities instigated by the helmintics. Botanical anthelmintics had secured considerable attention in technologically advanced as well as developing countries in the course of previous few years owing to its worthy usefulness and cost efficiency. Primarily, they were documented to be the reservoir of effective veterinary anti-helminthic (Getachew *et al*., 2012).

Even though weeds were considered as unwanted for a number of reasons, the most important one is that they interfere with food and fiber production in agriculture, but there are many weeds having ethno-medicinal and pharmacological value. The current investigation was an effort to investigate the anthelmintic capacity of root, stem, leaf and fruit of two local weeds of Punjab belonging to the carpet-weed family, known as Aizoaceae (Ficoidaceae) i.e., *Trianthema portulacastrum* L. and *Zaleya pentandra* (L.) C. Jeffrey. *T. portulacastrum* L. is an exotic weed that is contemplated to be the indigene of tropical America. At present, it has encroached 39 crops beyond 40 countries, frequently prevailing in maize, mustard, potato, onion, cotton, rice and sugarcane, eminently amid rainy periods (Holm *et al*., 1997). Horse purslane, Black pigweed, Narma, Bishkapra and Itsit are a few trivial nomenclatural terminologies utilized by the local people (Gledhill, 2008). *T. portulacastrum* L. is still employed in the Ayurvedic medication as anodyne, purgative, stomachic, cure of blood ailments, anemia, night blindness and tenderness; consequently, replenishing motivation for appropriate appraisal of the plant in medical prescriptions (Khare, 2006).

Zaleya pentandra (L.) C. Jeffrey is a prevalent weed found both on road edges and cultivated domains (Holm *et al.*, 1997). African purslane and Itcit are the aborignal nomenclatural terminologies utilized by natives (Gledhill, 2008).*Z. pentandra* (L.) C. Jeffrey is utilized in Ayurvedic medicine for stomach disorders, snake bite and as forage for cattle (Afzal *et al.*, 2013).

Materials and method

Test organisms

In vitro anthelmintic potential of *Trianthema portulacastrum* L. and *Zaleya pentandra* (L.) C. Jeffrey was determined in contradiction to roundworm i.e., *Haemonchus contortus*. The test entities were procured by dissecting abomasum of freshly butchered goat acquired from regional abattoir, after rinsing the abomasum with saline (0.9% NaCl) solution to discard entire filth. *H. contortus* were authenticated by Department of Zoology, GC University, Lahore and retained in 0.9% NaCl solution till subjecting them to further investigation.

Plant specimen

T. portulacastrum L. was collected from the Botanic Garden of Government College University, Lahore in October. While,*Z. pentandra* (L.) C. Jeffrey was collected from road edges of Changa Manga forest plantation in November. The healthy plant specimens were opted, as pathogenic contagions could lead to the alteration of metabolic profile of plant. The plant specimens were identified, assigned the authenticated voucher numberfollowed by their deposition in the Dr. Sultan Ahmed herbarium, Department of Botany, GC University, Lahore.

Maceration

The plant material was primarily rinsed with cold flowing tap water, mildly brushed to eliminate soil and remaining detritus, separated into its constituents including root, stem, leaf and fruit, dispersed uniformly on trays to ease consistent drying and then administrated to desiccation in shade under optimum conditions at room temperature, for 20-30 days. Subsequently, the dehydrated plant material was pulverized to powdered texture with pestle and mortar before subjecting it to maceration.

The crude or traditional extracts were formulated employing steady-state maceration (Seidel, 2006). The weighed quantity of the finely grated plant material was uniformly positioned in impenetrable glass container and was drenched in the solvent: menstruum. The solvents including *n*-hexane, chloroform, ethanol and distilled water were applied in accordance to their polarity gradient, initiating from non-polar solvents with gradual shift to polar solvents. Dissimilar quantity of plant components were engaged in maceration procedure as per their accessibility and the consummate quantity of the fluent was adjusted correspondingly (Table 1).

The glass container was positioned at the room temperature with repeated agitation for 7 days. Afterwards, the ingredients present within the glass container were filtrated via Whatmann filter paper no. 4. The fluid was poured into the Erlenmeyer flasks for the further processing, while the residue of the plant material left behind (known as marc) was air-dried for about 20 minutes and was then executed again to maceration with another fluent.

