



**SCHOOL OF GRADUATE STUDIES
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE
DEPARTMENT BIOLOGY (BIOMEDICAL SCIENCE)**

**EFFECTIVENESS OF *BUDDLEJA POLYSTACHYA* AND
NICOTIANA TABACUM AGAINST ADULT LEECHES
(*Limnatis nilotica*)**

MSc THESIS

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**JANUARY 2024
DILLA, ETHIOPIA**

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(*Limnatis nilotica*)**

**A THESIS RESEARCH SUBMITTED TO SCHOOL OF GRADUATE STUDIES,
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE DEPARTMENT
OF BIOLOGY FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENT
OF MASTER SCIENCE (MSC) IN BIOLOGY**

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**DEPARTMENT BIOLOGY (BIOMEDICAL SCIENCE)
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE
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ACKNOWLEDGMENT

Firstly, I want to express my gratitude to my Almighty God for giving me strength and for sending kind individuals into my life who support me in completing my work. I would like to sincerely thank my advisor, Dr. Belayhun Mandefro, for all of his support and guidance during this research project, from the beginning to the end. I appreciate his expert advice, encouragement, and follow up. My appreciation goes to GDA, whom support me in the financial matter and sponsoring to attend MCs class and Mr Abebe Demse, whom was the head of Repe wereda educational office, allowed me to get free time to complete the course and my thesis in line with my work. My special thanks go to Dilla University, for giving the chance to attend education. I acknowledge Biology and Chemistry department for permission to use the laboratory facilities. I express my deepest respect to Mr Brhanu and Mr Abebe Awel from chemistry department and Eyob Mgbaru form biology department for their support in providing me the necessary laboratory equipment and adjusting the machines and work with me in secondary metabolite test. I would like to acknowledge Dr Talimos Seta and Instructor Muluken Tadele for their help in identifying the plant species and Instructor Abiyot Gemechu for his help in identifying the leech species. My special thanks goes to my friend Tigist Teferi and Teresa Beyene for supporting me while am working in anti-leech assay for kipping the leeches in the solution, also my friends Melese Guro, Teshome Brhanu, Andargachew Tariku and Muluken Ayele since they collect leeches and plants samples together with me. Most significantly i would like to express my deepest thanks to my family for supporting me financially to finish this work. Finally, I am also grateful to all persons that have direct or indirect contribution for the accomplishment of this study.

DECLARATION

I certify the work titled Effectiveness of *Buddleja polystachya* and *Nicotiana tabacum* against adult leeches (*Limnatis nilotica*), is my original work, that it has not been submitted to any other institution for the award of academic degree that I adhered to all ethical and technical standards during the collection, analysis and preparation and preparation of the data and al source I used or quoted were credible.

Name: Meskerem Kodede

Date

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ABBREVIATIONS

ACh	Acetylcholine
AChR	Acetylcholine Receptors
BCE	Buddleja Chloroform Extract
BEE	Buddleja Ethanol Extract
BMC	<i>BioMed Central</i>
EDCs	Endocrine Disrupting Chemicals
GDA	Gedeo Development Association
GI	Gastro Intestinal
LD	Lethal Dose
LT	Lethal Time
TCE	Tobacco Chloroform Extract
TEE	Tobacco Ethanol Extract

ABSTRACT

*Hirudiniasis is condition when leech parasitize animals and/or human. Leech are belongs to the phylum Annelida and order Hirudina. Leech infestations cause blood loss, which leads to anemia and several other complications. Therefor this study is aimed to investigate the anti-leech (*Limnatis nilotica*) properties of *Nicotiana tabacum* (*N.tabacum*) and *Buddleja polystachya* (*B.polystachya*) in laboratory condition. Adult leech were collected from river and acclimatized in laboratory for one week before experiment. Extracts from the plants leaves were made by maceration technique using ethanol and chloroform solvents independently, 250g/1.25L of each plant powder were macerated in chloroform and ethanol. Then Stock solution was prepared by dissolving 1g/100ml of the extract using dissolvent 3% Tween 20 and then a concentration of 1ppm, 2ppm, 4ppm, 8ppm and 16ppm for *N. tabacum* and 200ppm, 400ppm, 600ppm and 800ppm for *B. polysthachya* were prepared by serial dilution. Following that, 10 adult leeches were exposed to each concentration for 12 hours. The test was set in triplicate with positive and negative controls. The results showed that *N.tabacum* chloroform and ethanol extracts showed 100% leech death in concentration of 16ppm and above and an LD 50 of 3.447 and LD90 of 4.563 in chloroform and LD 50 of 3.68 and LD 90 of 4.866 in ethanol extract solution while *B. polystachya* did not exhibits any leech death at even 1000ppm. While encountering the test solution the leech exhibited a violent reaction, tries to escape and produce high jelly like mucus as mechanism for neutralizing the effect of the extract solution in both of the plants and concentrations. Considering this violent leech reactions *B.polysthchya* might only have a repellent effect without killing. As compared to previous findings, the current study revealed that *N.tabacum* is a more efficacious plant than expected in killing leeches. In summary of the two plants tested *N.tabacum* exhibited strong anti-leech effect corroborating its traditional application in leech treatment and evidencing for its anti-leech potential. Different extraction technique for *B. polysthchya* and further toxicity test for *N.tabacum* is needed.*

Key words *B. polysthchya*, Hirudiniasis, hirudicidal, LD50, LD90, *L. nilotica*, *N.tabacum*

1. INTRODUCTION

1.1 Background of the study

The disease known as hirudiniasis is caused by leech parasite infestation in humans or animals. Leeches are segmented worms that feed on blood and are distributed all over the world. They belong to the phylum Annelida and order Hirudinea (Moreira *et al.* 2022). The majority of leech species live in water, however some are telluric. Marshes, lakes, ponds, springs, slow-moving streams and humid environments with moist vegetation are among the habitats of aquatic leeches. *Limnatis nilotica*, *Myxobdella africana*, *Dinobdella ferox*, *Hirudo medicinalis*, *Phytobdella catenifera*, and *Theromyzon tessulatum* are common aquatic leeches while, *Haemadipsa zeylanica*, *Haemadipsa sylvestris*, and *Haemadipsa picta* are among common telluric leeches (Mulilo *et al.*, 2020).

Leech infestation is a neglected waterborne parasitic disease that affects livestock and sometimes humans causing significant economic losses due to loss of productivity, mortality and treatment costs. Animals become infested when they drink water contaminated with leeches, while humans become infected when they drink, bathe, swim or walk through water infested with leeches (Bodena *et al.*, 2022).

Various body parts are parasitized by leeches. Some invade internal body surfaces (hence are called internal leeches), while others localize on external surfaces (skin) hence named as external leeches. Internal organs commonly parasitized include oral cavity, oesophagus, epiglottitis, nasopharynx, vagina, cervix, and ovaries (Mulilo *et al.*, 2020). Aquatic leeches frequently reside in the mucous membranes of the mouth and nose of domestic animals (Bodena *et al.*, 2022). Animals infected with leeches have been observed to exhibit lower milk supply, emaciation, anemia, blood in the mouth and nose and coughing (Bahmani *et al.*, 2013). The impacts of this parasite can drastically lower the profitability of livestock farm. In the report of Temesgen *et al.*, 2019, comparing to the severity of parasite infestation in farm animals, leech (*Limnatis nilotica*) was found to be higher (Temesgen *et al.*, 2019). The majority of reported cases and higher prevalence rate are from developing countries including Ethiopia and the problem occurs mainly during dry seasons (Cundall *et al.*, 1986; Iynen *et al.*, 2010; Kasuka *et al.*, 2022; Tadesse., 2010).

