

PHYTOCHEMICAL SCREENING, ACUTE TOXICITY, AND EFFECTS OF METHANOL EXTRACT OF *EUPHORBIA COTINIFOLIA* ON BLEEDING AND CLOTTING TIMES IN RATS ENVENOMATED WITH LD₉₉ OF *NAJA NIGRICOLLIS* VENOM

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ABSTRACT

Plants have long been used in medicine, offering diverse compounds with pharmacological potential. Herbal antivenoms can neutralize venom through multiple mechanisms, and species of *Euphorbia* are notable in this regard. *Euphorbia cotinifolia*, a member of the Euphorbiaceae family, was investigated for its phytochemical composition, acute toxicity, and antivenom activity. Methanol extracts of the whole plant revealed alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins, and terpenoids, while anthraquinones were absent. Acute toxicity studies in albino rats showed an LD₅₀ of 3807.8 mg/kg, indicating relative safety. Antivenom activity was tested against *Naja nigricollis* (LD₉₉ venom). Bleeding time decreased significantly with increasing extract doses (20, 40 and 80 mg/kg), indicating dose-dependent neutralization potential. At 80 mg/kg, bleeding time was reduced to 267.4±6.45 seconds, while clotting time increased to 387.2±5.56 seconds compared with controls. These findings suggest that *E. cotinifolia* extracts can counteract venom effects, particularly by reducing bleeding time and modulating clotting. The results highlight its potential as a plant-based treatment for snakebite, particularly *N. nigricollis* envenomation, and provide preliminary data on safe dosage ranges for in vivo use.

Keywords: Acute toxicity, Bleeding and clotting time, *Euphorbia cotinifolia*, Methanolic extracts and Phytochemical screening.

INTRODUCTION

The term “traditional medicine practice” refers to the knowledge, attitudes and beliefs related to health that use plant, animal and mineral-based methods and practices to diagnose, prevent and treat diseases or to promote overall well-being (Ngwogu *et al.*, 2019). Approximately 80% of the global population receives their main healthcare from traditional medicine. In developing nations, particularly in Asia and Africa, the percentage of people who use traditional medicine ranges from 70% to 95%. About 90 % of people in Africa, particularly in sub-Saharan Africa, rely on traditional medicine (Tizazu *et al.*, 2020). Traditional medicine, also known as alternative medicine, is the oldest healthcare system in the world and is used to prevent and treat physical and mental illnesses (Yuan and Piao, 2016). Medicinal plants are plants that possess therapeutic properties or exert beneficial pharmacological effects in the human or animal body. Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions such as defense

against insects, diseases, and herbivorous mammals (Gershenzon & Ullah, 2022). According to the World Health Organization (WHO, 2017), about 5.4 million snakebites occur each year, resulting in 1.8 to 2.7 million cases of poisonous envenomation. There are between 81,410 and 137,880 snakebite-related deaths and around three times as many amputations and other permanent disabilities each year (Yemane *et al.*, 2021). The use of medicinal plants with anti-snake venom activity may be useful in treating victims of snakebites (Sani *et al.*, 2018), which is particularly important in rural areas where antivenins are not readily available (Habib *et al.*, 2015). Envenomation from snakebite is one of the severe but neglected public health problems responsible for high morbidity and mortality rates across the globe, especially in rural areas where access to modern health facilities is very limited (Sani *et al.*, 2020b and Yusuf *et al.*, 2019). However, antivenins have some disadvantages, thus limiting their efficient use. For example, they can induce adverse reactions ranging from mild symptoms to serious anaphylaxis; in addition, they do not neutralize local tissue damage. Therefore, complementary therapeutics need to be investigated, with plants being considered as a major source (Sani *et al.*, 2020). The present study aimed to investigate the effects of methanol extracts of the whole plant of *E. cotinifolia* on bleeding and clotting times in *Naja nigricollis*-envenomed rats, with a view to either supporting or debunking the traditional claims of using the plant in the management of snake bites.

MATERIALS AND METHODS

Plant Collection and Identification

The collection of *Euphorbia cotinifolia* samples was carried out at PRA and Wusasa, Zaria, Kaduna State. The plants were identified and documented by a taxonomist in the herbarium section of the Department of Biological Sciences, Kaduna State University, Kaduna. Voucher specimen number 998 was assigned to it, and the specimens were deposited at the herbarium section for future reference.

