Journal of Complementary and Alternative Medical Research

Volume 22, Issue 3, Page 8-16, 2023; Article no.JOCAMR.100658
ISSN: 2456-6276

Phytochemical Composition and Anti-Motility Effects of *Napoleonae imperialis* Methanol Crude Leaf Extract and Fractions in Rats

Oguamanam, Kelechi C. [a*], Yusuf N. Omeh [a], Uhuo Emmanuel [a] and Raymond C. Ofoezie [b]

[a] Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
[b] Department of Science Technology, Institute of Management and Technology, Enugu State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2023/v22i3458

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/100658

Original Research Article

Received: 01/04/2023
Accepted: 01/06/2023
Published: 10/06/2023

ABSTRACT

Background: Extracts from that *Napoleonae imperialis* leaves have traditionally been used to treat wounds and diarrhoea in south east Nigeria.
Aim: The present study was carried out to evaluate following scientific principles, the phytochemical composition and anti-motility effect of leaf extract and fractions prepared from the plants in experimental rats.
Methods: Crude extract was prepared from the plant's leaves and was subjected to phytochemical and acute toxicity tests. Thereafter, the extract was fractionated using column and thin layer chromatographic techniques. Anti-motility effect of the crude extract and fractions was evaluated

**Corresponding author: E-mail: oguamanamcallybest@gmail.com;*

using 80 rats assigned to 16 groups of 5 rats each. While group 1 served as control and group 2 was treated with Loperamide (0.5 mg/kg), groups 3-16 were treated with specific dose levels of the crude extract or fraction before charcoal meal 30 minutes after treatments. The animals were sacrificed in a further 30 minutes to assess the extents of movement of the charcoal meal along the gastrointestinal tract.

Results: Alkaloids (31.09 +0.44 mg/100 g) were the most abundant phytochemical identified in the crude extract and was followed by flavonoids (17.19± 0.90 mg/100 g). Others were saponins (9.61±0.12 mg/100 g), tannins, (3.23±0.06 mg/100 g), phenolics (8.83±0.41 mg/100 g), steroids (5.24±0.10 mg/100 g), terpenoids (2.58±0.11 mg/100 g) and cardiac glycosides (4.95±0.08 mg/100 g). Results of acute toxicity (LD_{50}) test showed no mortality nor obvious signs of toxicity, even at an oral dose of 5000 mg/kg in rats. Results of the anti-motility studies showed that *Napoleonae imperialis* crude extract and fractions significantly inhibited charcoal meal transit in rat's gastrointestinal tract with 500 mg/kg of the extract producing an inhibitory effect of 51.44±3.53%. A repeat of the test on chromatographic fractions of the extract showed that fraction 5 had the highest anti-motility effect having produced an inhibitory effect of 87.70±6.04% on intestinal motility.

Conclusion: Our findings have shown that *Napoleonae imperialis* leaves contain pharmacologically active components with antidiarrhoeal activities and may be the reason for its antidiarrheoal application in traditional medicine. The observed effects of the extract may be due to its flavonoids and tannins contents and may be via the anticholinergic and osmotic pathway. Solvent combination for fraction 5 may be the most ideal for higher anti-motility effects. More work is required to further establish these findings.

Keywords: Antidiarrhoea; crude extract; fractions; gastrointestinal motility; Napoleonae imperialis.

1. INTRODUCTION

Globaly, diarrhoeal diseases are reported as the leading cause of mortality among children aged five years and below [1]. In some parts of the world, they account for higher mortality rates than all other causes combined [1,2]. Diarrhoea affecting children five years old and below accounts for approximately 63% of the global diarrhoea burden [3,4], and is the second significant cause of infant mortality in developing nations [5,6] where poor sanitation and insufficient potable water supply are key factors [7,8].

The incidence of diarrhoea disease still remains high despite the efforts of both local and international organizations. Unfortunately, however, some of the organizations responsible for diarrhoea are gradually becoming resistant to established antidiarrhoeal drugs. To this end, it is pertinent to harness a wide range of medicinal plants with antidiarrhoeal potentials to augment other antidiarrhoeal drugs.