Many farmers in the developing nations rely on traditional medicines to treat livestock ailments due to the lack of access to contemporary veterinary health care facilities and the high expense of modern medications (Asaye *et al.*, 2022). Since ancient times, Ethiopians have utilized traditional remedies, which are now widely accepted in the country owing to their effectiveness, cultural acceptability and affordability (Temesgen *et al.*, 2019b). Approximately 80% of human and 90% of animal treatments in Ethiopia are thought to be covered by these traditional cures, 95% of which have a botanical origin (Ofga and Regasa., 2021).

Ethiopian farmers and pastoralists rely on traditional knowledge, practices and locally available materials such as plants in particular, to control and manage livestock parasites. Different ethno botanical studies mentions a numbers of plant species which are traditionally used to treat leech infested animal. Of these the use of *Buddleja polystachya* (*B. polystachya*) and *Nicotina tabacum* (*N.tabacum*) are reported in different parts of the world including Ethiopia (Yared *et al.*, 2014; Ingtipi *et al.*, 2016; Ogallo *et al.*, 2016; Asmamaw., 2018; Mulugeta *et al.*, 2020; Megersa *et al.*, 2021; Niguse and Reta., 2022). Despite the rich traditional medical knowledge and significant contribution of medicinal plants to Ethiopia's animal healthcare system (Megersa *et al.*, 2021), little effort has so far been made to properly evaluate the efficacy of traditional medicine under scientific method. Although the efficacy of *N. tabacum* is studied earlier and show promising result (Bahmani *et al.*, 2010; Temesgen *et al.*, 2019b) further studies required using different solvents and concentration. But the efficacy and safety of *B. polystachya* against leeches has not been scientifically evaluated. Thus, the present study intended to investigate the *in vitro* anti-leech activity of leaves of *B. polystachya* and *N. tabacum*

1.2 Statement of the problem

Even though there are no studies on prevalence rate of human hirudiansis. According to cause studies, leech infestation in humans is becoming a serious health concern in developing nations like Tanzania and Kenya there are also case reports in different parts of Ethiopia. Human hirudiniasis described by more than 20 case reports in Ethiopia of them, vaginal leech infestation (Temesgen., 2015; Habtamu and Demisew., 2021), Oropharyngeal leech infestation (Abiey *et al.*, 2021; Abdulsemed and Zebeaman ., 2022), proximal trachea (Mekonnen., 2013), laryngopharynx (Nebiyu and Segenet., 2017). Viruses, bacteria and protozoa infectious to humans have been found to survive inside leech digestive tracts and there have been documented

cases of transmission of infectious agents. Infections that have been reported after repeated medicinal leech use include syphilis, puerperal fevers, erysipelas, and *Aeromonas hydrophila* (Jeremy Joslin *et al.*, 2017). Leeches may also regurgitate digestive material during feeding, whether from an accidental or medicinal attachment. Beside these leech infestation in cattle's is a major issue in animal health care system. Although Ethiopia has the greatest population of livestock, a lack of food and a range of animal diseases are the main causes of the low productivity in the nation (Desta and Eyob., 2018). Leeches are one type of parasite that significantly reduces livestock production. This can lead to shortage of dairy product for human consumption mostly low milk production expose children's to malnutrition especially for low income rural community. Although traditionally local people use different plant species, *N.tabacum*, *Phytolacca dodecandria*, *millettia ferruginta*, *Schinus molle*, *Buddleja polystachya* and many other plant species in treating leech infestation, not much research has been done to develop a controlling mechanism for this parasite scientifically, thus studies aimed at reducing the issue and providing scientific proof of safety and efficacy are needed.

The possible options that already have to alleviate the problem have a lot of constraints, of these tissue damage by physical removal and a negative impact on the environment and it is irritation to the mucus membrane of the animal from the hirudincidal chemicals. Its expensive cost and uneasy accessibility are also other problems of using chemicals (Tadesse *et al.*, 2010). Therefore it is important to focus on plant based treatment options since they have easy access and more or less negative impact. Despite investigating the anti-parasitic effects of *N.tabacum* on leeches there is still limited and inconsistent information in literature regarding the LD and time required to kill the parasitic leeches, thus further study is required. Hence this study will focus on determining the LD as well as the time required to kill leeches by *N.tabacum* crude extract and give scientific proof about the anti-leech properties of *B. polystachya*.

1.3. Research Questions

- ✓ Is *Nicotina tabacum* effective against adult leech parasite?
- ✓ Is *Buddleja polystachya* effective against adult leeches parasites?
- ✓ What are the LD 50 and LD 90 of this plant extract against leeches?
- ✓ Do this plant extract have a variation in killing leeches?
- ✓ Which solvent extract and plant species give high extract yield?

1.4. Objective of the study

1.4.1. General objective

To study the efficiency of *Buddleja polystachya* and *Nicotina tabacum* crude extract against adult leech parasites under laboratory condition.

1.4.2. Specific objective

- ✓ To measure the amount of extract yield of *N. tabacum* and *B. polysthchya* in chloroform and ethanol solvent extraction.
- ✓ To assess the efficacy of *Buddleja polystachya* leaf crude extract against leech parasite.
- ✓ To assess the efficacy of *Nicotiana tabacum* leaf crude extract against leech parasite.
- ✓ To determine LD 50 and LD 90 of the plants extracts.
- ✓ To compare the efficacy of *Buddleja polystachya* and *Nicotiana tabacum* crude extracts against leech.

1.5. Significant of the study

Herbal approach to manage leech infestation could be a feasible option as it is non-toxic to environment. Despite having large body of ethno botanical reports of medicinal plants in Ethiopia, scarcely evaluated for their anti-leech activity. The present report will contribute to the ethno botanical knowledge database of indigenous communities by about the effectiveness of *B.polystachya* and *N. tabacum* plants so it contribute for the scientific community as a source of knowledge for further investigation. Result of this study can be also useful for livestock owner since provide them which plant they really need to use so help to improve health of the animals and as well as for the improvement of diary product amount. Therefore this study addresses this particular animal and human health problem, using medicinal plants without any detrimental impact on the environment.

2. REVIEW OF LITREATURE

2.1. Biology of Leeches

Morphology and taxonomy of leeches

Segmented worms of the phylum Annelida, order Hirudinea: Euhirudinea, are referred to as real leeches (Lotfy., 2021). Their bodies are flattened and typically have a dark color, such as brown, black, or dark green. Some species have no markings at all, while others have stripes and dots. The smallest leeches can only reach a maximum length of 5 mm, while other larger species can reach lengths of up to 25 cm. On top of their heads, several leech species have one or more pairs of eyes that are visible. Constant number of body segments and body cavity largely filled with muscles and connective tissue (Temesgen *et al.*, 2019).

Though there are a number of issues with the systematics and taxonomy of euhirudins, a review by Lotfy (2021) states that euhirudins are commonly divided into two groups: Rhynchobdellida (proboscis leeches) and Arhynchobdellida (proboscisless leeches). Many arhynchobdellids have jaws, whereas all rhynchobdellids lack jaws. Phrengobdellae (worm-leeches) and Gnathobdellae (jawed leeches) are the two orders into which arhynchobdellids are classified. They can be either hematophagous or predaceous carnivores (Lotfy., 2021). Members of Pharyngobdellae cannot penetrate the tissue of a host and extract blood. As they are carnivores, they consume their food with their large, toothless mouth. Hundreds of tiny, sharp teeth are crammed into the jaws of Gnathobdellae members. They have developed a significant sanguivorous adaptation for engorgement with large volumes of blood. Almost all species of medically important leeches are found in the Gnathobdellae (Tubtimon *et al.*, 2014). These leeches mostly infect animals as their hosts; humans are just incidentally impacted (Lotfy., 2021).

