INTERNATIONAL JOURNAL OF APPLIED SCIENCE RESEARCH
FEDERAL POLYTECHNIC OKO, ANAMBRA STATE

DETERMINATION OF PHYTOCHEMICAL CONSTITUENTS AND ACUTE TOXICITY (LD_{50}) OF ETHANOL LEAF-EXTRACT OF *STACHYTARPHETA CAYENNENSIS*

[1]Ezebuilo Ugbala Ekpono, [2]Ernest Obaji Ezeh, [1]Perpetual Lovinda Ugwu and [1]Blessing Precious Egwu

[1]Department of Science Laboratory Technology, Biochemistry Option, Federal Polytechnic, Oko, Anambra State, Nigeria

[2]Department of Biochemistry, Faculty of Sciences, Ebonyi State University, Abakaliki, Nigeria

Phone Number: +2348136482643

Corresponding Author's E-mail Address: ekponoezebuilo@gmail.com; ekpono.ezebuilo@federalpolyoko.edu.ng

ABSTRACT

This study was designed to determine the LD_{50} and phytochemical constituents of ethanol leaf-extract of *Stachytarpheta cayennensis*. Twenty-four mice were used for the acute toxicity (LD_{50}) test. The mice were randomly assigned into seven groups of 1-7 with three mice in each group under the doses of 0, 100, 1000, 2000, 3000, 4000 and 5000 mg/kg body weight of the mice respectively. Group 1 served as the control. Groups 2-7 were administered with the extract with the corresponding doses and monitored closely for the periods of 72 hrs to ascertain the extract toxicity and/or lethal dose. Evaluation of phytochemicals composition and acute toxicity were known done using standard methods. The results revealed the following order of occurrence of phytochemicals (mg/100g): flavonoids > saponins > alkaloids > phenols > tannins > glycosides in the S*tachytarpheta cayennensis* ethanol leaf-extract. The acute toxicity result showed that even at extract dose of 5,000 mg/kg body weight of the mice, *Stachytarpheta cayennensis* ethanol leaf-extract did not cause death to any of the experimental mice, however, signs of toxicities were observed at the doses of 4000 and 5,000 mg/kg. This study buttresses the traditional uses of this plant in the management of some diseases conditions.

Keywords: Phytochemical, Acute Toxicity, *S. cayennensis,* Ethanol and Leaf-extract

Introduction

Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Plants have been an important source of medicine with qualities for thousands of years. They have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against predators such as insects, fungi and herbivorous mammals (Tapsell, Hemphill, Cobiac, 2006). Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs. Herbal medicines do not differ greatly from conventional drugs in terms of how they work (Tapsell *et al.,* 2006). This enables herbal medicines to be as effective as conventional medicines and also gives them the same potential to cause harmful side effects (Lai and Roy, 2004). Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including aspirin, digitalis, quinine, and opium (Tapsell *et al.*, 2006).

Furthermore, many of the common weeds that populate human settlements, such as nettle, dandelion and chickweed, also have medicinal properties (Stepp, 2004). Other non human primates are also known to ingest medicinal plants to treat illness (Stepp, 2004). Some of the

medicinal plants include, *Cannabis sativa* (marijuana) which is applied topically for inflammation, *Sphenocentrum jollyanum* which is used in the management of malaria (Ekpono *et al.*, 2018a) and the essential oil of *Thymus vulgaris* called thymol which is used as a powerful antiseptic and antifungal which is used in a variety of products (Pierce, 1999).