Ultimately, the extracts weredesiccated using rotary evaporator (for *n*-hexane, chloroform and ethanol extracts) and lyophilizer (for aqueous distillates). The concentrated extracts were afterwards stored at 20°C. The % extraction yield was calculated by following formula:

% Extraction yield = (Wt. of plant extract / Wt. of initial plant sample) ×100

Estimation of Anthelmintic prospective

Preparation of test solutions

0.9% NaCl and 10mg/mL piperazine citrate solution to be utilized during the inquisition was formulated freshly exactly before commencing the appraisal.

Preparation of plant extracts

Different concentrations (10, 20, 50, 100mg/mL) of each plant extract were prepared via serial dilution scheme.

Procedure

The anthelmintic screening of the plant macerates against the gastro-intestinal nematode in the abomasum of freshly butchered goat i.e.,

Haemonchus contortus at the various concentrations (10, 20, 50 and 100mg/mL) was conducted employing the methodology taken into consideration by Ajaiyeoba*et al.* (2001). The trial worms were allocated in six categories for evaluating the potential of a single constituent of a particular plant macerate. In each of the reaction plate, 10mL of plant macerate (saline solution for control and Piperazine citrate for standard) was dispensed followed by the inclusion of uniform-sized nematodes. All plates were stationed at room temperature up till 4 hours of the trial session. Time interval occupied by worms for paralysis (no motion was discerned other than when they were agitated strenuously) as well as death (neither movement was detected when stirred potently nor when immersed in 0.9% NaCl solution) was recorded.

Results

The percentage extraction yield of the plants under inquisition were considered as a measure of the efficiency of the solvents employed during maceration to extract specific components from the original material.The % extraction yield of *T. portulacastrum* L. (Fig. 1) ranges from 0.92-27.28% with maximum maceration capability reported in aqueous extract of fruit and minimum extract recovery obtained from chloroform macerate of root.

The percentage extraction yield of *Z. pentandra* (L.) C. Jaffrey (Fig. 2) was found within the range of 2.87-10.84% with highest yield obtained in chloroform extract of fruit and minimum macerate was produced by the aqueous extract of root.

Table 1. Quantity of plant material (g) of *T. portulacastrum* L. and *Z. pentandra* (L.) C. Jeffrey dissolved in test solvents (mL) during maceration.

Plant specimen	Plant part	Plant material used (g)	Solvent used (mL)
Trianthema portulacastrum L.	Root	17.71	100
	Stem	56.60	250
	Leaf	62.35	250
	Fruit	5.76	25
Zaleya pentandra (L.) C. Jeffrey	Root	14.23	75
	Stem	112.19	500
	Leaf	73.40	300
	Fruit	9.67	50

The ethno-veterinary appraisal of the plants under inquisition could serve as an affordable, easily available and effective agent to combat helminthes in comparison to the synthesized drugs in the developing countries. The anthelmintic activity of the macerates of *Trianthema portulacastrum* L. and *Zaleya pentandra* (L.) C. Jeffrey at four different consistencies including 100, 50, 20, 10mg/mL were recorded in minutes to demonstrate the time taken into account by the helminthes for paralysis eventually leading to their death. The time span considered for the respective evaluation was 4 hours that sum up to 240 minutes.

The time duration occupied by the extracts of *T. portulacastrum* L. (Table 2, Fig. 3) for paralysis ranged from 2.5-15.8 minutes at 100mg/mL with maximum time consumed by aqueous extract of root rendering it least effective, while minimum time was utilized by chloroform macerate of stem hence, proved to be most effective. As far as the entire plant components were analyzed, the fruit extracts had presented maximum potential followed by the stem, leaf and root macerates. The polar macerates had demonstrated less efficacy in comparison to the non-polar solvents with *n*-hexane providing promising potential followed by chloroform, ethanol and distilled water.

Table 2. Time duration (minutes) taken by extracts of *Trianthema portulacastrum* L. for paralysis and death of helminthes.