Preparation of Plant Materials

The whole plant material of *Euphorbia cotinifolia* plants was air-dried in the laboratory at room temperature (24°C to 28°C) for some weeks and then pulverized using a pestle and mortar (Kubmarawa *et al.*, 2009). The powder of whole plant materials was stored in clean, airtight containers and subsequently macerated with methanol.

Extraction of Plant Materials

200g of the plant material was macerated in methanol (2.5 litres) for 3 days, and the extract was filtered. The filtrate of the plant material was evaporated to dryness on a water bath, and the extract yield was calculated as a percentage. Plant extracts were used in acute toxicity and biological studies. The method described by Anas et al. (2010) was adopted.

Qualitative Phytochemical Screening

Phytochemical analysis was carried out on the plant material, with slight modifications, following Trease and Evans (1989). In this analysis, the presence or absence of several phytochemical compounds in the crude plant extracts was determined: alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids were detected, whereas anthraquinones were absent.

Quantitative Phytochemical Screening

The quantitative analysis of *E. cotinifolia* extract showed the presence of major constituents, moderate constituents and minor constituents, which contain the following bioactive constituents such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids, which were detected in most parts of the plant tested, except anthraquinones, which were absent in the plant extract.

Experimental Animals

A total of fifty-three (53) albino rats weighing 93- 178 g of both sexes (male and female) were used, out of which 13 were used for acute toxicity, 15 were used for determination of minimum lethal dose, and 25 were used for bleeding and clotting time of *N. nigricollis* venom. All the rats were acclimatized for fourteen days and allowed free access to water and food.

Acute Toxicity Studies

The method described by Lorke (1983) was adopted. A pilot study was carried out with methanolic extract of the plant on albino rats, using nine rats in three groups (three rats per group). Animals in each group were administered 10, 100 and 1000 mg/kg, respectively. Based on the results of the pilot studies, suitable dose intervals were selected and used to conduct the final toxicity studies. Four groups of four rats were used to assess the final toxicity of the plant's methanolic extract.

Bleeding and Clotting Time

The effect of plant extracts on bleeding and clotting times in *Naja nigricollis* rats was assessed. The modified method of Omale et al. (2012) was adopted. In this method, twenty-five (25) albino rats were used and equally divided into five groups accordingly.

Group one (n= 5) received normal saline as negative control, group two (n= 5) received LD₉₉ *Naja nigricollis* venom as positive control(n=5), group three (n=5) received doses of plant extracts and LD₉₉ *Naja nigricollis* venom, group four (n= 5) received doses of plant extracts and LD₉₉ *Naja nigricollis* venom (n=5) and group five(n= 5) received doses plant extracts and LD₉₉ *Naja nigricollis* venom.

All the albino rats treated with plant extract were injected intraperitoneally (ip). Rats in Group three (3), four (4) and five (5) were injected with different doses of the plant extract based on the results of acute toxicity studies (20, 40 and 80 mg/kg) of *Euphorbia*

cotinifolia extracts one hour (1 hr) after LD₉₉ *Naja nigricollis* venom was administered intraperitoneally (i.p). Bleeding and clotting times were determined two hours (2 hrs) after the treatment of the animals. For bleeding time, the tip of each rat's tail was cut to induce bleeding. A stopwatch was started as soon as the animal began to bleed. Cotton wool was used to wipe off the blood every 15 seconds. As soon as bleeding ceased, the stopwatch was stopped and the time was recorded as bleeding time for that particular animal. For the clotting time, a drop of blood from the cut tail of each rat was placed on a clean glass slide, and the stopwatch was started simultaneously. A pin was passed across the drop of blood once every 15 seconds. As soon as a fibrin thread was observed, the stopwatch was stopped, and the time was recorded as the clotting time for that particular rat.

Data Analysis

Results were statistically analyzed using analysis of variance (ANOVA) and expressed as mean $\pm$ SEM compared with the positive control group.

RESULTS

Qualitative Phytochemical Constituents

The phytochemical screening of methanolic whole plant extract shows the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids in most parts of the plant. Anthraquinones were not seen in the plant extract, as shown in Table 1.

