ETHNOBOTANICAL DOCUMENTATION, PHYTOCHEMISTRY, AND CYTOTOXICITY OF ANTI-SNAKEBITE ENVENOMATION PLANTS OF MWINGI WEST SUB- COUNTY, KENYA

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ABSTRACT

Snakebite envenomation (SBE) is a life-threatening Public health problem affecting over 2.7 million persons annually globally, and the greatest burden lies in the developing world. Despite the successful management of SBE by antivenom therapy in conventional medicine, it is of low efficacy due to the diverse venom composition across snake types, limiting its usefulness. As a result, inhabitants of the Sub-Sahara region, where Snakebite envenomation incidence is high, utilise medicinal plants as an alternative to manage SBE and associated complications. However, there is scanty of ethnomedical and empirical information available for many medicinal plants with longstanding usage in traditional medicine, especially those used against SBE. In Kenya, Kitui C5ounty records the highest incidence rates of SBE, attributable to the agricultural activities of inhabitants, their housing type, and semi-arid climate. Due to the unaivailability, unaffordability, and inaccessibility of conventional antivenom therapy for Snakebite envenomation for victims, the locals utilize medicinal plants as a viable alternative to manage Snakebite envenomation. However, there is no sufficient ethnomedical documentation of these plants, which can foster empirical investigations, and heritage. Therefore, the objective of the current study was to investigate and document plants used to manage snakebite in Migwani Ward, Mwingi West Sub County, Kitui County, and determine their qualitative phytochemical composition and their cytotoxicity. Ethnobotanical survey to document medicinal plants used to manage SBE in the study area was done between January and February 2021. Ethnobotanical data was collected from 45 purposefully selected respondents from the ward using semi-structured questionnaires, field walks, and oral interviews. Voucher specimens of cited plants were collected with the help of respondents, identified with the help of botanists, and deposited at the East Africa herbaria of the National Museum of Kenya. Using Relative Frequency of Citation criteria. The four

medicinal plants which were selected, includes *Entada leptostachya* Harms, *Senna sengueana* (Delile) Lock, *Securidaca longipendunculata,* and *Strychnos henningsii* Gilg were selected, extracted using water, methanol, and dichloromethane, and analysed for qualitative phytochemical composition and cytotoxicity on brine shrimp nauplii. In this study, 14 medicinal plants which are used to manage Snakebite envenomation were documented. Four plant materials with the highest Relative Frequency of Citation, included *Entada leptostachya* Harms-stem bark (0.58), *Senna singuenna*-Roots (0.53), *Securidaca longipendunculata*-Roots (0.36), *Strychnos henningsii*-Stem bark (0.46) were selected for phytochemical analysis and cytotoxicity assay. Qualitative phytochemistry revealed antiSnakebite envenomation associated phytochemicals such as alkaloids, saponins, tannins, phenols, and flavonoids in the aqueous and methanolic extracts of selected plants. However, the tested phytochemicals were not detected in dichloromethane extracts of all the extracts. The effects of the documented plants could be attributable to these phytochemicals. Furthermore, the aqueous and methanolic extracts of *Strychnos henningsii, Entada leptostachya,* and *Senna singuenna* had LC_{50}>1000µg/ml and were non-cytotoxic. However, *Securidaca longipendunculata* had an LC_{50}<1000µg/ml, which was considered slightly cytotoxic. Further empirical investigations to determine the anti-SBE efficacy of *Strychnos henningsii, Entada leptostachya, Senna singuenna,* and *Securidaca longipendunculata* should be conducted to validate their ethnomedicinal claims. Also, the bioactive phytochemicals of the studied plant extracts, their mode(s) of bioactivity and their safety should be investigated further.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Snakebite is a neglected Public Health problem affecting over 2.7 million individuals annually, especially in those living in the most remote, underdeveloped, and marginalised tropical and subtropical regions of the world (World Health Organization (WHO), 2021). Snakebite envenoming/envenomation (SBE) accounts for over 138,000 deaths while leaving over 400,000 survivors with long-term psychological and physical disabilities (Williams *et al*., 2019). Just like other poverty-associated diseases, there is insufficient public health policy frameworks, strategies, and investment in the affected regions, to sustainably reduce the medical and societal strain posed by SBE due to lack of political goodwill and the demographic nature of the affected communities (Williams, 2015; Williams *et al*., 2011, 2019).

Subcutaneous or intramuscular injection of venom, via a Snakebite, into the victim's body, elicits local and systemic toxic effects with profound sequela (Benjamin *et al*., 2020; Santhosh *et al*., 2013). Local effects associated with SBE include haemorrhage, oedema, myonecrosis, and extracellular matrix (ECM) degradation. On the other hand, neurotoxicity, myotoxicity, cardiotoxicity, and hemotoxic syndrome are associated with systemic SBE sequelae (Benjamin *et al*., 2020).

Currently, antivenom therapy is the standard and arguably reliable strategy for averting the adverse effects caused by snake venom (Bhaumik *et al*., 2020; WHO/Regional Office for South-East Asia, 2016). However, despite the benefits of antivenom therapy, it evokes immediate hypersensitivity reactions, among other adverse effects, exhibits limited efficacy against local tissue damage, and suffers a stability deficit (Alangode *et al*., 2020; Harrison *et al*., 2017).

Moreover, most antivenoms are ineffective to geographic variation in venom composition and antigenic reactivity attributable to the taxonomic diversity of venomous snake types (Alangode *et al*., 2020; Johnson *et al*., 2013). Besides, the high cost of antivenoms, especially in economically deprived settings, unavailability of enough antivenom stocks in various healthcare facilities, inaccessibility of hospitals impede timely antivenom access, thereby leading to high morbidity and mortality rates (Benjamin *et al*., 2020; Bhaumik *et al*., 2020; Tomaz *et al*., 2016a).

In Kenya, approximately 15,000 cases of SBE are recorded annually, with 6.7 deaths per 100,000 persons, in the rural settings, accounting for ~0·7% of all deaths (Williams *et al*., 2019). However, among the Kenyan Counties, Kitui County has a comparatively higher SBE caused by venomous snake species, including the black mamba (*Dendroaspis polylepis*), puff adder (*Bitis arietans*), and black-necked cobra (*Naja nigricolis*) (Kihiko, 2013). This is attributable to the hot-dry climate of Kitui County, housing type and agricultural activities which incline inhabitants towards snakebites (Kihiko, 2013). Additionally, the unavailability of effective SBE treatment in most health facilities, especially those in rural areas, and the unaffordability of antivenom treatments by most of the population further complicates effective management of SBE in Kenya (MoH, 2019).

Due to the bottlenecks of the conventional antivenom therapy, compounded by low supplies in sub-Saharan Africa, various communities use plants to manage SBE complications (Tomaz *et al*., 2016b). Medicinal plants are a critical component of maintaining human health, especially SBEs in rural regions where it is difficult to obtain specific antivenoms (Fernandes *et al*., 2014).

Despite the longstanding usage of medicinal plants against SBE in traditional medicine, it has not been accorded sufficient attention in the scientific arena (Tomaz *et al*., 2016b).

Research evidence has revealed the presence of various phytochemicals with antivenom properties, which present a viable alternative source of accessible, safe, and efficacious therapies for SBEs, especially in rural settings (Kumarapppan *et al.*, 2011). Various reports indicate that plant-derived extracts successfully inhibit and reverse snake venom-induced inflammation, haemorrhage, myotoxicity, and neurotoxicity (Bengal and Sarkhel, 2013; (Kihiko, 2013); Félix-Silva *et al*., 2017; Kumar and Khamar, 2010; Mors *et al*., 2000; Ricciardi *et al*., 2018; (Makhija and Khamar, 2010); Vaidya *et al*., 2018). Moreover, medicinal plants are used as prophylaxis for venomous Snakebites to protect themselves from SBE complications (Kumarapppan *et al*., 2011).

Based on this background, the current study was conducted to identify and document medicinal plants used to manage venomous snakebites in Mwingi West Subcounty, Kenya, and evaluate their phytochemical composition and safety.

1.2 Statement of the problem and justification of the study

Snakebite envenomation (SBE) is a life-threatening public health concern affecting over 2.7 million persons living in remote and resource-limited regions of sub-Saharan Africa yearly (Benjamin *et al*., 2020). In Kenya, Kitui County, especially Mwingi West sub-County, records the highest SBE incidence, owing to its geographic location and activities of the locals (Kihiko, 2013; MoH, 2019; Williams *et al*., 2019; World Health Organization (WHO), 2021).

Despite the successful management of SBE by antivenom therapy in conventional medicine, it is of low efficacy due to the diverse venom composition across snake species, limiting its usefulness (Alangode *et al*., 2020; Johnson *et al*., 2013). Besides, antivenom therapy has been shown to cause undesirable effects, such as evoking hypersensitivity reactions, its lability in certain conditions, and inefficacy, limiting its use (Alangode *et al*., 2020; Harrison *et al*., 2017). The unavailability, unaffordability, and inaccessibility of

timely antivenom treatment in local health centres in economically challenged remote areas, such as in the Mwingi West sub-County, has compromised the sustainable reduction of the burden posed by SBE to victims and caregivers (Benjamin *et al*., 2020; Kihiko, 2013). As a result, the affected persons often suffer long-term physical and psychological debilities, which interfere with their normal life, hence the need for efficacious, accessible, safe, and affordable therapies for SBE and associated syndromes.Despite the longstanding usage of medicinal plants by Mwingi West sub-County residents to manage SBE and associated complications, this information is yet to be documented, risking its loss. Furthermore, empirical data on the safety profile and phytochemical composition of medicinal plants used to manage SBE in the Mwingi West sub-County can spur further research and development of safe, accessible, efficacious, and affordable alternative therapies SBE is lacking. Therefore, the current study was designed to document medicinal plants used to manage snakebite in Mwingi West Subcounty and determine their phytochemical composition and safety.

1.3 Objectives

1.3.1 General objective

The aim of this study was to document the medicinal plants used to manage snakebite envenomation (SBE) in Mwingi West Sub-County, Kitui County, and determine their phytochemical composition and their cytotoxicity.

1.3.2 Specific objectives

The following were the specific objectives of the study;

i. To document medicinal plants used to manage Snakebite envenomation (SBE) in Mwingi West Sub County, Kenya.

ii. To determine the qualitative phytochemistry of the aqueous, methanolic, and dichloromethane extracts of selected medicinal plants used to manage Snakebite envenomation (SBE) in Mwingi West Sub County, Kenya.

iii. To investigate the cytotoxicity effects of the aqueous, methanolic, and dichloromethane extracts of selected medicinal plants used to manage Snakebite envenomation (SBE) in Mwingi West Sub County, Kenya, in brine shrimp nauplii.

1.4 Research questions

The following were the research questions guided the current study:

i. Which medicinal plants are used to manage Snakebite envenomation (SBE) in Mwingi West Sub County, Kenya?

ii. What is the qualitative phytochemical profile of the aqueous, methanolic, and dichloromethane extracts of selected medicinal plants used to manage Snakebite envenomation (SBE) in Mwingi West Sub County, Kenya?

iii. What are the effects of the aqueous, methanolic, and dichloromethane extracts of selected medicinal plants used to manage Snakebite envenomation (SBE) in Mwingi West Sub County, Kenya, in brine shrimp nauplii?

CHAPTER TWO: LITERATURE REVIEW

2.1 Venomous animal bites and stings

Venomous animals include spiders, venomous snakes, hymenopterans, cnidarians, scorpions, venomous fish, and venomous terrestrial snakes (Hifumi *et al*., 2015). These categories of animals produce toxic chemicals as defense mechanisms, delivered via special apparatus such as arrows, unique stings, hairs, special teeth, or nematocysts, into the victims. Besides, poisonous animals produce venom but lack venom injection devices (Junghanss and Bodio, 2006).

2.2 Venomous snakes of Kenya and clinical manifestation of their envenomation

Snakes are widely distributed in Kenya, from the savanna grasslands, forests, woodlands, with some of the species in the rivers, lakes, and the ocean (Ochola *et al*., 2018). There are about 140 known species of snakes that are found in Kenya, out of which 29 species are venomous. Moreover, only 13 species of the venomous snakes have medical significance since their envenomation causes tissue injury and even death in extreme cases. Additionally, only nine (9) of these venomous snake species cause bites that require medical attention. In Kenya, venomous snakes are categorised as predominantly neurotoxic, predominantly cytotoxic, the deadly back fanged, the symptomatically treated, and the myotoxic (sea snakes) (MOH-Kenya, 2019).

The predominantly neurotoxic snakes (the mambas and the non-spitting coras) cause bites that are characterised by moderate or absent local oedema, progressive descending paralysis-initially manifesting as ptosis and double visison. The victim usually vomits, becomes profuse and stringly, and later suffers difficulties in swallowing and breathing. Three out of the four neurotoxic mambas (*Dendroaspis*) in Africa are found in Kenya (MOH-Kenya, 2019). They include *Dendroaspis polylepis* (black mamba), which possess a fast-acting neurotoxic venom, requiring medical emergency, *Dendroaspis angusticeps*

(Eastern green mamba), which is found along the coast, in Kibwezi,and Meru areas, and *Dendroaspsis jemesoni* (Jameson's mamba), which which is only found in western Kenya. The non-spitting cobras with a predominantly neurotoxic venom found in Kenya include the Egyptian cobra (*Naja haje*) and the Eastern cobra (*Naja subfulva*), which are mostly found around Mount Kenya and at the coast, and the Gold's tree cobra (*Pseudohaje goldi*) which is only found in western Kenya (MOH-Kenya, 2019; Ochola *et al*., 2018).

The predominantly cytotoxic snakes of Kenya, cause bites which are characterised by painful and progressive oedema- with blood stained tissue fluid leakage from the bite wound, blistering, and hypovolemic shock, and tissue necrosis or gangrenes in the victim (MOH-Kenya, 2019). These snakes include the red spitting cobra (*Naja pallida*), the black-necked spitting cobra (*Naja nigricollis*), and the Ashe's spitting cobra (*Naja ashei*). Besides, four large African adders or vipers (*Bitis*) are found in Kenya. They include the puff adder (*Bitis arietans*), Gaboon viper *(Bitis gabonica*), Rhinoceros viper *(Bitis nasicornis*), the Kenya horned viper *(Bitis worthibtonii*), and the smaller one the North East-African carpet viper (*Echis pyramidium*), which is more aggressive and more lethal (MOH-Kenya, 2019).

The deadly back fanged snakes produce a relatively less lethal venoms to humans; however, their venom is deadly to their reptilian prey. They are widely sprad in Kenya, and include the Boomslang (*Dyspholidus typus*), the vine snake (*Thelotornis spp.*), and the Blanding's tree snake *(Toxicordryas blandingii*) (Ochola *et al*., 2018).

The symptomatically treated venomous snakes that re found in Kenya have so far either rarely caused human falatilities, and their bites are treated symptomatically due to the unavailability of their antivenoms (MOH-Kenya, 2019). They include the African burrowing Asps (*Atractaspis bibronii* and *Atractaspsis fallax A. micro-lepidota*), the African night adders (*Causus defilippii, Causus lichtensteini, Causus resimus*, and *Causus*

rhombteatus), the bush vipers (*Atheris hispida, Atherissquamigera,* and *Atheris desaixii*) and montane viper (*Montatheris hindii*-only found in Mount Kenya and Abadares), and the African Garter snakes (*Elapsoidea loveridgei* and *Elapsoidea nigra*) (MOH-Kenya, 2019; Ochola *et al*., 2018).

Only one species of the sea snakes (*Pelamis platuru*) has been recorded in Kenya. This serpent causes mytoxic envenomation, which is characterised by slight local swelling, myalgia and associated features of neurotoxicity, including paralysis (MOH-Kenya, 2019). In Mwingi region of Kitui County, the Boomslang (*Dispholipus typus*) (Plate1:Male; Plate 2: Female), which is locally known as "*Ndalanga*", the Puff-Adder (*Bitis arietans*), which is known by Akamba people as "*Kimbuva*" (Plate 3), the black necked spitting cobra *(Naja nigricollis*) (Plate 4)- locally known as "*Kiko-kiu",* the red spitting cobra (*Naja pallida*)- locally known as"*Kiko kitune/Kiko kya Nguku*" (Plate 5), the brown spitting cobra (*Naja ashei*) (Plate 6)- locally known as *"Kiko kya Nzaana"*, and the black mamba (*Denroapsis polylepis*) (Plate 7)- commonly known by the Akamba people as "*Ikuuwa*" are the commonest (Malonza and Bwong, n.d.). These serpents's venoms cause three major types of envemonation namely: haemotoxicity (Boomslang bites), cytotoxicity (Puff-Adder and spitiing cobra bites), and neurotoxicity (Mambas and non-spitting cobra bites) (Malonza and Bwong, n.d.; MOH-Kenya, 2019).

Plate 1: A photograph of the male Boomslang (*D. typus*) *Courtesy: Dr.Malonza and Dr. Bwong of the herpetology Department, the National Museums of Kenya.*

Plate 2: A photograph of the female Boomslang *(D. typus*) *Courtesy: Dr.Malonza and Dr. Bwong of the herpetology Department, the National Museums of Kenya.*

Plate 3: A photograph of the Puff-Adder (*B. arietans*) *Courtesy: Dr.Malonza and Dr. Bwong of the herpetology Department, the National Museums of Kenya.*

Plate 4: A photograph of the black-necked spitting cobra (*N. nigricollis*) *Courtesy: Dr.Malonza and Dr. Bwong of the herpetology Department, the National Museums of Kenya.*

Plate 5: A photograph of the red spitting cobra (*N. pallida*) Courtesy*: Dr.Malonza and Dr. Bwong of the herpetology Department, the National Museums of Kenya.*

Plate 6: A photograph of the brown spitting cobra (*N. ashei*) *Courtesy: Dr.Malonza and Dr. Bwong of the herpetology Department, the National Museums of Kenya.*

Plate 7: A photograph of the black mamba (*D. polylepsis*) *Courtesy: Dr. Malonza and Dr. Bwong of the herpetology Department, the National Museums of Kenya.*

2.3 Snake venoms, composition,and their mechanisms of envenomation

Venomous snakebites are the most common form of venomous animal injuries commonly reported globally (Fatah, 2014a; Ricciardi Verrastro *et al*., 2018). Snake venoms comprise diverse biotoxic amalgams which cause inflammation, haemorrhage, oedema, paralysis, myonecrosis, organ failures, and bleeding disorders, when injected into the human system (Fatah, 2014b). Therefore, SBE is a life-threatening medical emergency requiring immediate attention to avert its long-term debilitating sequelae (Benjamin *et al*., 2020; Williams *et al*., 2019; World Health Organization (WHO), 2021).

Globally, the incidences of venomous snakebites are estimated to be more than 3 million annually, with over 150,000 (Williams *et al*., 2019; World Health Organization (WHO), 2021). Most often, effective treatment and management of venomous bites are hindered by the lack of sufficient information among healthcare providers, the inaccessibility, unavailability, unaffordability, and inefficacy of antivenom therapy, especially in

resource-limited remote settings, thus warranting the need for better alternatives (Benjamin *et al*., 2020; Williams, 2015).

