Volume: 1, Issue: 4
Page: 36- 50
2019

ISSN 2664-7354

International Journal of Academic Research in Business, Arts and Science (IJARBAS.COM)

Phytochemical Evaluation, Toxicity Study and Graded Dose Response of the Methanol Crude Extract of Cassia singueana (Del.) on Experimental Animals

Author(s), [1]ALI MOHAMMED FULATA, [2]HAMIDU USMAN, [2]ABUBAKAR DALHATU, [2]SULE IBRAHIM, [2]ADAMU ISHAKU ZADVA, [2]MOHAMMED BABAKURA, [2]HALIMA ABDULSALAM. UMAR,

Abstract:

Cassia singueana (Del) is a shrub or small tree found in most part of northern Nigeria. Ethno medicinal survey reveals that the plant (root, leaf and stem bark) are being used in the treatment of malaria, syphilis, ulcer, pneumonia, snake bite and eye infection. Other uses include against mental disorder, swollen breast, fever, hernia, abdominal pain, convulsion etc. This paper seeks to examine phytochemicals, LD_{50} and intestinal contraction of the methanol stem bark extract of *Cassia singueana* (Del.) and acetylcholine on experimental animals. The results of the phytochemical evaluation revealed the presence of terpenoids, cardiac glycosides, flavonoids, saponins, cardiac glycosides and tannins. The lethal dose of the extract was found to be 2150 mg/kg bd. wt. ip, thus indicating that the plant is relatively safe. The grade dose response of this study revealed that acetylcholine produced a dose-dependent increase in contraction with the least response value of 24.1 mm to a maximum response value of 57.2mm; while the extract revealed a contraction response of 20.8 mm at the lowest concentration and 10.0 mm at the highest concentration. The normal response was 30.4 mm at the lowest concentration and 10.0 mm at the highest concentration. This sequence could be due to the presence of a competitive antagonist in the crude extract thereby inhibiting the agonist response to a lower limit.

IJARBAS
Accepted 1 December 2019
Published 31 December 2019
DOI: 10.5281/zenodo.3596058

Keywords: Phytochemical, Toxicity, Cassia singueana, Methanol,

About Author

Author(s), [1]Department of Remedial Science, Ramat Polytechnic Maiduguri, P.M.B. 1070 Maiduguri, Borno State– Nigeria.
[2]Department of Chemistry, University of Maiduguri, P.M.B. 1069 Maiduguri – Nigeria.

(Corresponding Author) Email: ibnmuhd.ali@gmail.com

Introduction

Cassia singueana (Del) is a shrub or small tree of about 15 m high with conspicuous yellow flower and dark grey bark (Ibrahim and Islam, 2014). The plant is characterized with leaflet of 5-12 pairs and cylindrical fruits with flat and dark brown seeds (Neuwinger, 1994). The plant is reported to be ethno-medicinally useful across Africa. The leaf is used in the treatment of malaria, syphilis, ulcer, pneumonia, snake bite and eye infection. Similarly, the decoction of the root bark is drunk against mental disorder, swollen breast, fever, hernia, abdominal pain, convulsion, other uses include the treatment of gonorrhoea, bilharziosis, women infertility, painful uterus, constipation, anti-emetic, painful menstruation and to prevent still birth (Schmelzer, 2008). Previously scholars reported the potential of the plant as antioxidant, anti-ulcer, antiplasmodial, antipyretic (Ifeanyi, 2012), and cytotoxic. The active principle such as flavonoids, anthraquinones and Terpenes were reported (Endo,1980).

