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Phytochemical Characterization, Acute Toxicity Studies and Anti-inflammatory Activities of Ethanolic Root Extract of *Agave Sasilana* in Albino Rats

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Abstract
The present study investigates the phytochemical and acute toxicity studies of the ethanolic root extract of *Agave sasilana* in albino rats. The lorke's method was used for the acute toxicity study of which twenty-seven (27) male albino rats were used. The extracts were administered at the doses of 50, 100, 150, 200, 250, 500 and 750 mg/kg body weight of the animals. The animals were observed for general signs and symptoms of toxicity including mortality over a period of 48 hours. The acute toxicity study (LD_{50}) showed no adverse effect in their general behavior and mortality at the dose level of 50 and 100 mg/kg body weight. However, death was recorded at the dose concentration of 150 to 750 mg/kg body weight using the Probit table. This observation that a dose of 150 to 750 mg/kg body weight of the extracts produced death is an indication that the ethanolicroot extract is not relatively safe for use at high doses despite its efficacy in the management of diseases. The phytochemical analysis showed the presence of flavonoid (15.390 ± 0.156), alkaloid (12.420 ± 0.158), saponins (4.433 ± 0.081), anthocyanins (0.733 ± 0.015), tannins (0.277 ± 0.059) andphenol (0.443 ± 0.035). The study also revealed that the ethanolic root extract possesses antiinflammatory activities which was evident in the studied inflammatory parameters. The presence of some of these biological molecules in this plant justify to a large extent, some of the ethno- medicinal applications of *Agavesasilana*.

Keywords: *Agave sasilana,Acute toxicity, Phytochemical analysis, Anti-inflammatory activity, Flavonoids, Saponins*

1. Introduction
Medicinal plants have been traditionally used in different kinds of ailments including infectious diseases, which account for approximately one-half of all deaths in tropical countries [1]. Pharmacological actions of medicinal plants include; antioxidant, antimalaria, antidiabetic, antidiarrhea, antitumoral or anticancer, antioxidant, diuretic, analgesic, antimicrobial, anti-

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inflammatory, antibacterial, antifungal, hepatoprotective, immune-modulatory, laxative and sedative activities etcetera [2]. Extracts from leaves, stems, roots of *Agave sasilana* are used locally to prevent ailments. According to the ethnic difference of populations and localities, medicinal plants are either used alone or in combination with other plants or with natural substances for the preparation, especially in decoction [3]. Oral route of administration is mostly used for this preparation. Medicinal plants also help in the alleviation of human suffering especially amongst those living in rural areas. Herbal medicine has a long history in the treatment of several kinds of diseases [4]. The use of medicinal plants for the treatment of diseases has been practiced by man for many years and is still being widely practiced even today [5]. For many years, people have developed a store of information concerning the therapeutic values of local plants before orthodox medical practice appeared. This traditional medicine uses numerous plants, among them is sisal (*Agave sasilana*), is an herbaceous monocotyledonous plant from the *agaveceae* family originating from Central America and Mexico, *Agave sasilana* grows in many tropical countries with Tanzania and Brazil being currently the main producer [6].

The chemistry of the *Agave* leaves, roots and stems is complex and only a few of the component compound have been identified. The most widely known are sapogenins including hecogenin, tigogenin and diosgenin. In pharmaceutical industry, these natural compounds are used for the semisynthesis of medicinal steroids corticosteroid sex hormones and steroid diuretic. According to the earlier research the plant also contains carbohydrates, uronic acids, flavonoids, aliphatic fatty acids and proteins [7].

Various species of the genus *Agave* are used in traditional medicine, according to the tradition of a particular region. In South Africa, leaves of *Agave* Americana are used for treatment of high blood pressure [8]. Their anti-hypertensive properties are related to the angiotensin converting enzymes which inhibit the conversion of angiotensin I to angiotensin II a potent vasoconstrictor [9].

2. Materials and Methods

Plant material collection

Fresh root part of the plant *Agave sasilana* were locally sourced in Umudike farm, Abia State, Nigeria and was identified by Prof. Garuba Omosun, a taxonomist, of the Plant Science and Biotechnology Department, Michael Okpara University of Agriculture, Umudike, Nigeria. The fresh roots were washed and dried under shade at room temperature, using a blender; the roots were blended into powder.

Extraction

The powdered roots of *Agave sasilana* (100 g) were soaked in ethanol for 48 hours and the extract filtered using a Whatman no. 1 filter paper, the filtrate was allowed to evaporate to dryness, under a water bath with a temperature set at 40°C.

Animals

Male albino rats of mean weight of 130 g were used for the study. The animals were kept in the animal house (Michael Okpara University of Agriculture, Umudike, Nigeria), allowed to

acclimatized for two (2) weeks, and were used, following the approval of institutional animal ethical committee. Commercial pellet diet (Vital growers mash; Grand Cereals and Oil Mills, Nigeria) and water were given to the animals ad libitum.

Phytochemical analysis of ethanolic root extract of *Agave sasilana*

The preliminary phytochemical screening of the ethanol extract of *Agave sasilana*were carried out, to ascertain the presence of phytochemicals, those detected were quantified. Both qualitative and quantitative analyses were done using standard methods previously described [10].