Stachytarpheta cayennensis is a weedy and sometimes perennial herbaceous flowering plant from the verbaenaceae family. They are evergreen sub shrub which can reach heights of 2.5 m. It has a woody glabrous stem with several branches. Their leaves are opposite, membranous, elliptic to broadly elliptic or ovate, 4-8 cm long, 2-4.5 cm wide, upper surface rugose. They have surfaces that are glabrous or occasionally lower with a few scattered hairs usually along the veins and margins. Their margins are sharply and coarsely serrate with the teeth conspicuously divergent, apex acute, base cuneate and petioles 0.5-2 cm long. They have spikes that are slender with the rachis flexuous to erect or somewhat nodding, 14-40 cm long, ca. 2.5 mm in diameter. The furrows are somewhat shallow, nearly as wide as the rachis (Wagner *et al.,* 1999). In Nigeria, it is called 'tsárkíyárkúúsù (Hausa), "àgógó ìgún"(Yoruba), ogwu-ugwa (Ibo), (Efik), opua-ali (Izzi, Ebonyi State). Names in other languages include "honagasō" (Japanese), "gervão-urticante" (Brazilian), "piche de gato, rabo de zorro" (Spanish), "herbe á chenille", "herbe bleue", "queue de rat" (French), *ōi*; "ōwī" (Hawaiian), and "tiāki" (Māori) (Hyde, 2013).

Stachytarpheta cayennensis extracts is used in parts of Southern Nigeria (Kvist *et al.*, 2006), Latin America, India and Peru for the treatment of malaria. The boiled juice or tea made from the leaves or the whole plant is taken to relieve fever and other symptoms. The whole plant is boiled and the liquid drunk as a tea to treat high blood pressure and thrush (Adebajo, 2007). A tea of the leaves is also taken to control diabetes in Peru and other areas and for treatment of inflammation, pain, hepatic and renal disorders, helminthiasis, constipation, hypertension and stress (Eliakim-Ikechukwu *et al.,* 2013). It is a remedy for dysentery in Belize, together with syphilis, gonorrhoea and catarrheal conditions (Gill, 1992). The roots, stems and leaves are used as a decoction in baths to remedy grippe, headache and fractures (Hyde, 2013). The plant is used externally to treat wounds and toothache. The leaves are astringent, cholagogue and purgative. They are used to make a resolutive cataplasm. An infusion is used to treat colic, dysentery pain, diabetes and malaria (Adebajo, 2007). The leaves and leaf-juice are used externally as a wash to treat a range of conditions including eye infections, bacterial infections, chicken pox, and measles. Latex obtained from the plant is used for treating venereal diseases, especially syphilis (Schapoval, 1998).

This study was designed to determine the LD_{50} and phytochemical constituents such as phenols, flavonoids, tannins, alkaloids, glycosides and saponins of ethanol leaf-extract of *Stachytarpheta cayennensis*.

Materials and Methods

Collection of Biological Materials

S. cayennensis leaves were collected from a farmland in Ekwe-Agbaja, Izzi, Ebonyi State. It was identified by a Taxonomist in Applied Biology Department, Ebonyi State, University, Abakaliki. Total of twenty four (24) albino mice weighing between 16-25 g and the age of 2-4 months

were procured from Animal Unit, University of Nigeria, Nsukka, Enugu, Nigeria. They were housed in a well ventilated cage in the Animal House of the Biochemistry Department, Ebonyi State University, Abakaliki, fed with normal rat chow.

Plate 3: *Stachytarpheta cayennensis* Leaf (Mg. ×3)

Preparation of Plant Extracts

Fresh leaves of *Stachytarpheta cayennensis* were air-dried at room temperature for 2-3 weeks and thereafter homogenized using a manual blender. A known quantity (325.10g) of the dried and homogenized sample of *Stachytarpheta cayennensis* was macerated in 400 ml of 96% ethanol for 48 hours and filtered with a 2 mm sieve cloth. It was allowed to evaporate to dryness at room temperature. The concentrated *S. cayennensis* were stored in the refrigerator for subsequent studies.

Acute Toxicity Studies (LD_{50}) Design

Acute toxicity studies (LD_{50}) was determined according to the modified method of Lorke (1983). Doses of 100 to 5,000 mg/kg body weight were used. Total of twenty four (24) albino mice were randomly assigned into seven groups of 1-7 with three mice in each group under the doses of 0, 100, 1000, 2000, 3000, 4000 and 5000 mg/kg body weight of the mice respectively. Group 1 served as the control. Groups 2-7 that were administered with the extract and monitored closely for the periods of 72 hrs to ascertain if the extract is toxic and/or lethal or not.

