



Adama Science and Technology University

School of Applied Natural Science

Department of Applied Biology

Anti Leech effect of Niger seed (*Guizotia abyssinica*) hull crude extract and it's Kinetics in selected stream water body ecosystems of Ethiopia.

Investigators:

Mrs. Genet Atsbeha

Mr. Getachew Bantihun

Mr. Yilkal Dessie

Mr. Solomon Girmay

February, 2019
Adama, Ethiopia

Abstract

Leech remains to be one of the health problems to livestock in Ethiopia. The objectives of this study were to carry out phytochemical screening and determine bioactivity of the crude extract of crop Niger hull which are traditionally used in the treatment and control of leeches. Phytochemical were screened in different solvent extracts using standard methods. Phytochemical detected in the extracts were, saponins, flavonoids and terpenoids. Paralyzing or killing time was determined by severity effects from 4⁺ - 1⁺ with timing zones from 1-720 minutes including negative paralysis. The crude extract of Niger seed hull showed anti leech activities from 1⁺ - 4⁺ severities. Niger hull crude extracts might provide a natural source of anti-leech activities, although the anti-leech actions of methanolic, hexane and chloroform extracts are equal or week to that of levamisole. All dose levels of standard reference drug Lavamisole caused a significant kill of leeches. There was a significant difference in mortality of a leech by the methanoloic, hxane and chloroform extracts of Niger hull. The crude extract was characterized using differential scanning calorimetry (DSC). Experimental results showed that in DSC curve, onset of melting temperature at ($T_{onset} = 45.4\text{ }^{\circ}\text{C}$ and $207\text{ }^{\circ}\text{C}$) was recorded and endothermic peak at 99°C is recognized to water lose from the crude extract. Step at 215°C peak temperature is associated with second order phase transition such as Glass Transition and it should be confirmed melting of pure compounds. Endothermic peak found at 180°C is broad, indicates the complex nature of the extract. From the curve at 300°C and higher than this temperature comprise thermal decomposition for the sample.

Keywords: Leeches identification, Niger seed hull Extraction, DSC analysis, phytochemical screening

Table of Content

Contents	Page
1. Introduction.....	1
1.1. Statement of the problem	2
1.2. Objective of the study	2
1.2.1. General Objective.....	2
1.2.2. Specific Objectives.....	2
1.3. Significance of the study.....	3
2. Literature review	3
2.1. Experimental animal, Leech.....	3
2.1.1. Distribution	4
2.1.2. Feeding habits	4
2.1.3. Medicinal Role	5
2.1.4. Reproduction.....	5
2.2. Plant material.....	6
2.2.1. Botanical description	6
2.2.2. Geographical distribution	6
2.2.3. Economic importance	7
3. Methodology.....	10
3.1. Study Design	10
3.1.1. Experimental Organism.....	10
3.2. The plant material collection and extraction	10
3.3. Preliminary Phytochemical Screening of Crude Extracts.....	11
3.4. Thermal Behavior	11
3.5. Preparation of the chemical drugs	12
3.6. Anti-leech assay.....	12
3.7. Data Analysis.....	13
4. Results and Discussion.....	13
5. Conclusion and Recommendations	17
6. References.....	20

1. Introduction

Ethiopia is well known country with the largest livestock population in Africa. Livestock are of economic and social importance both at the household and national levels. The country is ecologically diverse, featuring 18 distinct agro-climatic zones, but according to the report of Sintayehu GebreMariam, et al. (2013) it has two major recognized livestock production systems: highland with predominantly mixed farming; and lowland pastoral and agro-pastoral systems.

Despite Ethiopia's livestock substantial numbers, productivity is very low by the standards of other significant livestock producing African countries like Kenya and Senegal (Haan, 2003 cited in Sintayehu GebreMariam, et al. 2013). Low productivity is mainly related with zoonotic diseases or poor health care and feeding quality, etc. The involvement of private sectors in provision of drugs and treatment ailments is limited (Sintayehu GebreMariam, et al. 2013). Similarly, the report indicated that animal health services are mostly provided by government. As the livelihood of Ethiopian population depends on this sector, finding a solution for health related against spread of parasitic blood sucking organisms is highly vital. Parasites and parasitic diseases as a challenge in health systems have a widespread epidemic around the world (Bahmani, et al. 2014). Among parasitic organisms this study focuses on hematophagous leeches, which are segmented annelid worms with ability to extend or contract their bodies (Zaidi, et al., 2011). Leech (*Limnatis nilotica*) which is not true worm is a pathogenic parasite causing complications such as pain, itching, inflammation, bleeding and anaphylactic reaction on their host the main species that contaminates both humans and animals (Bahmani, et al. 2013).

Although reports are found for the parasitic severe complications of leeches, no standard drug has been registered in the pharmacopeia in order to treat effects of leech on cattle or generally, livestock. This explains that leech infestations are the most neglected among the health care systems. However, through indigenous practice different nations are using supportive and traditional treatments like: the use of putting tobacco leaves, placing some bicarbonate, using bag of ice water and putting Niger seed hulls in the water bodies of cattle drinking. These are traditional treatments used by people in different culture of the world in cases of infection with

the leech. Nevertheless, some of the treatment methods are not cost effective and environmentally friendly. It is true that the usefulness of medicinal value of such plants, which are utilized traditionally, must be proven through scientific assessments and research. Therefore, in present this research is aimed at evaluating the anti leech effects of Niger seed (*Guizotia abyssinica*) hull extracts to suggest scientifically possible control mechanisms against parasitic infection leech on cattle. Niger is an indigenous plant to Ethiopia where it is grown in rotation with cereals and pulses. Niger is a plant with a scientific name *Guizotia abyssinica* is an oilseed crop cultivated in Ethiopia and India. It constitutes about 50% of Ethiopian and 3% of Indian oilseed production.

1.1. Statement of the problem

Generally, the existing literature knowledge and research gap on such crops as ethnobotanical uses against parasitic leeches is not studied so far. This has resulted not only researching with accessible environmentally friendly crop, but also there is neglecting in a research focus the spread of leeches and mechanisms of treatment for a livestock. Although Niger seed has been used for centuries as a food supplement and even nowadays is part of popular in many cultures, but until recently there has been no scientific support of its therapeutics and pharmacological properties. Therefore, the current study focuses with Niger seed hull extract against leech spread and the crop was chosen on the basis of traditional use by Indigenous people.

1.2. Objective of the study

1.2.1. General Objective

The general objective of this study is extraction of Niger seed (*Guizotia abyssinica*) hull and its Kinetics to study its anti-leech activities.