Acute Toxicity Studies (LD_{50}) of ethanolic root extract of *Agave sasilana*

The acute toxicity was tested using albino rats, the method and calculation proposed by [11] was employed of which twenty-seven (27) male albino rats were used. The extracts were administered at the doses of 50, 100, 150, 200, 250, 500 and 750 mg/kg body weight based on the body weight of the animals. The animals were observed for general signs and symptoms of toxicity including mortality ov er a period of 48 hours. All doses were administered to the animals orally. The LD_{50} for the plant extract was calculated using probit table.

Anti-inflammatory activity of ethanolic root extract of *Agave sasilana*

Anti-inflammatory drug like Aspirin or plant extract inhibit the spread of erythema and or the size of oedema of the right hind paw or rats in response to inflammation to fresh egg albumen-induced acute inflammation [12].

3. Results

Table 3.1: Qualitative Phytochemical Characteristics of *Agave sasilana root*extracts

Phytochemicals	Indication in Root of *Agave sasilana*
Alkaloid	+
Flavonoid	+++
Lipids	-
Saponins	++
Tannins	+
Phenol	+
Anthocyanin	+

Key:
+++ = presence at bioactive compound in very high concentration.
++ = presence of bioactive compound in high concentration.
+ = presence of bioactive.
— = Absence of bioactive compound.

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The result of phenolic and polyphenolic content of the root of *Agave sasilana* is shown in Table 3.2. There was significant different (P≤0.05) in the phytochemical content. The values ranged from 0.277-15.390 for Tannins content and Flavonoid respectively.

Table 3.2: Results of Quantitative Analysis of *Agave sasilana* root extract

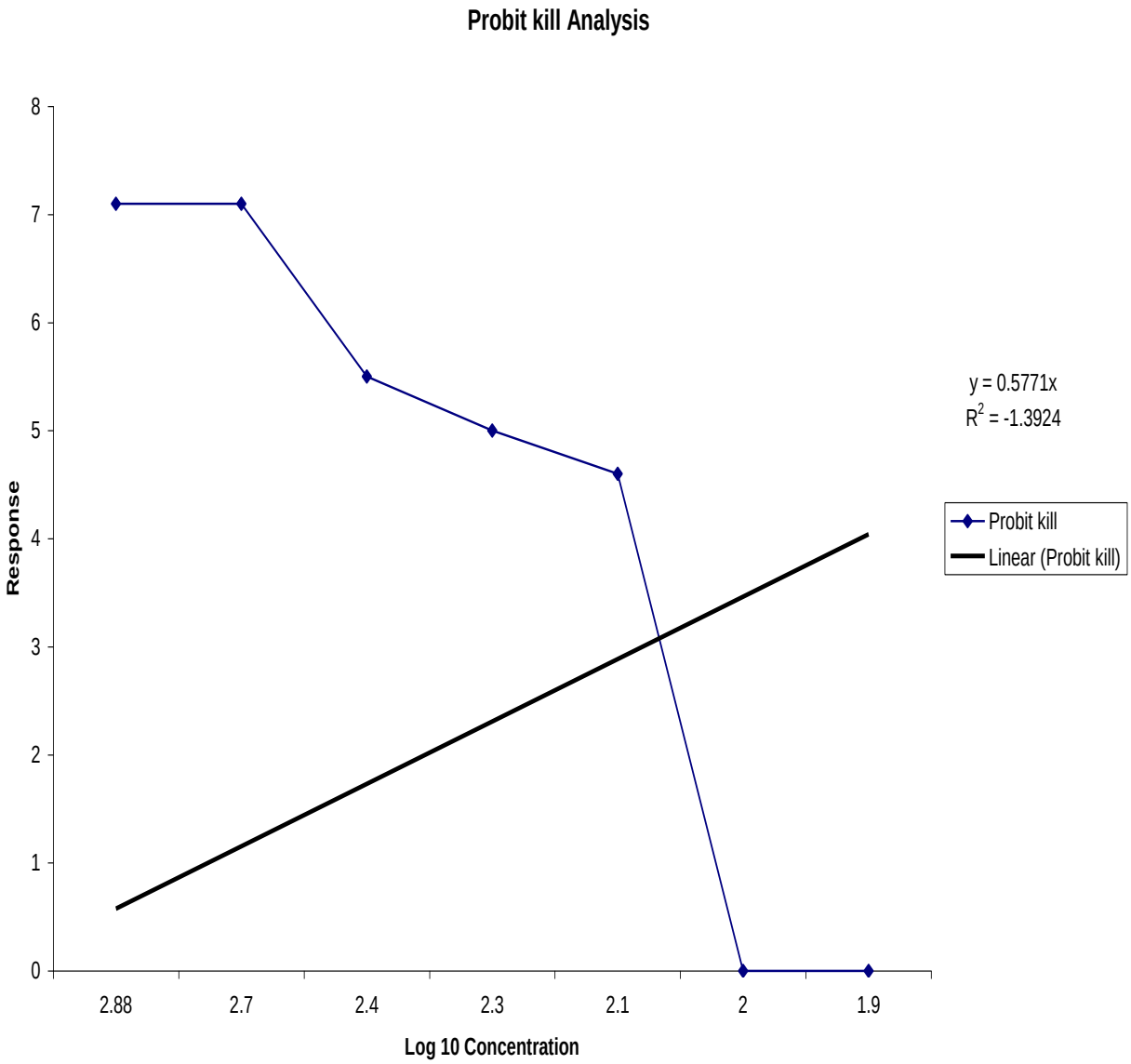
Phytochemical	Percentage Content
Flavonoid	15.390±0.156
Saponins	4.433±0.081
Anthocyanins	0.733±0.015
Tannins	0.277±0.059
Phenol	0.443±0.03
Alkaloid	12.420±0.158