Snake venoms have been described since the Cenozoic Era and are the most characterized of all animal venoms (Ferraz *et al.*, 2019). They comprise pharmacologically active peptide molecules, which alter biological systems leading to adverse effects (Casewell *et al*., 2014; Chan *et al*., 2016). Furthermore, research has shown that snake venom constituents are more diverse, with many pharmacologic and toxic properties, compared to those of other venomous animals (Zelanis and Tashima, 2014; Zhang, 2015).

2.3.1 Composition of snake venoms

Snake venoms contain biomolecules and inflammatory-associated molecules which possess varied biological activities. The major components of snake venoms involve secreted proteins, sythesised by the 12S and 20 S mRNAs in venomous glands (Ferraz *et al*., 2019). These proteins exhibit diverse biologic activities, such as interfering with metabolic functions and hydrolytic/digestive enzymes, some of which cause deleterious effects (Casewell *et al*., 2014; Chan *et al*., 2016). Snake venom enzymes damage biomembranes, vasculature, and induce coagulopathy in victims (Fry, 2015). In addition, venoms of Viperidae family snakes contain other molecules that act as proinflammatory mediators of blood coagulation, fibrinolysis, the complement, and kinin systems, producing profound inflammation (Ferraz *et al*., 2019; Fry, 2015).

2.3.2 Mechanisms of snake bite emvenomation

Snake venoms, like *Vipera russelli, Naja naja,* and *Trimeresurus flavoviridis* are rich sources of phospholipases (Gutiérrez and Lomonte, 2013; Harris *et al*., 2000). Snake venom-derived phospholipases are classified into two groups based on primary sequence and disulphide bridge position (Gutiérrez and Lomonte, 2013). The first group of venom phospholipases includes those of Elapidae and Hydrophidae snake families; whereas, the

second group includes venom phospholipases of Viperinae and Crotalinae snake families. Research shows that snake venom phospholipases are neurotoxic, myotoxic, oedematic, cardiotoxic, hemorrhage, cytotoxic, hemotoxic and induce coagulopathy and paralysis in victims (Hifumi *et al*., 2015; Williams *et al*., 2019).

A current study by (Warrell, 2019) indicates that SBE causes nausea, numbness, vomiting, swelling, muscle cramping, localized pain and inflammation, tingling of the body parts, dizziness, shock, dyspnoea, and life-threatening coagulopathy in affected patients. The SBE effects are attributed to venom phospholipases, which induce haemolysis and necrosis of the body's cells and tissues, leading to deadly consequences, if not managed adequately and timely (Williams *et al*., 2019). Additionally, phospholipase A_2 enzyme is responsible for the neurotoxic and myotoxic effects of snake venoms and exacerbates allergic responses by upregulating histamine and bradykinin secretion, thereby worsening the outcomes (Chan *et al*., 2016; Fry, 2015; Harris and Scott-Davey, 2013; Harrison *et al*., 2019; Kini, 2005, 2006; Warrell, 2019).

Proteolytic enzymes present in snake venoms have been shown to promote tissue necrosis, haemorrhage, and bleeding disorders in patients due to their fibrinolytic properties (Ferraz *et al*., 2019; Hifumi *et al*., 2015). Snake venom proteases are categorised as Snake Venom Serine Proteases (SVSPs) and Snake Venom Metalloproteinases (SVMPs) (Munawar *et al*., 2018; Slagboom *et al*., 2017). SVMPs are the main constituents of viper venoms and are exert their toxicity by altering the haemostatic equilibrium and induction of oedema and hyperalgesia in victims (Munawar *et al*., 2018). Additionally, SVMPs induce inflammation, myonecrosis, skin damage, degrade the extracellular matrix, inhibit platelet aggregation leading to massive extravasation of blood, and cause cardiovascular shock in patients (Kini, 2006; Kini and Koh, 2016; Munawar *et al*., 2018). Therefore, SVMPs are also known as haemorrhagins, due to their ability to induce systemic haemorrhage, leading

to severe health effects (Paine *et al*., 1992). On the other hand, SVSPs are known to rupture capillary vessels, leading to haemorrhage. Also, SVSPs induce hemotoxic effects, leading to life-threatening sequelae (Murakami and Arni, 2005).

Three-finger toxins are non-enzymatic neurotoxins with 58-81 residues, commonly present in the elapid and colubrid snake venoms (Kessler *et al*., 2017). They bind to the post-synaptic sites of the neuromuscular junction leading to flaccid paralysis (Kessler *et al*., 2017).

2.4 Traditional management of SBE

Traditional medicine and natural products play an essential role in treating and managing diseases (Aziz *et al*., 2017; SO *et al*., 2018; State *et al*., 2019). Traditional medicine practices, such as Traditional Chinese Medicine, Traditional Korean Medicine, are being integrated into the current medicine systems of disease management due to their proven potency and safety (Amri and Kisangau, 2012; Gao *et al*., 2019; Githinji and Kokwaro, 1993; Nankaya *et al*., 2020; Singh *et al*., 2020). In the prehistorical times, natural products such as marine organisms, plants, microorganisms, and animals were utilized for alleviating and treating diseases as revealed by fossil records (Fabricant and Farnsworth, 2001).

In the underdeveloped regions of the world, traditional management of SBE and associated complications using medicinal plants is common (Benjamin *et al*., 2020; WHO/Regional Office for South-East Asia, 2016). This is attributable to the high incidences of SBEs, high costs of conventional management, underdeveloped healthcare systems, unavailability and inaccessibility of antivenom therapy, and high poverty levels (Benjamin *et al*., 2020; Bhaumik *et al*., 2020). As a result, the inhabitants of these regions resort to utilising various medicinal plants to thwart SBE as an alternative.

Various medicinal plants, including *Bauhinia cumanensis, Nicotiana tabacum, Aristolchia rugosa, Cecropia peltata,* are traditionally used to manage SBE in various parts of India (Jammu *et al*., 2019). Elsewhere, many studies have reported various medicinal plants, their parts, and modes of preparation of remedies against SBE (Jammu *et al*., 2019; Okot *et al*., 2020; Tiwari and Gupta, 2016; Vaidya *et al*., 2018). However, ethnomedical reports of medicinal plants used to manage SBE in many rural settings are lacking. In this study,

2.5 Phytochemicals

Phytochemicals are secondary metabolites produced by plants through various chemical pathways (Budisan *et al*., 2017; Iqbal *et al*., 2017; Mendoza and Silva, 2018). The major classes of phytochemicals with pharmacologic activity include phenolic acids, flavonoids (flavanols, anthocyanidins, flavones, isoflavones, flavanols), lignans, tannins, stilbenes, quinones, coumarins, alkaloids, saponins, glycosides, steroids, lectins, terpenoids, and peptides (Kurmukov, 2013; Moriasi, *et al*., 2020). Despite the significance of phytochemicals in human health promotion, phytochemical research involving many medicinal plants is in its preliminary stages, which warrants the need for focused and extensive research in this area.

Several studies have demonstrated the importance of phytochemicals in treating diseases and improving health (Moriasi *et al*., 2021; Moriasi *et al*., 2020a). Moreover, there has been the development of neuropharmacological approaches for the provision of preliminary information on the medical importance of phytochemicals through *in vitro, ex vivo, in vivo,* and molecular analysis (Anosike *et al*., 2019; Rahman, 2013; Iqbal *et al*., 2017; Moriasi *et al*., 2020a; Moriasi *et al*., 2021; Moriasi *et al*., 2020b; Moriasi *et al*., 2021; Muchonjo *et al*., 2021).

Research shows that flavonoids and phenolic acids derived from vegetables and fruits are responsible for providing defensive mechanisms in the protection against both abiotic and biotic stress (Liu *et al*., 2018). Traditional vegetables have also been a major source of antioxidants that can protect against diseases such as diabetes and obesity (G. Moriasi, Ireri, *et al*., 2020b) In addition, carotenoids, the precursors for the synthesis of vitamins A, possess antioxidant activity, which protects against free radical damage, thus reducing the incidences of developing cardiovascular complications and autoimmune diseases (Arulselvan *et al*., 2016; Liu *et al*., 2018). Antioxidant-associated phytochemicals, such as flavonoids and phenols, exhibit the most comprehensive spectra of bioactivity, hence stand a better chance to offer safe and efficacious therapies for various diseases (G. Moriasi, Ireri, *et al*., 2020b, 2020a).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Area

Ethnobotanical documentation was done in Migwani Ward, Mwingi West Sub- County, in Kitui County, Kenya (Figure 3.1), which is located about 49.7 Km from Kitui town, and 176.2 Km from Nairobi city. Migwani ward comprises six villages: Kyamboo/Kaliluni, Migwani/Itoloni, Nzatani/Ilalambyu, Nzeluni/Mung'alu, Kisovo, and Katalwa/Mumbuni (Kitui County Intergrated plan 2018-2022, 2018; Nation, 2017). It the most populated ward of Kitui County, with a population of 79,255 persons in 39,096 households, according to the 2019 national census report (KNBS, 2019). This region experiences a sub-humid climate, hot and dry for almost the entire year, with an erratic and unreliable rainfall distribution. As a result, its lowest annual average temperature is 14℃, while the highest annual average temperature is 32℃. Most residents of the Migwani Ward (67.3%) are small-scale farmers, with family members being the primary source of labour in the agricultural production system (Kitui County Intergrated plan 2018-2022, 2018).

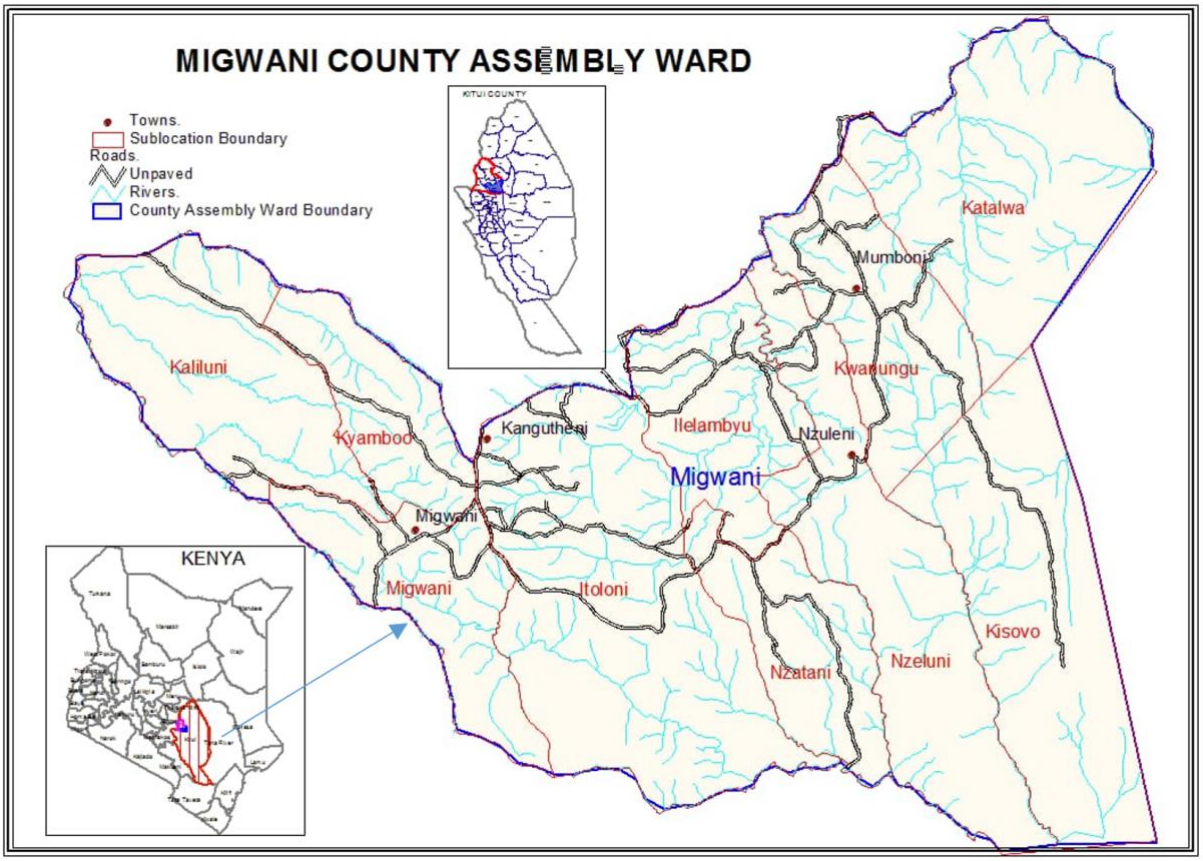


Figure 3.1: Map of Kenya Showing Migwani Ward.
(Kitui County Intergrated plan 2018-2022, 2018)

3.2 Ethnobotanical data collection and analysis

Ethnobotanical survey was done in January and February 2021. The purposive sampling technique described by (Palinkas *et al*., 2015) was used to select fourth five participants who were aged between 20 and 80 years and who were knowledgeable about medicinal plants used to treatment SBE in the study area. The inclusion criteria for selecting study respondents were based on their knowledge (Herbalists and local community members), natives who understood the local area, and the local names of the plants. The initial participants were selected with the help of local leaders, local dwellers, and herbalists, who referred others through their existing networks within the study area. Saturation was reached when new data collection did not yield any new information on the medicinal plants used (Palinkas *et al*., 2015).

Following this, relevant information (data) was collected through interviews (conducted either in the native language, Kamba or Swahili, depending on the participants` preference), administration of semi-structured questionnaires, and through guided field walks to plant collection sites (Cotton, 1996; Cunningham, 2014; Omwenga *et al*., 2009; Ouelbani *et al*., 2016).

The respondents' socio-demographic information and ethnobotanical data of plants used to manage SBE, and associated information were documented. In addition, the respondents filled informed consent forms before taking part in the study. The content of the consent form and data collection questionnaires are summarized in Appendices 1 and 2, respectively.

3.3 Collection and identification of the plants

Frequently cited plants used in managing SBE by the participants during the survey, were collected as voucher specimens by a team comprising herbalists and the researcher (University of Nairobi) and documented photographically. Identification of plant specimens was done at the East African Herbaria hosted at the National Museums of Kenya by botanists; Dr. Paul Kirika and Mr. Mathias M. Mbale, and Reference numbers assigned, voucher specimens deposited. The selected plant materials were anlysed at the Department of Public Health, Pharmacology, and Toxicology laboratory of the University of Nairobi.

3.3.1 Sample preparation and extraction

The plant materials were prepared according to the methods of (Abubakar and Haque, 2020) and (Moriasi *et al*., 2020b) with slight modifications. Briefly, the collected plant parts were washed with clean water, chopped into small pieces with a sharp knife, and dried at room temperature for two weeks. Upon drying, the plant materials were ground

using an electric mill to a coarse powder, packed in khaki bags, and stored on a shelf awaiting extraction.

3.3.2 Reagents and chemicals

Analytical grade Methanol and Dichloromethane and distilled water were used as extraction solvents for the powdered plant materials. Besides, hydrochloric acid, sulphuric acid, ammonia (30%), magnesium, chloroform, sodium hydroxide, acetic anhydride, ferric chloride, sodium nitrite, Dragendorff's, and Mayer's reagents were used for qualitative phytochemical screening.

3.3.3 Extraction with organic solvents

The procedure of (Harborne, 1998), as modified by (Moriasi *et al*., 2021), was followed. Briefly, two hundred grams (200g) of each powdered material were soaked in 1000 ml of 95%methanol and Dichloromethane, respectively, at room temperature for 48 hours and regularly shaken using a mechanical shaker. The extracts were then filtered twice, initially with cotton wool and later with a Whatman filter paper No.1. The filtrates were evaporated to dryness at 40℃ using evaporating dish. Resultant extracts were weighed and stored in capped glass bottles in a refrigerator (4℃) awaiting analysis.

3.3.4 Aqueous extract of the plants

Aqueous extraction was carried out according to the method of G. A. Moriasi *et al*., 2021) with slight modifications. Briefly, two hundred grams (200g) of the powdered plant materials were weighed and transferred into conical flasks, and 1000ml of distilled water was added and shaken. Afterward, the flasks were placed in a hot water bath (70^{o}C) and heated for 2 hours, and then the content filtered through a cotton gauze and Whatman filter paper No. 1. Next, the filtrates were transferred into freeze-drying flasks covered with dry carbon ice and acetone and freeze-dried for 48 hours. Finally, the extracts were weighed kept in capped glass bottles in a refrigerator (4℃) awaiting analysis.

3.4 Qualitative Phytochemical Screening of the selected plants

Qualitative phytochemical screening of the aqueous, methanolic, and dichloromethane extracts of the selected plants was performed using the methods described by (Trease and Evans, 2009), (Harborne, 1998), and (Moriasi, *et al.*, 2020a) with slight modifications, in order to detect the presence or absence of various bioactive compounds. The phytochemicals tested included alkaloids, tannins, phenols, saponins, and flavonoids. In addition, observations of color changes or precipates were done and used to appraise the presence or absence of respective phytochemicals in the study samples.

3.4.1 Test for alkaloids

A total of 0.2g of extract of each plant extract was dissolved in a 10 ml aqueous solution of 1% hydrochloric acid, boiled, and then filtered through a Whatman filter paper No.1. To 5ml of the filtrates, 2ml of dilute ammonia was added, followed by 5 ml of chloroform, and shaken. The chloroform layer was extracted with 10 ml of acetic acid and subdivided into two portions. Into the first portion, 2 to 3 drops of Mayer's reagent were added. Into the second portion, 2-3 drops of the Dragendoff reagent were added. The presence of red or orange precipitates indicates the presence of alkaloids in Dragendoff's test, while the formation of a cream precipitate in Mayer's test indicates the presence of alkaloids (Trease and Evans, 2009).

3.4.2 Test for Saponins

A total of 0.2 g of extract for each studied plant extract, 5ml of distilled water were added into a test tube. First, the solution was shaken and observed for a stable, persistent froth. Next, the frothing was mixed with three drops of olive oil and shaken vigorously, after which it was observed for the formation of an emulsion, which indicates the presence of saponins (Harborne, 1998).

3.4.3 Test for tannins

A total of 0.1g of each plant's extract was boiled in 10ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added. The development of a deep green or a blue-black colouration indicates the presence of tannins (Trease and Evans, 2009).

3.4.4 Test for Flavonoids

Dilute ammonia (5ml) was added to a portion of an aqueous filtrate of each studied plant extract. Then, 1 ml of concentrated sulphuric acid was also added and gently swirled. A yellow colouration that disappears on standing indicates the presence of flavonoids (Moriasi *et al*., 2020a).

3.4.5 Test for phenolics

A total of 0.1g extract of each plant was dissolved in distilled water, and then 2ml of 5%ferric chloride solution was added. The formation of a deep bluish-green solution indicates the presence of phenols (Moriasi *et al*., 2020a).