Quantitative Phytochemical Analysis of Ethanol Leaf-extract of *Stachytarpheta cayennensis*

Determination of Alkaloids

The determination of alkaloid was as described by Harborne (1973). Five gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid and ethanol was added, covered and allowed to stand for 2 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract till a precipitate was formed. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Determination of Flavonoids

This was determined according to the method of Harborne (1973). Five gram of the sample was boiled in 50 ml of 2M HCl solution for 30 minutes under reflux. It was allowed to cool and then filtered through Whatman No. 1 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The solution was filtered into a weighed crucible. The filtrate was heated to dryness in an oven at 60^0C. The dried crucible was weighed again and the difference in the weight gave the quantity of flavonoid present in the sample.

Determination of Glycosides

This was done according to the method of Harborne (1973). One gram of the sample was macerated with 20 ml of distilled water; 2.5 ml of 15 % lead acetate was added and then filtered. Exactly 2.5 ml of chloroform was added and the mixture shaken vigorously. The lower layer was collected and evaporated to dryness. The residue was dissolved with 3 ml of glacial acetic acid. Exactly 0.1 ml of 5% ferric chloride and 0.25 ml of conc. H_2SO_4 were added, shaken and incubated for 2 hours in the dark. The absorbance of the solution was read at 530 nm. The glycoside concentration was calculated using the equation:

$$\frac{100}{W} \times \frac{Au}{As} \times \frac{C}{1000} \times \frac{Vf \times D}{Va}$$

Where:
W=Weight of sample analyzed
Au=Absorbance of test sample
As=Absorbance of standard solution
C= Concentration of standard in mg/ml
Vf= Total Filtrate volume
Va= Volume of filtrate analyzed
D=Dilution factor

Determination of Saponins

The determination of alkaloid was as described by Sofowora (1993). Exactly one gram of the sample was weighed into a beaker and soaked with twenty mililitres of petroleum ether. The solution was decanted into a beaker and washed again with 10 ml of petroleum ether. The filtrate was allowed to evaporate using a water bath until it was completely dry. Exactly six mililitres of ethanol was used to dissolve the dried sample. Two miliitres of the sample was introduced into a test tube and two mililitres of chromogen solution was added and allowed to atand for thirty minutes. The absorbance was read at 550 nm.

Determination of Tannins

This was done according to the method of Pearson (1976). One gram of the sample was macerated with 50 ml of methanol and then filtered. To the filterate 5 ml was added to 0.3 ml of 0.1 M ferric chloride in 0.1 M HCl. To this was added 0.3 ml of 0.0008 M potassium ferricyanide. Absorbance was taken at 720 nm.

Results were expressed as mg/100g of tannic acid equivalent using the calibration curve:

$Y = 0.0593x - 0.0485$, $R^2 = 0.9826$

Where x is the absorbance, Y is the tannic acid equivalent, 0.0593 and 0.9826 is the conversion factor.

Determination of Phenols

This was determined by the method of Harbone (1973). The total phenol content of the sample was determined spectrophotometrically with folinciocalteaus reagent. An aliquot of the extract (0.5 g) was mixed with 2.5 ml of 10 % folin-ciocalteu reagent and 2 ml of Na_2CO_3 (755w/v). The resulting mixture vortexes for 15 seconds and incubated at 40 °C for 30 minutes for colour development. The absorbance was read at 765 nm. The total phenol content was gotten from the formular below;

$$\frac{100}{W} \times \frac{Au}{As} \times \frac{C}{1000} \times \frac{Vf \times D}{Va}$$

Where:
W=Weight of sample analyzed
Au=Absorbance of test sample
As=Absorbance of standard solution
C= Concentration of standard in mg/ml
Vf= Total Filtrate volume
Va= Volume of filtrate analyzed
D=Dilution factor

Results

Phytochemical Constituents of *Stachytarpheta cayennensis* Ethanol Leaf-Extract

The results of the phytochemical constituents of *Stachytarpheta cayennensis* leaf showed the presence of tannins, flavonoids, saponins, phenols, glycosides and alkaloids in the extract sample. The results revealed the following order of occurrence of the phytochemicals: flavonoids > saponins > alkaloids > phenols > tannins > glycosides as shown in Figure 1.