Values are expressed as mean ±standard deviation (n=3)

When the ethanolic extract was administered to the rats there was no death recorded at the dose concentration of 50-100mg/kg body weight. However, death was recorded at the dose concentration of 150-750mg/kg body weight when the extract was administered. LD_{50} was calculated to be 177.80mg/kg body weight using the probit Table. This was determined by the method cited in materials and methods. The result of acute toxicity is as presented on Table 3.3. Death was recorded at a dose at 150mg/kg body weight following the administration at the extracts. This suggests that there could be a particular bioactive compound resident in the extract that must have causedthe lethality. This observation that a dose of 150mg/kg body weight of the extracts produced death is an indication that the plant is not relatively safe for use at high doses despite its efficacy in the management of diseases.

Table 3.3: ResultofAcute Toxicity Testing (LD_{50})

Group	Conc. of Ext	Log. Concur	No. of Animal/grp	No of Animal killed	% Mortality	Probit kill
1	750	2.88	6	6	100	7.1
2	500	2.70	6	6	100	7.1
3	250	2.40	6	4	66.67	5.5
4	200	2.30	6	3	50	5.0
5	150	2.10	6	1	33.33	4.6
6	100	2.00	6	0	0	0
7	50	1.90	6	0	0	0



LD_{50} = 177.80mg/kg b.w
FIGURE 1: RESULT OF ACUTE TOXICITY TESTING (LD_{50})

The result of the inflammation (diameter) in time (mins) is presented in Figure 4.2. This shows that the inflammation is dependent of time and dose of fresh egg albumin. The percentage inflammation is presented in Figure 4.3. This shows that the percentage of inflammation is

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dependence on time and dose. The result of the anti-inflammatory activity is shown in Figure4.4. The inhibition of inflammation of the plant extract is time and concentration dependent.
The result of the inflammation using Arachidonic acid (2mg/ml) is shown in Figure4.5. The rate of inflammation depends on time and concentration.
The result of percentage inflammation is shown in Figure 4.6. Percentage inflammation depends on time and concentration.
The result of percentage inhibition of inflammation is shown in Figure 4.7. Percentage inhibition is time and concentration dependent.

