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Anthelminthic analysis of two important species of family Sterculiaceae *viz. Firmiana simplex* (L.) W.Wight and *Dombeya burgessiae* Gerrard ex Harv

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Abstract

The infections afflicted by helminthes are considered to be pervasive and detrimental, thereby distressing a major portion of the world's population. The macerates of two plants from family Sterculiaceae namely *Firmiana simplex* (L.) W.Wight and *Dombeya burgessiae* Gerrard ex Harv. prepared via steady-state maceration were compared from anthelmintic perspective at four concentrations (10, 20, 50 and 100mg/mL) employing *Haemonchus contortus* as test organism and Piperazine citrate as reference. The aqueous bark extract of *D. burgessiae* Gerrard ex Harv. extracted more phytochemical contents. The dose-dependent anthelmintic appraisal render the stem macerates of *F. simplex* (L.) W.Wight and the bark extracts of *D. burgessiae* Gerrard ex Harv. most effective. 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

The human population residing in developing countries heavily rely upon botanical wealth of their state for treating various ailments. Keeping in mind, the reliance of mankind on plants, a comprehensive inquisition with the aim to provide organic anthelmintic alternatives was conducted. The infections afflicted by helminthes are considered to be pervasive and detrimental, thereby distressing a major portion of the world's population. The gastro-intestinal tract is the abode of many helminths, although some also live in tissues, or their larvae migrate into tissues.

They harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins. Helminthiasis is rarely fatal, but is a major cause of morbidity (Bundy, 1994). Approximately 300 million people suffer severe morbidity and half of which are school-going children affected by massive infections (Tripathi, 2008).

Not only mankind, the gastrointestinal (GI) nematodes have posed serious economic losses to livestock professionals. For instance, haemonchosis is one of the most significant parasitic diseases of livestock worldwide, affecting hundreds of millions of small ruminants (including sheep and goats) and causing substantial losses to the livestock industry estimated at tens of billions of dollars per annum (Waller and Chandrawathani, 2005).

A number of control measures are broadly used to restrict extent of anthelmintic infections. There is no doubt that the consumption and discovery of chemosynthetic drugs owing good anthelmintic activity has become beneficial for the health of both animals as well as humans, but these types of control measures are associated with detrimental environmental effects (Cox, 1999; Knox, 2000; Dalton and Mulcahy, 2001). Moreover, the gastrointestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases (Subhasish *et al.*, 2010).

Natural compounds from plants provide a unique opportunity in the search for new, effective and safe anthelmintics (Hammond *et al.*, 1997). In the last few decades, there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects (Brahmachari, 2001).

Keeping in mind the medicinal significance of the plants, the present investigation was designed to test the anthelmintic efficacy of two members of family Sterculiaceae namely *Firmiana simplex* (L.) W.Wight and *Dombeya burgessiae* Gerrard ex Harv. *Firmiana simplex* (Linn.) W.F. Wight, commonly called Chinese parasol tree, is native to China and introduced as ornamental in Pakistan.

The tree was found to be the rich reservoir of coumarins, lignans as well as flavonoids and serve as the treatment of diarrhea and stomach disorders in Korea. Moreover, different parts of this tree were used as antiphlogistic, swelling reductant, carbuncles and hemorrhoids treatment (Bae, 2000). In addition, *Dombeya burgessiae* Gerrard ex Harv. is native to Africa but introduced in India and Pakistan. Medicinally, the bark of the respective plant is aphrodisiac while, leaf macerates were used to cure leprosy sores (Neuwinger, 2000).

Materials and methods

Test organisms

In vitro anthelmintic potential of *Firmiana simplex* (L.) W.Wight and *Dombeya burgessiae* Gerrard ex Harv. 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

The tree parts, *F. simplex* (L.) W.Wight and *D. burgessiae* Gerrard ex Harv. were collected from the Botanic Garden of Government College University, Lahore in November. While, the fruit and inflorescence of the respective test plants were collected in May. The plant specimens were identified, assigned the authenticated voucher number followed 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.