Aquatic leeches that threaten humans are divided into endo-parasites, attacking the mucous membranes and ecto-parasites attacking the skin. Members of the former group have weak jaws adapted for feeding on soft tissues, as the esophagus, conjunctiva, mouth, pharynx, larynx, nose, trachea/bronchi, vagina, bladder, and rectum (Basu *et al.*, 2004; Lotfy., 2021). The Nile leech, or *Limnatis nilotica*, is the most well-known species in this group and is found in southern Europe, North Africa, and the Middle East. *Limnatis nilotica* inhabits lakes and streams and rarely marshes. They usually crawl on the bottom or attach themselves to stones or plants. They swiftly swim in a snake-like fashion towards the prospective animal or human victim; it usually reaches

the mouth or nostril of the victim while drinking and then attaches itself to the mucosa. It normally attacks mammals. On attachment, *L. nilotica* adhere its anterior sucker to the soft parts of the mouth or to the bucco-pharyngeal cavities, leaving its body flopping freely. For periods ranging from a few days to a few weeks, the leech stays inside the host, inflicting mechanical damage and anemia. Breathing and swallowing may become difficult as a result of the mechanical harm causing edema in the invading organs. Leeches can ingest an amount of blood equivalent to ten times of their body weight. In addition to *L. nilotica*, several other species were reported to attack humans (Kumar and Joseph., 2019). This species rarely bites the skin (Harun., 2019).

The jaws or members of the ecto-parasite group are comparatively strong, allowing them to pierce skin and attach to any part of the body's exterior, as indicated in fig 1A. For years of age, this kind of leech was utilized in medicine. The first known use of leeches for local blood depletion dates back to ancient Egypt, however its exact origin is still unknown. In the past few decades, leech therapy for medical purposes has gained acceptance. As indicated in fig 1B and leech are used for drawing the bad blood out by their blood feeding behavior They release the proteins and peptides that thin blood and prevent clotting (Abdualkader *et al.*, 2013).

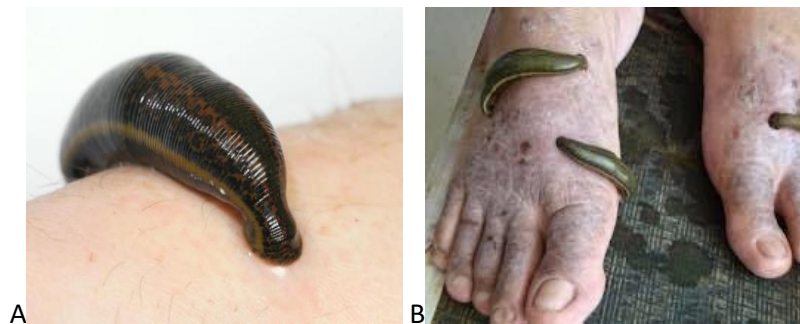


Fig. 1 external attachment of leech to human skin. (Source: Lotfy *et al.*, 2021).

Life cycle

Leech life cycle comprises three stages: egg, juvenile, and adult. Leeches are hermaphrodites that reproduce by reciprocal fertilization, in which two individuals align with their anterior parts facing each other's posterior parts. Each leech then shoots a spermatophore into the other's clitellar region, where the sperm travels to the female parts. Following copulation, a cocoon-containing egg is placed, fertilized internally, and hatches to release juvenile leeches. Until the eggs hatch, leeches cocoons, which can hold up to seven eggs depending on the species, are left

in muddy or dumpy soil, on rocks, vegetation, or other aquatic life. The cocoon serves as a protective capsule that creates the ideal environment for the growth of the embryo. Different leech species have different environmental conditions that affect how fertilized eggs develop in cocoons. For instance, some aquatic leeches attach to hosts like fish, whereas others prefer to fix in plants or submerged items. Before developing into adult leeches, immature leeches feed for a few days to several months, depending on the availability of hosts (Desta and Eyob., 2018). Leeches have a lifespan of two to 27 years (Mulilo *et al.*, 2020).

Feeding and digestion

In just 20 to 40 minutes, leeches can consume blood from their host up to ten times their body weight, demonstrating their remarkable feeding behavior. Nevertheless, they separate from the animal after consuming a blood meal and can remain without consuming any more blood for a period of 12 to 18 months (Mulilo *et al.*, 2020). The mouth is a tri radiate opening located in the anterior sucker's center. Two of the jaws are ventro-lateral, and the third is dorso-median. The papillae on the jaws contain the salivary gland aperture. The unicellular salivary gland that secretes hirudin, which stops host blood from clotting during feeding, is located on the outside edge of the pharynx, which stretches from the fifth to the eighth segment. The blood that is taken is held in the crop and gradually enters the stomach and intestine for digestion, taking a considerable amount of time.

The crop, which has 10 chambers altogether and one chamber in each of the nine to eighteen segments, is the biggest chamber in the alimentary canal. The crop leads to the stomach, which has transverse folds formed in its walls internally. The intestine is the next chamber. It is a thin, straight tube that is found in segments 20 and 22. At its posterior end, it narrows to become the rectum, which opens by the anus on the dorsal side of the 26th segment.

2.2 Medical importance of Leeches

2.2.1 Leech as a parasite

Pathology, sign and symptom

Leech saliva contains a wide range of compounds, including hyaluronidase, hirudin (a potent antithrombin), calin (a platelet aggregation inhibitor) and histamine-like vasodilator. Hirudin is an anticoagulant that takes hours to wear off and stops thrombin from converting fibrinogen into fibrin clots (Conley *et al.*, 2023). These results in a massive blood flow and the bleeding may last

for a while after the leech has dropped off, occasionally leading to anemia. Blood or bloody froth may often be seen exuding from the mouth or nostrils of the animals. In severe situations, the mouth is held open and the neck is stretched due to dyspnea. Edematous edema might be observed in the inter-mandibular and parotid spaces. They might inflame and traumatize the pharynx, which could result in subsequent microbiological complications and prevent swallowing. Asphyxia can potentially result in death, which can happen quickly due to glottis edema (Tadesse *et al.*, 2010).

Diagnosis of leech infestation

The diagnosis is typically made based on clinical symptom observation and parasite presence examination during the cow's rest period following or prior to grazing, when it is observed if the animals drool blood-stained saliva. Mucosal membranes in the mouth, nose, and nostrils are common locations of preference for aquatic leeches (Desta and Eyob, 2018). According to Taggesu (2018), dyspnea, neck extension with mouth held open, edematous areas in the parotid and inter mandibular regions and bloody coughing are the most prevalent clinical symptoms seen in infected humans and animals.