Table 1: Qualitative Phytochemical Constituents

S/No	Phytoconstituents	Test	<i>E. cotinifolia</i>
1	Alkaloids	Dragendorff test	+
2	Carbohydrate	Molisch test	+
3	Cardiac Glycosides	Keller-Kiliani test	+
4	Flavonoids	Shinoda test	+
5	Phenolic compounds	Lead acetate test	+
6	Saponins	Frothing test	+
7	Steroids	Salkowski test	+
8	Tannins	Ferric Chloride test	+
9	Terpenoids	Liebermann-Burchard test	+
10	Anthraquinones	Bontr�ger's test	-

Key: Present +
Absent -

Quantitative Phytochemical Constituents

The quantitative analysis of *E. cotinifolia* extract showed the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids in most of the plant parts tested. Anthraquinones were absent in the plant species. Major Constituents in *E. cotinifolia* are rich in carbohydrates, flavonoids and phenols, which were the three (3) most abundant bioactive constituents. High levels of flavonoid and phenol content in *E. cotinifolia* extract suggest it may possess stronger antioxidant and anti-inflammatory activity. Moderate amounts of steroids, tannins and triterpenes were detected in the

plant extract. This further suggests that *E. cotinifolia* has diverse therapeutic potential, including antimicrobial, wound-healing, and anti-inflammatory properties. Minor amounts of alkaloids, cardiac glycosides and saponins were also detected in the plant extract. The results indicate that *E. cotinifolia* is a rich source of bioactive compounds, with the plant extract being relatively richer in antioxidant-related phytochemicals. The absence of anthraquinones in the plant extracts implies that neither plant is expected to have strong laxative or purgative effects.

Table 2: Quantitative Phytochemical Screening Results

S/No	Phytoconstituents	<i>E. cotinifolia</i> (mg/kg)	<i>E. cotinifolia</i> (%)
1	Alkaloids	9.66±0.05	0.96
2	Carbohydrate	211.95±0.04	21.19
3	Cardiac Glycosides	18.76±0.02	1.87
4	Flavonoids	81.76±0.03	8.17
5	Phenolic compounds	72.73±0.01	7.27
6	Saponins	10.71±0.02	1.07
7	Steroids	21.38±0.07	2.14
8	Tannins	49.56±0.07	4.95
9	Terpenoids	23.76±0.00	2.38
10	Anthraquinones	0.00	0.00

Acute Toxicity Studies

The acute toxicity test of *E. cotinifolia* showed an LD₅₀ value of 3807.8 mg/kg. This value was very high, indicating that the extract was relatively safe and less toxic, and that the choice of dosage for the extract's in vivo activity should be high.

Table 3: LD₅₀ value of *E. cotinifolia* methanolic extract on Albino rats

Phase of toxicity test	Dose (mg/kg body weight)	Number of deaths
Phase I	10	0/3
	100	0/3
	1000	0/3
Phase II	1200	0/1
	1600	0/1
	2900	0/1
	5000	1/1

$$LD_{50} = \sqrt{2900 \times 5000} = 3807.8 \text{ mg/kg.}$$

Bleeding and clotting time

The results of *E. cotinifolia* extracts against LD₉₉ showed that the bleeding and clotting times of albino rats treated with different doses (20, 40 and 80 mg/kg) of the extract against LD₉₉ *N. nigricollis* venom were significantly reduced with increasing dosage compared with the positive group. This indicates that the extract had a significant effect on the neutralization potential against LD₉₉ *N. nigricollis*-envenomated rats, as shown in Table 4.

Clotting time increased with higher dosage compared with both

control groups. The results showed a slight effect of the venom, particularly at high doses, compared with the positive control.

Table 4: Bleeding and Clotting time of albino rats treated with different doses of *E. cotinifolia* extract against LD₉₉ *Naja nigricollis* venom.

Treatment group	Bleeding time (seconds)	Clotting time (seconds)
Group 1 Negative control	218.4±5.87	144.4±5.64
Group 2 Positive control	397.6±5.40	282.6±2.94
Group 3 20mg/kg+LD ₉₉ Venom	289.8±2.98 ^a	383.2±5.56 ^d
Group4 40mg/kg+LD ₉₉ Venom	275.8±2.15 ^{ab}	383.2±5.56 ^d
Group5 80mg/kg+LD ₉₉ Venom	267.4±6.45 ^{bc}	387.2±5.56 ^d

Results are the mean ± SEM (n= 5); Values in the same column with the superscripts are considered significant (P < 0.05) when compared with the positive control.