Plants are considered potent with huge number of phytochemicals embedded which can prevent and subsequently treat several disorders [9]. It has been scientifically proven that traditional medicinal practitioners have been making use of parts of medicinal plants like stems, leaves, barks and roots to cure various ailments. Various phytochemicals that are present that are present in plants and having therapeutic benefits are tagged to be active components of herbal medicines and provide the primary source to the development of drugs [10]. Therefore, medicinal plants are being considered of having great value to researchers especially Biotechnologists because, most of the pharmaceutical compounds are produced from the medicinal plants.

Napoleonae imperialis belong to the family of Lecythidaceae. It variously described in some Eastern Nigeria as "ike mkpudu" in Mbaise of Imo State and Okwe Oyibo in Nsukka of Enugu State. It is small, evergreen tropical West African tree [11]. It grows averagely to the height of 6m with a dense and low branching crown. They develop either as young trunks or from the ancient wood of the branch. The fruit is a berry, dark orange or reddish brown containing kidney shaped seed [12]. Idu, Asowata and Erhabor [13] equally showed that the extract of *Napoleonae imperialis* leaves exhibited significant broad spectrum inhibitory activity against bacterial and fungal isolates. The bark and fruit are used as medicine to treat respiratory tract infections while the fruit-pulp is used for food among the indigenous locals in Eastern Nigeria where the plant is found [14].

Table 1. Pooling of fractions based on retention factor values

S/N	Dist travelled by solvent	Dist travelled by spot	2	3	4	Rf_v	2	3	4	Fractions	Bath ratio
1.	12.5	7.6				0.61				F_1	
2.	12.5	7.6				0.61					
3.	12.5	7.5				0.60					100% Hexane
4.	12.0	9				0.75				F_2	
5.	12.0	9				0.75					
6.	12.0	9				0.75					100% Hexane
7.	12.0	9				0.75				F_3	
8.	12.0	9.2				0.77					
9.	12.0	9.4				0.78					
10.	12.0	9.6				0.80					85% Hexane + 15% methanol
11.	13.0	11.5				0.88				F_4	
12.	13.0	11.5				0.88					
13.	13.0	11.5				0.88					80% Hexane + 20% methanol
14.	13.0	11.9				0.91				F_5	
15.	16.0	12.5	13.0	13.5	14.5	0.78	0.81	0.84	0.91		
16.	16.0	12.4	13.0	13.5	14.5	0.78	0.81	0.84	0.91		80% Hexane + 2 drops of chloroform + 20% methanol
17.	16.0	12.5	13.0	13.5	14.5	0.78	0.81	0.84	0.91	F_6	
18.	16.0	13.0	14.5			0.81	0.91				
19.	16.0	13.0	14.5			0.81	0.91				75% Hexane + 25% methanol
20.	16.0	13.0	14.5			0.81	0.91				
21.	16.0	11.0				0.69					
22.	16.0	11.0				0.69					

Prior studies have shown that *Napoleonae imperialis* leaf extract and fractions generate powerful curative effects on diarrhoea and wound [15,16]. While acknowledging the prior scientific findings on this plant, the present study examined the phytotchemical composition, acute toxicity potential and anti-motility effect of *Napoleonae imperialis* leaf extracts and fractions in experimental rats.

2. MATERIALS AND METHODS

2.1 Collection and identification of *Napoleonae imperialis*

Mature and fresh leaves of *Napoleonae imperialis* were collected in Lude Ahiara in Ahiazu Mbaise Local Government Area of Imo State and subsequently identified and authenticated by Dr Garuba Omosun, a taxonomist of Plant Science and Biotechnology Department, Michael Okpara University of Agriculture, Umudike. The leaves were all collected between the periods of June – August 2019. The leaves were shade-dried for 7-14 days to attain a constant weight and then milled to powder. For reference purposes, the leaves were deposited in the herbarium with the voucher number MOUAU/ZEB/HERB/21/14.