Plant macerate		100mg/mL		50mg/mL		20mg/mL		10mg/mL	
		P (min)	D (min)	P (min)	D (min)	P (min)	D (min)	P (min)	D (min)
Root	*n*-hexane	14.9	24.8	16.2	46.4	19.3	52.8	71.5	129.3
	Chloroform	12.5	23.7	18.7	25.2	24.2	58.1	89.1	220.8
	Ethanol	14.6	22.1	19.1	35.6	23.7	63.4	32.8	141.2
	Aqueous	15.8	29.8	22.7	41.6	50.1	73.2	82.1	152.9
Stem	*n*-hexane	7.6	10.0	9.0	29.8	13.9	51.7	28.7	58.3
	Chloroform	2.5	9.43	11.2	16.7	25.9	52.8	36.7	104.6
	Ethanol	4.5	13.9	9.8	16.6	17.8	28.1	28.4	56.2
	Aqueous	9.7	14.9	16.9	29.9	68.2	121.0	145.6	Insig.
Leaf	*n*-hexane	7.8	11.6	10.8	14.0	12.8	29.4	45.5	58.2
	Chloroform	9.0	13.8	14.5	26.2	18.3	42.3	26.5	84.6
	Ethanol	8.2	12.4	14.2	23.6	15.2	34.2	32.7	68.4
	Aqueous	9.8	14.9	24.6	61.4	31.9	72.5	62.8	144.0
Fruit	*n*-hexane	7.2	10.6	14.8	17.5	19.3	29.4	23.8	58.8
	Chloroform	4.3	14.4	16.1	63.2	35.0	74.0	59.9	85.7
	Ethanol	2.6	11.7	5.1	16.8	9.6	19.7	21.7	48.7
	Aqueous	9.5	16.3	15.8	39.8	19.3	50.8	28.9	102.7
Piperazine citrate		0.3	6.8	1.3	11.9	2.4	19.1	25.6	45.3

*Key: P = Paralysis time, D = Death time, Insig. = Insignificant.

Table 3. Time duration (minutes) taken by extracts of *Zaleya pentandra* (L.) C. Jeffrey for paralysis and death of helminthes.

Plant macerate		100mg/mL		50mg/mL		20mg/mL		10mg/mL	
		P (min)	D (min)	P (min)	D (min)	P (min)	D (min)	P (min)	D (min)
Root	*n*-hexane	8.8	13.7	11.7	26.8	90.1	104.8	96.9	182.3
	Chloroform	9.6	19.2	14.6	38.6	62.9	81.6	74.6	164.9
	Ethanol	9.4	18.8	22.7	36.4	81.5	144.8	91.1	196.8
	Aqueous	12.8	25.6	16.6	51.2	112.2	202.9	191.6	Insig.
Stem	*n*-hexane	8.8	17.6	19.2	28.9	59.7	65.4	125.3	210.9
	Chloroform	4.1	8.2	18.1	26.4	86.4	153.7	205.8	Insig.
	Ethanol	8.8	17.6	16.5	34.6	37.6	68.9	75.6	134.6
	Aqueous	6.8	13.6	18.6	26.5	91.7	118.1	157.4	228.9
Leaf	*n*-hexane	6.7	13.4	9.9	27.9	45.5	91.2	98.5	197.4
	Chloroform	7.6	15.2	18.7	30.4	63.25	127.6	124.3	138.7
	Ethanol	8.8	17.6	11.2	34.2	54.2	108.9	89.3	122.5
	Aqueous	10.0	20.1	14.3	41.8	59.8	176.7	132.2	208.9
Fruit	*n*-hexane	3.8	7.6	5.6	11.2	8.6	17.5	29.6	55.4
	Chloroform	2.3	8.9	6.1	12.2	18.8	38.9	94.2	117.9
	Ethanol	2.8	7.4	6.6	14.8	14.1	48.7	28.1	87.4
	Aqueous	9.7	21.8	13.8	43.9	18.1	56.2	39.9	167.0
Piperazine citrate		0.3	6.8	1.3	11.9	2.4	19.1	25.6	45.3

*Key: P = Paralysis time, D = Death time, Insig. = Insignificant.