3.5 Evaluation of the effects of the selected plant extracts on brine shrimp nauplii

The brine shrimp lethality assay technique of (Meyer *et al*., 1982) was used to determine the cytotoxic effects of the studied plant extracts, in order to appraise their safety.

3.5.1 Hatching of Brine shrimp nauplii

Brine shrimp eggs were hatched in a rectangular box with two chambers having perforations. One chamber was dark, and a 40-watt electric bulb illuminated the other chamber. The box was filled with brine solution, after which 50g of brine shrimp eggs were sprinkled with a spatula into the dark chamber of the box. Five grams of yeast was added as feed for the hatched nauplii. After 48 hours, the nauplii were collected from the illuminated chamber and used for the brine shrimp lethality test.

3.5.2 Plant extracts solution preparation

Then 0.1g of the studied organic and aqueous plant extracts were weighed and dissolved in 1o ml of brine salt solution (38.5g marine salt in 1 litre distilled water) to make a stock concentration of 10,000µg/ml, which was then diluted serially.

3.5.3 Brine shrimp lethality assay

The cytotoxic effects of the selected plants' extracts were investigated according to the principle and protocol previously described by (Meyer *et al*., 1982), with slight modifications. Three dilutions were prepared by transferring 500µl, 50µl, and 5µl of plant extract *(Entada leptostachya, Senna singueana, Securidaca longipendunculata,* and *Strychnos henningsii*) into the set of five graduated tubes. Brine solution was added accordingly in order to obtain dilutions of 1000µg/ml, 100µg/ml, and 10µg/ml in five replicates. After that, 10 brine shrimp nauplii were transferred into each tube. Vincristine sulphate was used as a positive control. Test tubes were left to settle at room temperature(°C), and the surviving nauplii were counted after 24 hours. Probit regression analysis was performed in order to determine the medial lethal concentration (LC_{50}) of each studied plant extract in this assay.

3.6 Data management, analysis, and reporting

Ethnobotanical and extract yield data were organized and summarized using Microsoft office excel 2013 software, where descriptive statistics were performed.

The Relative Frequency of Citation (RFC) criteria was used in order to determine popularly used plants to manage SBE in the study area. The relative frequency of citation (RFC) of plant species was calculated by dividing the frequency of citation (FC) (the number of respondents who cited a particular species) by the total number of respondents in the survey (N=45). This RFC index ranges from 0 (when nobody refers to a plant as

useful) to 1(when all respondents mention the species as useful). The following formula described by (Vitalini *et al*., 2013) was used to calculate the RFC index.

$$RFC = \frac{FC}{N}$$

Where RFC is the relative frequency of citation, FC is the citation frequency, and N is the sample size (45 respondents).

Medicinal species with high RFC were selected for phytochemical analysis as per the recommendations of (Rahman *et al*., 2016) and (Teklehaymanot and Giday, 2010).

The brine shrimp lethality assay data of the studied plant extracts were analysed using probit regression analysis using SPSS v20 (Bliss, 1935; Finney, 1952). The cytotoxicity results (LC_{50} values) were interpreted based on the Meyer's and Clarkson's criteria (Clarkson *et al*., 2004; Meyer *et al*., 1982). The findings of this study were presented in bar graphs (drawn using GraphPad Prism version 9.1.2) and Tables.

3.7 Ethical consideration

The study was performed after obtaining Institutional ethical approval from the Biosafety Animal Use and Care Committee of the Faculty of Veterinary Medicine of the University of Nairobi (BACUC) (REF: BAUEC/2021/294) (Appendix 3) and a research permit from the National Commission for Science Technology and Innovation (NACOSTI) (NACOSTI/P/21/11115) (Appendix 4). Furthermore, the study participants signed a consent form, and confidentiality of their information was upheld.

CHAPTER FOUR: RESULTS

4.1 Ethnobotanical documentation of medicinal plants used to manage SBE in the study area

4.1.1 Socio-demographic characteristics of the study participants

This study included 45 participants, aged between 20 and 80 years, who provided ethnobotanical information of medicinal plants which are used to manage SBE in the study area. Most participants (53%) were aged between 41 and 60 years, followed by those aged between 20 and 40 years (31%), while those aged ≥61 years accounted for 16 %. In terms of gender,58% of the respondents were males, while 42% were Females. Only 2 % of the respondents were formally employed, with the majority (67%) practicing subsistence farming and other small-scale activities for livelihood.

The results further showed that 33 % of the respondents had not acquired any formal education, while 38% had a primary level, 18% had obtained a secondary level of education, and 11 % had tertiary education. Most respondents (91 %) were native kamba people, while 9 % of respondents comprised herbalists. Ethnomedical knowledge was mainly acquired from close family members and relatives (65%); 13% of the respondents acquired knowledge from herbalists, while 22 % learnt from dreams/ divine call/literature.

It was observed that most of the respondents (42%) had <5 years of ethnomedical experience, while those having 6-10 years of practice were 38 %, and only 20 % had practiced for ≥10 years. Table 4.1 presents the socio-demographic characteristics of the participants.

Table 4.1: Socio-demographic characteristics of the respondents

Variable	Category	N	Frequency
Gender	Male	26	58
	Female	19	42
Age(years)	20-40	14	31
	41-60	24	53
	61-80	7	16
Education	Primary	17	38
	Secondary	8	18
	Tertiary	5	11
	None	15	33
Practice specifications	Herbalist	4	9
	Local people	41	91
Source of income	Employment	1	2
	Business	14	31
	Others	30	67
Experience(years)	0-5	19	42
	6-10	17	38
	> 10	9	20
Source of knowledge	Relatives	29	65
	Herbalist	6	13
	Others	10	22

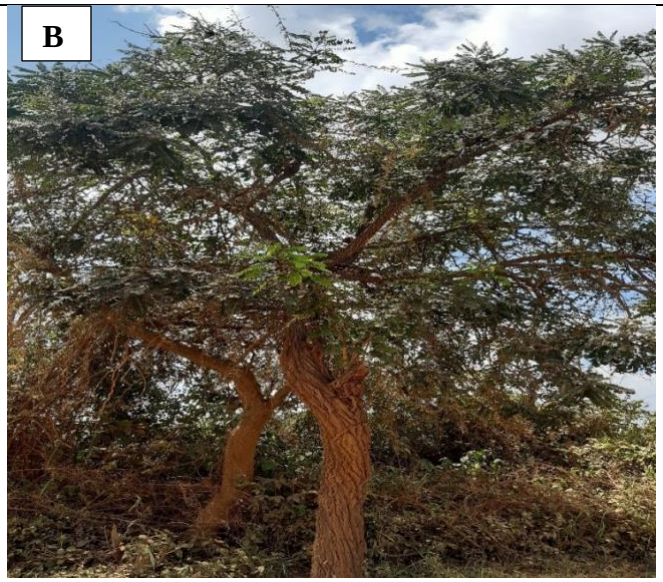
Total number of respondents(N) = 45

4.1.2 Ethnobotanical and ethnomedical information of documented plants and frequency of citation (FC and RFC)

Medicinal plants used to manage SBE in Migwani Ward were documented, and their relevant information, is summarised in Table 4.2. In this study, 14 medicinal plant species belonging to 12 families were documented. The most represented family was Asteraceae with three plant species, while Capparaceae, Fabaceae, Burseraceae, Loganiaceae, Musaceae, Polygalaceae, Vitaceae, Solanaceae, Euphorbiaceae, Leguminacea, and Opiliaceae were represented by one plant species each (Table 4.2).

The most frequently cited plant species included *Entada leptostachya* (RFC=0.56) (Plate 8A), *Senna singueana* (RFC=0.53) (Plate 8B), *Strychnos henningsii* (RFC=0.47) (Plate 8C), and *Securidaca longipendunculata* (RFC=0.36) (Plate 8D), respectively (Table 4.2;). Notably, all the documented plants were applied topically on the site of bite, while some were administered orally and topically (Table 4.2).

Entada leptostachya (*Captured in situ By Stella Mokua)*



Senna singuena (Captured in situ By Stella Mokua)

Strychnoss henningsii (Captured in situ By Stella Mokua)

Securidaca longipendunculata (Captured in situ By Stella Mokua)

Plate 8: Photographs of the four most frequently cited plants used for the management of snakebiteenvenomation in the study area

Table 4.2: Medicinal plants used to manage SBE in Migwani Ward, Mwingi West Sub County

Plant Species (V/N)	Local name	Family	Growth form	Part(s) used	Preparation the drugs	Dosage	Mode of Administration	FC	RFC
Securidaca longipendunculata (NMK/BOT/CTX/5/1)	*'Munguuka'*	Polygalaceae	Tree	Roots, leaves, barks	Soak the dried powdered root bark part in water.	Taken thrice a day	Topical or oral	16	0.36
Boscia salicifolia L. (NMK/BOT/CTX/5/2)	*'Ithangana'*	Capparaceae	Shrub	Barks, roots	Roots and barks burned into charcoal, crushed into fine powder.	Applied twice for six days	Topical	1	0.02
Notoria abyssinica A.Rich. (NMK/BOT/CTX/5/3)	*'Ngondu ya kimani'*	Asteraceae	Herb	Roots	Roots pounded, soaked in water, and infusion drunk two glasses twice a day.	Two glasses taken twice a day for six days or till the wound heals	Oral	1	0.02
Entada leptostachya Harms. (NMK/BOT/CTX/5/4)	*'Mwaitha'*	Fabaceae	Shrub	Stem, bark	Stem crushed, sap squeezed	Applied until the wound heals	Topical	26	0.56
Commiphora sp. (NMK/BOT/CTX/5/5)	*'Ithangu'*	Burseraceae	Shrub	leaves, fruit	The milky exudates from unripe fruits can be applied. Leaves crushed or pound.	Half a cu of the exudate taken once a day for three days. Pound leaf applied on the wound till healing.	Oral orTopical	2	0.04
Strychnos henningsii Gilg. (NMK/BOT/CTX/5/6)	*'Muteta'*	Loganiaceae	Tree	leaves, stem, bark, roots	Fresh roots can be chewed and swallowed to expel poison by vomiting. Leaves may be cooked	A quarter taken twice daily.	Oral Topical	21	0.47

					with water or mutton soup.				
Musa x paradisiaca L. (NMK/BOT/CTX/5/7)	*'Mathangu ma maiu'*	Musaceae	Tree	Leaves, stem	Sap squeezed out of leaves and stem for reducing swelling and pain	Applied on the wound immediately after the snake bite and thrice daily till recovery	Topical	5	0.11
Gutenbergia cordifolia Oliv. (NMK/BOT/CTX/5/8)	*'Ithungululu'*	Asteraceae	Herb	Leave	Leaves are sun-dried and burned to ash	Rubbed on the bitten site daily for five days	Topical	1	0.02
Solanum incanum(NMK/BOT/ CTX/1/1)	*'Kikondu' / 'Mutongu'*	Solanaceae	Shrub	Fruits, Leaves	The stem or fruits cut into small pieces, dried in the sun and pounded. and powder applied, or sap from the fruit may be applied directly	Applied on the wound immediately after the snake bite and thrice daily till recovery	Topical	11	0.24
Cissus rotundifolia (Forsk.) Vahl. (NMK/BOT/CTX/1/2)	*'Itulu'*	Vitaceae	Shrub	Leaves	Sap from pounded leave is squeezed	Applied irectly onto the wound four times daily for 10-14 days	Topical	8	0.18
Ricinus communis L. (NMK/BOT/CTX/1/3)	*'Kyaiki'/'Kivaiki'*	Euphorbiaceae	Shrub	Leaves	Fresh young leaves are pounded. The plant is cultivated at the homestead due to its strong smell that causes discomfort or disorientation to snakes.	Tied on the wound for 6 hours to accelerate healing	Topical/Relellant	2	0.04

Senna singueana (Delile.) Lock. (NMK/BOT/CTX/1/4)	*'Mukengeka'/ 'Mukengeta'*	Leguminosae	Shrub	leaves, roots	Roots dried in the sun, crushed into a fine powder and applied or mixed with mutton soup. Leaf infusion drunk as an antidote for puff adder bites.	One full glass of the soup drunk daily for five days. Powder applied on the wound for five days	Topical Oral	24	0.53
Opilia amentacea Roxb. (NMK/BOT/CTX/1/5)	*'Mutonga'*	Opiliaceae	Climber	Roots	Roots cut into pieces, sun-dried, then crushed into powder mixed with crushed snake teeth.	Applied on the snakebite wound for 7-10 days	Topical	1	0.02
Tagetes minuta L. (NMK/BOT/CTX/1/6)	*'Muvangi'*	Asteraceae	Herb	Leaves	Leaves crushed or chewed, and rubbed rubbed into snakebite wound as an antidote	Applied once on the wound following a bite	Topical	4	0.09

V/N = Voucher Number; FC = Frequency of citation; RFC =Relative Frequency of Citation

The results also shows that most of the documented plants were shrubs (50%), followed by herbs (21.4%), trees (21.4%), and climbers (7.2%) (Table 4.2; Figure 4.1).

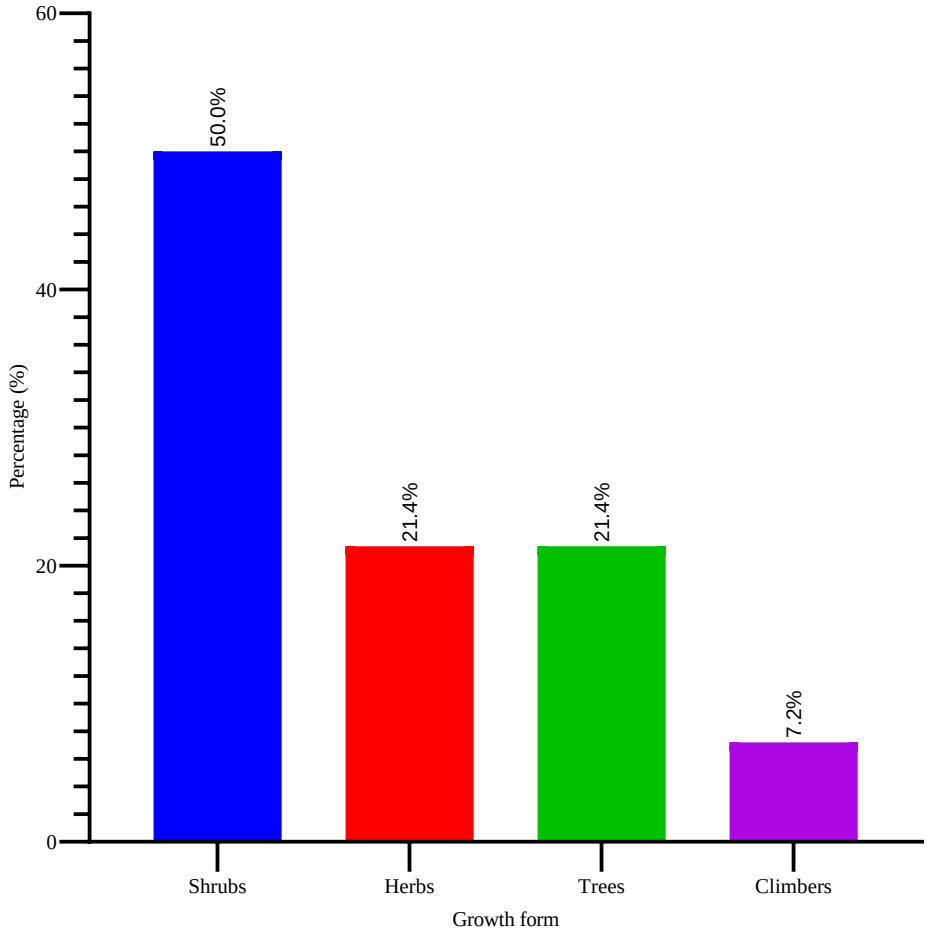


Figure 4.1: Growth form of the documented plants

The most used plant part (s) in the preparation of SBE remedies were the leaves (42%), roots (25%), stems/barks (25%), and fruit (8%), respectively (Table 4.2; Figure 4.2).

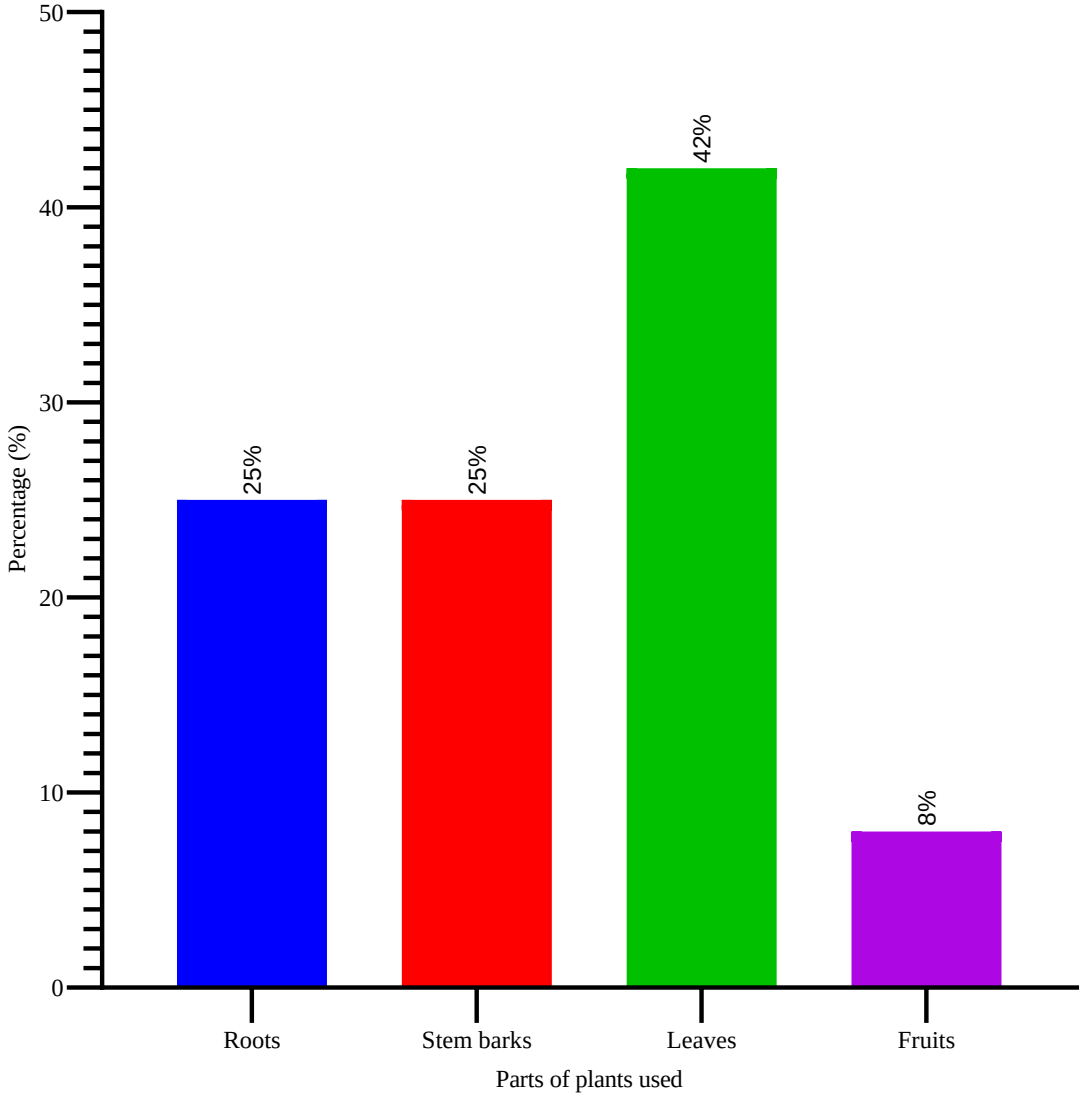


Figure 4.2: Parts of plants which are mostly used to prepare remedies for SBE in the study site

4.2 Extract yields of the selected plants

Four medicinal plants with the highest RFC values were selected and extracted using water, methanol, and dichloromethane for qualitative phytochemical screening and brine shrimp lethality assay.