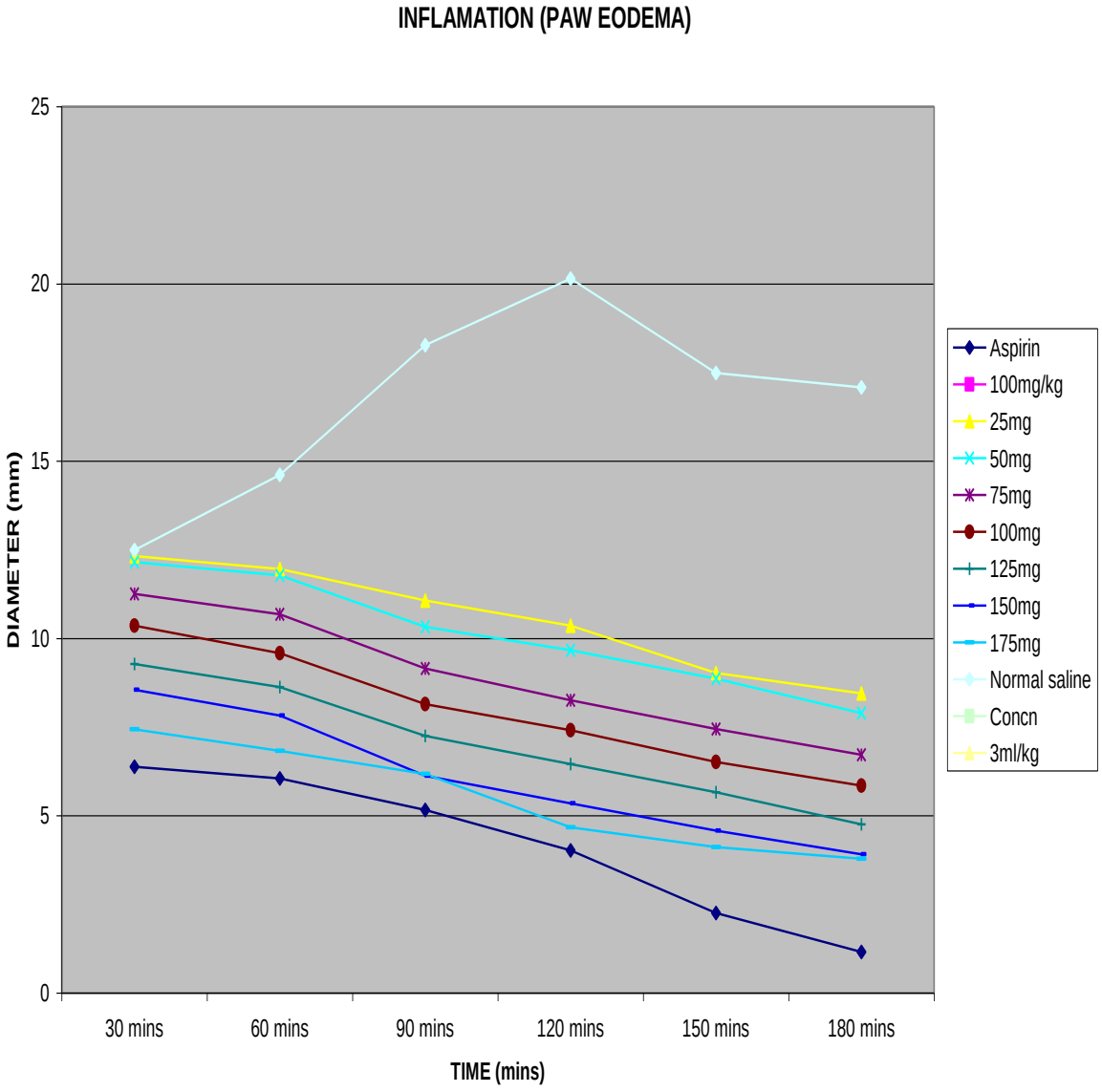

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FIGURE 4.2: RESULT OF TIME (MINUTES) AND LINEAR HIND PAW EODEMA DIAMETER (mm) USING 2mg FRESH EGG ALBUMIN

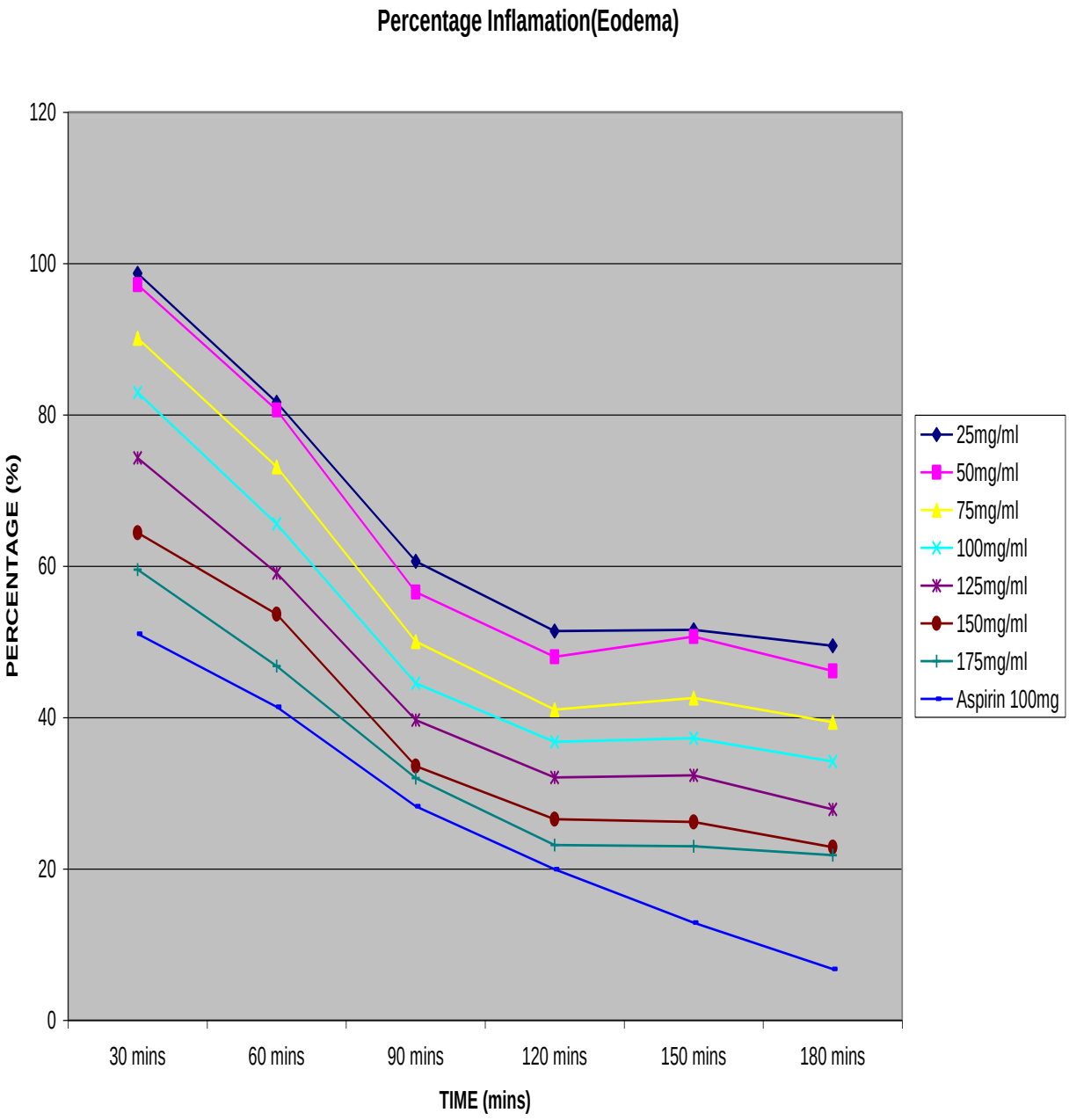

FIGURE 4.3: PERCENTAGE INFLAMMATION (EODEMA) PER TIME INTERVAL USING NON-TREATED ANIMAL AS CONTROL Using Fresh egg Albumin (2mg)