2.3. Economic impact of Leech infestation

Animal and human consequences from leech infestation are becoming a global problem. These annelids excrete which causes blood loss and wounds, using their particularly designed mouths that allow them to stick to hosts and draw blood from them (Ingtip *et al.*, 2016). One of the major health issues that can result from a leech bite is irritation, discomfort, site-specific complication, blood loss that can cause severe anemia and even death. Leeches are known to infect any hollow organ system in humans that has a natural point of entrance. The oral cavity, respiratory system, and external body surface of animals (Sarmah *et al.*, 2017). A developing country's socioeconomic losses from leech infestation (hirudiniasis) are indeterminate and result in cattle's poor health

According to reports, leech infestation is widespread in rural areas of Africa and Asia. It is particularly endemic during the dry or hot season when there are water pools for cattle to drink. Any domesticated animal can contract leech disease. In Ethiopia, leech prevalence increases throughout the dry season, which runs from December to early June. According to Taggesu (2018), the increase in leech prevalence and intensity in domestic animals during the dry months

is caused by a proportionately higher number of leeches in the water during the summer or hot months, as well as a decrease in water volume and flow rate (Taggesu ., 2018).

Not only has the prevalence of leech been documented in domestic animals but also in people. Human leech infestations have been documented in Tanzania, Kenya and Ethiopia in Eastern Africa. Humans are susceptible to leech contamination when they consume contaminated water. In rural areas of underdeveloped nations, leech is particularly widespread and can have a serious negative influence on children's health (Taggesu ., 2018).

2.4. Risk factors in Leech exposure

The extreme annelid is the leech. Extremes in temperature, wetness, salinity, pressure, light, and pollution are just a few of the harsh conditions that leech live in (Phillips *et al.*, 2020). However, the majority of leech species are normally found in protected, shallow waterways, hidden beneath stones, logs, and other detritus or amid aquatic vegetation. The disturbance of the water near docks and bathing places attracts them. Animals that consume contaminated water from leeches become infested and humans contract the disease by drinking, bathing in, swimming in, or coming into contact with polluted water (Temesgen *et al.*, 2019). On hot summer days leeches are most active. They burrow mud in the winter, just below the frost line. There are several factors affecting the distribution of leeches, the availability of suitable hosts and availability of food organisms is the major factor. In addition, the physical and chemical properties of the water substrate, water depth, water current, the size and type of the water body, water hardness, pH, and temperature, the minimum amount of dissolved oxygen in the water, siltation, turbidity, and salinity are the most significant factors influencing leech distribution (Kazanci *et al.*, 2015). Large lakes with dense stands of various macrophytes and with diverse types of bottom substrate seem to be especially rich habitats in terms of taxonomic diversity (Koperski., 2010). In contrast to rivers and ponds, lakes are therefore expected to have much greater levels of leech species richness; however, slow-moving water with a dense bottom substrate is also expected to have a high leech population. According to various researches, leech species can survive in a variety of trophic levels in lakes and saprophytic levels in streams, but they typically favor polluted areas (Kazanci *et al.*, 2015).

2.5. Prevention and Control of leech infestation

2.5.1. Chemical Method

It is feasible to treat contaminated water with commercial chemicals like copper sulfate, niclosamide, and N-trityl-morpholine and to give anthelmintic medications like levamisol and ivermectin, but these treatments are costly and hazardous to organisms that are not their intended targets (Desta and Eyob., 2018; Tagesu., 2018)

Endocrine-disrupting chemicals, or EDCs, can result in malformed leech embryos, aberrant leech progeny and problems with leech reproduction. Moreover, EDCs have the ability to sterilize leech cocoons and stop leeches from laying eggs. These things, however, can also harm other aquatic wildlife (Saglam., 2018).

2.5.2 Biological and environmental Method

Ducks can be used to manage leech populations, however too many ducks can lead to other, more significant issues. Traditional leech-repelling plants include *Nicotinum* plant, *Alluvium sativum*, *Phytolacca dodecandra*, and lighted cigarettes applied to sick animals. Leeches require a hide and an attachment, therefore maintaining clean lakes and ponds where animals drink water will also help reduce their prevalence (Tagesu., 2018).

The best way to prevent leech infestation in humans and domesticated animals is to supply safe and clean drinking water, especially in rural areas. It is advised that residents avoid leech-infested locations and use clean, filtered, or sieved water for human and animal consumption, especially in endemic areas. Swimming in deeper water will reduce the likelihood of a leech attaching itself to a swimmer because leeches like the shallow areas of lakes. Reaching the deeper water can be accomplished using a boat or float. By keeping cattle away from the contaminated water throughout the spring and summer breeding seasons, leech infestation can be avoided.

Treatment option

Applying insecticidal pesticides to keep water sources free of leeches or treating infected animals with chemicals or human removal of the parasites are two potential solutions to the problem of leech infestation. Even if using chemical treatment, such chloroform water, to get the desired effects is an efficient method, it is expensive. Commercial substances like niclosamide, N-trityl-

morpholine, and copper sulfate can also be used to disinfect contaminated water; however, this approach is expensive and hazardous to non-target organisms (Tadesse *et al.*, 2010). The other option utilized by most farmers to treat animal infested by leech is the use of traditional medicine particularly plants.

2.6. Medicinal plant used for treatment of leech infestation

Different ethno botanical study shows there are a number of plant species that are used in traditional medicine against leech. Of these *Guizotia scabra* (leaf part), *Celtis Africana*(leaf), *Nicotiana tabacum* (leaf part), *Phytolacca dodecandria*(seed part), *millettia ferruginta*(seed part), *Schinus molle* (leaf part), *Buddleja polystachya* (Leaf) (Aberha., 2015; Asaye *et al.*, 2022) *Maesa lanceolata* Forssk, *Capsicum annum*(seed), *Nigella sativa*, *Cyphostemma adenocaula*, *Ranunculus multifidus* (Asmamaw *et al.*, 2018; Temesgen *et al.*, 2019; Ijigu *et al.*, 2021; Megersa *et al.*, 2021). And from these species, *Guizotia scabra*, *Nicotiana tabacum*, *Phytolacca dodecandria*, *millettia ferruginta*, *Schinus molle*, (Temesgen *et al.*, 2019), *Achillea millefolium* L. (Bahmani *et al.*, 2014) are studied under laboratory condition to determine their efficacy but further studies are required for *N.tabacum* at different concentration and solvent since having a wide range cultural acceptability and even *B. polystachya* is extracted to investigate its potential against different human aliments there is no experimental evidence for leech infestation.

2.6.1. *Nicotina tabacum*

Taxonomy and distribution

Nicotiana tabacum, commonly known as tobacco is an herbaceous annual or perennial plant in the family Solanaceae grown for its leaves. As indicated in Fig 2 the tobacco plant has a thick, hairy stem and large, simple leaves which are oval in shape. The tobacco plant produces white, pink or red flowers which grow in large clusters (Leal *et al.*, 2023).

Tobacco is a plant in the genus *Nicotiana* (Ogello *et al.*, 2016). The genus includes more than 70 species with variable biological activities and most of them are indigenous to America. The plant known as *N. tabacum*, which is currently used for tobacco production on a commercial scale, is believed to have originated in South America. From the base of the plant to the top, the lamina has a higher nicotine level than the midrib. The amount of nicotine in tobacco plants is also greatly influenced by their age or ripening. Tobacco plants that are more mature turn yellow

more quickly, have a greater yield and quality index with a higher concentration of sugar and alkaloids (nicotine) and a lower percentage of total nitrogen (Zebasil and Chandravanshi., 2015). There are several populations in various parts of Ethiopia who use local tobacco in various ways, including pipe smoking, chewing, and snuffing (Gaya). This is evidence that tobacco has been present in Ethiopia for a very long time. Of the several varieties of tobacco, Virginia, Burley and Oriental are the most widely grown and typical in Ethiopia. About 74% of the production comes from Virginia, with 22% coming oriental, and 4% from barely (Zebasil and Chandravanshi., 2015).