For the aqueous extracts, *Securidaca longipendunculata* had the highest yield (10 %), followed by *Senna singueana* (5%) and *Strychnos henningsii* (5%), while *Entada leptostachya* had the lowest yield (4%) (Table 4.3; Appendix 6). The highest percentage yield of the methanolic extracts was recorded by *Securidaca longipendunculata* (1.97%)

followed by *Senna singueana* (1.43%), then *Entada leptostachya* (1.23%), and *Strychnos henningsii* (1.03%), respectively. On the other hand, the highest yield of dichloromethane extracts was obtained by *Senna singueana* (1.45%) followed by *Securidaca longipendunculata* (0.51%), *Strychnos henningsii*, (0.46%), and *Entada leptostachya* (0.5%), respectively (Table 4.3; Appendix 6).

Table 4.3: Yields of the Aqueous, Methanolic, and Dichloromethane extracts of the studied plants

Plant and part extracted	Percentage yield (%)		
	Aqueous extracts	**Methanolic extracts**	**Dichloromethane extracts**
Entada leptostachya (stem bark)	4	1.215	0.485
Senna singueana (Root)	5	1.425	1.45
Securidaca longipendunculata (Root)	10	1.965	0.51
Strychnos henningsii (Stem bark)	5	1.03	0.46

4.3 Qualitative phytochemical composition of the selected plant extracts

Qualitative phytochemical screening revealed the presence of alkaloids, phenols, and tannins in all the aqueous and methanolic extracts of the four plants which were studied (Table 4.4). Additionally, saponins and flavonoids were detected in all the aqueous and methanolic extracts, except in the methanolic extracts of *Senna singueana* and *Entada leptostachya* (Table 4.4). Conversely, alkaloids, phenols, flavonoids, tannins, and saponins were not detected in the dichloromethane extracts of all the studied plants (Table 4.4).

Table 4.4: Qualitative phytochemical composition of the aqueous, methanolic, and dichloromethane extracts of the studied plants

Phytochemical	*Entada leptostachya* stem bark			*Senna singueana* (Delile) Roots			*Securidaca longipendunculata* roots			*Strychnos henningsii* Gilg-stem bark		
	Aq.	**Me.**	**Dc.**	**Aq.**	**Me.**	**Dc.**	**Aq.**	**Me.**	**Dc.**	**Aq.**	**Me.**	**Dc.**
Alkaloids	+	+	-	+	+	-	+	+	-	+	+	-
Flavonoids	+	-	-	+	+	-	+	+	-	+	+	-
Saponins	+	+	-	+	-	-	+	+	-	+	+	-
Tannins	+	+	-	+	+	-	+	+	-	+	+	-
Phenols	+	+	-	+	+	-	+	+	-	+	+	-

+: Present; -: Absent; Aq.: Aqueous extract; Me.: Methanolic extract; Dc.: Dichloromethane extract

4.4 Cytotoxic effects of the aqueous, methanolic, and dichloromethane extracts of the studied plants

The results of the brine shrimp lethality assay showed that all the aqueous extracts of the plants which were investigated have high LC_{50} values (>1000µg/ml) except that of *Securidaca longipendunculata,* which posted an LC_{50} value of 170.66 µg/ml (Table 4.5). Similarly, the methanolic extracts of all the plants which were studied had high LC_{50} values (>1000µg/ml), except *Securidaca longipendunculata,* which had an LC_{50} value of 293.97µg/ml (Table 4.5). Besides, no LC_{50} values were predicted for all the dichloromethane extracts of the selected plants, as no nauplii mortalities were recorded in the respective setups. Overall, the positive control drug (Vincristine sulphate) showed the lowest LC_{50} value of 4.06 µg/ml in this study (Table 4.5). The information on the mortalities of brine shrimp nauplii recorded in this study is presented in Appendix 7.

Table 4.5: Cytotoxic effects of the aqueous, methanolic, and dichloromethane extracts of the studied plants

Drug	LC_{50} (µg/ml)		
	Aq.	**Me.**	**Dc.**
E. leptostachya	5789.69$^{\#*}$	16108.21$^{\#*}$	ND
S. singueana	24995.60$^{\#*}$	230149.13$^{\#*}$	ND
S. longipendunculata	170.66$^{\#\#**}$	293.93$^{\#\#**}$	ND
S. henningsii	1288.55$^{\#*}$	2180.37$^{\#*}$	ND
Vincristine sulphate		4.06$^{\#\#***}$	

The Superscript notations #, ##: represent non-cytotoxic and cytotoxic, respectively, based on Clarkson's criteria, while the superscript notations *, **, and *** represent non-cytotoxic, cytotoxic, and highly cytotoxic, respectively based on Meyer's criteria. ND: Not determined.

CHAPTER FIVE: DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

Herbal medicine plays a significant role in treating diverse diseases, especially in rural settings of less developed countries. A recent report by the WHO indicates that over 80 % of the world's human population relies on medicinal plants for their primary healthcare needs (World Health Organization, 2018). The increased popularity of herbal medicines is attributable to their easy accessibility, affordability, and presumed safety compared to conventional medicines (Othman and Farooqui, 2015; Ros *et al*., 2018; State *et al*., 2019; Wubetu *et al*., 2017). However, despite the longstanding usage of medicinal plants in traditional medicine, only a handful have been scientifically investigated. One of the hindrances to the appraisal of medicinal plants' potency and possible development is the lack of baseline ethnomedical information to spur empirical studies.

The management of snakebite envenomation using medicinal plants has been practiced since antiquity in many ethnic communities, especially those in rural settings (Okot *et al*., 2020). However, in most traditions, ethnomedical knowledge, including the traditional management of SBE, is undocumented and often passed across generations by word of mouth to trusted members of the family or relatives (Abbott, 2014; Abdullahi, 2011; Kewessa *et al*., 2015; Otieno and Analo, 2012). There is a high propensity to lose this critical information, especially if the family members are not interested and not appropriately documented (Biró *et al*., 2014). As a result, ethnomedical documentation is an important undertaking for heritage, conservational strategies, and the advancement of research.

The current study's findings revealed that most respondents (65%) acquired ethnomedical knowledge through their family members and relatives. This finding corroborates (Nadembega *et al*., 2011), who asserted that ethnomedical knowledge transmitted across

generations verbally to family members. However, most people who could inherit this art are often younger and disinterested, as they view it as archaic. As a result, valuable ethnomedical information is lost, especially when the bearer dies and no record of such information is available.

In the current study, shrub species were commonly used to manage SBE in Migwani Ward. Perhaps, this could be attributed to their relatively high resistance to drought conditions experienced in the study area, hence their unlimited availability throughout the year (Tolossa *et al*., 2013) made a similar observation. Furthermore, studies have shown that the abundance and availability of herbaceous plants in natural habitats such as forests largely influence their exploitation for medicinal purposes (Uniyal *et al*., 2006).

The most widely used plant part(s) in preparing SBE remedies were the leaves, perhaps due to their ease of harvest, and availability in large quantities, compared to other plant parts.This is in agreement with (Vitalini *et al*., 2013). Moreover, previous studies have indicated that the preference of leaves in traditional medicine to other parts is due to their perceived rich host of bioactive ingredients, such as alkaloids and tannins, associated with photosynthesis (Fortini *et al*., 2016). Furthermore, leaves produce and accumulate most phytochemical amalgams due to their involvement in photosynthesis (Ghorbani, 2005). Indeed, most phytochemicals possess pharmacologic activities, which are thought to confer therapeutic potency (Kurmukov, 2013; Moriasi *et al*., 2020a; Moriasi *et al*., 2021).

Notably, some respondents mentioned using single plant parts or a blend of many plant parts to thwart SBE complications. These findings are in agreement with previous reports on the use of various plant parts to mitigate ailments (Obakiro *et al*., 2020; Teklehaymanot and Giday, 2010). This practice could potentially be due to the synergistic effects of the combined plant parts, which produce amplified efficacy, and in a short time, hence helpful in cases of SBE (Obakiro *et al*., 2020; Teklehaymanot and Giday, 2010).

The most common herbal preparation methods included infusions, poultices, tinctures, decoctions, and powders administered orally, topically, or both to avert SBE (Cheikhyoussef *et al*., 2011; Muthee *et al*., 2011). It was observed that water was the primary medium for preparing most remedies, and additives like honey, cow milk, and sheep soup were added to enhance taste and palatability as reported by other authors (Amuka *et al*., 2014; Kamau *et al*., 2016; Kimondo *et al*., 2015; Odongo *et al*., 2018). This finding suggest that the administration of herbals through different routes could be due to increased bioavailability of the drug's bioactive constituents to counter SBE sequelae.

It was noticed that the mode of drug administration and type of treatment depended on the species of the snake, the age of the victim, and the presence of any other pre-existing conditions. This implies that the respondents were knowledgeable about the basic pharmacologic principles of their medicines' activity. Besides, it was evident that the study participants understood the dangers of a drug overdose and indicated that they use mutton soup, cow's milk, honey, activated charcoal, and water as antidotes. However, the herbalists claimed the potency of their herbal formulations would reduce if they revealed some specific adjuvants they add. Moreover, they argued that special offerings or rituals ought to be performed to reveal some of these adjuvants with the promise of utmost secrecy. This caveat is a tool employed by herbalists to protect their ethnomedical knowledge as noted by (Abel and Busia, 2005; Jeruto *et al*., 2010; Jima and Megersa, 2018), thus hindering knowledge sharing, especially in this study.

The relative frequency of citation (RFC) was used to determine the most used medicinal plant for managing SBE in the study area. The RFC index indicates the reliability and accuracy of the collected information, as it reveals the medicinal plants best known or with a long history of use by most of the participants (Faruque *et al*., 2018). Additionally, it is possible to preserve ethnomedical knowledge for heritage and the advancement of

scientific research. *Entada leptostachya, Senna singuena, Strychnos henningsii,* and *Securidaca longipendunculata* had the highest RFC indicating they were commonly utilized among the study area's population manage SBE. As a result, these plants were selected and screened for their qualitative phytochemical composition and cytotoxic effects on brine shrimp nauplii, in order to lay a framework for further characterisation and development of bioactive components, which can be used as therapies for SBE.

Upon extraction, the yields of studied plants' extracts were varied according to the solvent used. The variations were attributed to the different polarity indices of the solvents, which solubilise and extract amalgams of corresponding polarity (Dhanani *et al*., 2017). Previously, Moriasi *et al.* (2020a) highlighted that polar solvents such as methanol and water extract antioxidant-associated phytochemicals, which possess diverse pharmacological activities. Owing to the profound usage of *E. leptostachya, S. singuena, S. henningsii,* and *S. longipendunculata* to manage SBE in the study area, their pharmacologic efficacy could be due to the presence of polar phytocompounds, such as phenols and flavonoids (Truong *et al*., 2019). Besides, the absence of certain phytochemicals in one sample and their presence in the others can be attributed to the various physiological and biosynthetic reactions of the plant and the agroecological conditions of the study area (Moriasi *et al*., 2020c; Olela *et al*., 2020). Additionally, the absence of the tested phytochemicals in the dichloromethane extracts of the studied plants could be attributed to the low polarity of the solvent, which hindered their extraction (Dhanani *et al*., 2017).

Previous studies indicate that various phytochemicals, such as flavonoids, polyphenols, saponins, tannins, and alkaloids, inactivate toxic venom proteins (Sani *et al*., 2020). Flavonoids have been shown to inhibit phospholipases A_2, an important ingredient of snake venoms (Kadir *et al*., 2015). The presence of flavonoids in all crude plant extract

may confirm their use in SBE management. Additionally, flavonoids, phenols, tannins, and alkaloids have been shown to act as antidotes to snake venoms, with the ability to reverse the deleterious effects of SBE (Kulatunga and Arawwawala, 2019; Soares *et al*., 2005). Therefore, the studied plant extracts are a valuable reservoir of bioactive compounds of pharmacological significance, which warrant further investigations.

The brine shrimp lethality assay technique of (Meyer *et al*., 1982). was adopted to appraise the studied plant extracts' cytotoxicity. A drug agent or chemical which kills the exposed brine shrimp nauplii is considered a cytotoxic agent in this assay. Furthermore, median lethal concentration (LC_{50}) values are widely used to evaluate the cytotoxic efficacy of drugs and chemicals in bioassays, whereby low values indicate high cytotoxic efficacy. According to Meyer`s cytotoxicity classification criteria, plant extracts with $LC_{50}$<1000µg/ml are considered toxic, while those with LC50>1000µg/ml are considered non-toxic, hence safe (Meyer *et al*., 1982). Additionally, Clarkson`s toxicity criteria classify plant extracts non-toxic (LC_{50}>1000µg/ml), slightly toxic (LC_{50}= 500-999µg/ml), moderately toxic/toxic (LC_{50}= 99-499µg/ml), and highly toxic (LC50= 0-100µg/ml), respectively (Clarkson *et al*., 2004).

Accordingly, based on Meyer's and Clarkson's criteria, the aqueous, methanolic, and dichloromethane extracts *E. leptostachya, S. singueana,* and *S. henningsii* were non-toxic safe to brine shrimp nauplii since their LC_{50} values were higher than 1000µg/ml. The safety of these plants was attributed to the absence or low concentrations of toxicity-associated phytocompounds. Conversely, the aqueous and methanolic extracts of *S. longipendunculata* were moderately toxic, which calls for caution when they are used for SBE management. However, the extracts can be used as cytotoxic agents in appropriate settings. Nevertheless, further toxicological investigations should be performed, in order to establish the toxicity profile and safety of the studied plant extracts.

5.2 Conclusions

The following conclusions were made from the results obtained from the study:

i. In this study, fourteen (14) medicinal plants are used to manage SBE in Migwani Ward, Mwingi-West Sub-County, Kitui County, Kenya were documented. Out of all the documented plants, *Entada leptostachya, Strychnos henningsii, Securidaca longipendunculata,* and *Senna singueana* were most frequently cited as remedies for SBE.

ii. The mostly used plants against SBE in Migwani Ward, West Mwingi Sub-County (*Entada leptostachya, Strychnos henningsii, Securidaca longipendunculata,* and *Senna singueana*) contain phytochemicals associated with snake antivenom activity.

iii. Three of the aqueous, methanolic, and dichloromethane extracts of the commonly used plants against SBE in Migwani Ward, West Mwingi Sub-County that is *Entada leptostachya, Strychnos henningsii,* and *Senna singueana* were non-cytotoxic and safe, whereas, the aqueous and methanolic extracts of *Securidaca longipendunculata* were moderately toxic to brine shrimp nauplii.

Therefore, the research questions which were formulated in this study were answered in the affirmative.

5.3 Recommendations

Based on this study's findings, further empirical investigations of the plants which were studied and their extracts should be conducted, in order to determine their potential to avert SBE in *in vivo* models. Besides, further phytochemical analyses should be done on four selected plants, in order to identify the specific bioactive molecules and their mode(s) of pharmacologic activity against SBE. Further toxicological investigations involving the

studied plant extracts, using other models, should be performed in order to establish the toxicological profiles and safety.

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APPENDICES

Appendix 1: CONSENT FORM

Title of the Study: Ethnobotanical Documentation, Phytochemical Screening, and Cytotoxicity Evaluation of Medicinal Plants Used to Manage Snakebite Envenomation in Mwingi West Sub County, Kenya

Principal investigator: Stella Kwamboka Mokua

Study Location: Mwingi West Sub County, Kitui County, Kenya

Purpose of the Study: Academic (MSc. Thesis)

Dear Participant,

You have been selected to participate in this study. The study seeks; To help Ms. Stella Kwamboka Mokua, a postgraduate student undertaking an MSc. Degree in Pharmacology and Toxicology at the University of Nairobi.

To facilitate the collection of the relevant data, the investigator shall use a structured questionnaire containing questions about the plants you use or are used to manage snakebite envenomation in this area, the parts used, modes of preparation, and routes of administration.

Confidentiality: The information you provide is confidential and will only be used for research purposes. Your participation is entirely voluntary, and you can withdraw from the study even after having agreed to participate.

Potential Benefits of the study: Ethnomedical documentation of plants used to manage snakebite envenomation for heritage and advancement of science.

Potential Risk of the Study: There is no risk associated with your participation in this study.

Participant's declaration: I am a native resident/ herbalist of this region, have understood the purpose of this study, and am able to answer questions you have regarding the the use of traditional medicines used to manage snakebite envenomation.

Name:……..

Signature: …….

Contact information: ……..

NB: If you have any questions about this study, you may ask me now or contact me via this address: P.O BOX 30197-00100, Nairobi: Phone Number: 0718312929

Appendix 2: ETHNOBOTANICAL DATA COLLECTION QUESTIONNAIRE

INSTRUCTIONS

Please fill in the blank spaces and tick in the appropriate Box

Personal Information on Respondents

1) Name:

……...

2) Gender:

Male ☐

Female ☐

3) Please specify your age category ……..

20-35 years ☐

36-50 years ☐

50-65 years ☐

65 and above ☐

4) Education level: ……

Primary

Secondary

Tertiary

Others specify

5) Source of income;

Employment ☐

Business ☐

Others, specify……

Indicate Professional experience on the management of venomous bites

6) Practice specification

Herbalists ☐

Traditional practitioners ☐

Local people ☐

Others

7) Indicate years of experience? ……

8) What is the source of your knowledge?

……...

9) Are you aware of Snakebites in the area?

Yes ☐

No ☐

10) Have you come across somebody bitten by a snake?

Yes ☐

No ☐

If yes, what plant (s) or combination do you use in managing the bite? …….

……

…….

11) Where do you get the plant(s) from? ……

……...

12) Are the plants available?

Readily available ☐

Scarcely available ☐

Available in season:

Cold ☐

Hot ☐

13) Which part(s) of the plant did you use to prepare the remedy?

Leaves ☐

Bark ☐

Roots ☐

Flowers ☐

Pulp ☐

Fruit ☐

Seed ☐

14) How do you prepare the herbal plant?