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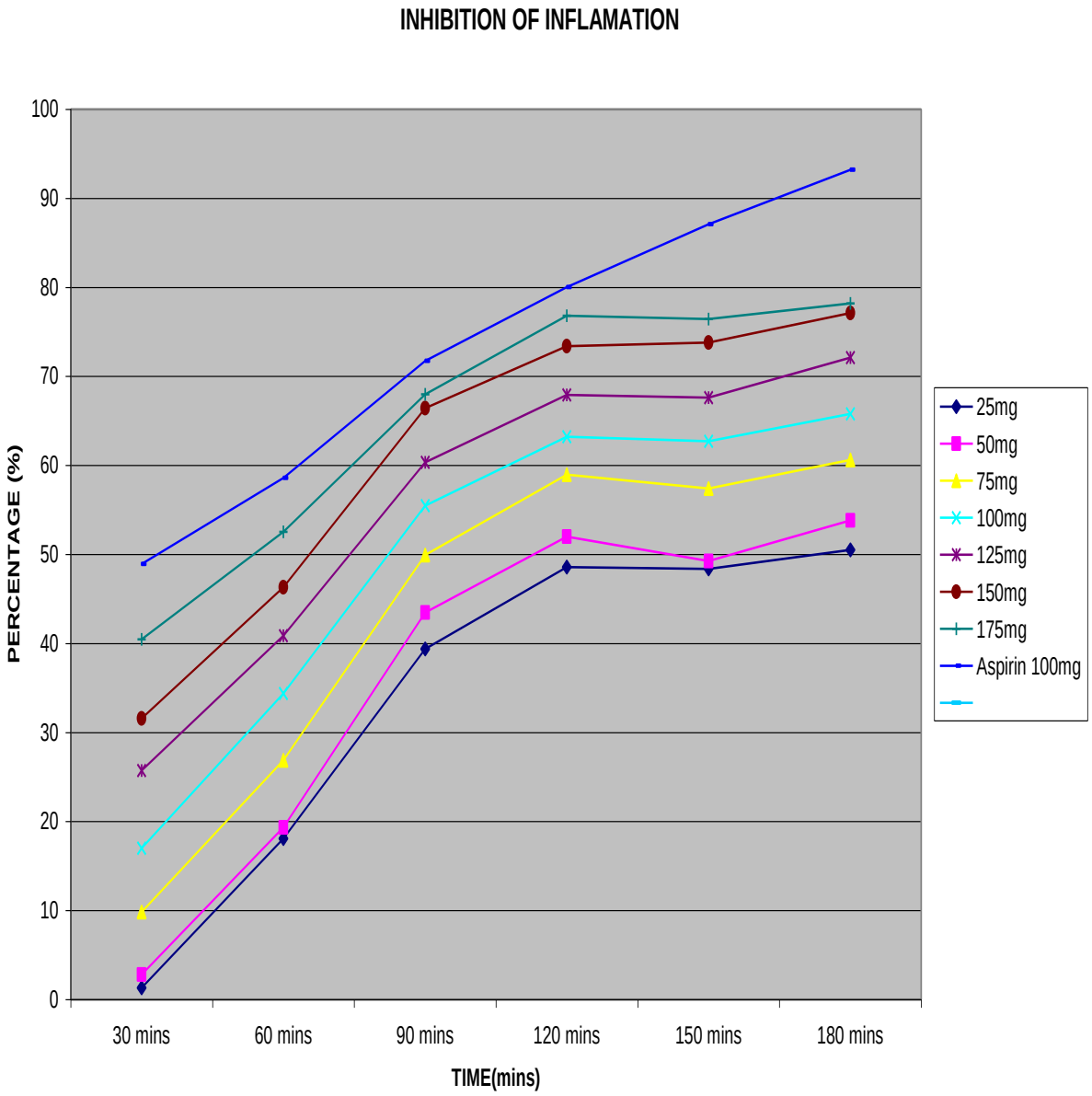

Figure 4.4: percentage inhibition of inflammation (EODEMA) per time interval 20mg/mL) fresh egg albumin