Fig. 2 *N. tabacum*. (Source: Leal *et al.*, 2023).

Medicinal value

The evidence that tobacco causes cardiovascular disease and lung disease took several hundred years to emerge. In the 15th century, when the use of *Nicotiana* by the indigenous populations in the New World was first observed by Columbus and the plant was brought to Europe, all herbs were considered to have potential therapeutic properties and this new one was used to treat a wide range of conditions (Charlton., 2004). Because of its potential to treat a wide range of illnesses, including dermatitis, constipation, urinary tract obstruction, abdominal discomfort, and dental pain, tobacco has been identified as one of the plants that are most frequently studied and a significant economic crop (Peter *et al.*, 2019). There doesn't seem to be any documentation regarding tobacco's medical use prior to the Civil War. Based on a review of relevant articles, medical professionals have long utilized this plant as part of conventional therapy. According to

Jani and Binorkar (2012), traditional tobacco plants have been used by Native American tribes for thousands of years for spiritual, ceremonial, and medical purposes throughout North America.

N. tabacum is very familiar plant in different countries culture in different way in using it for the treatment of various diseases according to a review by Jani, and Binorkar., 2012,. In Brazil, the leaf juice is consumed orally to cause narcosis and vomiting. Colombia: Crushed leaves are applied as a poultice to boils and infected wounds; they can also be used as a hair treatment to prevent balding. Cuba: Dysmenorrhea is treated by oral administration of the leaf extract. East Africa: To kill worms in wounds, a paste made from dried leaves of *N. tabacum* and *Securinega virosa* is applied externally. Ecuador: Leaf juice is used to cure respiratory conditions, cold, and snake bites. Fiji: Seed is used orally for rheumatism and to alleviate hoarseness; fresh root is applied topically as drops for bloodshot eyes and other issues; fresh root is eaten orally for asthma and indigestion (Jani and Binorkar., 2012).

The leaves in Guatemala used by adults, they apply the leaves topically for myiasis, headaches and wounds; for ringworms, fungal diseases of the skin, wounds, ulcers, bruises, sores, mouth lesions, stomatitis, and mucosa, an external extract of the dried leaf is applied; for renal problems, the leaf is eaten orally. Haiti: Oral decoction of dried leaf is used to treat pneumonia and bronchitis. Hong Kong: A remedy that is given to wounds to hasten their healing process is made by mashing fresh leaves with vegetable oil. There are many locations in China where this behavior is evident. Iran: Crushed leaves are used to make ointments for baldness, dermatitis, infectious ulcerations, and as a pediculicide. The dried leaf infusion is applied externally as an insect repellent. In the United States, *N. tabacum* extract is taken orally to relieve fatigue, prevent illness, and calm anxiety. Tanzania: *N. tabacum* leaves are inserted into the vagina to induce labor and Zimbabwe: The plant's leaves or roots are infused and consumed orally to treat respiratory conditions like asthma. Additionally, leaves and roots are applied as an ointment to wounds and warts (Jani and Binorkar., 2012).

2.6.2. *Buddleja polystachya*

Taxonomy and distribution

B. polystachya is a multi-branched shrub, it reaches a height of less than 5 meters, yet under ideal circumstances it can occasionally reach 12 meters (Mohammed *et al.*, 2016; Abiey *et al.*, 2021). This tree is native to the semi-arid highlands that border the Red Sea, Yemen, Somalia, Saudi Arabia, Eritrea, and Ethiopia. It belongs to family *Buddlejaceae* and was named in honor of the Reverend Adam Buddle. Eight genera make up the *Buddlejaceae* family; most of them are found in tropical climates, however couples are found in warm temperate zones. There are more than a hundred species of flowering plant in it. They are found in Southern Asia, East Africa and North America in both tropical and warm temperate climates, over a wide range of geographic locations. Several *Buddleja* species are reportedly utilized in traditional medicine (Ati *et al.*, 2015).

In Ethiopia, *Buddleja polystachya* is a medicinal plant used for the treatment of epilepsy in traditional medicine, where the dried leaves are crushed, mixed with local alcoholic beverage and taken orally (Tewodros *et al.*, 2020). In addition the dried or fresh leaves of the plant were used for the treatment of malaria, the leave powdered with Butter smeared on the skin topically for the treatment of wounds/diseases of the skin. Besides, the juice obtained by soaking with water used for eye infection applied by dropping at night time for Dingetegna applied in nasal and the infusion of the leaves is used topically for the treatment of burns, external and internal ulcers. It also used traditionally in the form of poultice or lotions to promote wound healing, able to stimulate to stimulate the growth of dermal fibroblasts and reduce inflammation (Berhanemeskel *et al.*, 2014).

It is locally known as known as “Anfare” in Amharic, Adaddoo in Afan Oromo (Megersa *et al.*, 2021). Madera in Afargna, Madere in Tigrigna, Metere in Eritrea and Kanfara (Dawuro), is a multi-branched shrub that grows to < 5 m, but can occasionally reach 12 m in favorable conditions. Its bark has deep grooves and is either grey or reddish-brown in color. The leaves are light grey-green on top and measure 15 centimeters in length, narrowness, and a pointy tip, Fig 3. Bright orange flowers surround a 20 centimeter long spike (Bussmann *et al.*, 2020). It is native to the semi-arid highlands that border the Red Sea, Yemen, Somalia, Saudi Arabia, Ethiopia and Eritrea (Berhanemeskel *et al.*, 2014). Usually grows at elevations of 2,200 to 3,600 meters above

sea level in secondary scrub of semi-arid upper highland forest and at forest borders in dry, moist, and wet climatic zone (Tewodros *et al.*, 2020).



Fig. 3 *B.polystachya*, leave and flower part. (Source: Busmann *et al.*, 2020).

Medicinal value

Many species of Buddleja have been recorded to have been utilized in folk medicine throughout the world for their roots, leaves and flowers. The use of Buddleia roots, leaves, and flowers in traditional medicine has been documented in numerous parts of the world. It is used as an antiprotozoal, for liver diseases, dysentery, eye disease, as diuretic, sedative, analgesic, wound healing, anti-inflammatory, antimicrobial, hypoglycemic, and antioxidant agent (Abiey *et al.*, 2021). In northwest Ethiopia, for instance, the powdered leaf of *B. polystachya* mixed with Tej was given orally to expel the intestinal parasite. Additionally, in north and south Ethiopia fresh leaves of this plant is used for treatments of eye disease and malaria. There are also reports that the plant used for treatments of headache and migraine (Tewodros *et al.*, 2020). Moreover, several experimental studies have been conducted to prove the traditional medicinal uses of Buddleja species. For instance, experimental studies revealed that *B. globosa*, *B. salviifolia* and *B. polystachya* have anti-inflammatory, analgesic and antioxidant properties (Backhouse *et al.*, 2007). *B.polystachya* also exhibited antimicrobial, hypoglycemic, anti-cancer, antimalarial antidiarrheal activities and for the treatment of many skin disorders in various experimental studies (Abiey *et al.*, 2021).