1.2.2. Specific Objectives

- ✓ To evaluate the crude extract of Niger seed hull for anti-leech activity
- ✓ To investigate the major photochemical of Niger seed hull
- ✓ To characterize the temperature and concentration effect of Niger seed hull on aquatic leech.

1.3. Significance of the study

The result of the current study is to be considered as a baseline phase for the next pharamcomia investigations. As the severe of leeches against to cause animals was presented, scientific investigations like with the selected study plant material which is environmentally friendly, economically important, can grow many part of Ethiopia and give medicinal value against leech prevention. The presences of secondary metabolites like flavonoids, terpenoids and saponins screened in the extract of Niger hull can significantly serve as a future novel drug discovery and therapeutics. In addition, the result of the current research is to be a continual for further investigation of treatment options and consequent effectiveness of the medicinal extract. The study had also tried addressing for immediate focus of livestock sector, which is not emphasizing parasitic diseases like leeches that it causes death for the cattle, while drinking water in natural areas of stream water bodies during the dry season.

2. Literature review

2.1. Experimental animal, Leech

Leeches are annelids which have 32 body segments, of which 4 form the head, 7 form the tail, and the remainder form 21 iterated mid body segments (Esch and Kristan, 2002). They have distinctive properties from other annelids by the presence of anterior and posterior suckers, and constant number of body segments and body cavity largely filled with muscles and connective tissues. The pattern of contraction and elongation of their bodies has resulted with sequential relationship of receptor neurons and muscles. Alternating bursts of longitudinal motor neurons results in contraction phase, in other words of circular motor neurons results in elongation phase (Kutschera and Wirtz, 2001; Esch and Kristan, 2002).

The anterior one surrounding the mouth contains saw-like teeth which are used to pierce the skin of the host and feed on blood of various animals to which they attach themselves at the nasal cavity. Similarly, leeches get into the mouth while the animals drink the water and attaches to the oropharyngeal by the help of powerful terminal suckers (Kutschera and Wirtz, 2001). Mechanosensors are well developed so that responds to noxious stimuli and immediately sucked with the animals (Esch and Kristan, 2002; Abowei and Ezekiel, 2011). Hungry leeches are more liable to stir around impulsively and respond to lower threshold stimuli. Furthermore, it seems hungry leeches vigorously place themselves at the water surface so that they will be more likely to receive stimulation when animals drink the water (Esch and Kristan, 2002; Ahangaran, et al. 2012).

2.1.1. Distribution

Leeches can live in a variety of environments, including aquatic and moist terrestrial regions. Some species live in freshwater, estuaries, rivers, ponds, lakes, and sea. Others are adapted with more mucous glands and larger nephridial vesicles (bladder) that retain and store extra water enabling leeches to tolerate the lack of water on damp land. Moreover, leeches have high physiological flexibility, which makes them able to withstand numerous environmental challenges, such as oxygen shortage and temperature fluctuations (Abdualkader, et al. 2013). Due to special adaptation they have they can easily be acclimatized a new environment.

2.1.2. Feeding habits

Leeches are both predators of many invertebrates and ectoparasites that feed on the blood of vertebrates including human, predacious and sanguivorous, respectively (Kutschera and Wirtz, 2001; Abdualkader, et al. 2013). The sanguivorous species which are the members of this study can store blood inside their body for months. Actually, the digestion process of blood in hematophagous leeches undergoes many slow stages allowing leeches to store the ingested blood for up to 18 months (Abdualkader, et al. 2013). Symbiotic bacteria named *Aeromonas* spp., located in the leech's gut, secrete enzymes that help not only in breaking down the components of the ingested blood, but also in producing antibiotics to prevent blood putrefaction after a long storage period in leech crop (Abdualkader, et al. 2013).

2.1.3. Medicinal Role

Our vivo study organism, leeches (*Limantis nilotica*) nowadays are used for a variety of medical purposes including providing useful treatments for arthritis, blood-clotting disorders, varicose veins and other circulatory disorders and are also used in modern plastic and reconstructive surgery. Hematophagous animals including leeches have been known to possess biologically active compounds in their secretions, especially in their saliva. The blood-sucking annelids, leeches have been used for therapeutic purposes since the beginning of civilization (Abdualkader, et al. 2013). Leeching is used for skin diseases, beneficial after surgical operations to improve blood flow, during the middle Ages medics depended on leech therapy, which was used for disorders like nervous systems, urinary and reproductive organ diseases, inflammatory diseases, eye illnesses, ear abnormalities and audiology and used as bloodletting. However, by the end of 19th century leeching gradually fell into disrepute and almost stopped by the early twentieth because hirudo therapy did not match the new requirements of the modern medical regulations and the great advancement in all medical fields. However, even though medical fields are advanced, bloodletting by leeches was still common and came back in the medical applications and treatments, which were proven and supported by a huge number of scientific studies (Abdualkader, et al. 2013).

Despite of their medicinal merits and other ecosystem services, leeches are a serious livestock and human health problem.

2.1.4. Reproduction

Aquatic leeches (*Limantis nilotica*) infestation starts at early dry season (November) and continues until May (Tadesse Eguale, et al. 2010). In early spring the leeches mate by true copulation an eversible male copulatory organ is inserted into the female gonopore of another leech. About 3 months after copulation the leeches produce cocoons. They deposit their spongy egg-capsules on land, i.e., above the shoreline, mostly among rotten leaves or under rocks. Sturdy cocoons can withstand desiccation and imbibed and rapidly re-gained their original shape produce juvenile leeches can be hatched within 4- 5 weeks (Kutschera and Wirtz, 2001).

2.2. Plant material

2.2.1. Botanical description

Guizotia abyssinica (L.f) Casso (Nug in Amharic and Niger in English) belongs to the family Asteraceae (Compositae), tribe Heliantheae, sub-tribe Coreopsidinae. The plant height diverges depending on the environmental conditions of the growing area. It grows to an average height of 1.2 m, though a height of 2.1 m is also observed subject on the growing condition. *Guizotia abyssinica*(*G. abyssinica*) is an annual plant with a hollow stem. The taxonomic revision of the genus was made by Baagoe (1974) who condensed the number of species to six. The major growing areas as edible oil seed are Ethiopia and India (Murthy, et al. 1993). Niger (*Guizotia abyssinica*) an oil seed crop belongs to the Compositae family. Reports by Getinet and Sharma (1996); Syume and Chandravanshi, (2015) and Bhavsar, et al. (2017) indicated that Ethiopia and India are the two major niger seed producing countries in the world. The crop is native to Ethiopia; however, it was also brought to India by traders before the Christian era (Getinet and Sharma, 1996). Studies showed that there is variation in composition of the seed among Ethiopian and Indian cultivation.