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 were desiccated 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

Percentage extraction yield

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 *F. simplex* (L.) W.Wight (Fig. 1) ranges from 0.08-11.16% with highest amount sequestered from *n*-hexane extract of leaf and minimum extract recovery obtained from chloroform macerate of flower.

In addition, the percentage extraction yield of *D. burgessiae* Gerrard ex Harv. (Fig. 2) was found within the range of 1.24-28.48% with greater quantity obtained from aqueous extract of bark and least macerate was produced by the *n*-hexane extract of bark.

Table 1. Time duration (minutes) taken by extracts of *Firmiana simplex* (L.) W.Wight 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)
Bark	*n*-hexane	2.58	9	6	15.37	28.28	38.16	32.27	54
	Chloroform	3.38	10.11	6.04	14	17.31	19.42	26.22	42.47
	Ethanol	3.36	10.17	5	12.19	10.21	20	29.12	76.54
	Aqueous	2	7.29	11.46	19.32	16.19	23.26	26.29	49.57
Stem	*n*-hexane	2	9.31	6.21	22.11	15.51	26.29	28.53	53
	Chloroform	9.41	13.21	15.34	18.17	18.11	21.22	31.24	94.31
	Ethanol	1	12.17	4	17.23	16.42	26.57	47.36	119.31
	Aqueous	3.57	7.21	6	36.29	20.17	56	31.13	118.41
Leaf	*n*-hexane	5.26	15.41	7.18	27.28	19	30.59	39.14	115.21
	Chloroform	6.34	15.41	12.16	23.27	18.29	51.08	41.39	118.21
	Ethanol	4.34	12.41	6.26	22.46	9.11	70.56	38.38	101.21
	Aqueous	4.35	11	6.47	47.49	15.18	58.35	39.34	117.41
Flower	*n*-hexane	5.46	16.11	7	41.33	18.21	52.15	41.33	117.21
	Chloroform	6.24	17.21	6.45	32.42	16.41	54.36	39.35	110.41
	Ethanol	4.15	11	5.17	27.48	19.42	61.27	55	113.31
	Aqueous	5.12	12.21	10.57	45.17	23.27	75.58	67.38	117.51
Fruit	*n*-hexane	2.46	14.19	5	18.41	7.24	52.36	36.36	109.21
	Chloroform	4.47	18.12	8.39	19.44	9.41	30.16	33.15	114.31
	Ethanol	7.41	10.25	9.16	48.34	18.29	60	51.38	119.21
	Aqueous	11.10	25.46	13.11	46.51	25.22	90.21	87.26	119.71
Piperazine citrate		0.3	6.18	1.03	11.29	2.46	19.17	25.46	45.33

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

Parameters for determination of anthelmintic activity

The anthelmintic medicines available in market never remained up to the mark or has developed resistance causing recurrent attacks.

For this purpose, the anthelmintic activity of the various parts of *Firmiana simplex* (L.) W.Wight and *Dombeya burgessiae* Gerrard ex Harv. was evaluated at four concentrations (10, 20, 50, 100 mg/ml). In four hour treatment period, the paralysis and death time was calculated.

Anthelmintic activity of Firmiana simplex (L.) W.Wight

The paralysis time duration in *F. simplex* (L.) W.Wight (Table 1) ranged from 1.0-11.10 minutes at 100mg/mL with maximum time consumed by aqueous extract of fruit indicating its least anthelmintic activity. The most potent results were shown by ethanol extracts of stem which paralyzed *Haemonchus contortus* within one minute (Fig. 3). The overall examination of various parts indicated that bark had highest potential followed by stem, leaf, fruit and flower.