2.2 Preparation of plant extract of *Napoleonae imperialis* leaves and fractions

Cool method approach was used to carry out the extraction. Four hundred grammes (400g) of powdered *Napoleonae imperialis* leaves were weighed and introduced into a 500cm^3 jar. 2.5L of methanol was introduced also to soak the sample. The resulting mixture was adequately stirred with a glass rod and allowed to stand for 48hrs covered with an airtight lid.

It was then filtered through Whatman no.1 filter paper and the residue rewashed with methanol. The combined filtrate was taken to a rotary evaporator where it was concentrated under pressure at 40^{o}c. The concentrate was allowed to stand for another 48hrs in order to release its moisture content. After that, it was then weighed to determine the weight of the extract.

The various fractions used were obtained by pooling closely related fractions based on their retention factor values as presented in Table 1.

2.3 Determination of Acute Toxicity Effect of the Extract

The acute toxicity test of the extract of *Napoleonae imperialis* leaves was determined in albino rats following a modification of the method outlined by Lorke [17]. The test was carried out in two batches. In each of the batches, nine animals were selected randomly and divided into three groups of three animals in a cage. The first batch received 10, 100 and 1000mg/kg body weight of the extract respectively and were observed for 6 hours post administration for signs of toxicity. They were scored for mortality and general behavior after 24hrs. Based on findings in batch one which recorded no death after 24h, the second batch of 9 rats also assigned to 3 groups of 3 rats each received 1600, 2900 and 5000 mg/kg of the extract respectively. The animals were also observed for general symptoms of toxicity and mortality within 24 hours and a further 7 days to establish toxicity level of the extract.

2.4 Determination of Phytochemical Composition of *Napoleonae imperialis* Leaves Extract

The qualitative and quantitative phytochemical screening of the plant extract was carried out according to the methods of Harborne (1998) and Trease and Evans [18] as was reported by Orieke et al. [19]).

2.5 Effect of Extract/ Fractions on Small Intestinal Transit Time of Charcoal Meal in Rats

The method used by Ijioma et al. [20] was adopted. A total of 80 adult male albino rats were used for the study. The rats were assigned to 16 groups of 5 each and were fasted for 18 hour before the commencement of the experiment but were allowed free access to water. Group 1 received no treatment and served as the negative control, group 2 was administered Loperamide (0.4 mg/kg body weight), group 3 received 250 mg/kg of crude extract, and group 4 received 500 mg/kg body weight of the crude extract. Groups 5-16 were treated with fractions of the plant extract as follows:

Group 5 received 250mg/kg of fraction 1(F1)
Group 6 received 500mg/kg of F1
Group 7 received 250mg/kg of F2
Group 8 received 500mg/kg of F2
Group 9 received 250mg/kg of F3
Group 10 received 500mg/kg of F3
Group 11 received 250mg/kg of F4
Group 12 received 500mg/kg of F4
Group 13 received 250mg/kg of F5
Group 14 received 500mg/kg of F5
Group 15 received 250mg/kg of F6
Group 16 received 500mg/kg of F6

All treatments were via the oral route. Thirty minutes after treatments, animals received 1ml of charcoal meal (10% charcoal suspended in 10% gum acacia) orally. In a further 30 minutes, the animals were sacrificed by cervical dislocation and the small intestine was carefully harvested and its full length measured from the pyloric sphincter to the ileocecal junction. For each animal, the distance travelled by the charcoal meal was also measured and expressed as a percentage of the full length using the relationship below:

$$\text{Gastrointestinal transit } (\%) = \frac{\text{Distance moved by charcoal meal}}{\text{Whole length of small intestine}} \text{ x } 100$$

The inhibitory effect of the extract on gastrointestinal transit was calculated relative to the control as:

$$\%\text{ inhibition } = \frac{\text{Gastrointestinal transit of control} - \text{ Gastrointestinal transit of test}}{\text{Gastrointestinal transit of control}} \text{ x } 100$$

2.6 Statistical Analysis

The data generated from the research were subjected to statistical analysis using SPSS version 25. Values are displayed as MEAN±SD at 95 % confidence level. P value greater or equal to 0.05 was taken as significant difference levels.