Guizotia abyssinica is the only cultivated species of the genus *Guizotia*. According to the report by Dagne (1994b) *G. abyssinica* is a diploid plant with a chromosome number of $2n = 2x = 30$. Its nature of cross pollination and self incompatibility is reported by Adda, et al. (1994). Thus growers may be suggested to have bee hives close to the growing area of the crop to simplify cross pollination by bees. It is a low yielding crop with low fertility needs. The low yield may be attributed to the self-incompatibility nature of the crop and the low input state under which it is normally grown.

2.2.2. Geographical distribution

Tropical Africa especially in East Africa with a greater concentration in Ethiopia are the grow zones of *Guizotia* species (Murthy and Hiremath, 1988). According to Mesfin (2004) throughout the Ethiopian highlands *G. abyssinica* is cultivated for its edible, oil producing fruits (cypselas) in Tigray, Gonder, Gojam, Welo, Shewa, Arsi, Wolega, Ilubabor, Kefa, GamoGofa, Sidamo, Bale and Hararge. In fact, five out of the six *Guizotia* species are originate in Ethiopia. *Guizota villosa*

is endemic to northern Ethiopia (Dagne, 1994b), and two *Guizotia* species that are not yet described and known only by the name of their areas, 'Ketcha' and 'Chelellu' are reported only from Ethiopia (Dagne, 2001). It is declared that *G. abyssinica* has its center of diversity in Ethiopia (Murthy, et al. 1993) and understood to have its center of origin also in Ethiopia (Murthy and Hiremath, 1988). Likewise *G. abyssinica* cultivated, introduced or naturalized elsewhere, including East Africa, Ghana, Republic Democratic of Congo, Sudan, South Africa; Yemen, Nepal, Malaysia, India, Hong Kong, Australia, British Isles and USA.

Although all the wild *Guizotia* species grow in tropical Africa, the extent of their distribution diverges greatly. Some of the species such as *G. villosa*, *G. arborescence*, *G. zavattarii* and *G. jacksoni* are restricted in their distribution, while others like *G. scabrassp scabra*, and *G. scabrassp schimperi* cover a relatively wide area in east Africa with a superior attentiveness in Ethiopia. *G. arborescence* is a component of the natural vegetation in the Imatong Mountains of Sudan and Uganda and southern Ethiopia (Friis, 1971; Hiremath and Murthy, 1988). *Guizotia zavattarii* is endemic to mount Mega in southern Ethiopia and the Huri hills in northern Kenya (Baagoe, 1974; Dagne, 1994b). The distribution of *G. scabrassp scabra* covers a widespread range ranging from east Africa to Nigeria with a distributional gap in the rain forest of Congo, while *G. scabrassp schimperi* is a common weed of cultivated crops and widely distributed in Ethiopia (Hiremath and Murthy, 1988; Dagne, 1994b). *Guizotia villosa* is endemic to the northern part of Ethiopia and *G. jacksoni* is endemic to mountains of Kenya, Aberdares and Elgon highlands in Uganda and Kenya (Dagne, 1994b).

2.2.3. Economic importance

Guizotia abyssinica has economic significance not only for domestic consumption, but also as an export commodity in many countries, where it is mainly sold as bird feed. The species is used in intercropping schemes, grows on poor but also enormously wet soils and contributes to soil conservation. While not fully domesticated and anguished from low yields and susceptibility to insect herbivores. Crop uses (soil improvement) – following the seed harvest, *G. abyssinica* crop residues are left behind in fields. This dried residue may be used to mulch fruit trees or merely spread out to decompose over the soil surface, serving as a source of organic matter. Due to a possible allelopathic weed-suppressing effect, *G. abyssinica* is recognized as an effective green manure cover crop. This crop seed constitutes about 50% of Ethiopian and 3% of

Indian oilseed production (Syume and Chandravanshi, 2015). The Ethiopian type of Niger seed is oil rich with 40% of oil with fatty acid composition of 75-80% linoleic acid, 7-8% palmitic and stearic acids and 5-8% oleic acid. Whereas the Indian type Niger seed is containing 55% and 25% of linoleic and oleic acid, respectively.

Oil seeds are vital sources of edible oils of nutritional, industrial and pharmaceutical importance. In addition to its role in edible uses, Ramadan and Morsel (2003) have pointed out that niger seed oil can be utilized in the manufacture of soaps, paints and lubricants and protein rich meal obtained after oil extraction can also be used as a feed and fuel. Niger seed is used as land rehabilitations (Getinet and Sharma, 1996), manufacture of soap, paints or as a lubricant or illuminant (Syume and Chandravanshi, 2015), medicinal properties as treatment of coughs and food for human consumption (Adarsh, et al. 2014).

In Ethiopia 50-60% of the edible oil requirement for domestic consumption is obtained from *G. abyssinica* seed (Riley and Belayneh, 1989). *Guziota abyssinica* seed oil is extracted through traditional extraction method that entails crushing, warming and traditional centrifugation (Getinet and Sharma, 1996). Nowadays, large oil mills located in the major cities and towns in Ethiopia extract *G. abyssinica* seed oil. The left-behind after oil extraction is also rich in protein and fiber and can be used as animal feed (Ramadan and Morsel, 2002; Kandel and Porter, 2002). In other countries like the United States, *G. abyssinica* seed is used as food finches. It is also used in the making of soap and as carrier of scent in perfume industry (Kandel and Porter, 2002). The higher percentage of linoleic acid gives the *G. abyssinica* seed oil from Ethiopian origin superior quality for use in paints (Kandel and Porter, 2002). *G. abyssinica* meal can also be used as a relatively cheaper growth medium for *Bacillus* species responsible for the production of alkaline protease (Gessese, 1997) Moreover, consuming *G. abyssinica* seed oil is beneficial from public health point of view because it contains minor quantities of substances such as copherols, phospholipids and sterols that offer protection against cardiovascular disorders and cancer (Ramadan and Morsel, 2002).

Niger seed can be used as dressings which can be applied to the surface of the body to relieve pain, itching, swelling and inflammation, abscesses, boils, etc. *G. abyssinica* seed poultice is the most effective treatment available for many types of disorders. Even today, they may provide effective, economic benefits. There are reports that *G. abyssinica* seed oil is used for birth

control and for the treatment of syphilis in Ethiopia (Belayneh, 1991), sprouts mixed with garlic and 'tej' are used for the treatment of coughs also.