Dried ☐

Fresh ☐

Others specify ……..……

……..…

15) Please indicate how the prepared drug is administered

Oral ☐

Topical application ☐

Others specify……

16) Indicate amount used …….

17) For how long? ……

18) Do you give verbal instructions?

19) When administering this remedy?

Yes ☐

No ☐

20) Are the plant(s) used safely?

Yes ☐

No ☐

Thank you.

Appendix 3: RESEARCH APPROVAL BY THE BIOSAFETY, ANIMAL USE AND ETHICS COMMITTEE

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY
P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2021/294

Ms. Stella Kwamboka Mokua,
University of Nairobi
Dept. of PHP & Toxicology,
11/03/2021

Dear Stella,

RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee

Ethnobotanical survey, phytochemical screening and safety of plants used for the management of venomous snakes bites.

Stella Mokua J56/35759/209

We refer to your MSc. proposal submitted to our committee for review and your application letter dated 20th February 2021. We have reviewed your application for ethical clearance for the study.

The phytochemical screening protocol and bioactivity of plants using brine shrimps meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.

We have also noted that KVB registered veterinary surgeons will supervise the study.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, Ph.D
Chairperson, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi

Appendix 4: RSEARCH PERMIT GRANTED BY THE NATIONAL COMMISION FOR SCIENCE,TECHNOLOGY, AND INNOVATION (NACOSTI)

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **867665**

Date of Issue: **17/June/2021**

RESEARCH LICENSE

This is to Certify that Dr.. STELLA KWAMBOKA MOKUA of University of Nairobi, has been licensed to conduct research in Kitui on the topic: ETHNOBOTANICAL SURVEY, PHYTOCHEMICAL SCREENING AND BIOACTIVITY OF PLANTS USED FOR MANAGEMENT OF VENOMOUS SNAKE BITES. for the period ending : 17/June/2022.

License No: **NACOSTI/P/21/11115**

867665

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix 5: YIELDS OF THE AQUEOUS, METHANOLIC, AND DICLOMETHANE EXTRACTS OF THE SELECTED PLANTS

Plant name	**Amount of powder (g) aqueous**	**Extract yield (g) aqueous**	**% Extract yield aqueous**	**Extract yield (g) Methanol**	**%Extract yield Methanol**	**Extract yield (g) DCM**	**% Extract yield DCM**
Entada leptostachya	200	8	4	2.43	1.215	0.97	0.485
Senna singueana	200	10	5	2.85	1.425	2.9	1.45
Securidaca longipendunculata	200	20	10	3.93	1.965	1.02	0.51
Strychnos henningsii	200	10	5	2.06	1.03	0.92	0.46

Appendix 6: BRINE SHRIMP LETHALITY ASSAY RESULTS OF THE AQUEOUS, METHANOLIC, AND DICHLOROMETHANE EXTRACTS OF THE SELECTED PLANTS

Sample	**Mortality per dose**			**Lethal concentration**	**Toxicity Appraisal**	
	10µg/ml	**100µg/ml**	**1000µg/ml**	**LC50**	**Meyer`s criteria**	**Clarkson`s criteria**
Vincristine sulphate	36	49	50	4.06	cytotoxic	Highly cytotoxic
The aqueous stem bark extract of *E.leptostachya*	0	11	13	5789.69	N0n cytotoxic	Non-cytotoxic
Aqueous roots extract of *S.singueana*	6	8	16	24995.60	Non cytotoxic	Non cytotoxic
Aqueous roots extracts *S.longipendunculata*	9	10	42	170.66	cytotoxic	Moderately cytotoxic
The aqueous stem bark of *S. henningsii*	9	10	42	1288.55	non-cytotoxic	Non-cytotoxic
The methanol stem bark of *E.leptostachya*	1	4	11	16108.21	Non-cytotoxic	Non-cytotoxic
Methanol roots extract of *S. singueana*	3	6	10	230149.13	Non-cytotoxic	Non-cytotoxic
Methanol roots extract of *S.longipendunculata*	7	8	39	293.93	cytotoxic	Moderately cytotoxic
Methanol stem bark extract of *S.henningsii*	6	4	24	2180.37	Non cytotoxic	Non cytotoxic
Dichloromethane stem bark extract of E. *leptostachya*	0	0	0	No death	Non-cytotoxic	Non-cytotoxic
Diclomethane roots extract of *S.singueana*	0	0	0	No death	Non cytotoxic	Non cytotoxic
Diclomethane roots extract of *S.longipendunculata*	0	0	0	No death	Non cytotoxic	Non cytotoxic
Diclomethane stem bark extract of *S.henningsii*	0	0	0	No death	Non cytotoxic	Non cytotoxic

Appendix 7: Aqueous Extract of *Strychnos henningsii*

```
PROBIT Response_frequency OF Total_observed_animals WITH Concentration
  /LOG 10
  /MODEL PROBIT
  /PRINT FREQ CI
  /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
```

Probit Analysis

Notes

Output Created		24-MAY-2021 17:36:58
Comments		
Input	Active Dataset	DataSet0
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	16
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		PROBIT Response_frequency OF Total_observed_animals WITH Concentration /LOG 10 /MODEL PROBIT /PRINT FREQ CI /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
Resources	Processor Time	00:00:00.72
	Elapsed Time	00:00:00.98

[DataSet0]

Warnings

Relative Median Potency Estimates are not displayed because there is no grouping variable in the model.

Data Information

		N of Cases
Valid		15
	Missing	1
Rejected	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	13	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	0.756	0.160	4.737	0.000	0.443	1.069
	Intercept	-2.352	0.384	-6.129	0.000	-2.736	-1.969

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	23.226	13	0.039[a]

a. Since the significance level is less than .050, a heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.000	10	1	.552	.448	.055
	2	1.000	10	0	.552	-.552	.055
	3	1.000	10	0	.552	-.552	.055
	4	1.000	10	2	.552	1.448	.055
	5	1.000	10	1	.552	.448	.055
	6	2.000	10	0	2.006	-2.006	.201
	7	2.000	10	0	2.006	-2.006	.201
	8	2.000	10	4	2.006	1.994	.201
	9	2.000	10	3	2.006	.994	.201
	10	2.000	10	0	2.006	-2.006	.201
	11	3.000	10	4	4.668	-.668	.467
	12	3.000	10	3	4.668	-1.668	.467
	13	3.000	10	6	4.668	1.332	.467
	14	3.000	10	4	4.668	-.668	.467
	15	3.000	10	8	4.668	3.332	.467

Confidence Limits

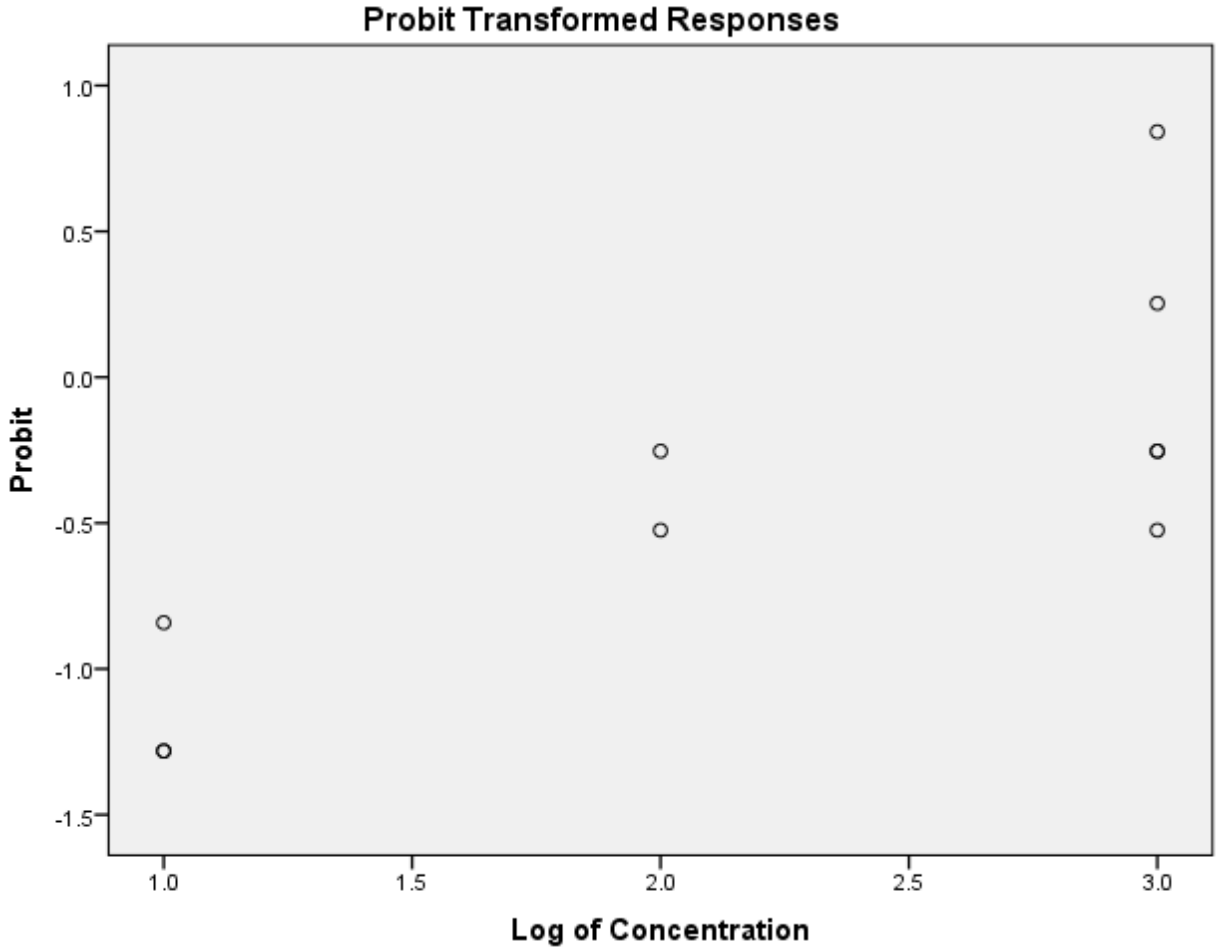
	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[b]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	.010	1.083	0.000	9.163	0.035	-3.549	0.962
	.020	2.483	0.002	15.848	0.395	-2.640	1.200

	.030	4.203	0.009	22.588	0.624	-2.066	1.354
	.040	6.245	.023	29.636	.796	-1.637	1.472
	.050	8.619	.051	37.119	.935	-1.289	1.570
	.060	11.339	.101	45.134	1.055	-.995	1.655
	.070	14.420	.182	53.772	1.159	-.739	1.731
	.080	17.884	.308	63.129	1.252	-.511	1.800
	.090	21.752	.495	73.310	1.337	-.305	1.865
	.100	26.047	.763	84.438	1.416	-.118	1.927
	.150	54.931	4.317	160.354	1.740	.635	2.205
	.200	99.394	15.297	298.791	1.997	1.185	2.475
	.250	165.315	39.305	587.169	2.218	1.594	2.769
	.300	261.056	79.161	1248.015	2.417	1.899	3.096
	.350	398.658	134.410	2828.149	2.601	2.128	3.452
	.400	595.760	204.968	6661.142	2.775	2.312	3.824
	.450	878.768	293.155	16047.126	2.944	2.467	4.205
	.500	1288.252	403.836	39357.877	3.110	2.606	4.595
	.550	1888.546	544.711	98585.172	3.276	2.736	4.994
	.600	2785.676	727.507	254331.559	3.445	2.862	5.405
	.650	4162.956	970.539	684738.414	3.619	2.987	5.836
	.700	6357.234	1303.908	1961071.724	3.803	3.115	6.292
	.750	10038.966	1780.679	6147250.191	4.002	3.251	6.789
	.800	16697.058	2503.834	22074708.834	4.223	3.399	7.344
	.850	30212.403	3703.195	98543771.158	4.480	3.569	7.994
	.900	63714.178	6021.198	651477171.261	4.804	3.780	8.814

.910	76296.423	6765.830	1028891735.678	4.883	3.830	9.012
.920	92796.550	7677.232	1690864557.125	4.968	3.885	9.228
.930	115086.475	8818.848	2920557840.757	5.061	3.945	9.465
.940	146366.174	10291.921	5379151108.559	5.165	4.012	9.731
.950	192543.210	12269.438	10799721901.280	5.285	4.089	10.033
.960	265730.056	15075.790	24505589793.090	5.424	4.178	10.389
.970	394861.943	19407.461	67146914973.807	5.596	4.288	10.827
.980	668490.784	27124.730	256665901315.840	5.825	4.433	11.409
.990	1532767.754	45893.270	2128095072790.879	6.185	4.662	12.328

a. A heterogeneity factor is used.

b. Logarithm base = 10.


Probit Transformed Responses
Probit
1.0
0.5
0.0
-0.5
-1.0
-1.5
1.0
1.5
2.0
2.5
3.0
Log of Concentration

Appendix 8: Data Output – Aqueous Extract of *Securidaca longipenduculata*

```
PROBIT Response_frequency OF Total_observed_animals WITH Concentration
  /LOG 10
  /MODEL PROBIT
  /PRINT FREQ CI
  /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
```

Probit Analysis

Notes

Output Created		24-MAY-2021 17:28:20
Comments		
Input	Active Dataset	DataSet0
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	15
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		PROBIT Response_frequency OF Total_observed_animals WITH Concentration /LOG 10 /MODEL PROBIT /PRINT FREQ CI /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
Resources	Processor Time	00:00:00.66
	Elapsed Time	00:00:01.12

[DataSet0]

Warnings

Relative Median Potency Estimates are not displayed because there is no grouping variable in the model.

Data Information

		N of Cases
Valid		15
	Missing	0
Rejected	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	10	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	0.807	0.141	5.724	0.000	0.531	1.083
	Intercept	-1.801	0.306	-5.879	0.000	-2.108	-1.495

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	34.210	13	.001[a]

a. Since the significance level is less than .050, a heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.000	10	0	1.600	-1.600	.160
	2	1.000	10	4	1.600	2.400	.160
	3	1.000	10	3	1.600	1.400	.160
	4	1.000	10	4	1.600	2.400	.160
	5	1.000	10	2	1.600	.400	.160
	6	2.000	10	3	4.257	-1.257	.426
	7	2.000	10	2	4.257	-2.257	.426
	8	2.000	10	0	4.257	-4.257	.426
	9	2.000	10	1	4.257	-3.257	.426
	10	2.000	10	4	4.257	-.257	.426
	11	3.000	10	8	7.323	.677	.732
	12	3.000	10	7	7.323	-.323	.732
	13	3.000	10	7	7.323	-.323	.732
	14	3.000	10	10	7.323	2.677	.732
	15	3.000	10	10	7.323	2.677	.732

Confidence Limits

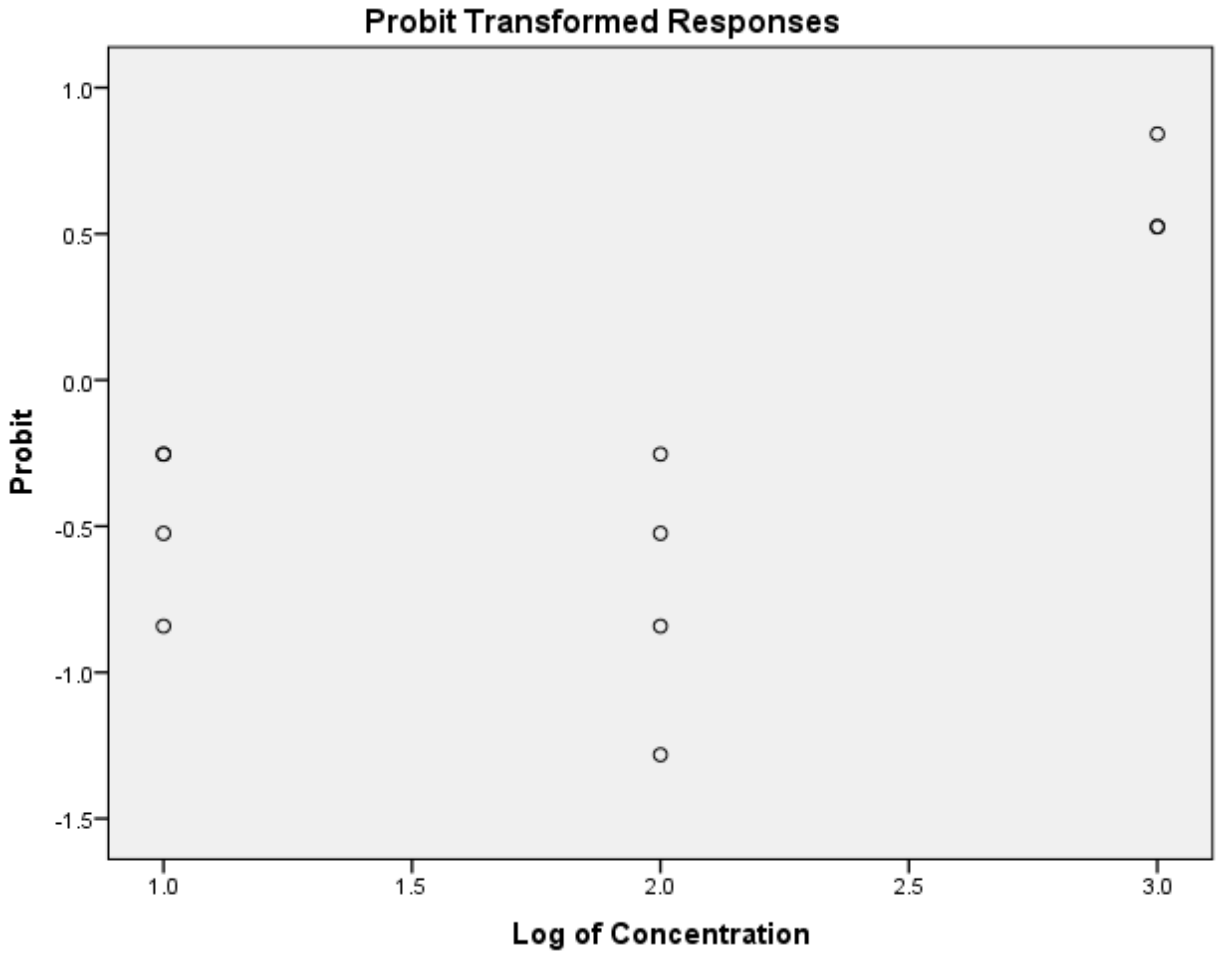
	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[b]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	.010	.223	.000	2.727	-.651	-4.946	.436
	.020	.487	.000	4.519	-.313	-4.085	.655
	.030	.797	.000	6.248	-.099	-3.540	.796

	.040	1.155	.001	7.991	.063	-3.131	.903
	.050	1.562	.002	9.781	.194	-2.799	.990
	.060	2.020	.003	11.636	.305	-2.517	1.066
	.070	2.531	.005	13.570	.403	-2.271	1.133
	.080	3.097	.009	15.593	.491	-2.051	1.193
	.090	3.721	.014	17.718	.571	-1.852	1.248
	.100	4.406	.021	19.952	.644	-1.668	1.300
	.150	8.867	.121	33.183	.948	-.917	1.521
	.200	15.459	.464	51.211	1.189	-.334	1.709
	.250	24.905	1.418	77.021	1.396	.152	1.887
	.300	38.219	3.695	116.376	1.582	.568	2.066
	.350	56.837	8.446	181.226	1.755	.927	2.258
	.400	82.828	17.153	297.704	1.918	1.234	2.474
	.450	119.236	31.211	524.877	2.076	1.494	2.720
	.500	170.659	51.606	999.723	2.232	1.713	3.000
	.550	244.259	79.207	2051.284	2.388	1.899	3.312
	.600	351.627	115.505	4512.558	2.546	2.063	3.654
	.650	512.419	163.363	10644.025	2.710	2.213	4.027
	.700	762.037	227.944	27154.491	2.882	2.358	4.434
	.750	1169.418	318.588	76471.342	3.068	2.503	4.883
	.800	1884.005	453.395	247103.004	3.275	2.656	5.393
	.850	3284.710	672.297	986657.663	3.516	2.828	5.994
	.900	6610.771	1085.054	5729237.891	3.820	3.035	6.758
	.910	7827.412	1215.601	8779726.799	3.894	3.085	6.943

.920	9404.170	1374.323	13969445.234	3.973	3.138	7.145
.930	11506.874	1571.690	23296070.456	4.061	3.196	7.367
.940	14415.644	1824.305	41275724.510	4.159	3.261	7.616
.950	18640.731	2160.323	79325680.085	4.270	3.335	7.899
.960	25212.302	2632.103	171089651.038	4.402	3.420	8.233
.970	36546.368	3350.916	440758587.197	4.563	3.525	8.644
.980	59865.100	4610.108	1553708296.772	4.777	3.664	9.191
.990	130311.729	7595.398	11358051434.396	5.115	3.881	10.055

a. A heterogeneity factor is used.

b. Logarithm base = 10.