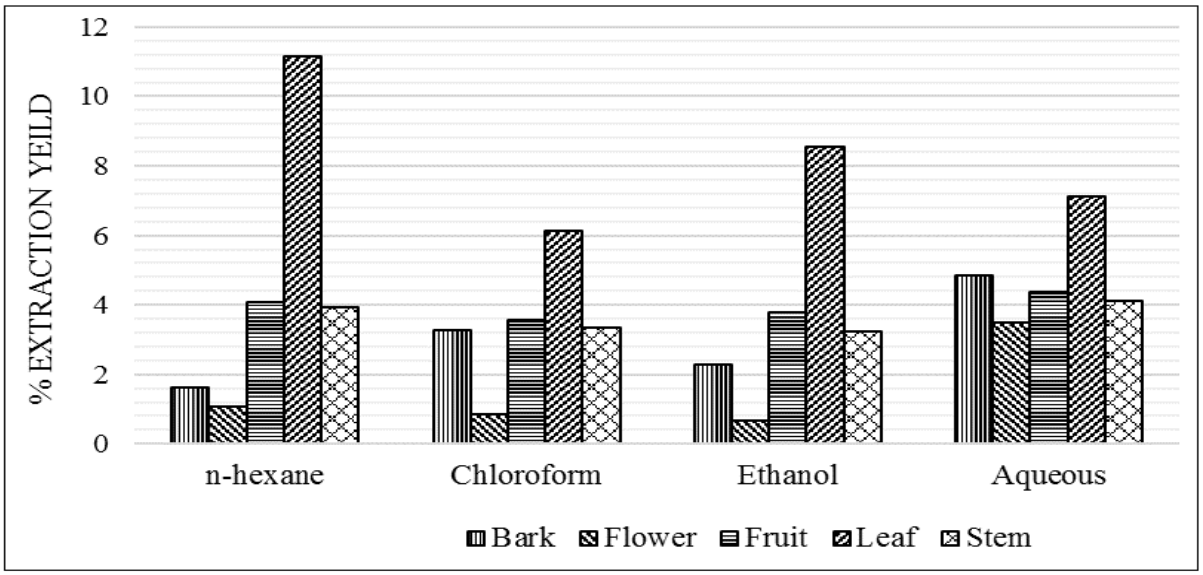

Fig. 1. % extraction yield of the different extracts of *Firmiana simplex* (L.) W.Wight.

The aqueous extract was documented as least efficient. The treatment period for death varied between 7.21-25.46 minutes at maximum used concentration. The shortest death period was shown by aqueous extract of stem while highest time duration were taken by aqueous extracts of fruit. The effectiveness of plant parts as a whole were noted as in descending order bark > stem > leaf > fruit > flower. Hexane extracted most of the anthelmintic compounds followed by chloroform, ethanol and aqueous.