Generally, there is not much work done on niger. An in-depth treatment of its taxonomy and distribution was done by Baagoe (1974). Cytological studies were done by some researchers (Dagne and Heneen 1992; Hiremath and Murthy 1992; Dagne et al. 2000; Dagne 2001) however; to date there is no scientific report of using *G. abyssinica* for leech treatments in cattle.

Moreover, existing literature knowledge on the anti-leech effect of Niger seed hull is neglected. This research report intends to draw consideration for the investigation of drug development process from the neglected environmentally friendly Niger seed hull. It is hoped that the report of this research would contribute as a baseline data of crude test drug for the further investigation of its toxic part, isolation of compounds and for future make use towards anti leech activities. Traditionally, the crop's hull after its post harvesting is used as anti leech, however there is no scientific study regarding the effects of this plant on leech. In addition photochemical investigation of Niger seed hull is not studied so far. Therefore, the study was necessitated to evaluate the anti-leech effect of Niger hull crude extracts and its photochemical studies.

3. Methodology

3.1. Study Design

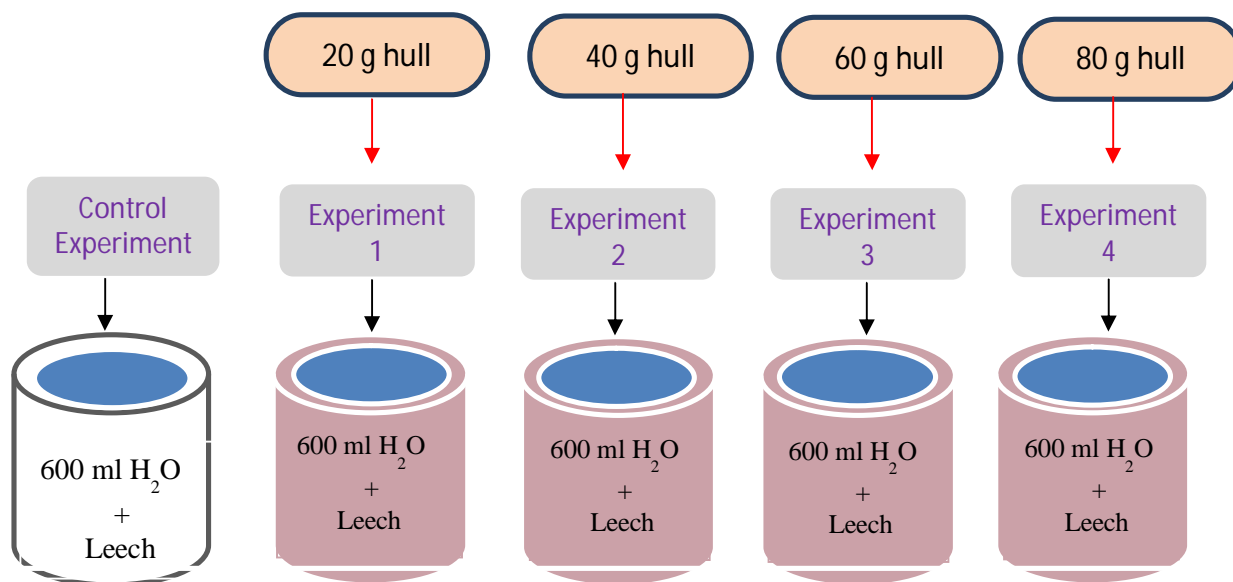
3.1.1. Experimental Organism

More than two hundred experimental leeches, *Limnatis nilotica* were collected in February 2016 from Northern Ethiopia; North eastern part Tigray regional state an area called "Quean" in 'Golagul' river. The strong muscular suckers at the anterior and posterior ends, dark green color surface with rows of green spots on the dorsal surface and yellowish orange and dark green bands on either side were the main signs for the revealing of the study species (Ahangaran, et al. 2012a).

The leech species, were reared in plastic open tank with 21 m depth of tap water as an indoor aquarium. The soil collected from their habitat was loaded into the tank and sand; small size stone were also placed. Even though leeches are poikilotherms, simple optimization experiment was conducted to check their adaptability with the new environment. Thus, the leeches were cultured in a plastic ater tank and acclimatized for extent of one month before using them for the experiment. To avoid contamination, water was changed once every 3 days till the experiment of bioassay has been started.

3.2. The plant material collection and extraction

The hull part of *Guizotia abyssinica* was collected in January 2016 from Ankesha Gugusa Woreda, Awi Zone, Amhara regional state Ethiopia. An oilseed crop, *Guizotia abyssinica* was identified and authenticated by a botanist Mrs. Genet Atsbeha. Before preliminary crude extraction, the indigenous practice pre-test was carried with same water amounts and different weights of the hull to confirm its positive result to be as an initial for the next experiments. Accordingly, experimental design was followed with control group and experimental as presented below. The evaluation of death of a leech was done.



The difference between the control group and treated group with Niger crop hull was examined. After the pre-test experiment the actual extraction protocol was followed. The collected plant material was washed with distilled water and air dried under shade. After the sample was dried, 2 kg of the hull of *Guizotia abyssinica* was powdered with mechanical grinder. The powdered sample was packed in polyethylene bags to avoid other mixing of surrounding material.

The pulverized 100 g of *G. abyssinica* hull was sequentially macerated by using 400 mL of chloroform, hexane and methanol for 24 hours. The respective extracts were filtered using Whatman filter paper No.1 and the solvents were evaporated in vacuum by using a rotary evaporator under reduced pressure at 40 °C and it was kept at 4 °C until analysis.

3.3. Preliminary Phytochemical Screening of Crude Extracts

The phytochemical screening test of Niger seed hull crude extract were conducted using three different solvents chloroform, hexane and methanol. The crude extract of Niger seed hull was conducted for the screening of phytochemicals to detect the presence or absence of secondary metabolites according to standard methods (Iqbal, et al., 2011; Dyana and Kanchana, 2012).

3.4. Thermal Behavior

Thermal behavior of air dried Niger seed hull extracts was carried out using differential scanning calorimeter (Pyris Manager DSC 400, Perkin Elmer instruments, Netherlands). 17 mg dried Niger seed hull crude extract was weighed into aluminum pan and then it was covered using pan

lid. DSC equipment was set to heat from 0 to 320°C with a linear heating rate of 10°C per minute. The test was performed under the atmosphere of nitrogen gas flow. An empty aluminum pan was used as the reference (Phaechamud *et al.*, 2012). The apparatus was calibrated with high purity zinc (419.5 °C) and Isothermal condition was maintained at 25°C for 5 min (Osorio and Carriazo, 2011; Matthew, et al., 2016). A peak transition onset temperature was recorded by means of an analyzer.