Figure 1: Phytochemical constituent in mg/100g of *Stachytarpheta cayennensis* ethanol leaf-extract

Acute Toxicity of the Mice

The acute toxicity testing showed that even at extract dose of 5,000 mg/Kg body weight of the mice, *Stachytarpheta cayennensis* ethanol leaf-extract did not cause death of any of the experimental mice. However, sluggish movement, foul smelling urine and stools, raised hair and loss of appetite were observed at the group administered with the dose of 4000 and 5000 mg/Kg body weight of the mice as shown in Table 1.

Table 1: The Acute Toxicity Study Result of *Stachytarpheta cayennensis* ethanol leaf-extract

GROUPS	DOSAGE (mg/Kg)	A/D	PERIOD	TOXICITY OBSERVED
Group 1	0	3/0	72 hours	----------
Group 2	100	3/0	72 hours	No sign of toxicity
Group 3	1000	3/0	72 hours	No sign of toxicity
Group 4	2000	3/0	72 hours	No sign of toxicity

Group 5	3000	3/0	72 hours	No sign of toxicity
Group 6	4000	3/0	72 hours	Dizziness, slugishness
Group 7	5000	3/0	72 hours	Weakness, foul smelling urine

KEY:
A/D = number of mice administered/number of deaths

Discussion

The results of the phytochemical constituents of leaf-extract of *Stachytarpheta cayennensis* clearly revealed the presence of tannins, flavonoids, saponins, phenols, glycosides and alkaloids in the sample in the following order of occurrence: flavonoids > saponins > alkaloids > phenols > tannins > glycosides as shown in Figure 1.

This finding correlate with the following report: Iwu *et al.* (2018) who reported the presence of phytochemical like alkaloids, tannins, saponins, glycosides, steroids and phenols in *Stachytarpheta cayennensis* root extract, Okoye *et al.* (2014) who reported that preliminary phytochemical analysis of leaf-extract of *Stachytarpheta cayennensis* revealed the presence of carbohydrates, glycosides, flavonoids, saponins, alkaloids, terpenoids and steroids. Ezeabara (2015) who reported that stem of *S. cayennensis* revealed the presence of alkaloid (3.46 ± 0.017%), sterol (0.44 ± 0.012%), saponin (2.85 ± 0.006%), flavonoid (1.89 ± 0.046%), tannin (1.83 ± 0.011%), and phenol (0.31 ± 0.012%). Ekpono *et al.,* 2018c, who reported the following order of occurrence of phytochemicals (mg/100g): alkaloids > phenols > terpenoids > flavonoids > steroids > tannins in ethanol root-extract of *Sphenocentrum jollyanum.*

These compounds have been shown to be active against some pathogens including those that are responsible for enteric infections (Owolabi *et al.,* 2007). These compounds have also been shown to have potential antibacterial and antimalarial activities and also act as analgesics agents and can equally act as stimulants. The presence of flavonoids suggested that the leave of this plant may be good for the management of cardiovascular diseases and oxidative stress, since flavonoids are biologic antioxidants (Ekpono *et al.,* 2018b). Several studies have shown that certain flavonoids can protect low density lipoprotein cholesterol from being oxidized (Donald and Cristobal, 2006). Alkaloid has been used as central nervous system stimulant, topical anaesthetic in ophthalmology, powerful pain relievers, as anti puretic, among other uses (Heikens *et al.,* 1995).