3. RESULTS

3.1 Results of Qualitative Phytochemical Screening of *Napoleonae imperialis* Crude Extract and Most Active Fractions

The results of the qualitative phytochemical screening carried out on Methanol crude extract and fractions of *Napoleonae imperialis* leaves are as presented in Table 2. These results clearly showed that in the Methanol crude extract, saponins, phenolics and flavonoids are moderately available (++); tannins, steroids, terpenoids and glycosides were present in low concentrations (+) while Alkaloids were present in high concentration (+++). However, in fractions 4, saponins and flavonoids were moderately available (++); tannins,phenolics, steroids, terpenoids and glycosides were present in low concentrations(+) while Alkaloids were present in high concentrations(+++). In fractions 5, Alkaloids and flavonoids were moderately present (++); tannins, phenolics, steroids, terpenoids and glycosides were present in low concentrations (+) while saponins were present in high concentrations (+++).

3.2 Results of Quantitative Phytochemical Screening of *Napoleonae imperialis* Crude Extract and Most Active Fractions

The results of the quantitative phytochemical analysis of the extracts and fractions of *Napoleonae imperialis* are shown in Table 3. The results show that the crude extract had significantly high content of Saponins, tannins, steroids, terpenoids and glycosides when compared to fractions 4 and 5. Fraction 4 had a significantly higher content of phenolics, flavonoids and Alkaloids when compared to crude extract and fraction 5. However, fraction 5 had a significantly higher content of saponins when compared to fraction 4. Fraction 5 equally had a significantly higher content of flavonoids when compared to crude extract.

Table 2. Qualitative phytochemicals

Parameters	Crude extract	Fraction 4	Fraction 5
Saponins	++	++	+++
Tannins	+	+	+
Phenolics	++	+	+
Flavonoids	++	++	++
Steroids	+	+	+
Terpenoids	+	+	+
Glycosides	+	+	+
Alkaloids	+++	+++	++

Keys: + = low availability; ++ = moderate availability;+++ = High availability

Table 3. Quantitative phytochemical screening

Phytochemicals	Crude extract	Fraction 4	Fraction 5
Saponins(mg/100g)	9.61 ± 0.12^{b}	7.71 ± 0.57^{a}	9.51 ± 0.24^{b}
Tannins(mg/100g)	3.23 ± 0.06^{c}	2.18 ± 0.02^{b}	1.71 ± 0.07^{a}
Phenolics(mg/100g)	8.83 ± 0.41^{a}	12.11 ± 0.83^{c}	10.32 ± 0.17^{b}
Flavonoids(mg/100g)	17.19 ± 0.90^{b}	19.79 ± 0.66^{c}	15.76 ± 0.20^{a}
Steroids(mg/100g)	5.24 ± 0.10^{c}	3.75 ± 0.12^{b}	2.28 ± 0.08^{a}
Terpenoids(mg/100g)	2.58 ± 0.11^{c}	1.72 ± 0.16^{b}	0.93 ± 0.06^{a}
Glycosides(mg/100g)	4.95 ± 0.08^{c}	1.45 ± 0.06^{b}	0.66 ± 0.04^{a}
Alkaloids(mg/100g)	27.82 ± 0.46^{a}	35.37 ± 0.67^{c}	31.09 ± 0.44^{b}

Values are presented as mean ± standard deviation (n = 3) and values with different letter superscripts are significantly ($p<0.05$) different from any paired mean across the row.