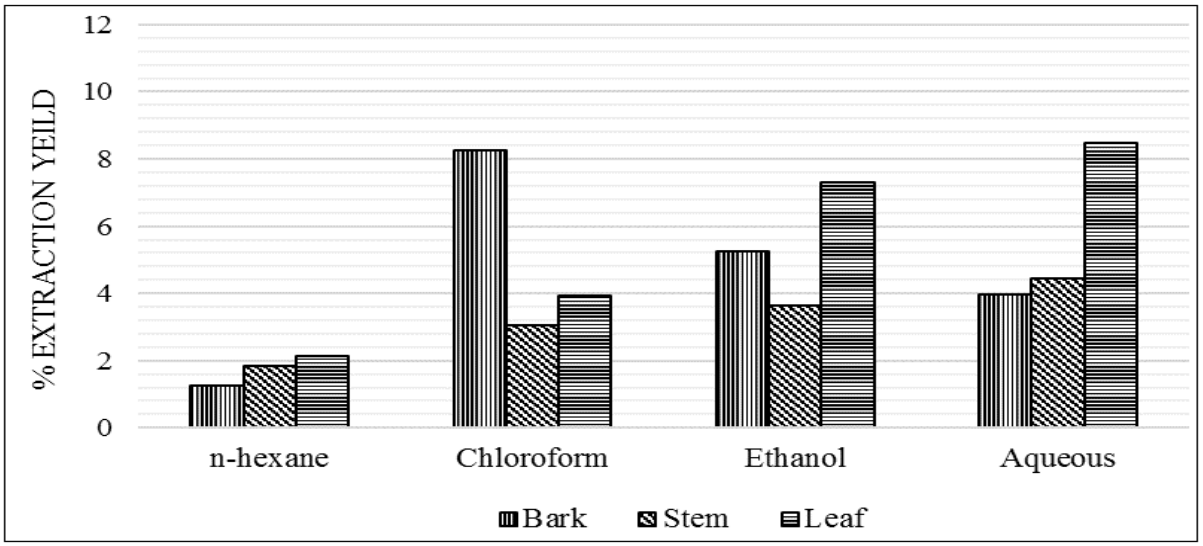

Fig. 2. % extraction yield of the different extracts of *Dombeya burgessiae* Gerrard ex Harv.

Anthelmintic activity of Dombeya burgessiae Gerrard ex Harv.

The time utilized by the stem, leaf and bark macerates of *D. burgessiae* Gerrard ex Harv.(Table 2) for paralysis ranged from 1.11-10 minutes at 100mg/mL, with the maximum time consumed by *n*-hexane extract of leaf and significant paralysis potential provided by chloroform macerate of stem.

The stem had shortest paralysis time followed by bark and leaf. Hexane extracted paralyzed compounds efficiently followed by chloroform, aqueous and ethanol (Fig. 4).

The time duration for deaths of helminthes employed by *D. burgessiae* Gerrard ex Harv. macerates ranged from 2.09-13.18 minutes with the good capability put forward by the ethanol extract of bark while, least action was provided by *n*-hexane extract of leaf. The presence of anthelminthic biochemicals was documented in decreasing order as bark > stem > leaf.

Correlation between concentration of plant macerates and time consumed till death of helminthes

The correlation between the concentrations of *Firmiana simplex* (L.) W.Wight and *Dombeya burgessiae* Gerrard ex Harv. 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.

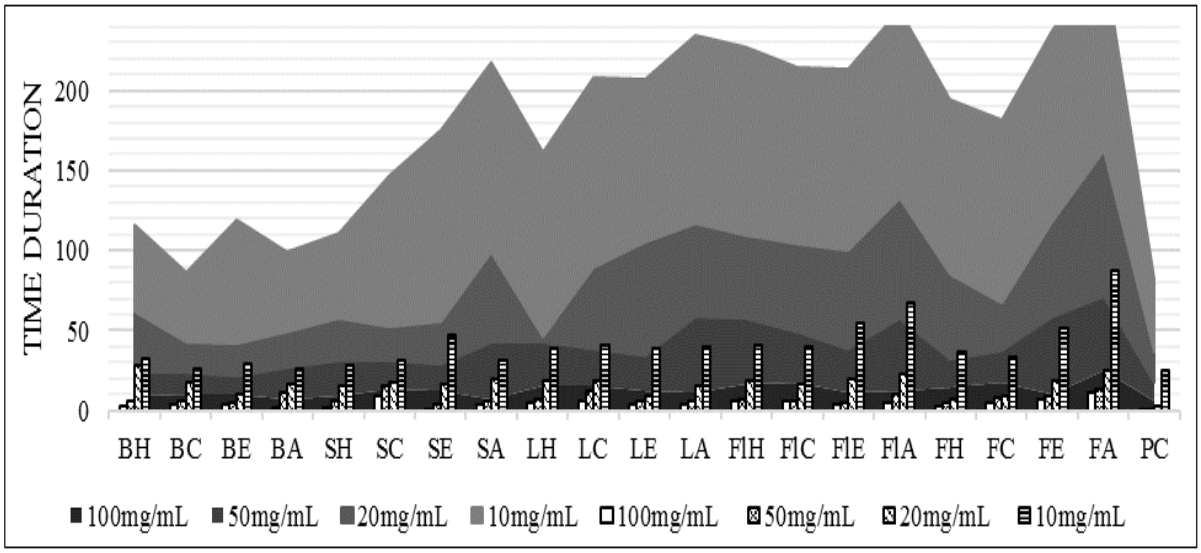

Fig. 3. Time duration (minutes) taken by extracts of *Firmiana simplex* (L.) W.Wightfor paralysis and death of helminthes.