Appendix 9: Data Output – Aqueous Stem bark extracts of *Entada lepstrachya*

```
PROBIT Response_frequency OF Total_observed_animals WITH Concentration
  /LOG 10
  /MODEL PROBIT
  /PRINT FREQ CI
  /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
```

Probit Analysis

Notes

Output Created		24-MAY-2021 16:52:34
Comments		
Input	Active Dataset	DataSet0
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	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	15
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		PROBIT Response_frequency OF Total_observed_animals WITH Concentration /LOG 10 /MODEL PROBIT /PRINT FREQ CI /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
Resources	Processor Time	00:00:03.28
	Elapsed Time	00:00:06.95

[DataSet0]

Warnings

Relative Median Potency Estimates are not displayed because there is no grouping variable in the model.

Data Information

		N of Cases
Valid		15
	Missing	0
Rejected	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	13	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	0.643	0.185	3.479	0.001	0.281	1.005
	Intercept	-2.419	0.454	-5.328	0.000	-2.873	-1.965

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	17.614	13	.173[a]

a. Since the significance level is greater than .050, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.000	10	0	.379	-.379	.038
	2	1.000	10	0	.379	-.379	.038
	3	1.000	10	0	.379	-.379	.038
	4	1.000	10	0	.379	-.379	.038
	5	1.000	10	0	.379	-.379	.038
	6	2.000	10	0	1.286	-1.286	.129
	7	2.000	10	3	1.286	1.714	.129
	8	2.000	10	3	1.286	1.714	.129
	9	2.000	10	2	1.286	.714	.129
	10	2.000	10	3	1.286	1.714	.129
	11	3.000	10	2	3.120	-1.120	.312
	12	3.000	10	0	3.120	-3.120	.312
	13	3.000	10	3	3.120	-.120	.312
	14	3.000	10	4	3.120	.880	.312
	15	3.000	10	4	3.120	.880	.312

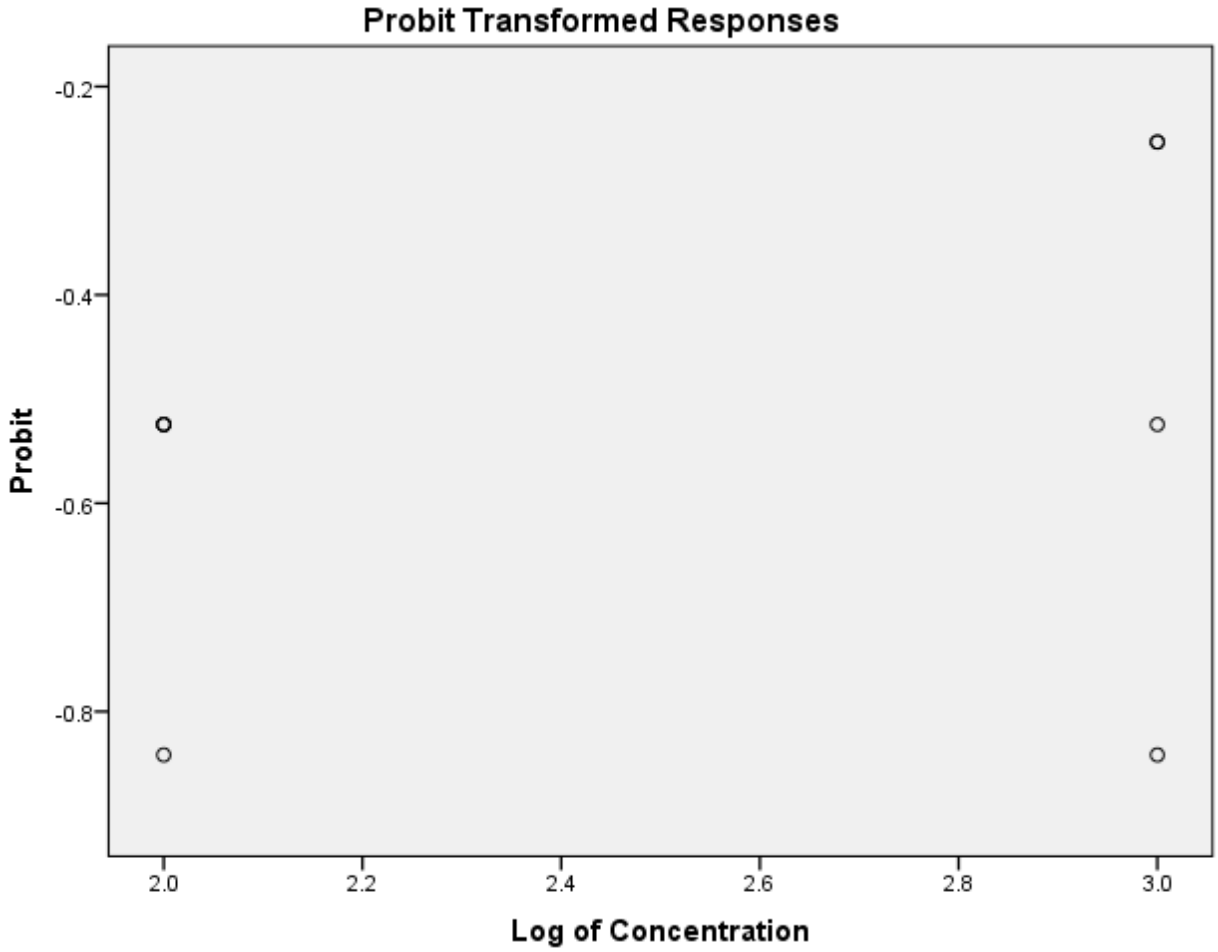
Confidence Limits

	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	1.393	.002	10.063	.144	-2.768	1.003
	.020	3.699	.015	19.419	.568	-1.811	1.288
	.030	6.872	.062	29.726	.837	-1.208	1.473
	.040	10.951	.175	41.240	1.039	-.757	1.615
	.050	15.998	.404	54.176	1.204	-.394	1.734
	.060	22.090	.819	68.779	1.344	-.087	1.837
	.070	29.312	1.512	85.349	1.467	.179	1.931
	.080	37.762	2.599	104.268	1.577	.415	2.018
	.090	47.544	4.222	126.025	1.677	.626	2.100
	.100	58.775	6.548	151.257	1.769	.816	2.180
	.150	141.408	35.132	369.071	2.150	1.546	2.567
	.200	284.123	102.464	977.276	2.454	2.011	2.990
	.250	516.989	206.118	2806.086	2.713	2.314	3.448
	.300	885.006	342.897	8147.376	2.947	2.535	3.911
	.350	1456.419	519.146	23157.336	3.163	2.715	4.365
	.400	2336.532	747.388	64245.453	3.369	2.874	4.808
	.450	3691.373	1045.798	175312.232	3.567	3.019	5.244
	.500	5789.688	1440.500	475765.626	3.763	3.159	5.677
	.550	9080.764	1970.172	1300313.818	3.958	3.295	6.114
	.600	14346.257	2694.322	3630567.457	4.157	3.430	6.560
	.650	23015.701	3708.788	10534219.126	4.362	3.569	7.023

	.700	37876.017	5176.989	32475300.142	4.578	3.714	7.512
	.750	64837.952	7398.941	109755525.435	4.812	3.869	8.040
	.800	117978.704	10983.796	427042967.825	5.072	4.041	8.630
	.850	237047.542	17363.610	2086193423.488	5.375	4.240	9.319
	.900	570320.131	30806.315	15394758757.537	5.756	4.489	10.187
	.910	705034.160	35368.560	24956804926.643	5.848	4.549	10.397
	.920	887677.110	41087.910	42187766812.843	5.948	4.614	10.625
	.930	1143561.980	48442.213	75153534616.546	6.058	4.685	10.876
	.940	1517461.598	58212.073	143247998551.862	6.181	4.765	11.156
	.950	2095259.922	71766.816	299004683245.264	6.321	4.856	11.476
	.960	3060984.305	91753.524	710008647882.359	6.486	4.963	11.851
	.970	4878029.209	124065.940	2056560909815.606	6.688	5.094	12.313
	.980	9063119.899	185192.192	8459389837948.688	6.957	5.268	12.927

.990	24060660.780	347870.435	786672234606 88.610	7.381	5.541	13.896

a. Logarithm base = 10.


Probit Transformed Responses
Probit
-0.2
-0.4
-0.6
-0.8
2.0
2.2
2.4
2.6
2.8
3.0
Log of Concentration

Appendix 10: DATA OUTPUT – AQUEOUS EXTRACTS OF *Senna singuena*

```
PROBIT Response_frequency OF Total_observed_animals WITH Concentration
  /LOG 10
  /MODEL PROBIT
  /PRINT FREQ CI
  /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
```

Probit Analysis

Notes

Output Created		24-MAY-2021 17:24:52
Comments		
Input	Active Dataset	DataSet0
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	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	15
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		PROBIT Response_frequency OF Total_observed_animals WITH Concentration /LOG 10 /MODEL PROBIT /PRINT FREQ CI /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
Resources	Processor Time	00:00:00.59
	Elapsed Time	00:00:00.97

[DataSet0]

Warnings

Relative Median Potency Estimates are not displayed because there is no grouping variable in the model.

Data Information

		N of Cases
Valid		15
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	10	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	0.367	0.148	2.472	0.013	0.076	0.657
	Intercept	-1.612	0.341	-4.725	0.000	-1.954	-1.271

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	19.106	13	.120[a]

a. Since the significance level is greater than .050, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.000	10	0	1.064	-1.064	.106
	2	1.000	10	0	1.064	-1.064	.106
	3	1.000	10	1	1.064	-.064	.106
	4	1.000	10	4	1.064	2.936	.106
	5	1.000	10	1	1.064	-.064	.106
	6	2.000	10	0	1.897	-1.897	.190
	7	2.000	10	0	1.897	-1.897	.190
	8	2.000	10	3	1.897	1.103	.190
	9	2.000	10	2	1.897	.103	.190
	10	2.000	10	3	1.897	1.103	.190
	11	3.000	10	2	3.042	-1.042	.304
	12	3.000	10	4	3.042	.958	.304
	13	3.000	10	3	3.042	-.042	.304
	14	3.000	10	4	3.042	.958	.304
	15	3.000	10	3	3.042	-.042	.304

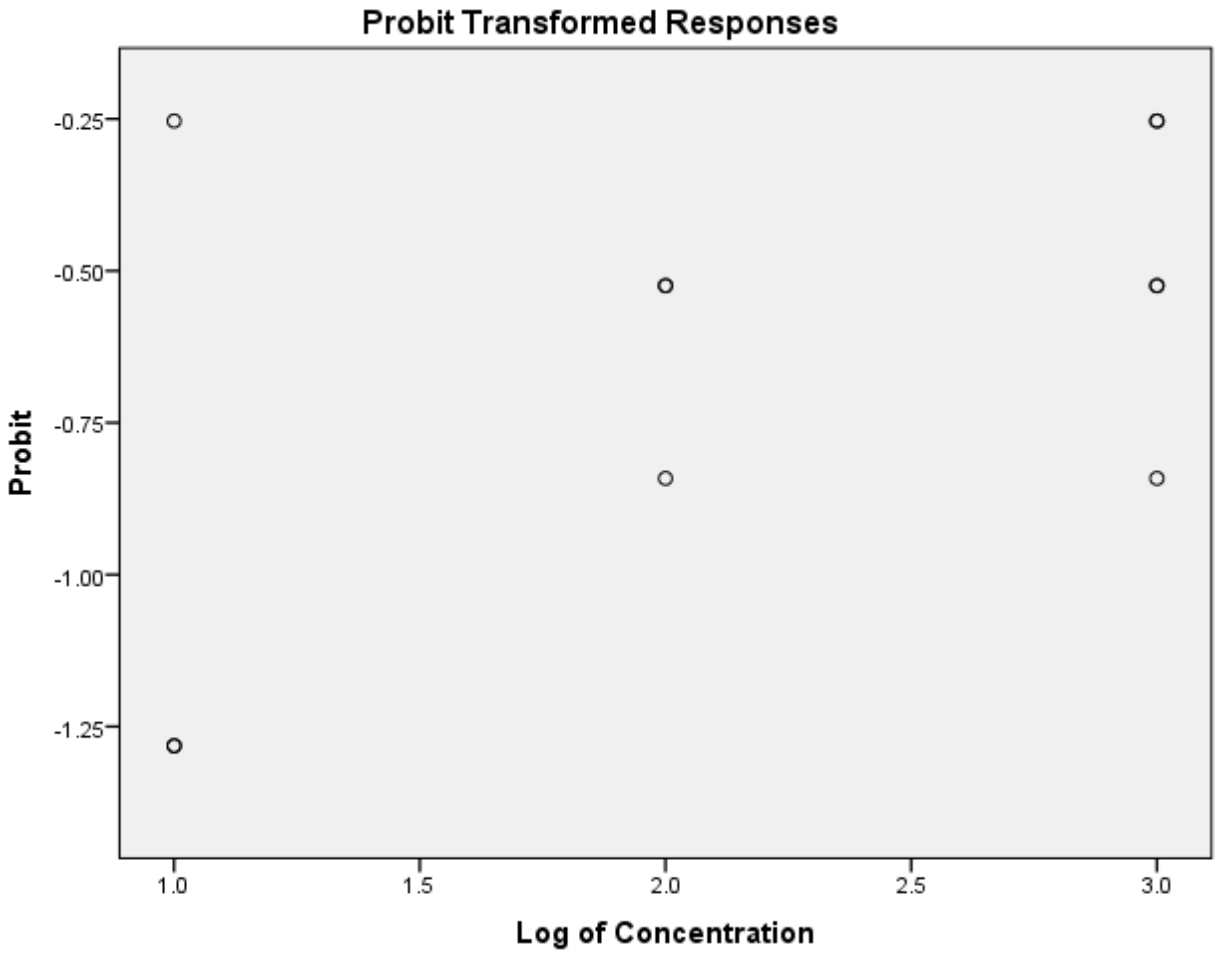
Confidence Limits

	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	.011	.000	.850	-1.948	-17.725	-.071
	.020	.062	.000	2.278	-1.204	-14.148	.358
	.030	.185	.000	4.294	-.732	-11.882	.633
	.040	.419	.000	6.965	-.378	-10.180	.843
	.050	.815	.000	10.387	-.089	-8.798	1.017
	.060	1.435	.000	14.689	.157	-7.625	1.167
	.070	2.357	.000	20.038	.372	-6.599	1.302
	.080	3.675	.000	26.656	.565	-5.684	1.426
	.090	5.504	.000	34.842	.741	-4.855	1.542
	.100	7.982	.000	45.009	.902	-4.096	1.653
	.150	37.218	.089	161.724	1.571	-1.050	2.209
	.200	126.516	9.112	1153.609	2.102	.960	3.062
	.250	361.440	76.993	39013.812	2.558	1.886	4.591
	.300	927.768	207.956	2318674.332	2.967	2.318	6.365
	.350	2222.351	411.071	129734821.470	3.347	2.614	8.113
	.400	5090.970	724.549	6400843853.827	3.707	2.860	9.806
	.450	11352.542	1210.060	288286198415.961	4.055	3.083	11.460

	.500	24995.601	1966.651	124589268093 99.713	4.398	3.294	13.095
	.550	55034.378	3159.146	544772902333 282.400	4.741	3.500	14.736
	.600	122723.175	5073.397	255159662599 74896.000	5.089	3.705	16.407
	.650	281134.763	8230.623	136775928659 5149570.000	5.449	3.915	18.136
	.700	673422.534	13643.530	912596740490 20580000.000	5.828	4.135	19.960
	.750	1728585.485	23451.312	852276399087 7305000000.0 00	6.238	4.370	21.931
	.800	4938337.403	42723.585	133676616675 414320000000 0.000	6.694	4.631	24.126
	.850	16787116.814	85685.882	485907158947 023000000000 000.000	7.225	4.933	26.687
	.900	78269544.683	204958.419	812412703094 911500000000 000000.000	7.894	5.312	29.910
	.910	113523037.41 0	252900.412	488067893221 180150000000 0000000.000	8.055	5.403	30.688

.920	170028706.505	317719.761	342380159324119550000000000000000.000	8.231	5.502	31.535
.930	265109937.379	408248.001	291636706543946260000000000000000.000	8.423	5.611	32.465
.940	435380808.721	540051.915	319131255259293260000000000000000.000	8.639	5.732	33.504
.950	766625867.959	742873.578	488882357436583900000000000000000.000	8.885	5.871	34.689
.960	1490273625.044	1080146.299	1.207E+036	9.173	6.033	36.082
.970	3374150954.795	1710694.130	6.224E+037	9.528	6.233	37.794
.980	9998623682.806	3150511.685	1.176E+040	10.000	6.498	40.070
.990	55400941001.775	8239870.929	4.557E+043	10.744	6.916	43.659

a. Logarithm base = 10.