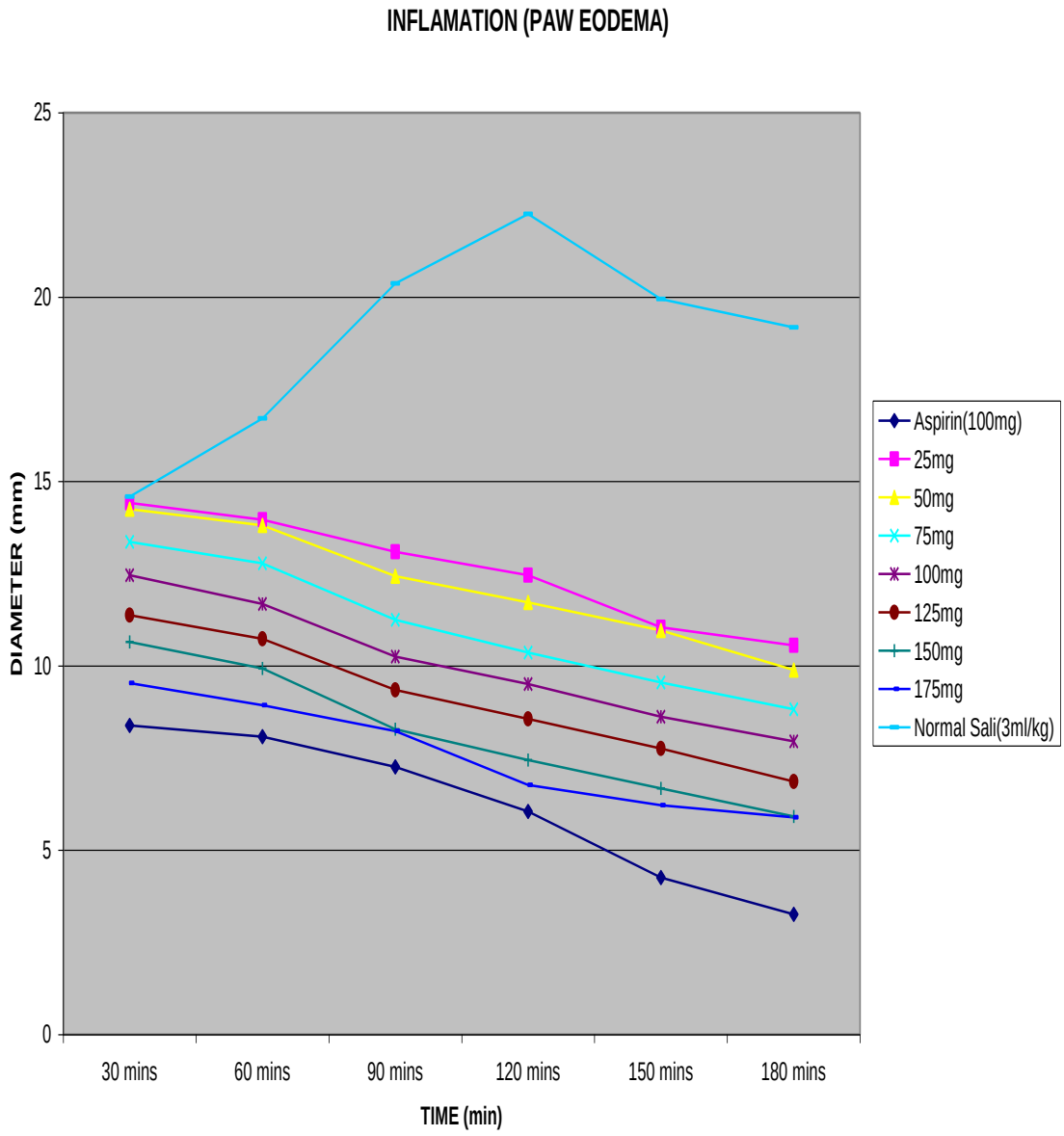

FIGURE 4.5: RESULT OF TIME (IN MINUTES) AND LINEAR HIND RAW EODEMA DIAMETER USING ARACHIDONIC ACID (2 mg/mL)