Toxicologist used to develop extrapolations and hypotheses to explain the adverse effects of chemical agents in situations where there is little or no information (Casarette *et al.,* 1996). Any agent (chemical, drug, food or plant) capable of producing deleterious response in a biological system and seriously injuring functions or causing death is regarded as poison. Similarly, the continuous administration of uncontrolled doses of medicinal plant may possibly lead to any of the following four categories of exposure to poison: Acute, Sub acute, Sub chronic and Chronic. Thus, the evaluation of LD_{50} of methanol stem bark extract of *Cassia singueana* (Del) is essentially critical. The characteristics of exposure and the spectrum of effects come together in a correlative relationship customarily referred to as the dose-response relationship. This is a relationship between exposure and health effect, which can be established by measuring the response relative to an increasing dose. This relationship is important in determining the toxicity of a particular substance. It relies on the concept that a dose, or a time of exposure (to a chemical, drug, or toxic substance), will cause an effect (response) on the exposed organism. Hence this study is aimed at evaluating the phytochemical constituents, lethal dose $LD_{50}$and graded dose response of the methanol stem bark extract of *Cassia singuaena* using Wister albino rats and the intestine of rabbit.

Methodology of Research

Plant Collection

The plant material was collected in Dass local government area of Bauchi State, Nigeria, and was identified by a Taxonomist at the Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria and a specimen voucher no. 18/08808 was allocated to it.

Sample Preparation and Extraction

The stem bark of *Cassia singuaena* was shade dried and pulverized using wooden mortar and pestle. One thousand five hundred grams (1500 g) of the pulverized material was extracted with 80% methanol using soxhlet apparatus at 55-65 ºC. The extract was concentrated under reduced pressure and temperature kept in a desiccator until required for analysis.

Phytochemical Screening

Preliminary phytochemical screening was carried out to confirm the secondary metabolites present using standard procedures earlier described by Harbone (1973); Trease and Evans (1989); Sofowora (1993); Bruneton (1999); Markham (1982);Silva*et al.,* (1998); Vishnoi (1979) for the presence of Alkaloids, Flavonoids, Anthraquinone, Saponins, Tannins, and Cardiac Glycosides.

Lork's Method of Determining Acute *i.p.* Toxicity

Procedure

The experiment was conducted in two phases:

Phase 1: The animals were divided into three groups consisting of three animals per group. Doses of 10, 100 and 1000 mg/kg body weight were administered to each group respectively. The animals were observed for 24 hours for any sign of toxicity, and change of behavior and mortality.

PHASE II

Phase two was divided into three groups consisting of only one animal per group. Doses administered in this phase depend on the mortality pattern recorded in phase one (1) within 24 hours of administration. The geometric mean method was used to calculate the LD_{50} (median lethal dose from the outcome of the phase two experiment. The geometric mean of the least dose that kill the animals and highest dose that does not kill the animals is referred to as LD_{50}.

$LD_{50} = \sqrt{a*b}$

Where a = least dose that kill the animal

b = least dose that does not kill animal

Graded Dose Response: An adult rabbit was starved overnight and was sacrifice the next morning. The throat was cut to drains the blood. It abdomen was open to locate ileocaecal junction. And a portion of the ileum about 25 mm. long from a point 10 cm proximal to the ileocaecal junction was removed. The mesentery carefully dissected as the ileum is lifted free. A piece of ileum, about 2 to 3 cm long was set up in an organ bath by means of two threads, one tied to each end of the ileum. The thread on the either end should be kept long for tying the liver. The bath containing Tyrode solution was thermostatically controlled at 37°C. The writing point of the lever will record contractions of the piece of ileum on a moving surface when drugs

are added to the bath. Contractions may be recorded for 10 or 15 seconds. Drugs may be added to the bath either by a tuberculin syringe. A rigid time cycle of 3-5 minutes was maintained. Drugs are removed from the organ bath by the wash out procedure. By opening the appropriate spring clip allow the Tyrode solution to enter the organ bath from the reservoir until the fluid over flows. The main part of the experiment consists of recording contracture with three or more progressively increasing doses of acetylcholine. The usually contractures are recorded with each dose. The doses may be selected at random or multiples of the first dose.

Results

Table 1. Phytochemical Constituents of the *Methanol stem bark extract of C. singueana D.*

Phytochemical Evaluation	*Methanol stem bark extract of Cassia singueana*
Phlobatannins	-
Anthraquinone	-
Carbohydrates	+
Flavonoids	+
Alkaloids	-
Terpenoids	+
Tannins	-
Saponins	+
Cardiac glycosides	+

Key: (+) Present, (–) Not detected.