Probit Transformed Responses
Probit
-0.25
-0.50
-0.75
-1.00
-1.25
1.0
1.5
2.0
2.5
3.0
Log of Concentration

Appendix 11: DATA OUTPUT – METHANOL EXTRACT OF *Strychnos henningsii*

```
PROBIT Response_frequency OF Total_observed_animals WITH Concentration
  /LOG 10
  /MODEL PROBIT
  /PRINT FREQ CI
  /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
```

Probit Analysis

Notes

Output Created		24-MAY-2021 17:50:55
Comments		
Input	Active Dataset	DataSet0
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	16
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		PROBIT Response_frequency OF Total_observed_animals WITH Concentration /LOG 10 /MODEL PROBIT /PRINT FREQ CI /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
Resources	Processor Time	00:00:00.64
	Elapsed Time	00:00:00.95

[DataSet0]

Warnings

Relative Median Potency Estimates are not displayed because there is no grouping variable in the model.

Data Information

		N of Cases
Valid		15
	Missing	1
Rejected	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	12	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	0.632	0.152	4.153	0.000	0.334	0.931
	Intercept	-2.111	0.361	-5.849	0.000	-2.472	-1.750

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	27.283	13	.011[a]

a. Since the significance level is less than .050, a heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.000	10	0	.696	-.696	.070
	2	1.000	10	1	.696	.304	.070
	3	1.000	10	0	.696	-.696	.070
	4	1.000	10	2	.696	1.304	.070
	5	1.000	10	3	.696	2.304	.070
	6	2.000	10	0	1.987	-1.987	.199
	7	2.000	10	0	1.987	-1.987	.199
	8	2.000	10	3	1.987	1.013	.199
	9	2.000	10	1	1.987	-.987	.199
	10	2.000	10	0	1.987	-1.987	.199
	11	3.000	10	3	4.153	-1.153	.415
	12	3.000	10	6	4.153	1.847	.415
	13	3.000	10	5	4.153	.847	.415
	14	3.000	10	7	4.153	2.847	.415
	15	3.000	10	3	4.153	-1.153	.415

Confidence Limits

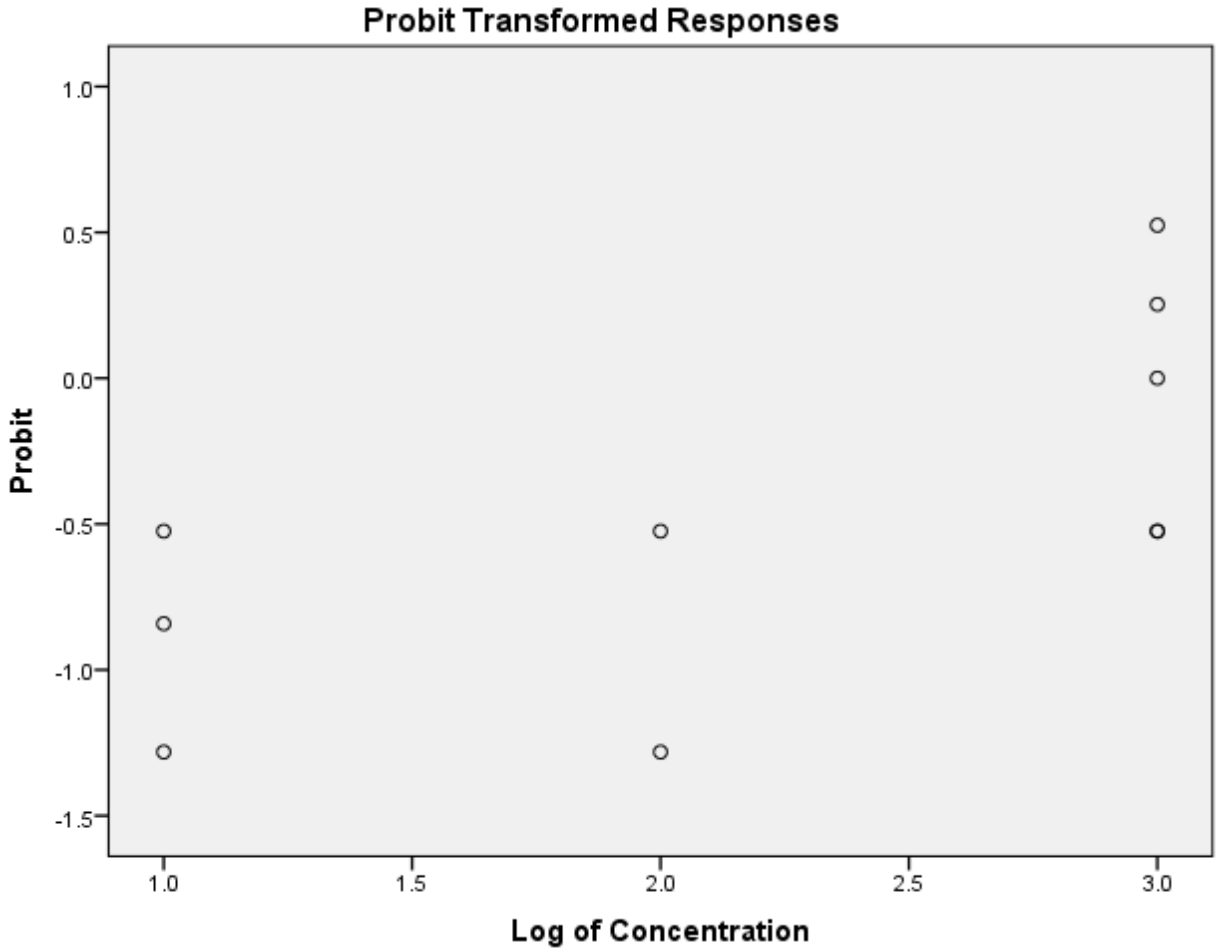
	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[b]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	.010	.456	.000	7.182	-.341	-8.313	.856
	.020	1.231	.000	13.177	.090	-6.580	1.120
	.030	2.310	.000	19.546	.364	-5.485	1.291

	.040	3.711	.000	26.484	.569	-4.664	1.423
	.050	5.456	.000	34.121	.737	-3.999	1.533
	.060	7.574	.000	42.587	.879	-3.435	1.629
	.070	10.099	.001	52.026	1.004	-2.944	1.716
	.080	13.065	.003	62.615	1.116	-2.507	1.797
	.090	16.514	.008	74.570	1.218	-2.111	1.873
	.100	20.488	.018	88.167	1.311	-1.751	1.945
	.150	50.026	.494	197.712	1.699	-.306	2.296
	.200	101.703	5.243	497.407	2.007	.720	2.697
	.250	186.933	25.849	1689.391	2.272	1.412	3.228
	.300	322.912	70.090	7827.749	2.509	1.846	3.894
	.350	535.880	134.479	42572.211	2.729	2.129	4.629
	.400	866.583	217.635	243498.484	2.938	2.338	5.386
	.450	1379.658	323.371	1411150.247	3.140	2.510	6.150
	.500	2180.373	459.186	8270046.752	3.339	2.662	6.918
	.550	3445.801	636.548	49646474.383	3.537	2.804	7.696
	.600	5485.945	872.884	311753108.769	3.739	2.941	8.494
	.650	8871.447	1195.741	2106573546.448	3.948	3.078	9.324
	.700	14722.384	1651.155	15918943845.321	4.168	3.218	10.202
	.750	25431.687	2321.885	142223343764.775	4.405	3.366	11.153

.800	46744.227	3372.105	1639913390585.212	4.670	3.528	12.215
.850	95030.604	5177.610	28523953127541.965	4.978	3.714	13.455
.900	232042.915	8822.109	1044183074588050.400	5.366	3.946	15.019
.910	287879.524	10025.520	2493397295861565.000	5.459	4.001	15.397
.920	363865.261	11516.056	6420769574058917.000	5.561	4.061	15.808
.930	470758.296	13407.463	1817296087091926 8.000	5.673	4.127	16.259
.940	627661.022	15883.317	5810634104849308 8.000	5.798	4.201	16.764
.950	871375.335	19261.279	2188463012979544 32.000	5.940	4.285	17.340
.960	1281153.142	24145.820	1039914226823838080.000	6.108	4.383	18.017
.970	2057751.289	31857.607	7069829300349500400.000	6.313	4.503	18.849
.980	3863289.687	46003.212	9044663059229883000 0.000	6.587	4.663	19.956
.990	10426261.574	81935.999	5034527292806385000000.000	7.018	4.913	21.702

a. A heterogeneity factor is used.

b. Logarithm base = 10.


Probit Transformed Responses
Probit
1.0
0.5
0.0
-0.5
-1.0
-1.5
1.0
1.5
2.0
2.5
3.0
Log of Concentration

Appendix 12: DATA OUTPUT – METHANOL EXTRACT OF *Securidaca longipenduculata*

```
PROBIT Response_frequency OF Total_observed_animals WITH Concentration
  /LOG 10
  /MODEL PROBIT
  /PRINT FREQ CI
  /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
```

Probit Analysis

Notes

Output Created		24-MAY-2021 17:45:27
Comments		
Input	Active Dataset	DataSet0
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	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	16
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		PROBIT Response_frequency OF Total_observed_animals WITH Concentration /LOG 10 /MODEL PROBIT /PRINT FREQ CI /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
Resources	Processor Time	00:00:00.64
	Elapsed Time	00:00:00.99

[DataSet0]

Warnings

Relative Median Potency Estimates are not displayed because there is no grouping variable in the model.

Data Information

		N of Cases
Valid		15
	Missing	1
Rejected	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	13	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	0.982	0.154	6.398	0.000	0.681	1.283
	Intercept	-2.425	0.352	-6.890	0.000	-2.777	-2.073

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	44.049	13	.000[a]

a. Since the significance level is less than .050, a heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.000	10	4	.746	3.254	.075
	2	1.000	10	0	.746	-.746	.075
	3	1.000	10	0	.746	-.746	.075
	4	1.000	10	3	.746	2.254	.075
	5	1.000	10	0	.746	-.746	.075
	6	2.000	10	3	3.227	-.227	.323
	7	2.000	10	2	3.227	-1.227	.323
	8	2.000	10	0	3.227	-3.227	.323
	9	2.000	10	1	3.227	-2.227	.323
	10	2.000	10	2	3.227	-1.227	.323
	11	3.000	10	9	6.993	2.007	.699
	12	3.000	10	10	6.993	3.007	.699
	13	3.000	10	5	6.993	-1.993	.699
	14	3.000	10	6	6.993	-.993	.699
	15	3.000	10	9	6.993	2.007	.699

Confidence Limits

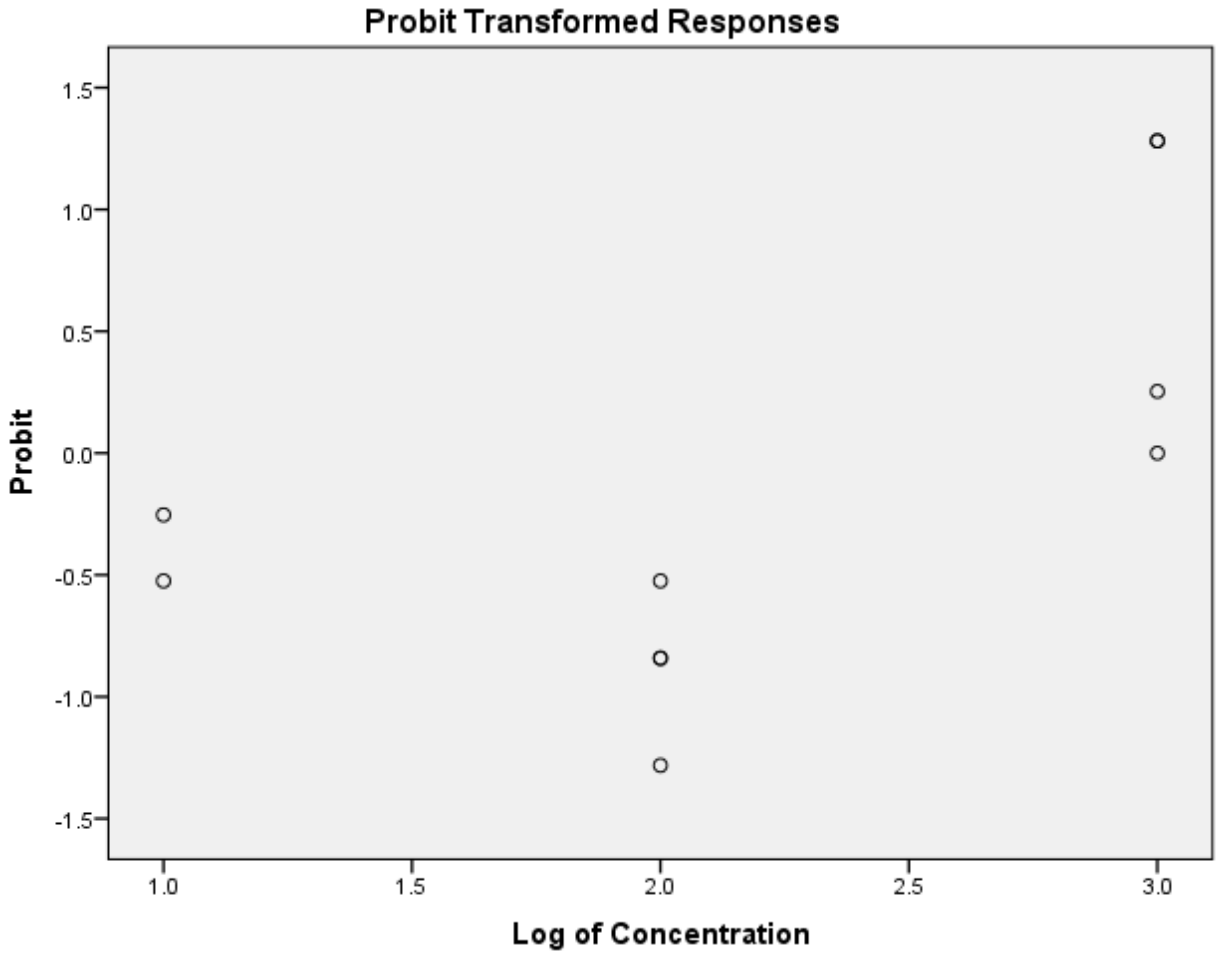
	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[b]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	.010	1.260	.000	9.479	.100	-3.369	.977
	.020	2.387	.002	14.479	.378	-2.649	1.161
	.030	3.580	.006	19.036	.554	-2.194	1.280

	.040	4.856	.014	23.467	.686	-1.853	1.370
	.050	6.223	.026	27.895	.794	-1.577	1.446
	.060	7.686	.045	32.391	.886	-1.343	1.510
	.070	9.249	.073	37.002	.966	-1.139	1.568
	.080	10.917	.110	41.765	1.038	-.957	1.621
	.090	12.693	.161	46.714	1.104	-.792	1.669
	.100	14.582	.228	51.877	1.164	-.642	1.715
	.150	25.901	.939	82.032	1.413	-.027	1.914
	.200	40.890	2.770	123.123	1.612	.443	2.090
	.250	60.498	6.677	183.137	1.782	.825	2.263
	.300	86.003	13.887	277.107	1.935	1.143	2.443
	.350	119.147	25.634	434.347	2.076	1.409	2.638
	.400	162.337	42.803	712.866	2.210	1.631	2.853
	.450	218.971	65.849	1228.938	2.340	1.819	3.090
	.500	293.966	95.140	2221.240	2.468	1.978	3.347
	.550	394.645	131.449	4198.415	2.596	2.119	3.623
	.600	532.325	176.355	8299.495	2.726	2.246	3.919
	.650	725.290	232.656	17240.381	2.861	2.367	4.237
	.700	1004.801	305.069	38041.914	3.002	2.484	4.580
	.750	1428.416	401.744	90914.198	3.155	2.604	4.959
	.800	2113.374	537.874	243422.196	3.325	2.731	5.386
	.850	3336.369	745.587	777735.951	3.523	2.872	5.891
	.900	5926.221	1108.778	3401467.738	3.773	3.045	6.532
	.910	6808.330	1218.210	4866275.720	3.833	3.086	6.687

	.920	7916.037	1348.535	7184999.326	3.899	3.130	6.856
	.930	9343.135	1506.978	11035556.239	3.970	3.178	7.043
	.940	11243.158	1704.774	17834431.154	4.051	3.232	7.251
	.950	13886.092	1960.578	30859059.175	4.143	3.292	7.489
	.960	17795.329	2308.235	58828318.503	4.250	3.363	7.770
	.970	24140.231	2817.583	130203273.930	4.383	3.450	8.115
	.980	36206.889	3665.980	375049301.754	4.559	3.564	8.574
	.990	68591.053	5532.080	1994058943.537	4.836	3.743	9.300

a. A heterogeneity factor is used.

b. Logarithm base = 10.



Appendix 13: DATA OUTPUT – METHANOL EXTRACT OF *Senna singueana*

```
PROBIT Response_frequency OF Total_observed_animals WITH Concentration
  /LOG 10
  /MODEL PROBIT
  /PRINT FREQ CI
  /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
```

Probit Analysis

Notes

Output Created		24-MAY-2021 17:42:08
Comments		
Input	Active Dataset	DataSet0
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	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	16
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		PROBIT Response_frequency OF Total_observed_animals WITH Concentration /LOG 10 /MODEL PROBIT /PRINT FREQ CI /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
Resources	Processor Time	00:00:00.59
	Elapsed Time	00:00:00.98

[DataSet0]

Warnings

Relative Median Potency Estimates are not displayed because there is no grouping variable in the model.