3. MATERIALS AND METHOD

3.1. Study design

It is laboratory based study that was conducted at Dilla University Biology department laboratory from April to August 2023 by collecting the required samples from their habitat. The plant sample or leaves of the plants were collected from Gedeo Zone Repe Village and Dilla town. The leaf of *B. polystachya* were solely collected from Repe Village which is found in south east of the capital city of Gedeo Zone, Dilla town, at 35km away and at altitude 2400m above sea level, which is expected to be the favorable environment for *B. polystachya* growth. The trees where the leaf samples were collected were around 10m. However *N. tabacum* was collected from both Dilla town and Repe woreda since this plant can grow both in high and low area but the researcher takes from both site is just to get enough amount of sample since *N. tabacum* is cultivated for market purpose while it dries make it difficult to take enough amount fresh leaf from a single farm.

Leeches were collected from Mejege River which is found in Abaya, west Guji Zone at Oromiya Regional State bordering Walem kebele of Dilla town, the capital city of Gedeo Zone in south Ethiopia Regional State. This river is selected because it is more stable and slow moving than those river found in Dilla town, which enable the researcher to find these huge number of parasite at the same habitat.

3.3. Plant materials collection and extract preparation.

Fresh leaves of mature *B. polystachya*, were collected from Repe village, which described above in study design, in April 2023 and *N. tabacum* in June 2022 from both Dilla town and Repe Village. The identity of each plant specimen was confirmed by botanist at Dilla University. The collected plant leave were transported in plastic bags to the laboratory. In the laboratory the samples was washed under running tap water to remove dust particles and then placed on clean magazine on flat table and air dried at room temperature under the shade at for 14 days in case of *B. polystachya* and 21 days in case of *N. tabacum*. Then the dried samples have been grounded to fine powder using electric grinder, sieved by 0.5mm sized mesh and stored in sterile bottle at room temperature until the extraction (Gemechu *et al.*, 2017; Geremew Tafese., 2018; Kim and Chin., 2020).

The extraction was done by maceration technique by dissolving fine plant powders separately in 1:5 powders to solvent ratios of 99% chloroform and 97% ethanol for 48h using orbital shaker at 145rpm. Thereafter, each extract was filtered using Whitman number.1 filter paper and the solvents from extracts were evaporated using Rota vapor at 40⁰C. The resultant extract was transferred in a beaker with a known mass and left in an oven overnight at 40⁰C in order for the solvent to evaporate entirely. The beaker was then measured again, and its final mass was deducted from its initial mass to determine the amount of extract. Then after packed with aluminum foil and stored around 0⁰C until further investigation According to (Abubakar., and Haque., 2020 ; Mohammed *et al.*, 2016) with some modification.

3.5. Collection of leeches

A total of 900 adult leeches were collected. Leeches were collected by hand and using plastic scoop. Woody stick was also used to make disturbance of water. Then transferred to transparent plastic buckets half filled with water of the collected leech habitat and transported to Dilla University laboratory. In laboratory they were housed in well-aerated plastic buckets containing gravel at its bottom 2 inch thickness and their natural fresh water at the room temperature (25⁰C). Water was regularly changed every two days until the experimentation. The leeches' species were identified by zoologist at Dilla University.

3.6. Experimentation of anti-leech assay

Stock solution containing of 10000ppm was prepared by dissolving 1g of extract in 100 ml 3% tween twenty. Next, different concentrations of extract were made in volumes of 100 ml for each concentration. The different concentration prepared from ethanol and chloroform extract of *N. tabacum* were 1, 2, 4, 8 and 16ppm and concentrations prepared from that of *B. polysthycha* 200, 400, 600, 800 and 1000 concentrations were selected based on the result of the range finding test done by the researcher. A positive control made of 0.25% formalin and a negative control made of (aged) dichlorinated water only were also prepared according to Gholami-Ahangaran *et al.*,(2012). Following the preparation of serial dilutions, ten adult leeches were submerged in each test solution and kept exposed for 12 hours. The whole set of experiment was prepared in triplicate. Careful bench side observation was employed for any changes in body condition, irritation paralysis, death signs etc. Notes on room temperature of the laboratory, pH and temperature of test solutions were also taken. After 12 hours of exposure leeches of each

experimental group were picked out of the solutions, washed by gentle flow of tap water in child care manner and kept in pure aged water for the next 12 hours of recovery. Finally the number of dead leeches was recorded. A method by Bahmani *et al.*, 2014 was used with some modification.

3.8. Statistical analysis

Comparison of means between control and experimental groups and among the concentration gradients was analyzed using one-way ANOVA. To determine LD50 and LD 90 P value of less than 0.05 was considered as significant difference.

4. RESULT

The amount of extract obtained from each test plant is presented in table1 and the percentage yields is calculated by dividing weight of extract to dry weight of sample and multiplying it by hundred. The leaves of *B. polystachya* presented the highest yield, a mean value of 9.6% than *N. tabacum* which having 4.55% mean yield and in both of the plant extract the ethanol extract give high amount of yield.

Table1. Percentage Yield of *N. tabacum* and *B. polystachya* extract

Plant type	Extract yield (%)	
	Ethanol	Chloroform
<i>N. tabacum</i>	5.9%	3.2%
<i>B. polystachya</i>	14.4%	4.8%

As shown in table2, the leeches were exposed to various concentrations of *B. polystachya* and *N. tabacum*. Upon encountering the test solution, the leeches exhibited violent reactions and attempted to flee. When compared to the *N. tabacum* solution, the leech-exposed *B. polystachya* were particularly difficult to sustain in the solution, but the researcher had to keep coming up to gently re-immerses them. They are also disturbed in the *N. tabacum* solution. Initially, they moved very erratically and attempted to adhere to the beaker wall in an effort to escape the solution but as they tried to do so, they became paralyzed, unable to move out; produced jell-like foam and eventually their bodies became hardened and died. Those leeches exposed in the higher concentrations of *N. tabacum*, 16 ppm and above, displayed bleeding in their upper part. The researcher confirmed their deaths by gently pressing the leeches, if no response consider as they are died and the time was noted but for the total death count 12h exposure and another 12h recovery time was kept. While there was no leech death in the *B. polystachya* solution, the leeches' movements were extremely erratic and black jail mucus production was noted.

In the positive control, the leeches produced a very thick and high amount of jell substance, showed very vigorous movement and soon died, but their bodies did not harden; instead, they became very soft while they died. Most of them also displayed bleeding. In the negative control, they did not disturbed, did not produce any jell, but even though they did not fast as the test group, they attempted to escape, since no hiding were there at the bottom part However, no deaths were recorded.

Table2. Raw data of the experiment

Plants		<i>N. tabacum</i>					<i>B. polysthachya</i>					
Solvents		TCE		TEE		BCE			BEE			
Leech no		exposed	Die d	Exposed	Died		Exposed	Die d	exposed	Died		
Concentrations	1 ppm	1		0	10	0	200ppm	10	0	10	0	
		2	10	0	10	0		10	0	10	0	
		3	10	0	10	0		3	10	0	10	0
	2 ppm	1	10	0	10	0	400 ppm	1	10	0	10	0
		2	10	0	10	0		2	10	0	10	0
		3	10	0	10	0		3	10	0	10	0
	4 ppm	1	10	1	10	1	600 ppm	1	10	0	10	0
		2	10	1	10	0		2	10	0	10	0
		3	10	0	10	0		3	10	0	10	0
	8 ppm	1	10	9	10	7	800 ppm	1	10	0	10	0
		2	10	9	10	7		2	10	0	10	0
		3	10	10	10	8		3	10	0	10	0
	16 ppm	1	10	10	10	10	1000 ppm	1	10	0	10	0
		2	10	10	10	10		2	10	0	10	0
		3	10	10	10	10		3	10	0	10	0

TCC (Tobacco Chloroform extract), TEE (Tobacco Ethanol extract), BCE (Buddlejia Chloroform Extract), BEE (Buddlejia Ethanol extract).