3.5. Preparation of the chemical drugs

In the present study, 600 mg of an anthelmintic Levamisole tablet was purchased and used as a positive control and compared with distilled water and crude extract. The tablets were powdered and diluted in 600 ml water (Bahmani, et al, 2012).

3.6. Anti-leech assay

Leeches were placed individually in a 1000 ml beaker with 600 ml distilled water. Lavamisole tablets were used as a positive control and compared with distilled water. Before actual replication experimentation, solvent selection experiment was conducted and level of efficacy of best solvent was determined with parameters like paralysis-death time of leech. Six different serial concentrations (18.8, 37.5, 75,150,300 and 600mg/mL) of each crude extract (in hexane, chloroform and methanol) were used for the determination of anti-leech activities of the Niger seed hull.

Then, the extract and drugs were added and their effects were screened for 720 min and the time to paralyze or kill each *L. nilotica* was recorded. The experimentation was repeated three times for each solvent. The evaluation of death of leech was based on immobility after stimulation with needle. The low average paralyzing or killing time of these compounds reflects anti leech properties.

Accordingly, the severity of effects of these candidate drugs were categorized into different timing groups, 4⁺ severity: paralysis and death of each leech within 1–60 min after addition of the drug, 3⁺ severity: paralysis and death of each leech within 61- 120 min after addition of the drug, 2⁺ severity: paralysis and death of each leech within 121- 180 min after addition of the drug, 1⁺ severity: paralysis and death of each leech within 181– 240 min after addition of the

drug, and Negative: paralysis and death of each leech within 241- 720 min after addition of the drug (Bahmani, et al. 2010).

3.7. Data Analysis

The variation between the control and treated groups were analyzed by using one- way ANOVA test.

4. Results and Discussion

Preliminary photochemical analysis revealed that the presences of flavonoids and terpenoids. Saponins were detected only in methanol crude extract (Table 1). The logic in using different solvents when screening for phytochemicals in plant materials was clearly validated in this study. For instance, the results show that saponins were exceptionally present in methanol extracts but absent in hexane and chloroform extracts. The presences of secondary metabolites like flavonoids, terpenoids and saponins screened in the hull of Niger used in this study might be responsible for the anti-leech activity. Photochemical found in hull extracts of Niger indicates their potential as a source of novel medicines.

Table 1. Photochemical screening of Niger hull crude extract

Phytochemical	Method of Test	Results		
		MeOH Extract	CHCl ₃ Extract	Hexane Extract
Alkaloids	Wagner's Test	-	-	-
Saponins	Foam Test	+	-	-
Tannins	Gelatin solution	-	-	-
	Iron Chloride Test	-	-	-
Flavonoids	NaOHTest	+	+	+
Terpenoids	Salkowski Test	+	+	+

Note: '+' indicates presence '-' indicates absence

The crude extract of Niger seed hull showed anti leech activities from 1⁺- 4⁺ severities. Among the different crude extracts of Niger seed hull, the maximum anti-leech activity was obtained in

the methanol crude extract and the minimum was obtained in the chloroform extract. This might be due to the presence of saponins in methanolic extracts. However, chloroform crude extract at the concentration of 75, 37.5 and 18.8 mg/ml did not showed any activity against *L. nilotica* with followed standard severity time zones. In this study, anti- parasitic drug, levamisole which was taken as a positive experimental control group was very effective with 4⁺ influences. The detail of results is presented in table 2. This study showed that higher concentration of crude extracts of Niger hull with all type of solvents showed paralytic effect much earlier and the time to death was shorter for almost all the leeches.

Table 2: In vivo anti-leech activities of methanol, n-hexane and chloroform extracts of Niger seed hull

Components and crude extracts	Dose concentration (mg/mL)	Death time (min) Mean±SD	Severity
Levamisole (Positive control)	600	3.7±0.03	4 ⁺
	300	3.3±0.03	4 ⁺
	150	5.7±0.03	4 ⁺
	75	8.3±0.77	4 ⁺
	37.5	15±0	4 ⁺
	18.8	35.3±0.03	4 ⁺
Distilled water (negative control)	-	-	-
Methanolic Niger seed hull extract	600	9.3±0.03	4 ⁺
	300	19.7±1.8	4 ⁺
	150	25±4.7	4 ⁺
	75	59.3±0.03	4 ⁺
	37.5	138±1.33	2 ⁺
	18.8	183±1	1 ⁺
n-hexane Niger seed hull extract	600	20±3.3	4 ⁺
	300	35±3	4 ⁺
	150	151.7±0.6	2 ⁺
	75	177.3±0.03	2 ⁺
	37.5	180.3±0.1	1 ⁺
	18.8	260.3±1.4	1 ⁺

Chloroform Niger	600	29±0.7	4 ⁺
seed hull extract	300	63.7±0.43	3 ⁺
	150	169±0.1	2 ⁺
	75	242.7±0.03	-
	37.5	309±2.7	-
	18.8	512±2	-

Based on the results presented above, Niger hull crude extracts provide a natural source of anti-leech activities, although the anti-leech actions of methanolic, hexane and chloroform extracts are equal or week to that of levamisole. All dose levels of standard reference drug Lavamisole caused a significant kill of leeches ($\chi^2 = 62.9$, $df=5$, $p<0.05$). Similar finding was reported for standard reference drug levamisole which caused leech death with 4⁺ influences (Gholami-Ahangaran, et al. 2012b).

There was a significant difference in mortality of a leech by the methanoloic, hxane and chloroform extracts of Niger hull ($\chi^2 = 355$, $df=5$, $p<0.05$, $\chi^2 = 299.3$, $df=5$, $p<0.05$ and $\chi^2 = 711.7$, $df=5$, $p<0.05$, respectively). The results of this study are in agreement with several studies of anti leech activities of different medicinal plants; however, present study is the first report and focus of anti-leech activities of Niger hull crude extract with different solvents. The findings of other studies showed that methanol extract of tobacco with a dose of 600 mg and its nicotine as active ingredient with 5, 10, and 20 mg killed leeches with the 4⁺ intensity and had strong effect on mortality of *Limnatis nilotica* (Bahmani, et al., 2012 and Bahmani, et al, 2014). Bahmani, et al. (2014) in other study reported that ethanolic extract of plant *Achillea millefolium* showed anti leech effect at 600mg dose with a mean time of 90±17 caused leech death with 3⁺ influences.