Time duration taken by extracts of *T. portulacastrum* L. for the death of helminthes ranged from 9.43-29.8 minutes with good potential provided by chloroform stem extract while, least action was provided by root aqueous extract. Stem had analyzed to exhibit promising activity with gradual decline in leaf, fruit and root.

The *n*-hexane had proved to be effective for paralyzing helminthes in comparison, however, the trend changed with ascending polarity with ethanol being the most effective. It was noticed that increase in the time duration as concentration of macerate decline from 100mg/mL, did not follow linear trend.

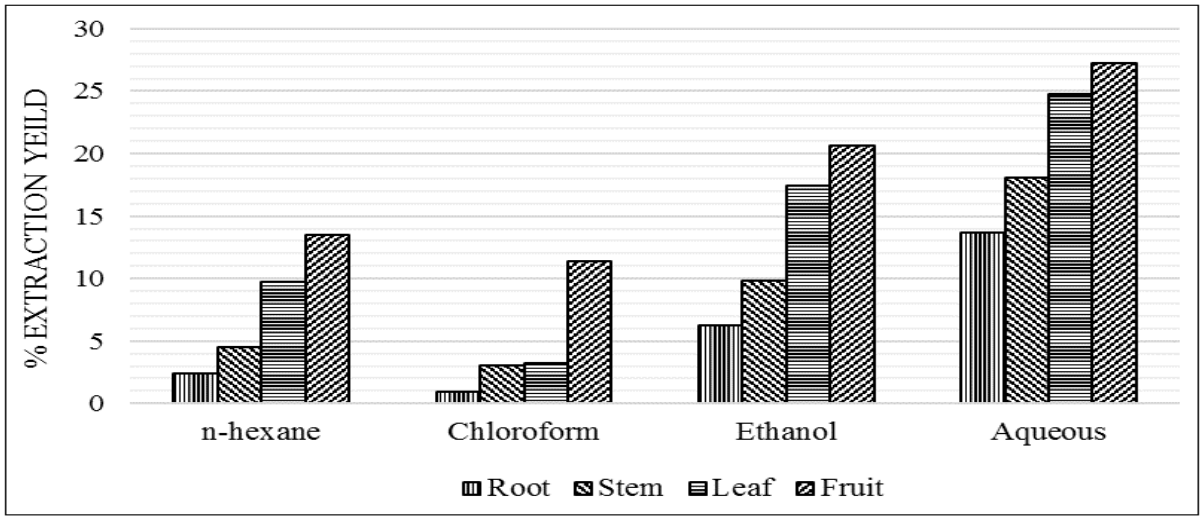

Fig. 1. Percentage **of** extraction yield of the different extracts of *Trianthema portulacastrum* L.