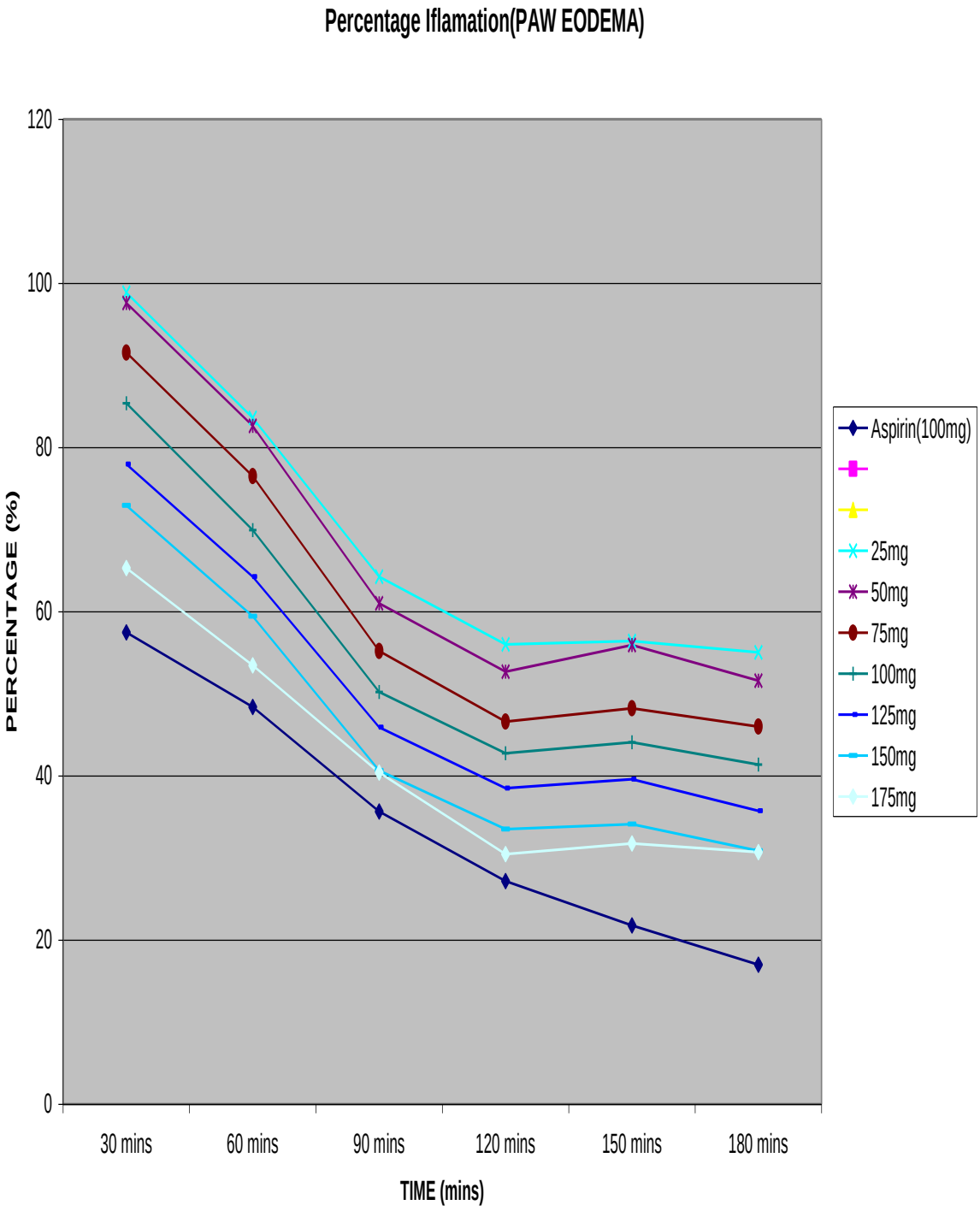

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FIGURE 4.6: PERCENTAGE INFLAMMATION (Eodema) USING ARACHIDONIC ACID (2mg/mL)