Table 2 LD_{50} of methanol stem bark of *Cassia singuenea*

PHASE I

Group	Dose (Mg/Kg)	Number of Rats (*i.p.*)	Mortality
A	10	3	0/3
B	100	3	0/3
C	1000	3	0/3

PHASE II

Group	Dose (Mg/Kg)	Number of Rats (*i.p.*)	Mortality
D	1600	1	0/3
E	2900	1	1/1
F	5000	1	1/1

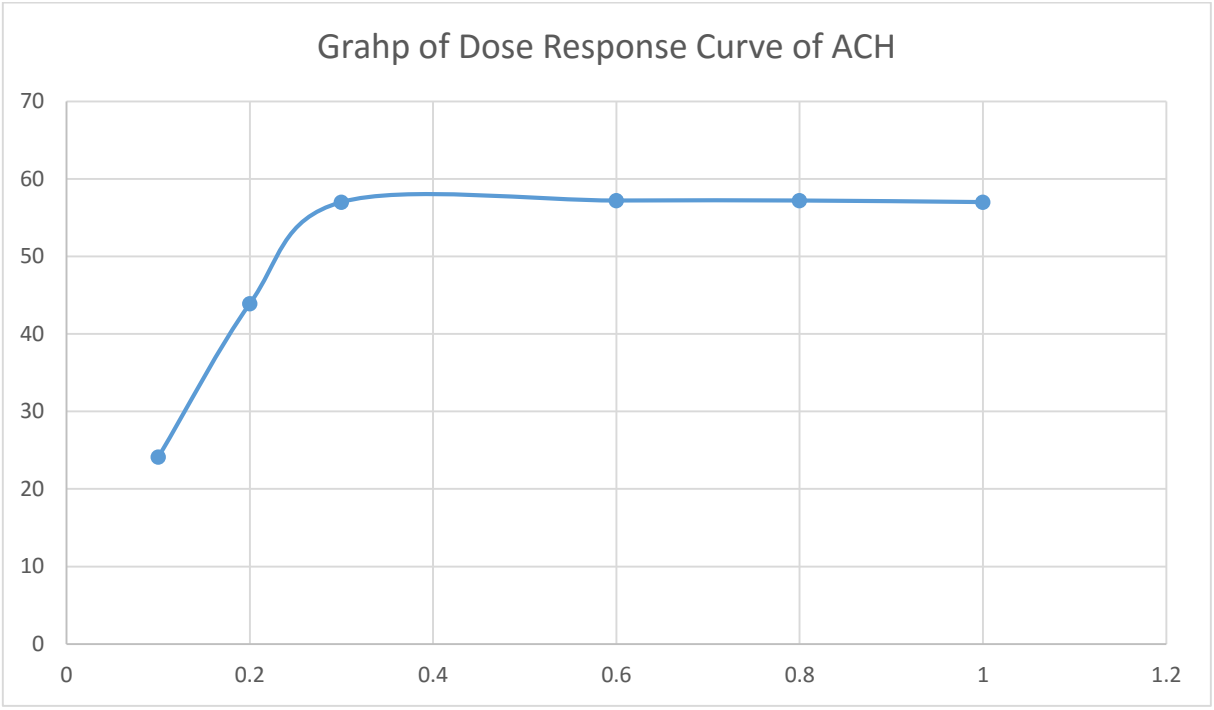
Intraperitoneal $LD_{50} = \sqrt{(a*b)} \longrightarrow \sqrt{(1600 * 2900)} = \sqrt{4640000}$

LD_{50} = 2150 mg/kg

According to the Organization for Economic Co-operation and Development (Walum, 1998) classification of acute systemic toxicity based LD_{50} as >500 ≤ 2000 mg/kg as non-toxic or harmful. Based on this classification, the LD_{50} of 2150 mg/kg established in rats indicated relative safety. Other toxicity scales (Hodge and Sterner, 1943) reported that compound with an LD_{50} of between 500 – 2000 mg/kg should be considered practically non-toxic. Hence, the methanol stem bark extract of *Cassia singueana* may be considered safe.