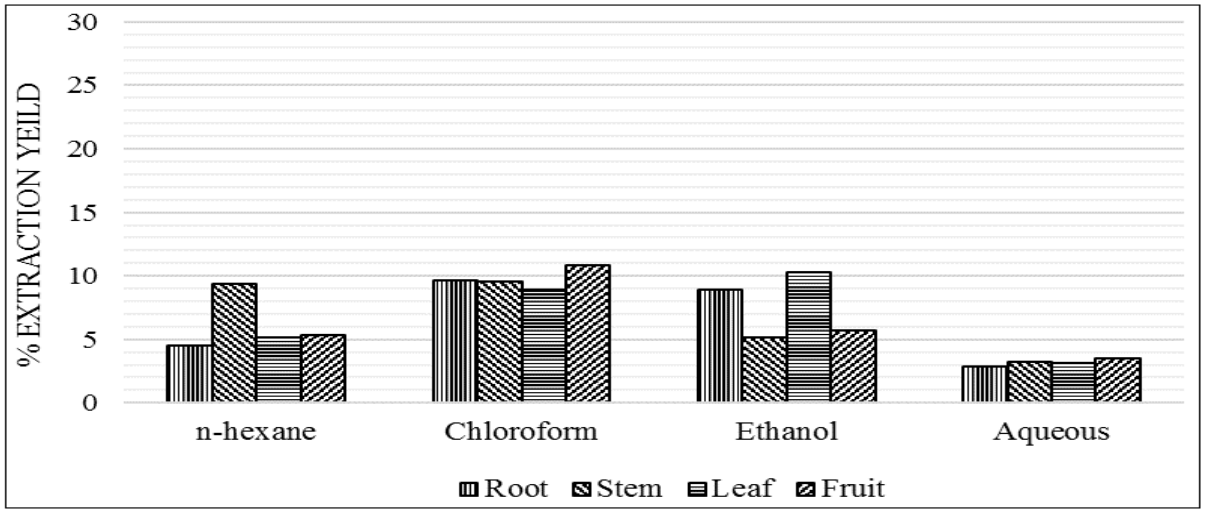

Fig. 2. Percentageofextraction yield of the different extracts of *Zaleya pentandra* (L.) C. Jaffrey.

The time utilized by the root, stem, leaf and fruit macerates of *Z. pentandra* (L.) C. Jeffrey (Table 3, Fig. 4) for paralysis ranged from 2.3-12.8 minutes at 100mg/mL plant macerate consistency, with the maximum time consumed by aqueous extract of root rendering it least effective and the minimum time utilized by chloroform macerate of fruit, hence proving to be most effective.

As far as the entire plant components were analyzed, fruit extracts had presented maximum potential followed by stem, leaf and root macerates, in agreement with the results concluded in *T. portulacastrum* L. Moreover, chloroform had provided promising potential followed by *n*-hexane, ethanol and distilled water extracts.

The time duration for deaths of helminthes employed by *Z. pentandra* (L.) C. Jeffrey macerates ranged from 7.4-25.6 minutes with the good capability put forward by the ethanol extract of fruit while, least action was provided by aqueous extract of root. The fruit extracts had exhibited promising activity with gradual decline of potential in stem, leaf and root extracts, similar to the trend reported during the paralysis time analysis. The chloroform macerates had demonstrated most effectiveness followed by the *n*-hexane, ethanol and aqueous extracts. However, it was observed that increase in the time duration as the concentration of macerate decline from 100mg/mL to 10mg/mL, did not follow linear trend.

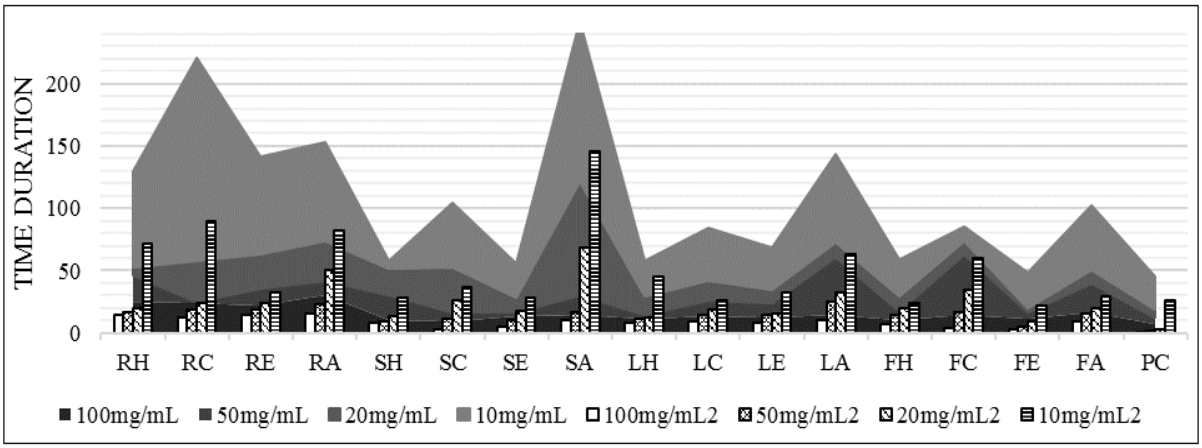

Fig. 3. Time duration (minutes) taken by extracts of *Trianthema portulacastrum* L. for paralysis and death of helminthes.

*Column graph: Time taken for paralysis

*Area graph: Time taken for death

*Macerates [Plant part]: R = Root, S = Stem, L = Leaf, F = Fruit

*Macerates [Solvents]: H = *n*-hexane, C = Chloroform, E = Ethanol, A = Aqueous

*PC: Piperazine citrate (standard).