Against with the present finding Gholami-Ahangaran, et al. (2012a) reported that Olive methanol extract at a dose of 600 mg, with a mean time of 210 ± 24.1 minutes, caused paralysis with 1⁺ severity. More power full activity of Niger hull crude extract with methanol might be due to existence of a myriad of compounds which resulted death of leech with 4⁺ severities with 600 mg (Table 1). The presence of secondary metabolite saponins in methanolic crude extract might be caused increased mortality of leeches. Reports showed that the existence of spaonins have been associated with haemolytic action, growth impairment and destabilize the cell membrane of

parasites (Athanasiadou and Kyriazakis, 2004; Delfin, et al. 2017). As per the study reported by Ellen, et al. (2007) saponins possess clear insecticidal activities; exert a strong and rapid working action against a broad range of pest insects and cause cellular toxicity effects. Photochemical saponins are also known to have side effect central nervous system activities of helminthes (Muthee, et al. 2016).

Bahmani, et al. (2013) in other finding reported that garlic methanolic extract showed anti - leech activities with a mean time of 68.44 ± 28.39 min (3^+ severity) with a dose of $600 \mu\text{g/ml}$, which is comparable with the chloroform extracts of Niger hull of the present finding, 3^+ severity against leech, even though variation of the dose and type of crude extract is in consideration. In other words, existence of secondary metabolites such as flavonoids and terpenoids in all extracts might be a reason for the paralysis and death of leeches with severity ranges from 1^+ - 4^+ , except concentrations below 75 mg/ml at chloroform extract of Niger hull. Photochemicals like flavonoids and terpenoids have been shown to have an anthelmintic property from plant crude extracts (Muthee, et al. 2016).

Differential scanning calorimetry (DSC) is a versatile technique that based on heat flow into and out of a material as a function of temperature. The instrument was calibrated with zinc reference (Melting Onset temperature = $419.5 \text{ }^\circ\text{C}$) as internal standard shown from Figure 1. Thus, from DSC thermogram four characteristic endothermic transitions (with melting onset temperature at $45.4 \text{ }^\circ\text{C}$ and 207°C) were characterizing the whole thermogram of the dried crude sample (Figure 2). Figure 2(A) shows the DSC thermogram of the crud extract Niger seed hull in the temperature range ($0\text{-}320^\circ\text{C}$) at a temperature heating rate $20 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$. Three endothermic transition peaks were formed at 49.5°C , 99°C , and 215°C peak temperature. Peak at 99°C due to water loss, and peak at 49.5°C and 215°C melting of pure compounds while one of the endothermic peaks found at 180°C is broad, this indicates the complex nature of the extract. From the curve at 300°C and higher than this temperature comprise thermal decomposition for the sample (Ram, et al., 2014; Matthew, et al. 2016). In Figure 2(B) DSC curve shows the heating rate decreases the feasibility of broadening peak at 180°C increases this designated that

as the heating rate increases the heat flow into the sample or heat absorbed by the sample increases and supports to observe clear endothermic transitions peaks.

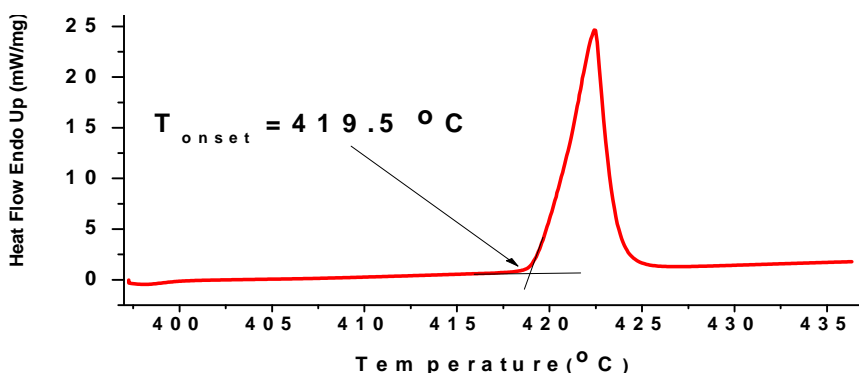


Figure 1: DSC thermogram for Zinc Calibration recorded in a dynamic nitrogen atmosphere (50 mL min^{-1}), at a heating rate of $20^\circ\text{C min}^{-1}$.

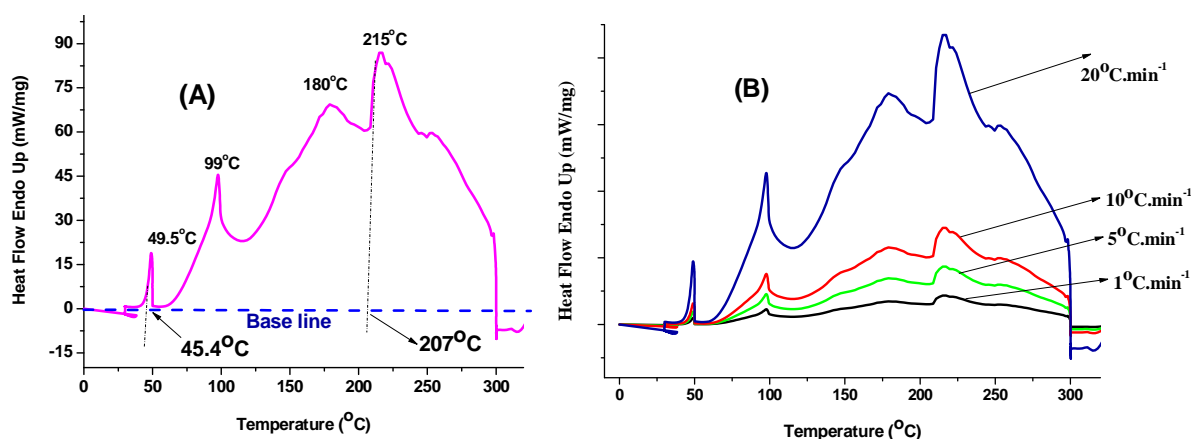


Figure 2: DSC thermogram for Niger seed hull crude extract (17 mg) recorded in a dynamic nitrogen atmosphere (50 mL min^{-1}) (A) at $20^\circ\text{C.min}^{-1}$ scan temperature, and (B) at a heating rate of 1, 5, 10 and $20^\circ\text{C.min}^{-1}$