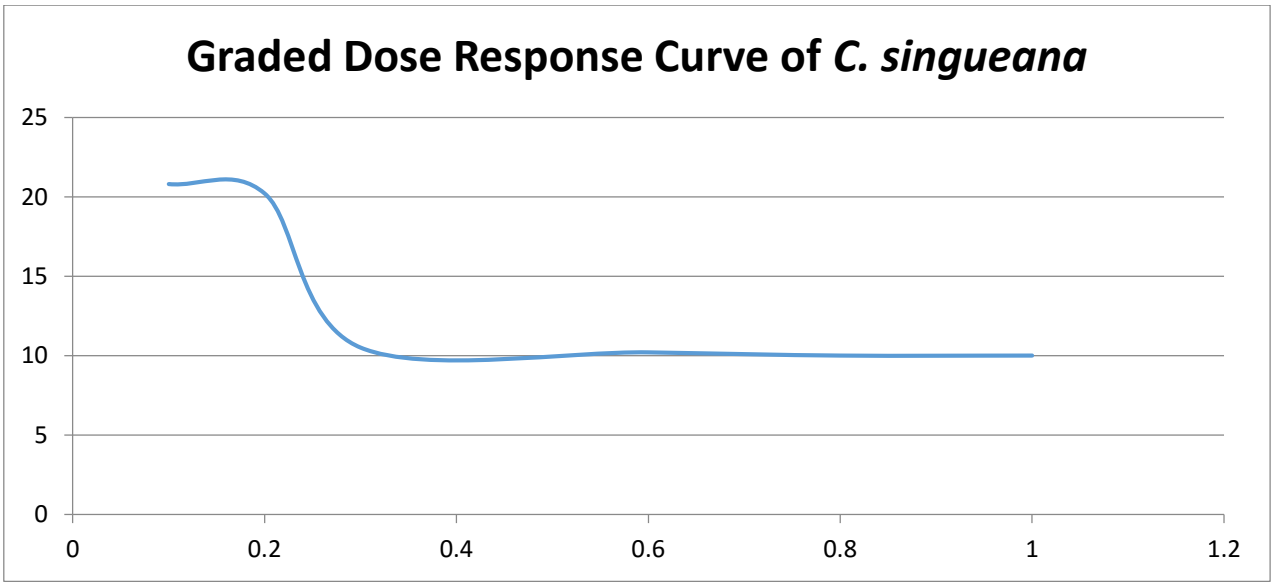
Mean Amplitude of Concentration (mm) 10 µg of Rabbit jejunal segment to graded exposed to graded bath concentration of the Acetylcholine (ACH).

Dose	Bath Concentration	Normal	Acetylcholine Segment
0.1	0.01	17.3	24.1
0.2	0.03	44.4	43.9
0.3	0.04	17.16	57.0
0.6	0.08	17.00	57.2
0.8	0.10	10.33	57.2
1.0	0.13	13.70	57.0



Mean Amplitude of Concentration (mm) 200 µg of Rabbit jejunal segment to graded exposed to graded bath concentration of the *Cassiciasinguenea* stem bark.

Dose	Bath Concentration	Normal	Extract Segment
0.1	0.26	30.4	20.8
0.2	0.53	20.6	20.2
0.3	0.80	20.9	10.5
0.6	1.60	20.5	10.2
0.8	2.13	20.5	10.0
1.0	2.66	10.0	10.0