DISCUSSION

Plants provide an inexhaustible source of various herbal compounds with pharmacological potential (Sani *et al.*, 2020). Herbal antivenoms have been reported to neutralize toxic venom through several mechanisms. These include inhibition of venom enzymes, inactivation of venom toxins, adjuvant action and combinations of these activities (Sani *et al.*, 2018).

Plants, especially those derived from natural products, have been used to help humanity maintain its health since the dawn of medicine. Over the past century, phytochemicals in plants have emerged as important for pharmaceutical discovery. The importance of plant active components in medicine has inspired scientific interest in the biological activities of these substances (Moghadamtousi *et al.*, 2015).

Various phytochemicals with protein-binding properties that are active against snake envenomation include alkaloids, carbohydrates, cardiac glycosides, flavonoids, polyphenols, saponins, steroids, tannins, terpenoids and anthraquinones. These bind to toxic venom proteins, thereby inactivating them (Sani *et al.*, 2019). The phytochemical components of plant materials have been established in previous studies, and these studies have linked the presence of bioactive compounds to anti-snake venom activities (Sani *et al.*, 2018).

From the results of the present research, the whole powdered materials of *Euphorbia cotinifolia* were successively extracted with methanol and it had yielding value of (8.02 %). The yields of the solvent extractions indicated that the methanol extraction of *Euphorbia cotinifolia* yielded a high quantity of extract. This showed that the whole plant parts of *Euphorbia cotinifolia* contained more constituents soluble in methanol.

The results obtained conform with the findings of Saidu (2016), which showed that *Indigofera conferta*, after extraction with petroleum ether, ethanol and aqueous. The average yield of extracts (percentage) was petroleum ether (4.21 %), ethanol (5.31 %), and aqueous (6.21 %). From the various results obtained, aqueous yielded the highest quantity of the extract.

The phytochemical screening of *Euphorbia cotinifolia* showed the presence of phytochemical constituents like alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids in most of

the plant parts. Anthraquinones were not seen in the plant extracts.

The quantitative phytochemical screening of *E. cotinifolia* revealed the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids, with anthraquinones absent from the plant species. This observation is consistent with the findings of Patil (2021), who also reported the absence of anthraquinones in ethanolic extracts of the *E. milii* stem. The plants were found to be rich in carbohydrates, phenols, and flavonoids, which were the three (3) most abundant constituents. *E. cotinifolia* recorded high concentrations of carbohydrates (211.95 ± 0.04 mg/g), flavonoids (81.76 ± 0.03 mg/g), and phenols (72.73 ± 0.01 mg/g). The high phenolic and flavonoid content in *E. cotinifolia* is in line with the work of Grazielle *et al.* (2015), who reported notable contents of phenolic compounds, flavonoids and tannins in all parts of *E. cotinifolia* and with Kaur *et al.* (2017), who recorded total phenols of 105.68 mg/g and total flavonoids of 62.34 mg/g in the extract of *E. cotinifolia*. Phenols and flavonoids are well documented for their antioxidant and anti-inflammatory properties. The relatively higher levels of these compounds in *E. cotinifolia* suggest that it may possess stronger antioxidant potential. Moderate amounts of tannins, triterpenes, and steroids were detected in the plant species. *E. cotinifolia* had a high tannin content of (49.56 ± 0.07 mg/g). This indicates anti-inflammatory and wound-healing properties. This agrees with Kaur *et al.* (2017). The presence of C-glycosides and saponins in moderate to low amounts in the species further supports their potential antimicrobial and immunomodulating properties. The findings of this study indicate that *E. cotinifolia* is a rich source of bioactive compounds.

The results of acute toxicity studies of the plant extracts indicated that *E. cotinifolia* had an LD₅₀ value of 3807.8 mg/kg. This value was high enough to classify it as less toxic. In this research, *E. cotinifolia* can be regarded as the less toxic plant. This finding was supported by Lorke (1983), which indicated that a lower value obtained in the acute toxicity study is an indication that the plant extract, when used as a drug, may have a high toxic effect, and that a higher value obtained is an indication that the plant extract, when used as a drug, may have a low toxic effect.

Bleeding and clotting times provide sufficient information about platelet activation and function and may serve as a means of assessing clinical conditions such as intravascular coagulation, thrombocytopenia and end-stage liver failure (hematological abnormalities) (Saliu *et al.*, 2012).