The acute toxicity results showed that even at extract dose of 5,000 mg/Kg body weight of the mice, *Stachytarpheta cayennensis* ethanol leaf-extract did not cause death to any of the experimental mice. This implies that LD_{50} of this plant extract could be greater than 5,000

mg/Kg in mice (Table I). This work agrees with the finding of Idu *et al.* (2007) who reported that acute toxicity of the aqueous leaf-extract of *Stachytarpheta cayennensis* on Wistar rats revealed no mortality even up to dose of 4 g kg^{-1} body weight and no significant changes in body weight $p>0.05$. Also, eye color was normal and loss of hair was absence. This finding also correlate with that of Okoye *et al.* (2014) who reported that LD_{50} of the leaf-extract of *Stachytarpheta cayennensis* was greater than 5000 mg/Kg (24 h) suggesting that the extract may be non-toxic. However, this finding disagrees with the report of Okokon *et al.* (2008) who reported that ethanolic leaf extract of *Stachytarpheta cayennensis* produced physical signs of toxicity such as writhing, gasping, palpitation, decreased respiratory rate, body and limb tone and death depending on the dose. However, all the mice treated with 4000 mg/kg dose of the extract and above died.

Conclusion

In toxicity rating by joint FAO/WHO Expert Committee on Food Additives (WHO, 1966), if at 2 g/Kg oral dose no death occurred, it is sufficient to assume the substance to be non lethal. The non-lethality through oral route justified therapeutic use of this plant parts by the traditional healers. This study provides scientific evidence in the use of this plant in management of some diseases conditions due to its phytochemical constituents.

Recommendations

The individual phytochemical constituents of this plant can be isolated and their biological activities and effects tested on different models in order to bring about the discovery of novel and effective antibiotics. Secondly, other parts of this plant such as stern bark, flowers, seed oil and root bark can also be tested for their anti-malaria potentials.

References

Adebajo, A. C. (2007). Hypoglycaemic Constituents of *Stachytarpheta cayennensis* Leaf. *Planta Medicine,* **73**(3): 241-250.

Donald, R. B. and Cristobal, M. (2006). Antiodant Activities of Flavonoids. *Journal of Agnaitine,* **52**: 125-757.

Ekpono, E. U., Aja, P. M. and Ugwu, Okechukwu P. C. (2018a). Effect of Ethanol Root-Extract of *Sphenocentrum jollyanum* on $CD4^+$ count and some Oxidative Stress Indices in *Plasmodium berghei*-infected mice. *Journal of Biochemistry, Biotechnology and Allied Fields,* **3**(1): 48-60.

Ekpono, E. U., Aja, P. M., Ugwu Okechukwu, P. C., Ibiam, U.A., Offor, C. E. and Udeozor P. A. (2018b). Alterations of Lipid Profile in *Plasmodium berghei*-infected Mice Treated with Ethanol Root Extract of *Sphenocentrum jollyanum. IDOSR Journal of Biology, Chemistry and Pharmacy,* **2**(1): 18-33.

Ekpono, E. U., Aja, P. M., Ugwu Okechukwu, P. C., Udeozor, P. A., Uwa, C. U. and Nweke, O. L. (2018c). Phytochemical and Proximate Analysis of *Sphenocentrum jollyanum* Ethanol root Extract and Dry Sample Collected from Ebonyi State, Nigeria. *IDOSR Journal of Biology, Chemistry and Pharmacy,* **2**(1): 8-17.

Eliakim-Ikechukwu, C., Obiri, A. and Igiri, A. (2009). The Effect of Aqueous Ethanolic Extract of *Stachytarpheta cayennensis* on the Histology of the Lines and Fasting Blood Sugar of Non-diabetic and Diabetic Instar Nets. *The Internet Journal of Nutrition and Wellness*, **10**(1): 12-14.

Ezeabara, C. A. (2015). Comparative Study of Phytochemical, Proximate and Mineral Compositions of *Stachytarpheta cayannensis* (L.C. Rich.). Chapman and Hall. Thompson Science, Vahl. pp 34-40.

Gill, L. P. (1992). Remedy for Dysentery, Syphillis, Gonorrhoea and Catarrhal Conditions. *African Journal of Biotechnology,* **4**(7): 685-688.

Harborne, J. B. (1973). *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis*. Chapman and Hall. Thompson Science, London. pp 107.

Heikens, H., Fliers, E., Endert, E., Ackermans, M. and Van Mont Frans, (1995). Liquorice-induced Hypertension, a New Understanding of an Old Disease. *Journal of Medicine,* **5**: 230-234.