Data Information

		N of Cases
Valid		15
	Missing	1
Rejected	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	12	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	0.354	0.170	2.087	0.037	0.021	0.686
	Intercept	-1.898	0.398	-4.766	0.000	-2.296	-1.499

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	20.842	13	.076[a]

a. Since the significance level is greater than .050, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.000	10	0	.613	-.613	.061
	2	1.000	10	2	.613	1.387	.061
	3	1.000	10	0	.613	-.613	.061
	4	1.000	10	1	.613	.387	.061
	5	1.000	10	0	.613	-.613	.061
	6	2.000	10	0	1.171	-1.171	.117
	7	2.000	10	0	1.171	-1.171	.117
	8	2.000	10	2	1.171	.829	.117
	9	2.000	10	1	1.171	-.171	.117
	10	2.000	10	3	1.171	1.829	.117
	11	3.000	10	0	2.016	-2.016	.202
	12	3.000	10	3	2.016	.984	.202
	13	3.000	10	3	2.016	.984	.202
	14	3.000	10	0	2.016	-2.016	.202
	15	3.000	10	4	2.016	1.984	.202

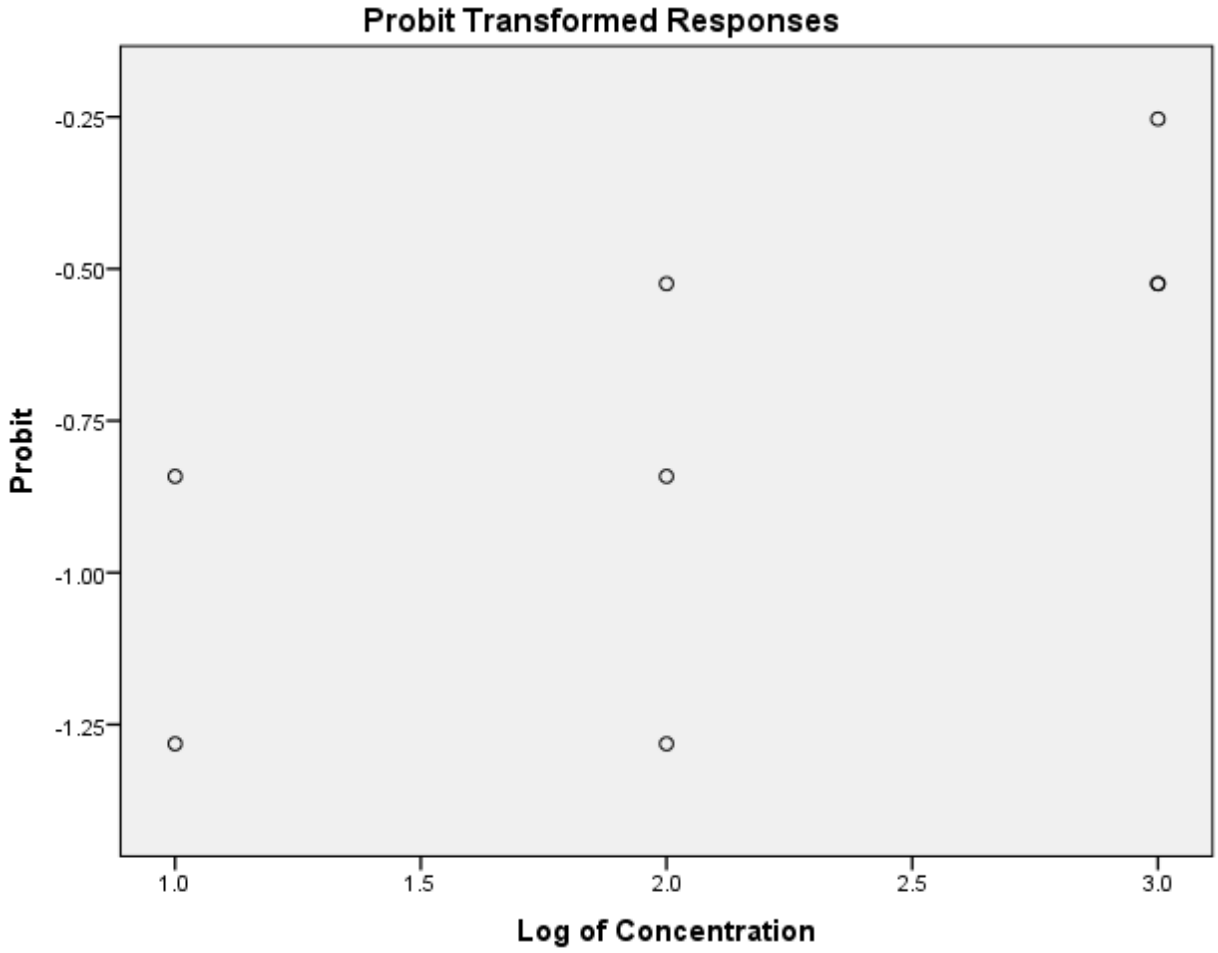
Confidence Limits

	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	.061	.000	3.400	-1.211	-54.288	.531
	.020	.362	.000	8.978	-.441	-41.617	.953

	.030	1.116	.000	16.972	.048	-33.586	1.230
	.040	2.601	.000	27.957	.415	-27.554	1.446
	.050	5.179	.000	42.899	.714	-22.657	1.632
	.060	9.306	.000	63.448	.969	-18.500	1.802
	.070	15.558	.000	92.618	1.192	-14.871	1.967
	.080	24.648	.000	136.495	1.392	-11.643	2.135
	.090	37.456	.000	209.154	1.574	-8.739	2.320
	.100	55.056	.000	349.906	1.741	-6.119	2.544
	.150	271.282	20.264	7781820.666	2.433	1.307	6.891
	.200	963.585	175.145	204750873591 9289.000	2.984	2.243	15.311
	.250	2858.535	418.618	912255023049 02830000000. 000	3.456	2.622	22.960
	.300	7590.001	791.444	780345520223 129500000000 000000.000	3.880	2.898	29.892
	.350	18759.968	1360.857	2.172E+036	4.273	3.134	36.337
	.400	44273.251	2225.919	2.896E+042	4.646	3.348	42.462
	.450	101609.777	3538.924	2.473E+048	5.007	3.549	48.393
	.500	230149.128	5541.611	1.714E+054	5.362	3.744	54.234
	.550	521294.528	8630.738	1.194E+060	5.717	3.936	60.077
	.600	1196402.341	13483.827	1.038E+066	6.078	4.130	66.016
	.650	2823492.113	21316.103	1.432E+072	6.451	4.329	72.156
	.700	6978737.094	34448.068	4.240E+078	6.844	4.537	78.627

	.750	18529987.086	57691.419	4.094E+085	7.268	4.761	85.612
	.800	54970370.181	102220.444	2.461E+093	7.740	5.010	93.391
	.850	195253058.423	198676.946	2.879E+102	8.291	5.298	102.459
	.900	962085732.092	457283.375	7.417E+113	8.983	5.660	113.870
	.910	1414169646.698	559090.597	4.231E+116	9.151	5.747	116.626
	.920	2149002475.148	695445.606	4.177E+119	9.332	5.842	119.621
	.930	3404589318.286	883931.575	8.191E+122	9.532	5.946	122.913
	.940	5691688157.965	1155245.260	3.896E+126	9.755	6.063	126.591
	.950	10227627817.739	1567408.329	6.090E+130	10.010	6.195	130.785
	.960	20361951208.165	2242646.721	5.154E+135	10.309	6.351	135.712
	.970	47474080535.219	3482504.908	5.890E+141	10.676	6.542	141.770
	.980	146273659992.153	6248395.926	6.656E+149	11.165	6.796	149.823
	.990	861852393327.790	15686531.712	3.284E+162	11.935	7.196	162.516

a. Logarithm base = 10.


Probit Transformed Responses
Probit
-0.25
-0.50
-0.75
-1.00
-1.25
1.0
1.5
2.0
2.5
3.0
Log of Concentration

Appendix 14: DATA OUTPUT – METHANOL STEM BARK EXTRACT OF *Entada lepstrachya*

```
PROBIT Response_frequency OF Total_observed_animals WITH Concentration
  /LOG 10
  /MODEL PROBIT
  /PRINT FREQ CI
  /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
```

Probit Analysis

Notes

Output Created		24-MAY-2021 17:47:49
Comments		
Input	Active Dataset	DataSet0
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	16
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		PROBIT Response_frequency OF Total_observed_animals WITH Concentration /LOG 10 /MODEL PROBIT /PRINT FREQ CI /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
Resources	Processor Time	00:00:00.56
	Elapsed Time	00:00:01.05

[DataSet0]

Warnings

Relative Median Potency Estimates are not displayed because there is no grouping variable in the model.

Data Information

		N of Cases
Valid		15
	Missing	1
Rejected	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	15	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	0.639	0.207	3.089	0.002	0.233	1.044
	Intercept	-2.687	0.521	-5.154	0.000	-3.208	-2.166

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	11.820	13	.542[a]

a. Since the significance level is greater than .050, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.000	10	0	.203	-.203	.020
	2	1.000	10	0	.203	-.203	.020
	3	1.000	10	0	.203	-.203	.020
	4	1.000	10	0	.203	-.203	.020
	5	1.000	10	1	.203	.797	.020
	6	2.000	10	0	.793	-.793	.079
	7	2.000	10	1	.793	.207	.079
	8	2.000	10	1	.793	.207	.079
	9	2.000	10	0	.793	-.793	.079
	10	2.000	10	2	.793	1.207	.079
	11	3.000	10	3	2.204	.796	.220
	12	3.000	10	2	2.204	-.204	.220
	13	3.000	10	0	2.204	-2.204	.220
	14	3.000	10	3	2.204	.796	.220
	15	3.000	10	3	2.204	.796	.220

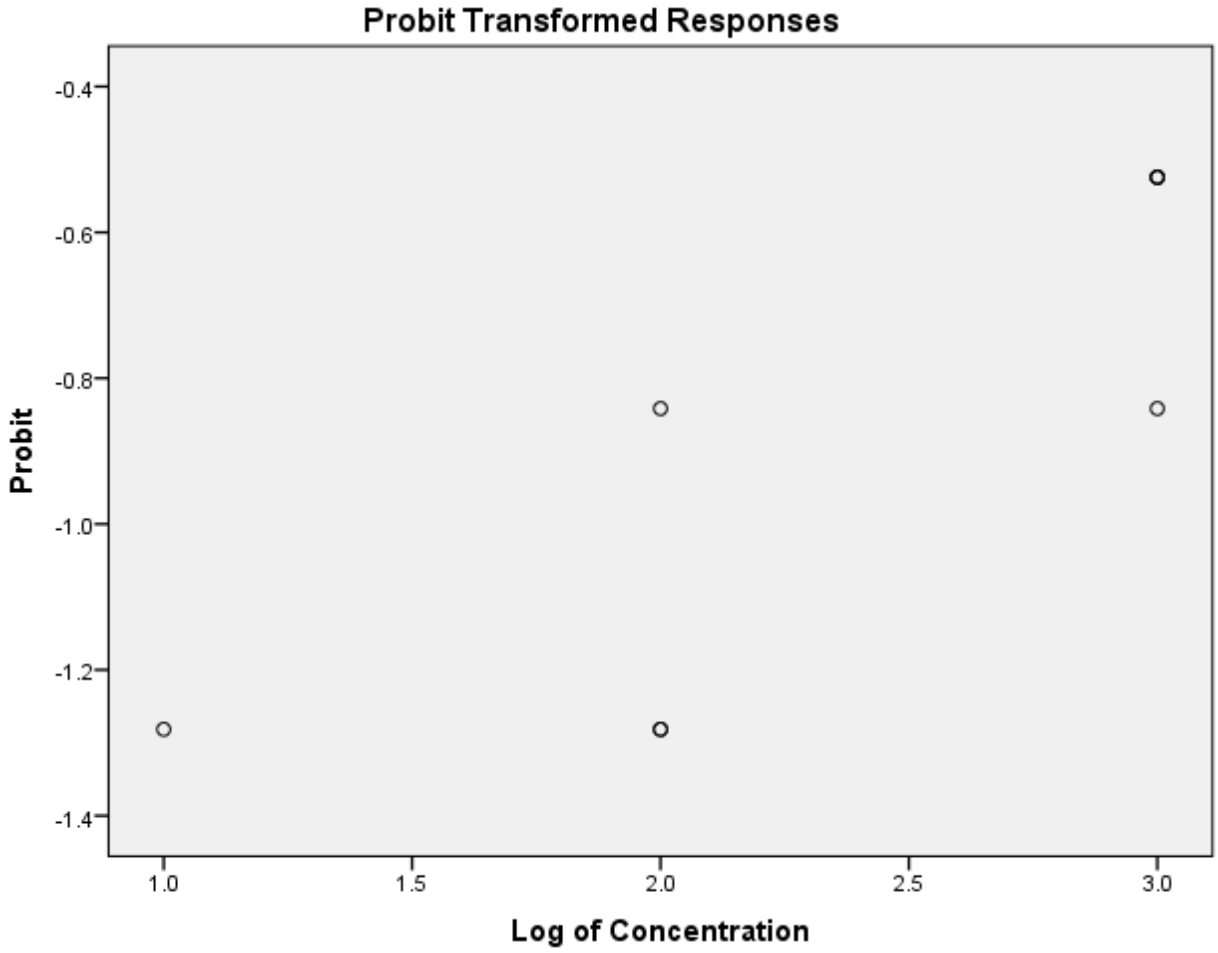
Confidence Limits

	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	3.668	.002	23.377	.564	-2.741	1.369
	.020	9.801	.025	45.038	.991	-1.597	1.654
	.030	18.285	.132	69.543	1.262	-.879	1.842

	.040	29.230	.451	98.032	1.466	-.346	1.991
	.050	42.809	1.204	131.801	1.632	.081	2.120
	.060	59.236	2.728	172.619	1.773	.436	2.237
	.070	78.751	5.478	223.027	1.896	.739	2.348
	.080	101.622	10.006	286.725	2.007	1.000	2.457
	.090	128.143	16.894	369.126	2.108	1.228	2.567
	.100	158.634	26.654	478.094	2.200	1.426	2.680
	.150	383.886	122.303	2008.548	2.584	2.087	3.303
	.200	774.887	274.936	9384.225	2.889	2.439	3.972
	.250	1415.574	471.176	41175.752	3.151	2.673	4.615
	.300	2431.879	719.910	164967.125	3.386	2.857	5.217
	.350	4015.256	1037.287	613635.857	3.604	3.016	5.788
	.400	6461.850	1445.226	2166460.350	3.810	3.160	6.336
	.450	10239.692	1973.946	7408836.232	4.010	3.295	6.870
	.500	16108.205	2666.480	24999591.181	4.207	3.426	7.398
	.550	25340.045	3586.188	84727426.005	4.404	3.555	7.928
	.600	40154.798	4829.934	293842686.641	4.604	3.684	8.468
	.650	64622.095	6552.585	1065418794.631	4.810	3.816	9.028
	.700	106697.009	9016.051	4150025313.287	5.028	3.955	9.618
	.750	183299.645	12696.826	18039405738.671	5.263	4.104	10.256

.800	334854.170	18552.584	92834224642.418	5.525	4.268	10.968
.850	675915.075	28808.396	627922291084.428	5.830	4.460	11.798
.900	1635677.207	50000.589	6974195421447.448	6.214	4.699	12.843
.910	2024875.168	57105.178	12478744030140.154	6.306	4.757	13.096
.920	2553318.090	65964.129	23481245916270.633	6.407	4.819	13.371
.930	3294862.536	77289.108	47060984676702.760	6.518	4.888	13.673
.940	4380341.991	92236.659	102316525255607.000	6.642	4.965	14.010
.950	6061151.283	112823.782	248140557333572.780	6.783	5.052	14.395
.960	8877022.462	142923.474	702791843362176.000	6.948	5.155	14.847
.970	14190228.775	191090.085	2528059966034820.500	7.152	5.281	15.403
.980	26472954.135	281011.946	13868437754834388.000	7.423	5.449	16.142
.990	70735467.721	515634.067	203013249021296896.000	7.850	5.712	17.308

a. Logarithm base = 10.


Probit Transformed Responses
Probit
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
1.0
1.5
2.0
2.5
3.0
Log of Concentration

Appendix 15: DATA OUTPUT – VINCRISTINE SULPHATE

```
PROBIT Response_frequency OF Total_observed_animals WITH Concentration
  /LOG 10
  /MODEL PROBIT
  /PRINT FREQ CI
  /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
```

Probit Analysis

Notes

Output Created		24-MAY-2021 17:55:28
Comments		
Input	Active Dataset	DataSet0
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	16
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		PROBIT Response_frequency OF Total_observed_animals WITH Concentration /LOG 10 /MODEL PROBIT /PRINT FREQ CI /CRITERIA P(.05) ITERATE(20) STEPLIMIT(.1).
Resources	Processor Time	00:00:00.53
	Elapsed Time	00:00:00.90

[DataSet0]

Warnings

Relative Median Potency Estimates are not displayed because there is no grouping variable in the model.

Data Information

		N of Cases
Valid		15
	Missing	1
Rejected	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	17	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	1.485	.435	3.415	.001	.633	2.337
	Intercept	-.903	.541	-1.670	.095	-1.444	-.362

a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	8.574	13	.804[a]

a. Since the significance level is greater than .050, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Cell Counts and Residuals

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	1.000	10	5	7.196	-2.196	.720
	2	1.000	10	7	7.196	-.196	.720
	3	1.000	10	8	7.196	.804	.720
	4	1.000	10	7	7.196	-.196	.720
	5	1.000	10	9	7.196	1.804	.720
	6	2.000	10	10	9.806	.194	.981
	7	2.000	10	9	9.806	-.806	.981
	8	2.000	10	10	9.806	.194	.981
	9	2.000	10	10	9.806	.194	.981
	10	2.000	10	10	9.806	.194	.981
	11	3.000	10	10	9.998	.002	1.000
	12	3.000	10	10	9.998	.002	1.000
	13	3.000	10	10	9.998	.002	1.000
	14	3.000	10	10	9.998	.002	1.000
	15	3.000	10	10	9.998	.002	1.000

Confidence Limits

	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	.110	.000	.695	-.959	-3.860	-.158
	.020	.168	.000	.913	-.775	-3.431	-.040

.030	.219	.001	1.086	-.659	-3.159	.036
.040	.269	.001	1.238	-.571	-2.955	.093
.050	.316	.002	1.377	-.500	-2.788	.139
.060	.364	.002	1.508	-.439	-2.647	.178
.070	.411	.003	1.634	-.386	-2.523	.213
.080	.459	.004	1.755	-.338	-2.412	.244
.090	.507	.005	1.874	-.295	-2.311	.273
.100	.556	.006	1.990	-.255	-2.218	.299
.150	.813	.015	2.556	-.090	-1.835	.408
.200	1.100	.029	3.125	.041	-1.531	.495
.250	1.425	.054	3.719	.154	-1.270	.570
.300	1.799	.092	4.355	.255	-1.038	.639
.350	2.232	.150	5.052	.349	-.823	.703
.400	2.739	.240	5.830	.438	-.620	.766
.450	3.339	.376	6.715	.524	-.425	.827
.500	4.058	.583	7.748	.608	-.234	.889
.550	4.931	.899	8.986	.693	-.046	.954
.600	6.011	1.386	10.528	.779	.142	1.022
.650	7.376	2.140	12.549	.868	.331	1.099
.700	9.152	3.319	15.402	.962	.521	1.188
.750	11.551	5.145	19.894	1.063	.711	1.299
.800	14.969	7.886	28.116	1.175	.897	1.449
.850	20.250	11.806	46.251	1.306	1.072	1.665
.900	29.617	17.484	97.051	1.472	1.243	1.987

	.910	32.465	18.985	117.537	1.511	1.278	2.070
	.920	35.871	20.685	145.263	1.555	1.316	2.162
	.930	40.029	22.647	184.026	1.602	1.355	2.265
	.940	45.246	24.967	240.542	1.656	1.397	2.381
	.950	52.031	27.802	327.685	1.716	1.444	2.515
	.960	61.313	31.423	473.040	1.788	1.497	2.675
	.970	75.023	36.370	746.092	1.875	1.561	2.873
	.980	98.107	43.938	1374.555	1.992	1.643	3.138
	.990	149.737	58.712	3630.180	2.175	1.769	3.560

a. Logarithm base = 10.