Table 4. Result of acute toxicity test evaluation of the extract

Group of rats	Dose of methanol Extract in mg/kg Weight	No of deaths recorded
Batch 1	10	Nil
	100	Nil
	1000	Nil
Batch 2	1600	Nil
	2900	Nil
	5000	Nil

3.3 Results of Acute Toxicity Evaluation of *Napoleonae imperialis* Crude Extract

The results of the acute toxicity test of *Napoleonae imperialis* are as presented in Table 3. The oral administration of *Napoleonae imperialis* methanol leaf extract to rats up to 5000 mg/kg caused no mortality in any of the treatment groups. Signs of toxicity like agitations, writhing reflexes, tremor, calmness, convulsions and roughness of hairs were also not observed in any of the treated groups. Therefore acute toxicity value for the extract was established to be >5000 mg/kg body weight. Results for acute toxicity evaluation of the extract is presented in Table 4.

3.4 Effect of *Napoleonae imperialis* Crude Extract and Fractions on Intestinal Charcoal Meal Transit in Rats

Table 5 depicts the result of the effect of crude & fractions of *Napoleonae imperialis* leaves on small intestinal motility of charcoal meal transit time. Two doses each of the crude and fraction were used to compare the positive and negative control. The crude extract at 250mg/kg significantly ($p<0.05$) reduced the distance covered by the charcoal meal. The percentage reduction for the crude at 250mg/kg is 40.09+ 2.09 as against loperamide treated group 33.52+2.85 and the negative control 0.00+0.00. The crude at 500mg/kg recorded a greater inhibition of 51.44+3.5 when compared to the

Table 5. Effect of methanol crude and fractions of *Napoleonae imperialis* leaves on gastrointestinal motility in rats

Treatment groups	Length of intestine (cm)	Distance moved (cm)	% movement	% inhibition
Control	96.40±5.68[a,b]	83.60±10.31[h]	86.46±6.05[h]	0.00±0.00[a]
Loperamide 0.4 mg/kg	96.40±3.36[a,b]	57.00±6.52[e]	59.17±6.93[e]	33.52±2.85[d]
Crude extract 250 mg/kg	97.20±4.32[a,b]	49.40±1.67[d]	50.84±0.61[d]	40.09±2.09[e]
Crude extract 500 mg/kg	99.60±2.70[b]	39.20±2.17[c]	39.34±1.29[c]	51.44±3.53[f]
F1 250 mg/kg	94.40±5.23[a,b]	65.20±4.15[f]	69.13±4.35[f]	20.04±2.86[c]
F1 500 mg/kg	98.20±5.27[b]	57.40±4.92[e]	58.33±3.93[e]	31.19±3.19[d]
F2 250 mg/kg	96.20±3.03[a,b]	57.60±3.36[e]	59.83±1.84[e]	30.14±1.63[d]
F2 500 mg/kg	95.60±4.22[a,b]	47.20±2.59[d]	49.48±4.11[d]	40.94±2.46[e]
F3 250 mg/kg	96.20±3.42[a,b]	81.20±1.31[h]	86.07±2.61[h]	2.68±0.70[a]
F3 50 0mg/kg	96.80±3.27[a,b]	80.40±3.07[g,h]	83.10±2.38[h]	3.93±0.57[a]
F4 250 mg/kg	98.60±4.83[b]	75.006±.04[g]	76.05±2.85[g]	11.31±3.54[b]
F4 500 mg/kg	97.40±3.51[a,b]	64.88±4.04[f]	66.49±2.04[f]	24.05±2.86[c]
F5 250 mg/kg	94.20±2.77[a,b]	25.66±3.22[b]	27.27±3.67[b]	69.59±5.55[g]
F5 500 mg/kg	98.80±3.49[b]	8.96±3.10[a]	9.11±3.22[a]	87.70±6.04[g]
F6 250 mg/kg	91.60±2.70[a]	61.20±1.92[e,f]	66.84±2.23[f]	23.46±2.54[c]
F6 500 mg/kg	96.80±4.76[a,b]	46.20±3.56[d]	48.21±4.26[d]	43.95±3.85[e]

Values are displayed as mean ± standard deviation (n = 5), and values with different letter superscripts are significantly (p<0.05) different from any paired mean in the corresponding column

positive & negative control. Groups 5-16 were administered the fractions at doses of 250 and 500mg/kg. A maximum percentage inhibition (87.70+ 6.04, p<0.05) was recorded by F5 at 250 mg/kg.