Discussion

The results of the phytochemical screening in this study has shown that the methanol stem bark extract of *Cassia sigueana* (Del) contained tannins, alkaloids, saponins, cardiac glycosides, flavonoids, carbohydrates, reducing sugars and combined sugars. This is in line with the findings of (Adzu*et al*.,2003) ;(Adeyanju *et al*., 2011) ;(Ottu*et al*., 2011) ;(Gebrelibanos *et al*., 2007) ; (Bangou*et al*., 2011);(Ode and Asuzu, 2011) respectively. Phytochemical are compounds of plant origin, they are also known as secondary plant metabolites and are reported to possessed wide range of biological activities such as antioxidant activity, antimicrobial effects Usman et al, (2018) analgesic and anti-inflammatory Ibrahim et al (2018) and Babakura et al (2018)

Recent study on the plants include the work of Ottu *et al*., 2011on the in *vivo* antioxidant and hepatoprotective properties, likewise Gebrelibanos *et al*., 2007 studied it's in *vitro* free radical scavenging activity, other work on the plants are the works of Ode and Asuzu, 2011 on the anti-ulcer potential as well as the work of Adzu and Gamaniel, (2003) who reported that the plant contained agent with sedative/anxiolytic properties; which merits further investigation.

Following the administration of the extract in the first phase, the animals exhibited no clinical signs of weakness, depression, insomnia or dizziness in the first phase. This findings agrees with Ohaeri and Agaoru, (2012) who observed similar behavior in the same phase, while in the second phase deaths were recorded at 2900 and 5000 mg/kg *i,p,* which could be attributed to intravascular obstruction of the circulatory system resulting into asphyxia as earlier reported by Ohaeri and Agaoru. The result of the acute toxicity testing also corroborate with the work of Alkali et al. (2018),who reported the oral acute toxicity of the leaf extract of the same plant extract we calculate the median lethal dose to be 2154 mg/kg in mice and >5000 mg/kg in rats. The findings of this study is in line with the findings of Alkali et al. (2018), who reported that after 28 days of oral administration the LD_{50} of the methanol leaf extract of *Cassia singueana* in rats and mice, their respective LD_{50} were2154 and >5000 mg/kg, hence they classify it as safe at lower dose only.

The graded dose response relationship is a fundamental aspect of pharmacology. In this relationship, it is employed to study the effects of increase of drug dose and its response in a

system. For instance, the individual fibres of the skeletal muscle are capable of eliciting progressively increasing response with increasing the dose of a particular drug. The increase in response can be measured. With increase in response of dose, at first there is considerable increase in response and then, there are smaller increments as the dose approaches the maximum limit. After maximum response has been reached, no further increase in response can be obtained with further increase of dose. The dose response curve, in general, assumes a shape or sigmoid pattern, as a rule, the relationship between the dose and response is linear and this is well pronounced in the main body of the curve. This part of the curve (between 25% and 75% of the curve). This part of the curve is importance in analytical and practical pharmacology. The boundaries of the linearity can be extended by means of certain mathematical transformation of other dose or response.

The findings of this study revealed that acetylcholine produced a dose-dependent increase in contraction with the least response value of 24.1 to a maximum response value of 57.2 after which there wasn't any further increase in response with corresponding increase in dose. Hence this result is in support of the previous work of Jain et al. (2012),whose work suggested that acetylcholine induce contractility of the intestinal strip. The normal at this phase gave a minimum response value of 17.3 and a maximum response value of 44.4. According to Aziba et al. (2011), in homologous series of drug which have affinity for the same receptor, it is found that agonistic activity diminishes as the series is ascended. This variation in agonistic activity observed with the extract may likely be due to variation in intrinsic activity alone. The concentration response curve of is presented in the graph below.

The extract revealed a less contraction behavior than what was observed with the acetylcholine in the first presentation. The extract revealed a response of 20.8 at the lowest concentration and 10 at the highest concentration. The normal response was 30.4 at the lowest concentration and a10.0 at the highest concentration. This result may be due to the presence of a competitive antagonist in the crude extract thereby inhibiting the agonist a lower limit value as was the case in the work of Braverman et al. (2009) on dual receptor occupational effect.

Conclusion

In conclusion, the methanol stem bark extract of *cassia singueana* (del) contains active metabolites and its lethal dose is considered relatively safe, hence, supportive of its wide acceptability by traditional healers.