As indicated in table 3 and table 4 *N. tabacum* showed high lethal effect on leeches which showed 6.67%, 93.3% and 100 % death at 4, 8 and 16ppm respectively in chloroform extract test solution and 3.33%, 73.33% and 100% death at 4, 8 and 16ppm respectively in ethanol extract test solution with LD 50 of 3.447 and LT of 3.895 in chloroform extract and LD 50 of 3.68 and LT of 4.046 ethanol extract solution but *B. polysthachya* had not showed any leech death.

Table3. Mean mortality rate of leeches

Concentration	Mean mortality rate (M+SE)	
	TCE	TEE
Negative control	0.00+0.00 ^A	0.00+0.00 ^A
1ppm	0.00+0.00 ^A	0.00+0.00 ^A
2ppm	0.00+0.00 ^A	0.00+0.00 ^A
4ppm	6.7+0.33 ^A	3.3+0.33 ^A
8ppm	93.3+0.33 ^B	73.3+0.33 ^B
16ppm	100+0.00 ^B	100+0.00 ^C
Positive control	100+0.00 ^B	100+0.00 ^C

The same alphabet indicates insignificant difference within each solvent. Mean mortality rate is the average of triplicate mean per 100% plus or minus the average standard error of the triplicate. M+SE = mean mortality of standard error

TCE= Tobacco chloroform extract

TEE= Tobacco ethanol extract

Table 4. Lethal dosage of extract concentration

Confidence limit at 95%							
Extracts	L50	LB	UB	L90	LB	UB	X ² (Df=3)
Ethanol	3.684	3.473	3.887	4.396	4.134	4.866	0.987
Chloroform	3.447	3.246	3.647	4.095	3.842	4.563	2.993

L90 and L50= lethal dosage of the extract required to kill 50% and 90% of the experimental parasite respectively.

UP= upper boundary, LB= lower boundary, X²= chi-square, Df= degree of freedom

Table5. Lethal time of leeches in the different test solution *N. tabacum* chloroform extract in hour per minute

concentration	TTDOICENT at subset for alpha = 0.05						
	N	1	2	3	4	5	6
400ppm	3	0:17					
200ppm	3	0:22	0:22				
positive control	3		0:30				
100ppm	3		0:31				
50ppm	3			0:44			
24ppml	3				1:45		
16ppm	3					2:07	
8ppm	3						3:27
Sig.		.773	.51	1.000	1.000	1.000	1.000

TTDOICENT (time of total death observed in chloroform extract of *N.tabacum*), N (number of treatment group). The same column indicates insignificant difference between the different treatments and or concentration.

Table6. Lethal time of leeches in the different test solution *N. tabacum* ethanol extract in hour per minute

concentration	TTDOIEENT Subset for alpha = 0.05						
	N	1	2	3	4	5	6
positive control	3	0:30					
100ppm	3		0:35				
50ppm	3			0:55			
24ppm	3				2:11		
16ppm	3					2:37	
8ppm	3						4:10
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

TTDOIEENT (time of total death observed in ethanol extract of *N.tabacum*), N (number of treatment group). The same column indicates insignificant difference between the different treatments and or concentration

Table7. PH and temperature value of the treatment solution

Sample	PH	Temperature
1ppm	7.42	23.1
2ppm	7.2	22.6
4ppm	7.00	22.0
8ppm	7.04	22.1
16ppm	7.07	22.7

Taxonomy of the experimental leeches

The leech species that were used are identified by zoologist and all are belongs to the species *Limnatis nilotica*. It is the most common species of leech in mhrab abaya district (Seyoum *et al.*, 2023). And is one of the 14 leech species found in Ethiopia (Sciacchitano., 1967). They are also mostly found in the Middle East, North Africa, and southern Europe (Arfuso *et al.*, 2019)

Limnatis nilotica inhabits lakes and streams, and rarely marshes. They usually crawl on the bottom or attach themselves to stones or plants. They swiftly swim in a snake-like fashion towards the prospective animal or human victim. This species rarely bites the skin; it usually reaches the mouth or nostrils of the victim while drinking, and then attaches itself to the mucosa. On attachment, *L. nilotica* adheres its anterior sucker to the soft parts of the mouth or to the bucco-pharyngeal cavities, leaving its body flopping freely (Lotfy *et al.*, 2021). In heavy infections, especially in animal hosts, leeches attach themselves to the gums, under the tongue or the upper parts of the respiratory tracts as well. In the human body, usually, a single leech settles in the pharynx or in the upper part of the respiratory tract. The leech remains in the host for periods varying from several days to several weeks causing mechanical injury and anemia The mechanical injury may result in swelling of the invaded organs, interfering with swallowing or breathing (Harun., 2019).

5. DISCUSSION

Using behavioral and survival changes as indicators, this study examined the anti-parasitic effects of *B.polysthachya* and *N.tabacum* against freshwater leeches at varying doses and extraction solvents. The leeches showed signs of irritation vigorously riggling, coiling and stretching. They constantly tried to escape out of the solutions and also produced mucus like secretions. The leeches may have used the foamy gel material they exuded as a defense against the stinging effects of nicotine (Ogello *et al.*, 2016).

This test showed, of the two plant examined, *N. tabacum* exhibited lethal effect in very low concentration, 4ppm and good lethal effect in 8ppm showing 93.3% and 73.3% death in the chloroform and ethanol extract respectively and both of solvent extract shows 100% mortality in 16ppm and above. However the extract from *B. polysthachya* leaves tested in the range 200ppm to 1000ppm didn't kill any leech. Such high concentration for test is selected based on the preliminary test, since no leech death was observed in the range 25-400 ppm. But there was no limitation in the extraction or test procedure since same methodology and procedure was done for both *N. tabacum* and *B. polysthachya*, the only reason that can be is based on the nature of the plant, it may require different extraction solvent, methodology or it may not have enough amount of compound needed to kill leeches due to different edaphic factor. Along with this, it might only have a repellent effect because, at high concentrations of *B. polysthachya*, the leeches were moving vigorously and producing a frothy, gaily substance similar to that of *N. tabacum*, and the positive control, they were attempting to counteract the effects of the drug/extract solution/ during the experiment. Given that the ethno botanical data does not specify how the plant material is used to treat leeches infestation and having information that there are studies on repellent activities of some plants for leeches (Ingtip *et al.*, 2016; Pelini., 2016), the researcher speculated that *B. polysthachya* may be used by the locals to simply drive leeches away without killing them by causing them to separate from the attachment site; however, controlled repellent experiments are necessary. Even if toxic plant can have a replant impact in those studies but all replant didn't show toxic effect. According to a study done by Deletre *et al.*, 2013 repellency, irritancy and toxicity, involve different physiological mechanisms

Other several studies have been done on the effects of other medicinal plants on leeches such as the methanol extract of Grape at doses of 300 and 600 mg at a mean time 260 ± 63 min

(extremely negative impact) caused the death of Leeches (Gholami-Ahangaran *et.al* 2012), Olive methanol extract at a dose of 600 mg, with a mean time of 210 ± 24.1 minutes, caused paralysis and death of *Limnatis nilotica* (Bahmani *et al.*, 2013), yarrow extract at a dose of 600 mg, with a mean time of 87 minutes, killed the leeches (Mohamed *et al.* , 2014).