The bleeding and clotting time results indicated that the plant species *Euphorbia cotinifolia* showed significant ($P < 0.05$) activity, as it reduced bleeding time and increased clotting time compared with the positive control group. This showed that these extracts could be used effectively to arrest bleeding caused by snake bites; across all extract-treated groups, bleeding times were significantly shorter than in the positive control. There is a dose-dependent reduction: as the dose increases, the bleeding time decreases. The highest dose yielded the greatest reduction and the value closest to the negative control.

Clotting time increased in all treated groups compared to both controls; the extract caused a marked, significant increase relative to the positive group. These results indicate a slight effect against the venom, especially at higher doses and increasing extract doses

also increases protection or neutralization potential against the LD₉₉ *N. nigricollis* venom.

This can be supported by the finding of Ushanandini *et al.* (2006), which showed that the aqueous extract of the aerial parts of *Bauhinia forticata* is a promising source of natural inhibitors, including proteases involved in blood-clotting disturbances induced by *Bothrops* and *Crotalus* crude venoms.

Conclusion

The present studies have established that the methanolic extracts of the plant species have significant ($p < 0.05$) concentrations and antivenom activity. The yield for the methanol extract of the plant species indicated that the plants had a high-yielding value of (8.02%).

Qualitative phytochemical constituents, namely alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids, were detected in most plant parts. Anthraquinones were not seen in the plant extracts.

The quantitative phytochemical study revealed that *E. cotinifolia* plant extract is a rich source of bioactive phytochemicals, with carbohydrates, phenols, and flavonoids being the predominant constituents. *E. cotinifolia* exhibited higher levels of antioxidant-related compounds. The presence of tannins, saponins, triterpenes, and other secondary metabolites further suggests potential antimicrobial, anti-inflammatory, and wound-healing properties. The absence of anthraquinones in the plant species indicates a lack of significant laxative effect. These findings provide scientific support for the traditional medicinal uses of *E. cotinifolia* and recommend further pharmacological and toxicological studies to explore the therapeutic potential.

The acute toxicity study of *Euphorbia cotinifolia* extracts helped determine the safe dose range for the plant extract's activities. It also helped to determine the safety of various plant extracts when used as drugs. The study showed that *Euphorbia cotinifolia* had an LD₅₀ of 3807.8 mg/kg (ip), indicating that extracts with high LD₅₀ values, when used as drugs, are likely to have low toxicity.

The methanolic extracts of the plants had significant ($p < 0.05$) and dose-dependent activity against LD₉₉ *Naja nigricollis* venom, the extracts of the plants reduced the bleeding time and increases the clotting time in envenomed rats and the effects were more effective at higher dose of *E. cotinifolia* extract of (80mg/kg) with bleeding time of 267.4 ± 6.45^{bc} (second) with increased in clotting time of 387.2 ± 5.56^d (second) when compared with the positive control group.

This study documents the use of medicinal plants by traditional medicine practitioners to treat snakebites. Medicinal plants were reported to be used in the treatment of snakebite envenomation. However, further pharmacological and phytochemical studies are needed to demonstrate the plants' efficacy.

REFERENCES

- Anas, K., Durga, J., Ashutosh, M., Dinesh, C., Indranil, K.Y. & Jain, D. A. (2010). International Journal of Chemical Technology Research. 2:744-751.
- Antony, G., Rinku, D., Sumana, S., Roshnara, M, Sanghamitra, M., & Shanik, B (2010). Herbs and herbal constituents active against snakebite. Indian Journal of Experimental Biology.