*Column graph: Time taken for paralysis , *Area graph: Time taken for death , *Macerates [Plant part]: B = Bark, S = Stem, L = Leaf, Fl = Flower, F = Fruit , *Macerates [Solvents]: H = *n*-hexane, C = Chloroform, E = Ethanol, A = Aqueous , *PC: Piperazine citrate (standard).

The extracts obtained from *Firmiana simplex* (L.) W.Wight (Fig. 5) had demonstrated strong inverse linkage within the *n*-hexane extract of bark (R^2 = 0.8138), aqueous macerate of stem (R^2 = 0.9856), ethanol macerate of leaf (R^2 = 0.8359), aqueous extract of flower (R^2 = 0.8825) and aqueous extract of fruit (R^2 = 0.8729). While, ethanol extract of bark (R^2 = 0.4489), chloroform macerate of stem (R^2 = 0.4063) in addition to chloroform macerate of fruit (R^2 = 0.4275) exhibited weak correlation. The strong inverse linkage was demonstrated by *Dombeya burgessiae* Gerrard ex Harv. (Fig. 6) aqueous macerates of stem (R^2 = 0.7726). However, *n*-hexane extract of bark (R^2 = 0.3959) exhibited weak correlation.

Discussion

The maceration technique was used for the extraction of various bioactive chemicals from aerial part of plants. It was opted because of its cost effectiveness, good percentage and quality yield of extracts (Gardner, 1975; Khan *et al.*, 2012). The percentage yield of aerial parts ranged from 0.68 to 11.16. A promising positive correlation was found increase in extraction yield with polarity index. The percentage extraction yield depends on plant material, nature of chemicals and mode of extraction as well agreed with Tian *et al.* (2009).

The helminthes are causing a number of diseases in animals as well as in human beings.

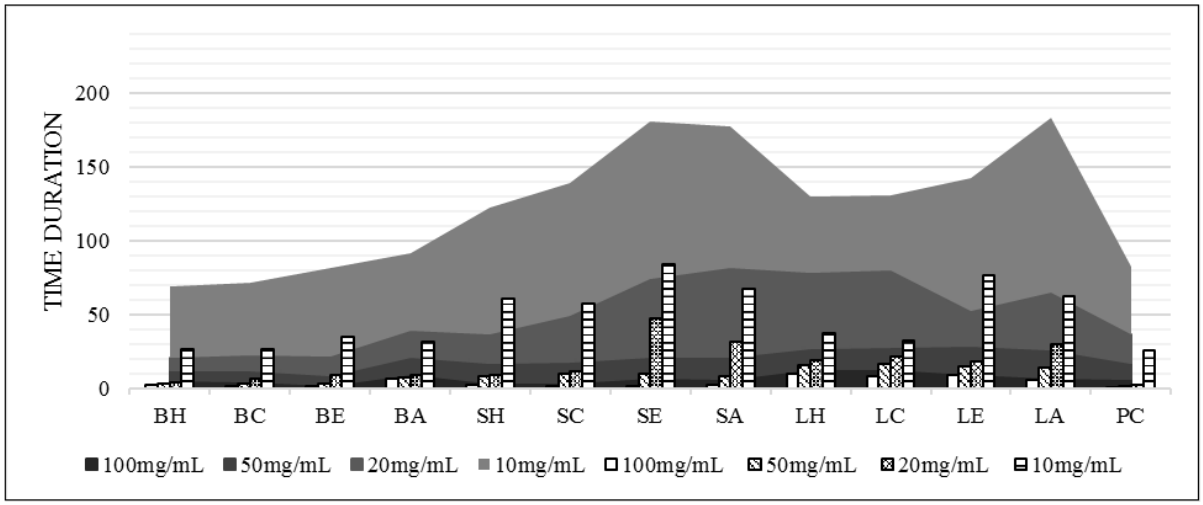

Fig. 4. Time duration (minutes) taken by extracts of *Dombeya burgessiae* Gerrard ex Harv.for paralysis and death of helminthes.