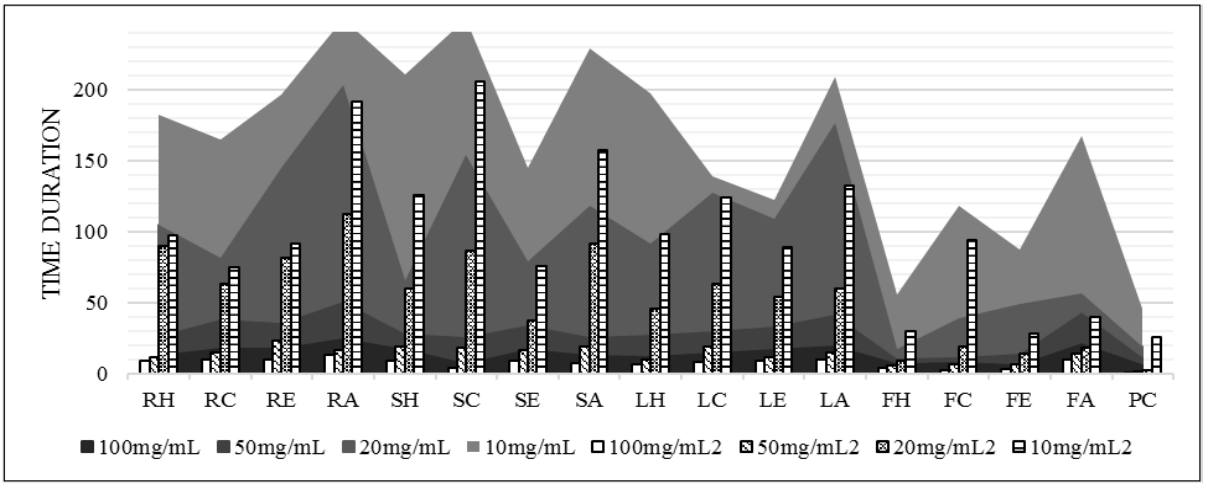

Fig. 4. Time duration (minutes) taken by extracts of *Zaleya pentandra* (L.) C. Jeffrey for paralysis and death of helminthes.

*Column graph: Time taken for paralysis

*Area graph: Time taken for death

*Macerates [Plant part]: R = Root, S = Stem, L = Leaf, F = Fruit

*Macerates [Solvents]: H = *n*-hexane, C = Chloroform, E = Ethanol, A = Aqueous

*PC: Piperazine citrate (standard).

The correlation between the concentrations of *Trianthema portulacastrum* L. and *Zaleya pentandra* (L.) C. Jeffrey utilized and time duration consumed till the death of helminthes was established to conclude that negative association was presented in the results with the time duration decreasing with the increase in the consistency of the plant macerates and vice versa.

The extracts obtained from *T. portulacastrum* L. (Fig. 5) had demonstrated strong inverse linkage within the *n*-hexane extract of stem (R^2 = 0.9766), aqueous macerate of leaf (R^2 = 0.7825), chloroform macerate of fruit (R^2 = 0.9703) and aqueous extract (R^2 = 0.7257) of fruit. While, chloroform extract of root (R^2 = 0.4633), in addition to alcoholic macerate of fruit (R^2 = 0.53) exhibited weak correlation.