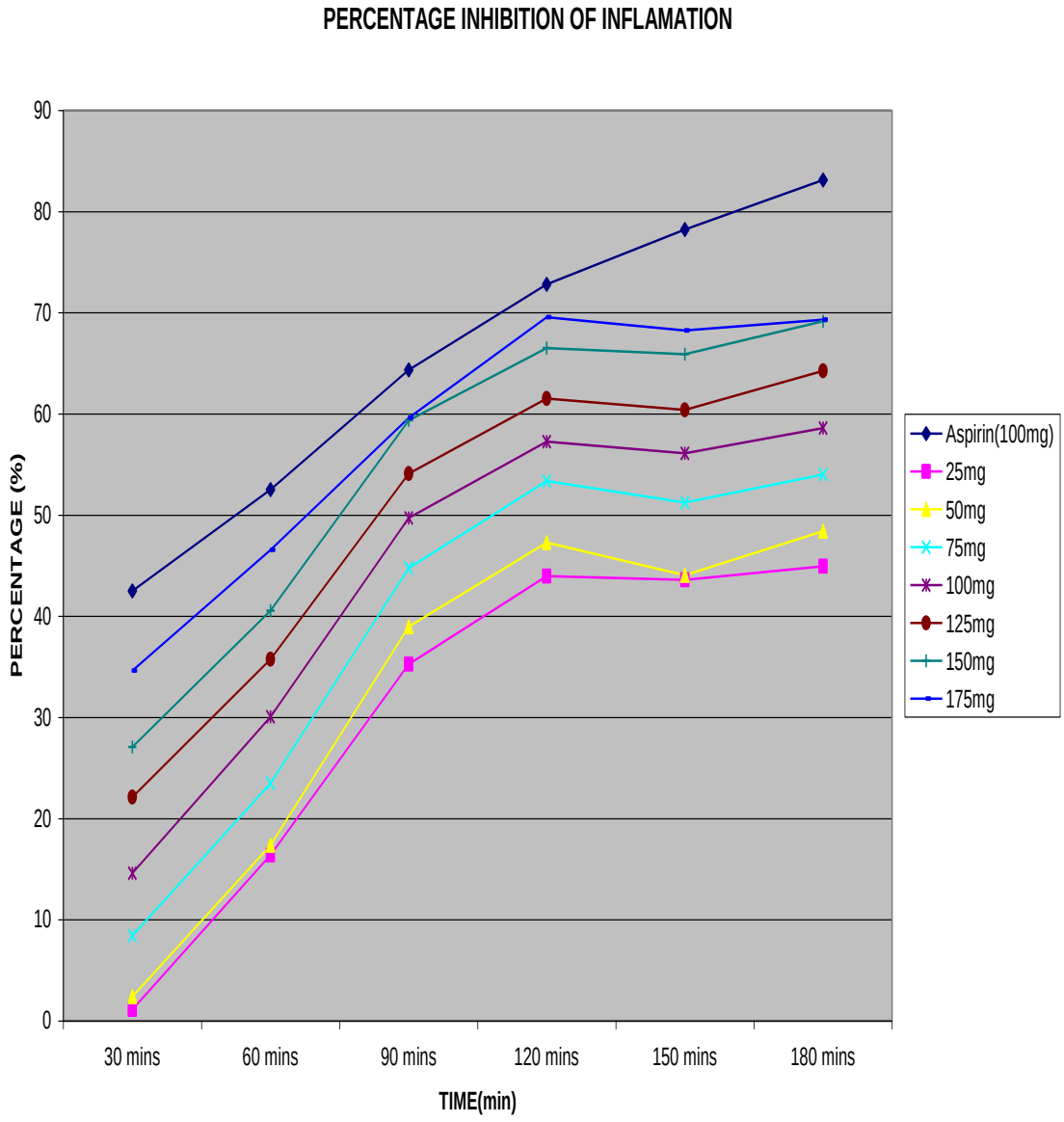

FIGURE 4.7: RESULT OF PERCENTAGE INHIBITION OF INFLAMMATION (EODEMA) USING 2mg/mL OF ARACHIDOMIC ACID

4. Discussion

The results of this study clearly indicate that *Agave sasilana* contains bioactive compound that are pharmacologically beneficial to human health, and that could be useful in disease conditions if use at a therapeutics dose. The pharmacological beneficial effects of these bioactive compounds were

demonstrated to be mainly through acting as antioxidants, antibacterial and antifungal properties, as well as having diuretic and antinflammatory properties. These were demonstrated and confirmed using acceptable chemical, physical and biochemical analysis[13].

The result of the phytochemical analysis revealed the presence of flavonoids, saponins,tannins alkaloid, phenol and anthocyanins. This result showed that the root of the plant is rich in phytochemicals [14].

Saponin is known to have hypocholesterolemic activities. This indicates that the use of this medicinal plant could help the protection and management of coronary heart diseases [15]. Tannins are polyphenols and have antimicrobial properties as well as ability to stabilize membrane against heart of lypotanic stresses. The presence of tannins in this plant could confer chemoprotective advantage to users [16]. Tannins may be employed mechanically in anti-diarrheal and anti-hemorhadal preparations. Tannins are also inhibitors of HIV replications. It therefore means that this plant has a great potential of becoming a reputable drug candidate [17]. Detected also in this plant is flavonoid. Flavonoids are polyphenolic molecules common to most flavoring plants. Flavonoid may help provide protection against some diseases such asatherosclerosis, Ischemic injury, inflammation, diabetes, cancer, liver cirrhosis, by contributing along with antioxidants, vitamins and enzymes to the defense system of the human body [18]. Epidemiological studies have shown that flavonoids intake is inversely related to mortality from coronary heart diseases and to the incidence of heart attacks [19]. The alkaloids are responsible for reducing the blood pressure, and are thus known for the hypotensive effect [20]. This phytochemical could be contributory to the efficacy of *Agave sasilana* in the management of arthritis and in inflammation as claimed by the herbal practitioner. Most diseases etiological factor is the biological oxidations that occur regularly in biological systems. Flavonoids can scavenge biological free radicals, and chemoprevents biological oxidation as etiological factor.

The presences of these biological molecules justify some of the reasons, for the ethno-medicinal applications of *Agave sasilana.*

The result of this study also shows that the ethanolic extract of *Agave sasilana* root is relatively safe and provides some scientific basis for the administration of *Agave sasilana* extract by the herbal medical practitioner in Yagba, Kogi state of Nigeria for the treatment of diverse disease conditions; the ethanolic root extract produced LD_{50} of 177.80mg/kg body weight in mice. This indicates the relative safety in the use of the plant as medicinal preparation. This implies that toxicity could only be achieved at a very high dosage and prolonged use [21].

From the results presented in figures 4.2- 4.7, it showed that the extract is highly potent as an anti-inflammatory agent. The anti-inflammatory activity of the extract was comparable to that of the standard anti-inflammatory drug (Aspirin), and it has been revealed that flavonoid exert profound anti-inflammatory effects invitro and in vivo in experimental animals [22]. Tannins and saponins also possess similar activity [23]. The high anti-inflammatory activity exhibited by the root of *Agave sasilana* in this study may be due to its flavonoids, tannins and anthocyanins content.

Mechanisms that possibly underline this anti-inflammatory activity may include inhibition of the actions of inflammatory mediators such as histamine, prostaglandin, nitric oxide, cytokines, platelet activating factor and substance P_1, effects on adrenocorticoid hormones and

immunosuppression. Earlier investigations have revealed that various herbal preparations are capable of exerting anti-inflammatory activity [24].

5. Conclusion

The present study investigated the acute toxicity, phytochemical, and anti-inflammatoryactivities of ethanolic root extract of*Agave sasilana*. The study establishes toxicity in the ethanolic extract of *Agave sasilana* when administered at a higher dose. The use of phytochemicals is of particular interests in several disease management. Many of these phytochemicals establish their disease curative potency via multiple processes and mechanisms. These characteristics, as well as their generally low toxicity, position these phytocompounds as crucial in attempts to find less expensive curative drugs in both resource poor environment and in developed economics. Also, the results from this investigation showed that the ethanolic root extract of *Agave sasilana* had some worth for future utilization study, either to be developed as anti-inflammatory drug or a source of natural antioxidants and antimicrobial agents.

Moving forward, there are needs for more functional studies to understand the biochemistry that would reveal the mechanisms and pathways of these bioactive compounds.

Ethical Approval

All authors hereby declare that “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropiate ethics committe.

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We sincerely appreciate all that made this work successful.

Conflict of Interests

We declare that there is no conflict of interests.

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