*Column graph: Time taken for paralysis , *Area graph: Time taken for death , *Macerates [Plant part]: B = Bark, S = Stem, L = Leaf, *Macerates [Solvents]: H = *n*-hexane, C = Chloroform, E = Ethanol, A = Aqueous , *PC: Piperazine citrate (standard).

The medicines available in market never remained up to the mark or has developed resistance causing recurrent attacks. For this purpose the anthelmintic activity was taken into consideration. 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).

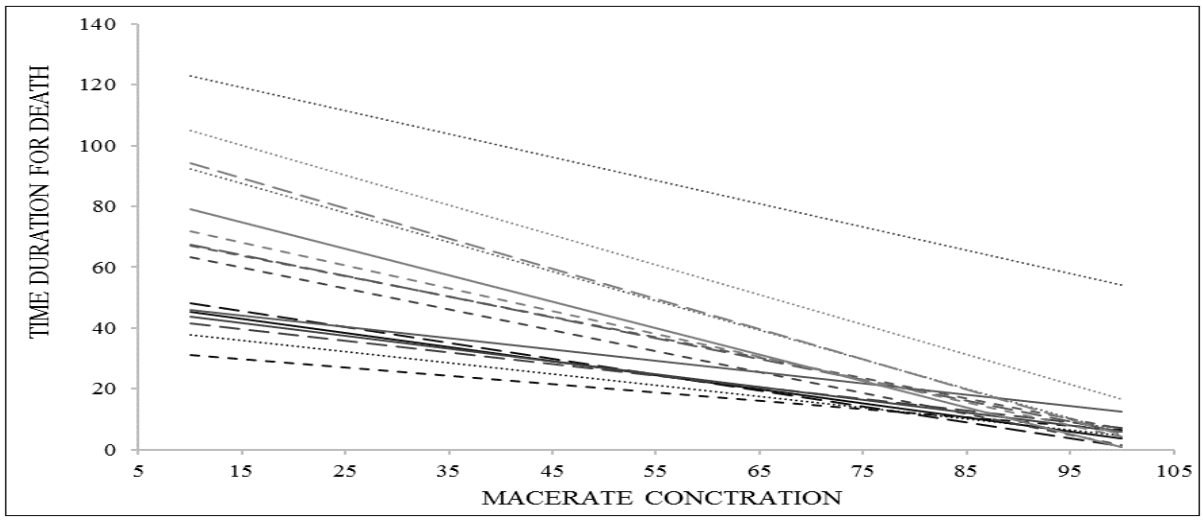

Fig. 5. Correlation between the concentrations of plant extract (mg/mL) and time duration (minutes) taken by extracts of *Firmiana simplex* (L.) W.Wight for death of helminthes.

*Extract (R^2): _____(BH: 0.8138), ...(BC: 0.5868), -----(BE: 0.4489), ...(BA: 0.7088), _____(SH: 0.6755), (SC: 0.4063), -----(SE: 0.617), (SA: 0.9856), _____(LH: 0.6861), (LC: 0.7429), -----(LE: 0.8359), (LA: 0.7793), _____(FlH: 0.6822), (FlC: 0.6909), -----(FlE: 0.7539), (FlA: 0.8825), _____(FH: 0.6401), (FC: 0.4275), -----(FE: 0.7904), (FA: 0.8729),*Macerates [Plant part]: B = Bark, S = Stem, L = Leaf, Fl = Flower, F = Fruit,*Macerates [Solvents]: H = *n*-hexane, C = Chloroform, E = Ethanol, A = Aqueous.