This particular study demonstrated a potent leech killing property of *N.tabacum*. The LD 50 and LD 90 of its ethanol extract are 3.68 and 4.40 ppm and that of chloroform extract are 3.45 and 4.10 ppm. Few researchers have investigated the anti-leech efficacy of *N. tabacum*, in different settings. In Behaman *et al* (2012) an LD 50 of 13×10^4 ppm within 30minute exposure was reported. Ogallo reported LD 50 of about 15g/L-1 and the lethal time (LT50) of 73 hours. On the other hand, Temesgen and colleagues tested around 12 medicinal plant species and reported that *N. tabacum* showed greater efficacy which killed the leech within twenty one hours and ten minutes (21:10) and four minutes (00:04) at a concentration of 100 ppm and 1000 ppm, respectively (Temesgen *et al.*,2019). Beside these Latifi *et al*, 2022 reported that tobacco ethanol extract shows a promising effect in controlling of protoscolices at a concentration of 100 mg/ml, led to the death of 91.19 % of protoscolices following 10 minutes of contact and 100% elimination of protoscolices at 20-60minute. And also *N. tabacum* showed good *in vitro* adult *Haemonchus contortus* killing effect within a range 125mg/ml-500mg/ml in study by Mumed *et al.*, 2022.

As compared to these previous findings, the current study revealed that *N.tabacum* is a more efficacious plant than expected in killing leeches with LC90 of less than 5 ppm and at 100ppm; it causes 100% mortality in 30-50 minutes. This concentration and time variation may be due to extraction technique variation since most of them done the experiment just taking the powder and immersing it in water this misleads the amount of extract (the secondary metabolites) in the powder but the current finding is based on the extract only so it is expected to require less concentration for the anti-leech assay. The other thing that can be considered is the influence of environmental variation.

The anti-leech effect of *N. tabacum* is probably due to presence of nicotine corroborating the finding in Bahmani *et al.*, 2014, that reported nicotine at 5, 10 and 20 mg doses had extremely strong effect. Nicotine at dose of 5 mg with mean of 2.44 ± 0.52 min and effect intensity of 4 + was determined as the best treatment for leech infestation (Bahamani *et al.*, 2014). Differences in

quantitative composition of nicotine and other secondary metabolites may exist among different tobacco varieties and landraces and hence the high potency in this particular test might be attributed to this variation.

It appears that anti-leech effects of *N. tabacum* is likely as a result of a cumulative effect of several active ingredients the major one is the presence the alkaloid called nicotine in the plant that interacts with nicotinic acetylcholine receptors leading to muscle weakness (Narahashi *et al.* 2000). Multicellular parasites have a nervous system with a number of important neurotransmitter/neuron receptor systems, such as acetylcholine (ACh) and ACh-receptors (AChR). If the ACh-receptors, which control muscular activity, are inhibited or overstimulated, muscular paralysis can occur. This also happens if sodium and potassium channels are either blocked or stimulated, because ion signaling is essential in neuronal activity (Wink., 2012) Muscular arrest can lead to direct death or in case of worms which are attached to the intestinal gut walls, they no longer can stick to the walls and are easily removed with the faces after laxative treatment. The problem is to find a dose which affects the parasite but which is still not toxic for the patients (Wink., 2012). Both vertebrate and invertebrate species have nicotinic receptors with many similar structural and functional properties, but they exhibit some different physiological and pharmacological characterization. The affinity of nicotine to vertebrate nicotinic receptors is lower than insect nicotinic receptors (Ballivet *et al.* 1996).

In regarding to the efficacy of *B. polystachya* further research is required in different way like repellent test or in different extraction technique, solvent and different water medium for solution preparation since researchers reported that different types of water medium affect the efficacy of plant solutions in killing leech parasites. In this regard Tagesu., 2003, reported that the efficacy of *P. dodecandra* varied based on the type of water medium used. Accordingly, the highest efficacy was recorded in solutions prepared from distilled water, medium efficacy from tape water and lowest efficacy from lake water. In the current study, tape water was used which may have medium efficacy according the report of Tagesu *et al.*, 2003.

Efficiency and amount of extract yield is strongly affected by the extraction method, temperature, extraction time, the composition of phytochemicals and the solvent used (Truong *et al.*, 2019). According to the results of these studies, under the same extraction conditions, solvent is recognized as one of the most important parameters for amount of extract yield. The present

study used solvents ethanol and chloroform to extract bioactive compounds from *N. tabacum* and *B. polystachya*. Results showed that different solvents resulted in various extraction yields. This is because differences in the polarity of the extraction solvents could cause a wide variation in the level of bioactive compounds in the extract. A higher extraction yield was observed in ethanol extract compared to chloroform indicating that the extraction efficiency favors to the highly polar solvents. This result is consistent with the extraction yield of (Abiey *et al.*, 2021; Prommaban *et al.*, 2022), so can confirm that as polarity increase yield also yield in the cause of these plants. This could be because the plant material contains high levels of polar compounds that are soluble in solvents with high polarity (ethanol). In order to better understand the solvents effect on extraction yield, further analysis need to be performed to measure the content of bioactive compounds in the extract. But in cause of anti-leech activity the chloroform extract showed a little beet better efficacy than the ethanol extract in *N. tabacum* plant and no difference were observed in cause of *B. polystachya*.

In general this study confirms the knowledge of traditional medicine in anti-parasitic effects of *N. tabacum*, it can be stated that *N. tabacum* is able to kill leeches and can be used as a natural anti-leech medicinal product and further investigation is required for the efficacy of *B. polystachya* since it has the important bioactive compound in and showed a promising antibacterial and parasitical activity in prewise in vitro investigations and have a wide range of ethno botanical data about the traditional use of it for leech infestation at different region of the country.

6. CONCLUSION

Serious consequences such as inflammation, severe bleeding and hematemesis, which can lead to anemia and even death in severe cases, are caused by leech infestation (Hirudiniasis). Despite the fact that parasites can be managed with commercial synthetic hirudincidal chemicals, medicinal plants are preferred because accessibility and affordability for poor farmers, less hazardous and cause less negative effect. Therefore this study done to prove scientifically the efficacy of the medicinal plants, *N. tabacum* and *B. polysthycha*, against adult leeches

The results of the current study indicated that of the two plants tested *N. tabacum* is found to be the efficacy plant in killing leeches which resulted hundred percent deaths in 16ppm concentration in both chloroform and ethanol solvent extraction and with LD50 value of less than 5. In regarding to the effectiveness of *B. polysthachya* it doesn't show any leech death in both of solvents extract. Therefore it's possible that the diversity in effectiveness is quantitative rather than qualitative and more research is necessary to determine this. Ultimately, these investigations validate the traditional use of *N. tabacum* against leeches, but not *B. polysthachya*.

7. RECOMMENDATION

- ✓ Even though toxicity test for *N. tabacum* was not performed can be used for treating leech infestation since it is effective in minimum of dosage and is less impact than chemical treatment because it is natural product.
- ✓ Further investigation is needed on the efficacy of *B. polysthachya* leaf using different methodology for conforming about its anti-leech properties.
- ✓ Further investigation is needed on different parts of the plants
- ✓ Awareness creation to the community is required for further protection and conservation of this plant species.
- ✓ Toxicity test is needed to assure the use *N.tabacum* as anti-leech compound for internal infestation.

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