5. Conclusion and Recommendations

From the results of this study, it is concluded that crude extracts of Niger hull showed anti leech activity and could be apply as an effective source of drug after further exploration. Phytochemical screening revealed presence of saponins, flavonoids and terpenoids whose intensity of causing death of leech a little varied with solvent used for extraction. The present study suggested the ani-leech potential of Niger hull crude extract although *in vivo* efficacy evaluation and toxicity studies need to be carried to ascertain their bioavailability and safety to

the animals. From this study, Niger seed hull crude extract changes its phase from solid to liquid (Melting), and the sample is going to be effective to resist heat by absorbing energy up to a maximum peak temperature at 215°C. After this temperature all stable molecules found in this sample begin to lost and break its bond structure and finally the material loses its effective applicability towards the targeted anti-activity. Photochemical found in hull extracts of Niger indicates their potential as a source of novel medicines

Therefore, based on the results obtained during the present study, the following recommendations are suggested:

- Studies should be needed in next steps of the undertaken work for understanding the mechanism of action by using in vivo models to figure out the effectiveness and pharmacological rationale of using Niger hull as an anti leech drug.
- Further detailed studies are required to isolate the possible active phytoconstitutents responsible for the anti -leech activity and study its future pharmacological actions
- Plant species are the potential stock for future genetic resources, and would have great implications for the environment and biological diversity as well as for such ethno-botanical uses accordingly to serve as endower sustainable bases it is better to conserve the wild and domesticated Guizotia species.
- Further in vivo study might be another future study space
- Finally, the traditional medical practices can be strongly endorsed for these plants as well as efforts should be geared up to further work out to isolate, purify and characterize the active constituents responsible for the activity of this crop hull.

Acknowledgements

The team of this study project would to like acknowledge Adama Science and Technology University, for financial support. The School of Applied Natural Science, ASTU is also acknowledged for financial release and continuous follow ups. We would like than Mr. Tolosa Dugma for his laboratory assistance. Mr. Dereje Birhanu is also thankful for collection of the sample and caring of leeches under laboratory. We would like to thank Department of Applied Biology and applied chemistry ASTU for the provision laboratory and some facilities.

6. References

- Abdualkader, A. M., Ghawi, A. M., Alaama, M. . Awang, M. and Merzouk, A. (2013): Leech therapeutic applications. *Indian Journal of Pharmaceutical Sciences* **75(2)**:127-137.
- Abowei, J. F. N. and Ezekiel, E. N. (2011): A review of Acanthocephla, Leeches, parasite Crustaceans and some other parasites of miscellaneous taxa infections in African fish. *International Journal of Animal and Veterinary Advances* **3(5)**: 337-351.
- Adarsh, M. N., Kumari, P. and Devi, S. (2014): A review of *guizotia abyssinica*: a multipurpose plant with an economic prospective. *Journal of Industrial Pollution Control* **30** (2):277 – 280.
- Adda, S., Reddy, T. and Kishor, P. (1994): Somatic embryogenesis and organogenesis in *Guizotia abyssinica*. *In Vitro. Cell. Dev. Biol.* **30**:104-107.
- Ahangaran, M. G. Bahmani, M. and Jahromi, N. Z.(2012a): In-vitro anti-Leech effects of *Vitis vinifera* L., Niclosamide and Ivermectin on mature and immature forms of *Leech Limnatis nilotica*. *Global Veterinaria* **8 (3)**: 229-232.
- Ahangaran, M. G., Bahmani, M., Jahromi, N. Z. (2012b). Comparative and evaluation of anti- leech (*Limnatis Nilotica*) effect of Olive (*Olea Europaea* L.) with Levamisol and Tiabendazole. *Asian Pacific Journal of Tropical Disease*. S101-S103.

- Athanasiadou, S. and Kyriazakis, I. (2004): Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems. *Proceedings of the Nutrition Society* **63**:631 -639.
- Baagoe, J. (1974) : The Genus *Guizotia* (Compositae). A taxonomic revision. *Bot. Tidskr.* **69**:139.
- Bahmani M., Abdi F., Adineh A., Hassanzadazar H., Eghbali B., Ahangaran M. and Kopaei M. (2014): The anti-leech effect of ethanolic extract of *achilleamillefolium* L. compared to levamisole and niclosamide on *limnatis nilotica*. *Studia Universitatis Vasile Goldis, Seria Stiintele Vietii* **24**:293-297.
- Bahmani, M., Banihabib, E., Saki, K., Ghoshji, B. K., Heydari, A. and Hashemi, M. (2012): Anti-Leech and Disinfection Activities of Methanolic Extracts of Walnut (*Juglans regia* L.) and Oleander (*Nerium oleander* L.) on *Limnatis nilotica*. *World Journal of Zoology* **7** (3): 267-272
- Bahmani, M., Hosseini, S. Avijgan, M. and Qorbani, M. (2010): Evaluating the anti-leech effects of tobacco methanolic extract compared with succinyle choline and some other anti-parasite drugs. *Journal of Sharekord University of Medical Sciences* **12**(3):53 – 59
- Bahmani, M., Kopael M. R., Eftekhari, Z., Banihabib E., Hajigholizadeh, G., Bahmani, F., Azadzeh, J., Abdollahi, R., Kheyri, A., Sotoudeh, A., Karamati, S. A. and Jelodari, M. (2013): Evaluating the Anti-Leech Effects of Methanolic Extracts of *Peganum harmala* L. and *Olea europaea* L. on *Limnatis nilotica*. *World's Vet. J.* **3**: 33-37.
- Belayneh H. (1991): Source-sink study on niger. *Oil Crops News.* **3**: 63-6.
- Bhavsar, C. J., Syed, H.M. and Andhale, R. R. (2017): Characterization and quality assessment of mechanically and solvent extracted Niger (*Guizotia abyssinica*) Seed oil. *Journal of Pharmacognosy and Phytochemistry* **6**(2): 17 – 21.
- Dagne, K. (1994b). *Cytology, Phylogeny and Oil Quality of Guizotia Cass. (Compositae)*. Ph.D. thesis, Addis Ababauniversity, Ethiopia.
- Dagne, K. (2001). Meiotic properties of induced autopoly-ploid *Guizotia abyssinica* (L.f) Cass. *J. Genet. Breed.* **55**: 11-16.