The anthelmintic activity of crude extracts of *Firmiana simplex* (L.) W.Wight and *Dombeya burgessiae* Gerrard ex Harv. was time and concentration dependent (Partap *et al.*, 2012). The result of the activity was according to the findings of Kumar *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. Based on these facts, it could be assumed that tannins, phenolic compounds and flavonoids present in the plant macerates under inquisition that could be responsible for the anthelmintic activity (Athnasiadou *et al.*, 2001).

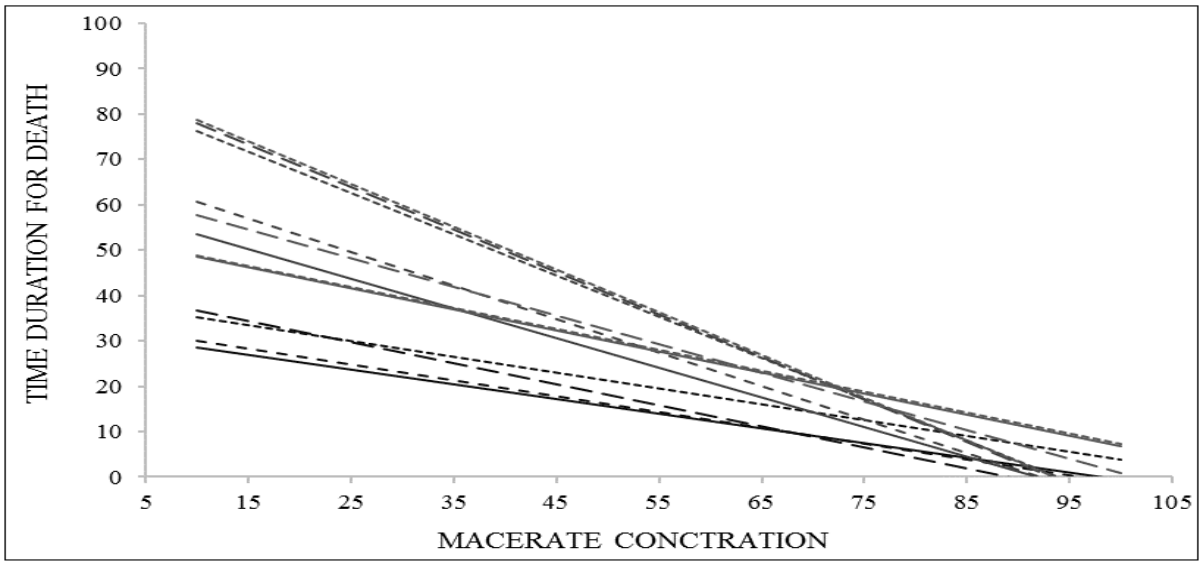

Fig. 6. Correlation between the concentrations of plant extract (mg/mL) and time duration (minutes) taken by extracts of *Dombeya burgessiae* Gerrard ex Harv.for death of helminthes.

*Extract (R^2): _____(BH: 0.3959), ... (BC: 0.4415), -----(BE: 0.4937), ... (BA: 0.4824), _____(SH: 0.497), (SC: 0.5997), -----(SE: 0.6824), (SA: 0.7726), _____(LH: 0.7542), (LC: 0.7646), -----(LE: 0.4824), (LA: 0.5761)

*Macerates [Plant part]: B = Bark, S = Stem, L = Leaf, *Macerates [Solvents]: H = *n*-hexane, C = Chloroform, E = Ethanol, A = Aqueous.

Conclusion

The anthelmintic potential of *Firmiana simplex* (L.) W.Wight and *Dombeya burgessiae* Gerrard ex Harv. provided the paralysis time duration of 1.0-11.1 minutes at 100 µg/ml. The most potent results were shown by ethanol extracts of the stem which paralyzed the *Haemonchus contortus* within one minute. Yet, the preliminary cytotoxicity inquisition is deemed essential before further pharmaceutical analysis.