Hyde, M. A. (2013). *Species Information*: *Stachytarpheta cayennensis*. Flora of Zimbabwe. pp 56-60.

Idu, M., Omogbai, E. K. I., Aghimien, G. E., Amaechina, F., Timothy, O. and Omonigho, S. E. (2007). Preliminary Phytochemistry, Antimicrobial Properties and Acute Toxicity of *Stachytarpheta jamaicensis* (L.) Vahl. Leaves. *Trends in Medical Research,* **2**: 193-198.

Iwu, I. C., Onu, U. L., Ukaoma, A. A. and Oze, R. N. (2018). Phytochemical, Antimicrobial and Gc/Ms Analysis of the Root of *Stachytarpheta Cayennensis* (L.Vahl) Grown in Eastern Nigeria. *International Research Journal of Natural Sciences,* **6**(2): 1-14.

Kvist, L. P., Christensen, S. B., Rasmusen, H. B., Mejia, R. and Gonzalez, A. (2006). Identification and Evaluation of Peruvian Plant used to Treat Malaria and Leishmaniasis. *Journal of Ethnopharmacology*, **106**: 390-402.

Lai, P. K. and Roy, J. (2004). Antimicrobial and Chemopreventive Protective Properties of Herb and Spices. *Journal of Current Medical Chemistry,* **11**(11)**:** 1451-1460.

Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. *Archive of Toxicology,* **54:** 275-287.

Okokon, J. E., Ettebong, E., and Antia, B. S. (2008). *In vivo* Antimalarial Activity of Ethanolic Leaf Extract of *Stachytarpheta cayennensis. Indian Journal of Pharmacology,* **40**(3): 111–113.

Okoye, T. C., Akah, P. A., Ezike, A. C., Uzor, P. F., Odoh, U. E., Igboeme, S. O., Onwuka, U. B. and Okafor, S. N. (2014). Immunomodulatory Effects of *Stachytarpheta cayennensis* Leaf Extract and its Synergistic Effect with Artesunate. *Complementary and Alternative Medicine*, **14**: 376-380.

Owolabi, O. J., Omogbai, E. and Obasuyi, O. (2007). Antifungal and Antibacterial Activities of the Ethanolic and Aqueous Extracts of *Kigelia africana* (Bignoniaceae) Stem Bark. *African Journal of Biotechnology,* **6**: 1677-1680.

Pearson, D. (1976). *The Chemical Analysis of Foods*. (7th Edition), Edinburgh, Churchill Livingstone. pp 575.

Pierce, A. (1999). American Pharmaceutical Association Pratical Guide to Natural Medicines. New York: Stonesong Press. pp 338-340.

Schapoval, E. E., Vargas, M. R., Chaves, C. G., Bridi, R., Zuanazzi, J. A., Henriques, A. T. (1998). Antiinflammatory and Antinociceptive Activities of Extracts and Isolated Compounds from *Stachytarpheta cayennensis*. *Journal of Ethnopharmacology,* **60**: 53–59.

Sofowora, A. E. (1993). *Medicinal Plants and Traditional Medicine in Africa*. (2nd Edition). Ibadan, Nigeria, Spectrum Book Ltd. pp 45-67.

Stepp, J. R. (2004). The Role of Weeds as Sources of Pharmaceuticals. *Journal of Ethnopharmacology,* **92**(2-3): 163-166.

Tapsell, L. C., Hemphill, I. and Cobiac, L. (2006). Health Benefits of Herbs and Spices. *Medical Journal of Australia*, **185**(4): 24-54.

Wagner, W. L., Herbst, D. R. and Sohmer, S. H. (1999). *Manual of the Flowering Plants of Hawaii.* (Revised Edition). Honolulu, Hawaii, USA: University of Hawaii Press/Bishop Museum Press. pp 23-30.

WHO, (1966). Specifications for Identity and Purity and Toxicological Evaluation of Food Colours. WHO/Food Add/66.25 Geneva WHO.