Acknowledgement

We will like to acknowledge the assistance of Mr. Fine Akawo of Chemistry Research Laboratory University of Maiduguri and Mr. Bitrus of Veterinary Medicine Laboratory University of Maiduguri, Borno state Nigeria for their technical support.

REFERENCES

Adeyanju O, Olutayo, Michael A, Khan IZ (2011). Preliminary phytochemical and antimicrobial screening of the leaf extract of Cassia singueana Del, African Journal of Pure and Applied Chemistry. 5(4):65-68.

Adoum, O.A. (2010). Screening of medicinal plants native to Kano and Jigawa states of northern Nigeria, using Artemiacysts (brine shrimp test). Int. J.Chem., 20, 67-70.

Adzu, B. and Gamaniel, K. (2003). Sedative Effects of Cassia Singueana Root Bark. Journal of Natural Remedies3:(2) 134 - 137

Alkali YI, Jimoh A O, Muhammad U (2018) Acute and Sub-chronic Toxicity Studies of Methanol Leaf Extract of Cassia singueana F. (Fresen) in Wistar Rats. Herb Med Vol.4 No.2:06.

Aziba, P. I. 1, Sokan, J. O., Ifedayo, O. and Kasim L. S. (2011). The pharmacological studies on intrinsic activities of acetylcholine, methacholine, and carbachol (Homologous Drugs) on isolated rat ileum preparation. Journal of Medicine and Medical Science 2:(8) pp. 1047-1049.

M. Babakura, H. Usman, Y. A. Gaidam, U. A. Halima and A. M. Fulata (2019). Preliminary Phytochemical Screening, Analgesic and Anti-inflammatory Effects of the Hydroethanol and n-Hexane Leaf Extracts of Maytenus senegalensis Lam. Excell. (Celestraceae). South Asian Research Journal of Natural Products 2(1): 1-9, 2019; Article no.SARJNP.47461

Bangou M J, Kiendrebeogo M, Compaore M, Coulibaly AY, Meda N-TR, Abarca NA, Zeba B, Millogo-Rasolodimby J, Nacoulma G. (2011). Enzyme Inhibition Effect and Polyphenolic Content of Medicinal Plant Extractsfrom Burkina Faso, Journal of Biological sciences.11(1):31-38.

Brain, K.R., & Turner, T. D. (1975). The practical evaluation of phytopharmaceuticals. Wright Science Technical. Bristol. pp.140-144, 152-154.

Braver man, A. S., Miller, L. S., Vegans, A. K., Tiana, M. I., Telluride, R. J. and Michael, Ruggeri, R. S. (2009). Quantitation of the Contractile Response Mediated by Two Receptors: M2 and M3 Muscarinic Receptor-Mediated Contractions of Human Gastroesophageal Smooth Muscle. JPET 329:218–224.

Cassette, I., Kaasen, C.D., Amur, M.O. and Dolls, J. (1996). Principles of Toxicology In: Cassarett and Doul's Pharmacology, The Basic Science of Poison Edited by Curtis, D. Klaassen, 5th edition copyright McGraw – Hill (USA) Health Professional Division New York. Pp 13 – 33; 403-414.

Endo, M., and Naoki, H. (1980). Antimicrobial and antispasmodic tetrahydroanthracenes from Cassia singueana. Tetrahedron, 36, 2449-2452.

Evans, W.C. (2002). Trease and Evans pharmacognosy.15th Edition, Elsevier India: 3-554.

Garba, I., Abdurahman, E.M., Ibrahim, H., Ibrahim, N.D.G. and Magaji, M.G. (2011). Toxicity and Analgesic effects of Vernoniaamygdalina Del. (Asteraceae) leaf extract on Mice. International Journal of Advanced Pharmaceutical and Biological Sciences, 1(1): 1-4.

Gebrelibanos M, Asres K, Veeresham C. In Vitro Radical Scavenging Activity of the Leaf and Bark Extracts ofCassia Singueana. Ethiop pharm J. 2007; 25(2):77-84.