- Dagne, K. and Heneen, W. (1992): The karyotypes and nucleoli of *Guizotia abyssinica* (Compositae). *Hereditas* **117**:73-83.
- Dagne, K., Cheng, B. and Heneen, W. K. (2000): Number and sites of rDNA loci of *Guizotia abyssinica* (L.f) Cass. As determined by florescence in situ hybridization. *Hereditas* **132**: 63-65.
- Delfin, E. Cabardo, J. R., Harvie, P. Portugaliza (2017). Anthelmintic activity of *Moringa oleifera* seed aqueous and ethanolic extracts against *Haemonchus contortus* eggs and third stage larvae. *International Journal of Veterinary Science and Medicine* **5**:30-34
- Dyana J.P. and Kanchana G., (2012): Preliminary phytochemical screening of *Cocosnucifera* Flowers, *Int. J.Curr.Pharma. Res.* **4**: 35.
- Ellen, D. G., Ellen, L. Danny, G. and Guy, S. (2007): Novel advances with plant saponins as natural insecticides to control pest insects. *Pest Technology* **1(2)** 96-105.
- Esch, T. and Kristan, W. B. (2002): Decision-Making in the Leech Nervous System. *Integ. and Comp.Biol.*, **42**:716–724.
- Friis, I. (1971): A new species of *Guizotia* (Compositae) from north east tropical Africa. *Norwegian Journal of Botany* **18**: 231-234.
- Gessesse, A. (1997): The use of nug meal as a low-cost substrate for the production of alkaline protease by the alkaliphilic *Bacillus* sp. AR-009 and some properties of the enzyme. *Bio-resource Technology* **62**:59-61.
- Getinet A. and Sharma, S. (1996): *Niger. Guizotia abyssinica* (L.f) Cass. *Promoting the Conservation and use of Underutilized and Neglected Crops*. 5. Int. Genet. Resour. Inst., Gatersleben, Germany.
- Hiremath S. and Murthy, H. (1992): Cytogenetical studies in *Guizotia* (Asteraceae). *Caryologia* **45**:69-82.
- Iqbal H, Moneeb U.R., Rehman K., Riaz U., Zia M., Naeem K., Farhat A., Zahoor U. and Sajjad H. (2011): Phytochemicals screening and antimicrobial activities of selected medicinal plants of Khyberpakhtunkhwa Pakistan, *Africa. J. Pharm. Pharmacol.* **5(6)**: 746-750.

- Kandel, H. and Porter, P. (2002): Niger: *Guizotia abyssinica* (L.f.) Cass. Production in northwest Minnesota. University of Minnesota extension service.
- Kutschera, U. and Wirtz, P. (2001): The evolution of parental care in freshwater Leeches. *Theory Biosci.* **120**:115 – 137.
- Matthew, A. I., Augustine, O. O., Finizia, A., Claudio, D. and Rocco, D. (2016): Microencapsulated *Garcinia kola* and *Hunteria umbellata* seeds aqueous extracts – part 1: effect of microencapsulation process. *International Journal of Phytopharmacy* **6(1)**: 01-09.
- Mesfin T. (2004): *Flora of Ethiopia and Eritrea Volume 4, Part 2* (Eds: IngaHedberg, IbFriis& Sue Edwards) Addis Ababa-Ethiopia and Uppsala-Sweden.
- Murthy H. and Hiremath, S. (1988): Domestication of niger (*Guizotia abyssinica*). *Euphytica***37**: 225-228.
- Murthy, H., Hiremath, S. and Salimath, S. (1993): Origin, evolution and genome differentiation in *Guizotia abyssinica* and its wild species. *Theor. Appl.* **1**: 587-592.
- Muthee, J. K., Gakuya, D. W., Mbaria, J. M. and Mulei, C.M. (2016): Phytochemical screening and cytotoxicity of selected plants used as anthelmintics in Loitoktok Sub-County, Kenya. *The Journal of Phytopharmacology* **5(1)**:15-19.
- Osorio, C. and Carriazo, J. G. (2011): Thermal and structural study of Guava (*Psidium guajava* L) powders obtained by two dehydration methods. *Quim.* **34(4)**: 636-640.
- Phaechamud, T., Yodkhum, K., Limmatvapirat, C. and Wetwitayaklung, P. (2012): Morphology, thermal and antioxidative properties of water extracts from *Sonneratia caseolaris* (L.) Engl. prepared with freeze drying and spray drying. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **3(1)**:725-739.
- Ram, V. R., Ram, P. N., Khatri, T. T., Vyas, S. J. and Dave, P. N. (2014): Thermal analytical characteristics by TGA-DTA-DSC analysis of *Carica papaya* leaves from Kachchh. *International Letters of Natural Science* **21**:12-20.
- Ramadan, M. and Morsel, J. (2002): Proximate neutral lipid composition of niger. *Czech Journal of Food Science* **20**:98-104.

- Ramadan, M. F. and Morsel J. T. (2003): Determination of the lipid classes and fatty acid profile of Niger (*Guizotia abyssinica* Cass.) seed oil. *Photochemical Analysis* **14(6)**: 366-370.
- Sintayehu GebreMariam, Samuel Amare, Derek Baker, Ayele Solomon and Davies, R. (2013): Study of the Ethiopian live cattle and beef value chain. ILRI Discussion Paper 23. Nairobi: International Livestock Research Institute.
- Syume, M. and Chandravanshi, B. S. (2015): Nutrient composition of niger seed (*guizotia abyssinica* (l. f.) cass.) cultivated in different parts of Ethiopia. *Bulletin of the Chemical Society of Ethiopia* **29(3)**: 341-355.
- Tadesse Eguale, Getnet Abie, Mesfin Sahile and Daniel Gizaw (2010). Control of aquatic leeches (*Lymnatis nilotica*) using *Phytolacca dodecandra* (Endod) in Sodo District, Gurage Zone, Southern Nations, Nationalities and Peoples Region, Ethiopia. *Ethiop. Vet. J.* **14** (2):125-135.
- Zaidi, S. M. A., Jameel, S. S., Zaman, F., Jilani, S., Sultana, A. and Khan, S. A. (2011). A Systematic overview of the medicinal importance of Sanguivorous Leeches. *Alternative Medicine Review* **16(1)**: 59 – 65.

We certify that the information and figures given in the report are correct and complete to the best of our knowledge.

Principal Investigator Name	Signature	Date
<u>Genet Atsbeha</u>	_____	_____

Co-Investigator's Name	Signature	Date
Mr. Getachewt Bantihun	_____	_____

Approved by:

1. Program Chair's	Signature	Date
_____	_____	_____

2. School/Asso. Dean for RTT' Name	Signature	Date
_____	_____	_____