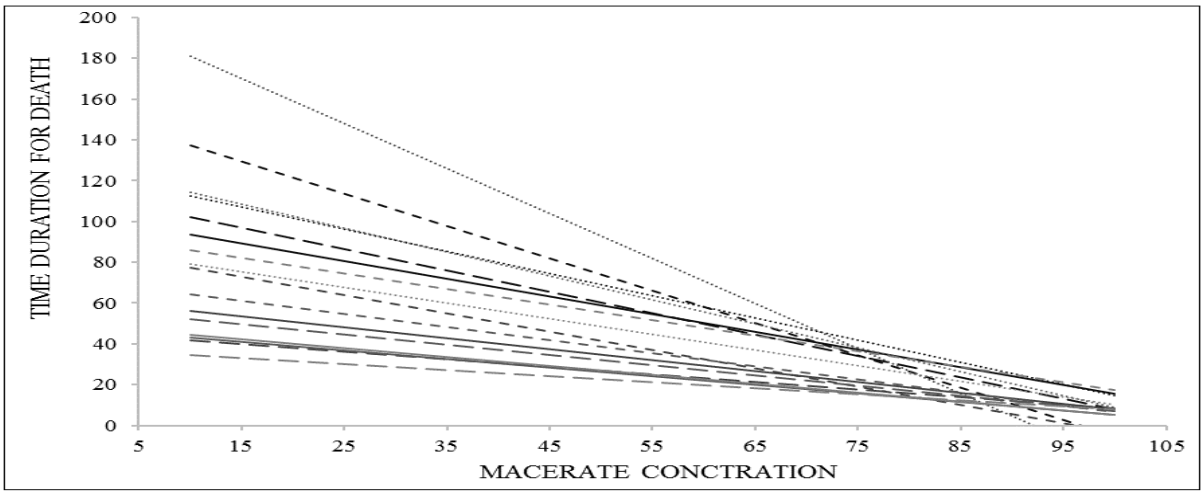

Fig. 5. Correlation between the concentrations of plant extract (mg/mL) and time duration (minutes) taken by extracts of *Trianthema portulacastrum* L. for death of helminthes.

*Extract (R^2): _____(RH: 0.5912), ...(RC: 0.4633), -----(RE: 0.6318), ...(RA: 0.6293)

_____(SH: 0.9766), (SC: 0.6894), -----(SE: 0.6038), (SA: 0.6802)

_____(LH: 0.6306), (LC: 0.6985), -----(LE: 0.6969), (LA: 0.7825)

_____(FH (0.6765), (FC: 0.9703), -----(FE: 0.53), (FA: 0.7257)

*Macerates [Plant part]: R = Root, S = Stem, L = Leaf, F = Fruit

*Macerates [Solvents]: H = *n*-hexane, C = Chloroform, E = Ethanol, A = Aqueous.

The strong inverse linkage was demonstrated by *Z. pentandra* (L.) C. Jeffrey (Fig. 6) macerates within ethanol (R^2 = 0.8005) and aqueous (R^2 = 0.8198) extract of root as well as chloroform (R^2 = 0.8226), ethanol (R^2 = 0.8481) and aqueous (R^2 = 0.8214) macerates of leaf. However, *n*-hexane extract of stem (R^2 = 0.5346), in addition to *n*-hexane (R^2 = 0.5072), chloroform (R^2 = 0.5462) and aqueous (R^2 = 0.5515) extract of fruit exhibited weak correlation.

Discussion

The scheme adopted for the extraction of the components was considered to be an important factor in the determination of the commercial feasibility, yield and the quality of the extract obtained.

The maceration procedure considered for the research was because of its cost effectiveness and good solute yield in comparison to other techniques.

The extraction yield of the macerates of *Trianthema portulacastrum* L. and *Zaleya pentandra* (L.) C. Jeffrey demonstrated as the amount of extract recovered in mass compared with the initial amount of whole plant ranged from 0.92-27.28% and found to be in agreement with Iqbal *et al.* (2012), Bari *et al.* (2012) and Anwar *et al.* (2009). The variation in the % maceration efficacy might be due to the use of different solvents, different plant parts, time, temperature, mode of extraction as well as on the chemical nature of the sample (Priya *et al.*, 2012).

Helminthic infections of the gastrointestinal tract of human beings and animals had been acknowledged to have adverse effects on the health standards with a consequent lowering of resistance to other diseases (Chandrashekhar *et al.*, 2008). The anthelmintic activity of plant extracts can be either due to direct action of extract on the worms or through induction of GI irritation and diarrhoea, which cause dislodgment of resident worms. However, the mechanisms whereby the consumption of certain plants and plant extracts could affect parasite viability, mobility and fecundity both *in vitro* and *in vivo* were largely unknown (Athanasiadou and Kyriazakis, 2004).