Hodge, H. C. and Sterner J.H., (1943). Determination of substances acute toxicity by LD50 British SSSAmericanIndustrial Hygiene Association, 10: 93-98

Ibrahim, M.A., and Islam, M.S. (2014). Anti-diabetic effects of the acetone fraction of Senna Singueana stem bark in a type2 diabetes rat model. J. Ethnopharmacol., 153, 392-399.

Ifeanyi, I.M., and Ode, O.J. (2012). In vitro and in vivo antioxidant potentialof the methanolic extract of Cassia singueanaDelile (Fabaceae) Lock leaves. Comp. Clin. Pathol., 21, 1565-1569.

Jain G, Bodakse SH, Namdev K, Rajput MS, Mishra S. (2012). Development of an ex vivo model for pharmacological experimentation on isolated tissue preparation. J Adv Pharm Tech Res;3:176-81.

Lorke, D. (1983). A new approach to Acute Toxicity Testing. Archives of Toxicology, 54**:** 275-287.

Markham, K.R. (1982). Techniques of flavoniods identification. Academic press London. pp. 38-39.

Neuwinger, H.D.(1994). African Ethnobotany: Poisons and Drugs: chemistry, Pharmacology, Toxicology. London, Chapman and Hall, 136.

OECD (2008) Test guideline 452. chronic toxicity studies. In: Draft OECD 76 guideline for testing chemicals 2: 1.

Ode, O. J and Asuzu, O. V. (2011). Investigation of Cassia Singueanaleaf extract for antiulcer effects using ethanolinducedgastric ulcer model in rats. International Journal of Plant, Animal and Environmental Sciences. 1(1):1-7.

Ohaeri, C. C and Agaoru, C. G. (2012). Antitrypanosoma effects of neem (Azadirachta indica) extract in rats infected with Tryponosama brucei. International Journal Advanced Semantic and Technical Research 2 (5):309-315.

Schmelzer, G.H., Gurib-Fakim, A., Arroo, R., Bosch, C.H., Ruijter, A., and Simmonds, M.S.J. (2008). Plant resources of tropical Africa II (1)-Medicinal palnts I. Wageningen-Netherlands, Backhuys Publisher, 49-60.

Silva, O., Duarte, A., Cabrita, J., Pimentel, M., Diniz, A. & Gomes, E. (1998). Antimicrobial activity of Guinea-Bissau Traditional Remedies. J. Ethnopharmacol. 50: 55-59.

Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.

Usman,H., Abubakar U Kaigama, Olajide O Ibisagba, Ali M Fulata, Ibrahim A. Ahmed (2018). Phytoconstituents Evaluation and Anti-microbial Efficacy of the Crude Flavonoids and Saponins Root Bark Extracts of Terminalia aviccinoide and Ficus polita. Journal of Herbmed Pharmacol.; 7(2): 106-111.

Walum E. (1998). Acute Oral Toxicity. Environmental Health Perspectives, 106: 497- 503.

Vishnoi, N.R. (1979) Advanced Practical Chemistry. Ghazaibadindia: Yikas publication house PVT Ltd. pp 447-449.

Cite this article:

Author(s), [1]Ali Mohammed Fulata, [2]Hamidu Usman, [2]Abubakar Dalhatu, [2]Sule Ibrahim, [2]Adamu Ishaku Zadva, [2]Mohammed Babakura, [2]Halima Abdulsalam. Umar, **(2019).** "Phytochemical Evaluation, Toxicity Study and Graded Dose Response of the Methanol Crude Extract of Cassia singueana (Del.) on Experimental Animals". Name of the Journal: International Journal of Academic Research in Business, Arts and Science, (IJARBAS.COM), P, 36- 50. DOI: 10.5281/zenodo.3596058, Issue: 4, Vol.: 1, Article: 3, Month: December, Year: 2019. Retrieved from https://www.ijarbas.com/all-issues/current-articles/

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