References

Ajaiyeoba EO, Onocha PA, Olarenwaju OT. 2001. In vitro anthelmintic properties of Buchholzia coiaceaeand Gynandropsis gynandra extract. Pharmaceutical Biology **39(3),** 217-220. http://dx.doi.org/10.1076/phbi.39.3.217.5936

Athanasiadou S, Kyriazakis I. 2004. Plant secondary metabolities: Antiparasitic effects and their role in ruminant production systems. The Proceedings of the Nutrition Society **63(4),** 631-639. http://dx.doi.org/10.1079/PNS2004396

Athnasiadou S, Kyriazakis I, Jackson F, Coop RL. 2001. Direct anthelmintic effects of condensed tannins towards different gastro intestinal nematodes of sheep: In vitro and in vivo studies. Veterinary Parasitology **99,** 205-219. http://dx.doi.org/10.1016/S0304-4017(01)00467-8

Bae KH. 2000. Medicinal Plants of Korea. KyoHak Publishing Co., Ltd., Seoul.

Brahmachari UN.2001. Herbal drugs. Current Science **81(1),** 15-16.

Bundy DA. 1994. Immunoepidemiology of intestinal helmintic infection I: The global burden of intestinal nematode disease. Transactions of the Royal Society of Tropical Medicine and Hygiene **8,** 259-261.

Cox J. 1999. The nature conservation importance of dung. British Wildlife **11**, 28-36.

Dalton JP, Mulcahy G. 2001. Parasite vaccines – a reality? Veterinary Parasitology **98,** 149-167. https://doi.org/10.1016/S0304-4017(01)00430-7

Gardner RO. 1975. An overview of botanical technique. Biotechnic and Histochemistry **50(2),** 99-105.

Hammond JA, Fielding D, Bishop SC. 1997. Prospects for plant anthelmintics in tropical veterinary medicine. Veterinary Research Communications **21(3),** 213-228.

Khan BA, Akhtar N, Rasul A, Khan H, Murtaza G, Ali A, Khan KA, Zaman S, Jameel A, Waseem K, Mahmood T. 2012. Human skin, aging and antioxidants. Journal of Medicinal Plants Research **6(1),** 1-6.

Kumar G, Karthik L, Rao KVB. 2010. Antimicrobial activity of latex of Calotropis gigantea against pathogenic microorganisms - An In-Vitro study. Pharmacologyonline **3,** 155-163.

Knox DP. 2000. Development of vaccines against gastrointestinal nematodes. Parasitology **120,** 43-61.

Neuwinger HD. 2000. African Traditional Medicine: A Dictionary of Plant Use and Applications. Medpharm Scientific, Stuttgart, Germany, 589.

Partap S, Kumar S, Kumar A, Sharma NK, Jha KK. 2012. In-vitro anthelmintic activity of Luffa cylindrica leaves in Indian adult earthworm. Journal of Pharmacognosy and Phytochemistry **1(2),** 27-30.

Seidel V. 2006. Initial and bulk extraction. In: Sarker SD, Latif Z, Grey AI, Eds. Natural Products Isolation. (2nd Ed), Humana Press Inc., New Jersy, 27-46.

Subhasish M, Rabiul H, Parag G, Debasish D. 2010. Investigation of in vitro anthelmintic activity of Clerodendron inerme. International Journal of Drug Development and Research **2(4),** 826-829.

Tian F, Li B, Ji B, Yang J, Zhang G, Chen Y, Lou Y. 2009. Antioxidant and antimicrobial activities of consecutive extracts from Galla chinensis: The polarity affects the bioactivities. Food Chemistry **113(1),** 173-179.

Tripathi KD. 2008. Essentials of Medical Pharmacology. (6th Ed.), Jaypee Brothers Medical Publishers, New Delhi, 808-810.

Waller PJ, Chandrawathani P. 2005. Haemonchus contortus: Parasite problem No. 1 from tropics - Polar Circle; Problems and prospects for control based on epidemiology. Tropical Biomedicine **22(2),** 131-137.