- 48:865 – 878.
- Grazielle, E., Natalia, M. N, Ingridy, S. R., Gabriel, D. O., Marcos, J. M. (2015). Phytochemical profile, antibacterial, antioxidant and cytotoxicity activities of *Euphorbia cotinifolia*. *African Journal of Pharmacy and Pharmacology*, 14(10).
- Gershenzon, J. & Ullah, C. (2022). Plants protect themselves from herbivores by optimizing the distribution of chemical defenses—Proceedings of the National Academy of Sciences, 119(4): 2120277119.
- Habib AG. (2015). Venomous Snakes and Snake Envenomation in Nigeria. In: *Clinical Toxicology in Asia Pacific and Africa*, 2, Springer, 275-298.
- Kaur, N., Bhaitia, A., & Sidhu, S. (2017). Antimicrobial activity and phytochemical screening of extracts of *Euphorbia cotinifolia*. *International Journal of Research in Pharmacy and Pharmaceutical Sciences*, 2(6), 83–85.
- Kubmarawa, D., Khan, M.E., Punnah, A.M. & Hassan, M. (2009). "Phytochemical and Antimicrobial Screening of Ficus against Human/Animal pathogens". *Pacific Journal of Science and Technology*, 10 (1): 382–386.
- Lorke. A new approach to practical acute toxicity testing. *Archives of Toxicology* 1983 54:275-87.
- Moghadamtousi S, Fadaeinasab M, Nikzad S, Mohan G, Ali H, Kadir H. (2015). *Annona muricata* (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities, *International Journal of Molecular Science*. 16:15625-15658.
- Ngwogu CA, Uzoma IG, Emah L (2019). Prevalence and attitude towards traditional medical practices among mothers of under-five children in Aba—Abia State University of Medical Studies Associated *Journal* 12.
- Omale, J., Ebiloma, U. G. and Grace, O. (2012). Plant extracts neutralized some Biological Effects of *Naja nigricollis* venom in rats. *British Journal of Pharmacology and Toxicology*, 4(2): 41–50.
- Patil, S. M. (2021). Phytochemical study and antioxidant properties of ethanolic extracts of *Euphorbia milii*. *Indian Journal of Pharmaceutical Sciences*.
- Saidu, A. The study of Antibacterial activity of Indigofera capitata and Indigofera conferta on albino rats (2016).
- Saliu, J. A., Elekotehinti, O., Komolafe, K. & Oboh, G. (2012). Effects of selected green leafy vegetables on haematological parameters in diabetic rats. *Journal of National Product and Plant Resource* 2(4): 20–28.
- Sani I, Umar RA, Hassan SW, Faruq UZ, Bello F and Abdulhamid A. (2020). Inhibition of Snake Venom Enzymes and Antivenom Adjuvant Effects of *Azadirachta indica* A. Juss. (Meliaceae) Leaf Extracts. *European Journal of Medicinal Plants*, 31(10), 114–128.
- Sani I, Umar RA, Hassan SW & Faruq UZ. (2018). Antisnake Venoms and their Mechanisms of Action: A Review. *Saudi Journal of Medical and Pharmaceutical Science*, 4(5), 512-520.
- Sani, I., Umar, A. A., Jiga, S. A., Bello, F., Abdulhamid, A., & Fakai, I. M. (2020). Isolation, Purification and Partial Characterization of Antisnake Venom Plant Peptide (BRS-P19) from *Bauhinia rufescens* (LAM FAM) Seed as Potential Alternative to Serum-Based Antivenin. *Journal of Biotechnology Research*, 6(4): 18–26.
- Sani, I., Umar, R.A., Hassan, S.W., Faruq, U.Z., & Bello, F. (2020). Isolation of Antisnake Venom Agents from *Azadirachta indica* (A. Juss) Leaf Extracts. *Academic Journal of Chemistry*. 5(2): 10–16.
- Sani, I., Umar, R.A., Hassan, S.W., Faruq, U.Z., Bello, F., & Abdulhamid, A. (2019). Protective Effect of *Azadirachta indica* Leaf Fractionated Extracts on Renal and Haematological Indices against Snake Venom Toxicity in Albino Rats. *Res. Journal of Medicinal Plants* 13(3): 103–108.
- Tizazu D, Workineh Y, Ayalew Y (2020). Parental traditional medicine use for children and associated factors in the North Mechanical District, North West Ethiopia. *Pediatric Health Medication Therapy* 11: 505–512.
- Trease, G. & Evans, W. (1989). *Pharmacognosy* London: Bailliere (11th ed., pp. 45–50).
- World Health Organization. (WHO). (2017). Snakebite envenoming, fact sheet.
- Ushanandini, S., Nagaraju, S., Kumar, K., Vedauathi, M., Machiah, D. & Kemeparaju, K.(2006).The anti-snake venom properties of *Tamarindus indica* seed extract—*Journal of Ethnopharmacology* 20:851-858.
- World Health Organization (WHO). (2017). Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants. WHO Press.
- Yemane, Y. & Russom, M. (2021). Spontaneous massive hemothorax following snakebite envenomation: a case report. *Authorea Preprints. Journal of Hepatology*, 53(2), pp.357–361.
- Yuan, H., Liye, Q., Piao, G. (2016). Review: Traditional medicine and modern medicine from natural products. *Molecules*, 29(59):1–8.