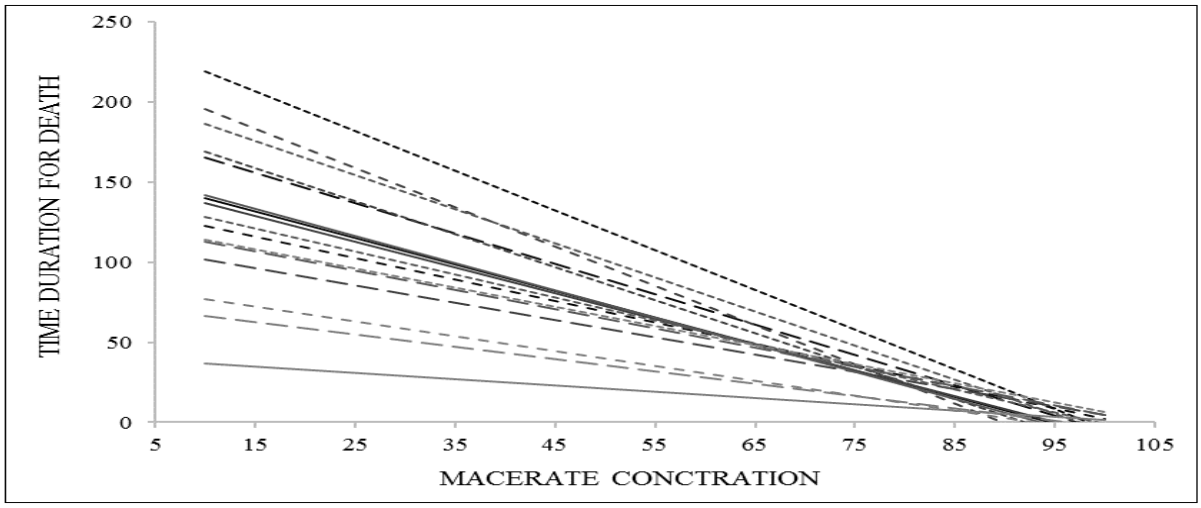

Fig. 6. Correlation between the concentrations of plant extract (mg/mL) and time duration (minutes) taken by extracts of *Z. pentandra* (L.) C. Jeffrey for death of helminthes.

*Extract (R^2): _____(RH: 0.5912), ...(RC: 0.4633), -----(RE: 0.6318), ...(RA: 0.6293)

_____(SH: 0.9766), (SC: 0.6894), -----(SE: 0.6038), (SA: 0.6802)

_____(LH: 0.6306), (LC: 0.6985), -----(LE: 0.6969), (LA: 0.7825)

_____(FH (0.6765), (FC: 0.9703), -----(FE: 0.53), (FA: 0.7257)

*Macerates [Plant part]: R = Root, S = Stem, L = Leaf, F = Fruit

*Macerates [Solvents]: H = *n*-hexane, C = Chloroform, E = Ethanol, A = Aqueous.

The results obtained from the anthelmintic activity of *Trianthema portulacastrum* L. and *Zaleya pentandra* (L.) C. Jeffrey revealed dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli which eventually progressed to death, in agreement with Sreejith *et al.* (2013), Pal and Dey (2011), Kumar *et al.* (2010) and Bhattacharjee *et al.* (2010).

Tannins and phenolics were known to interfere with the energy generation in helminthic parasites by uncoupling oxidative phosphorylation and also bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite, leading to death (Athnasiadou *et al.*, 2001). Based on these facts, it could be assumed that tannins, phenolic compounds and flavonoids present in the macerates of plants under inquisition that could be responsible for the anthelmintic activity.

Conclusion

The steady-state maceration scheme adopted had comparatively extracted more components from fruit and root of *T. portulacastrum* L. and *Z. pentandra* (L.) C. Jeffrey, respectively. These extracts had displayed wide array of odours, colours and texture thus providing an indication of variations in their therapeutic characterization.

Although, the anthelmintic appraisal render the stem macerates of *T. portulacastrum* L. and the fruit extracts of *Z. pentandra* (L.) C. Jeffrey most effective yet,

the preliminary cytotoxicity analysis should be taken into consideration before subjecting them to further pharmaceutical assessments.

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