4. DISCUSSION AND CONCLUSION

The fact that *Napoleonae imperialis* crude extract and fractions significantly inhibited charcoal meal transit in the rat's gastrointestinal tract suggests that the extract may contain substances with antidiarrhoeal activity and may have acted via the anticholinergic pathway.

As much as 5000mg/kg body weight of the extract of *Napoleonae imperialis* leaves, no death was recorded and the normal behaviours of the animals were never altered. According to Lorke [17], any substance that is not toxic up to 5000mg/kg body weight is considered to be relatively safe. It is well established that the gastrointestinal tract is host to numerous muscarinic receptors of all types (especially m_1 and m_2) which are involved in gastrointestinal peristaltic contractions and motility (Madubuike *et al*., 2015). Acetylcholine released due to parasympathetic innervations usually binds to these receptors to bring about increased intestinal motility [21]. Activation of M3 receptors usually achieves gastrointestinal motility by triggering phosphoinositide hydrolysis, calcium ion mobilization and direct contractile responses while activation of M2 receptors does same by inhibiting adenylcyclase and potentiating calcium dependent on selective conductance. A combination of these biochemical mechanisms usually brings about gastrointestinal motility [22].

Agents like atropine can bind to these receptors to inhibit acetylcholine activity and in the process inhibit gastrointestinal motility. Loperamide usually achieves antidiarrhoeal effect by binding to opiate receptors in the gastrointestinal wall to inhibit the release of acetylcholine and prostaglandins, thereby reducing propulsive peristalsis and also increasing intestinal transit time (Madubuike *et al., 2015*). *Napoleonae imperialis* crude extract and fractions in this study may have acted via a combination of atropine-like and loperamide-like effects on the gastrointestinal tract. These effects of the extracts may be due to their phenols, flavonoids and alkaloids contents. Findings in this study show that significant amounts of these phytochemical agents are present in *Napoleonae imperialis* crude extract and fractions. The antimotility activities of these phytochemical agents on the gastrointestinal tract (GIT) are well documented [20]. The higher antimotility effects exerted by fractions 4 and 5 suggest that solvent combinations that yielded these fractions

collected higher amounts of phytochemical agents with established antimotility effect on the GIT. The relationship between extraction solvent and quantity of extracted phytochemical agents is also well known [23].

The findings in this study agree with previous studies that *Napoleonae imperialis* leaves possesses antidiarrhoel features.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. United Nations International Children's Emergency Fund; 2018. Available:http://www.data.unicef.org/topic/child-health/diarrhoeal-disease/
2. Petri WA, Jr, Miller M, Binder HJ, Levine MM, Dillingham R, Guerrant RL. Enteric infections, diarrhoea, and their impact on function and development. J. Clin. Invest. 2008;118(4):1277.
3. Walker CLF, Perin J, Aryee MJ, Boschi-Pinto C, Black RE. Diarrhoea incidence in low-and middle-income countries in 1990 and 2010: A systematic review. BMC Publ. Health. 2012;12(1):220.
4. Zhang SX, Zhou YM, Xu W, Tian LG, Chen JX, Chen SH. Impact of co-infections with enteric pathogens on children suffering from acute diarrhoea in southwest China. Infect. Dis. Poverty. 2016;5(1):64
5. Platts-Mills JA, Babji S, Bodhidatta L, Gratz J, Haque R, Havt A. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED) Lancet Global Health. 2015;3(9):e564–e575.
6. Kotloff KL. The burden and aetiology of diarrheal illness in developing countries. Pediatr. Clin. 2017;64(4):799–814.
7. Chakravarty I, Bhattacharya A, Das SK. Water, sanitation and hygiene: The unfinished agenda in the world health organization south-east Asia region. WHO S. East Asia J. Public Health. 2017;6(2):22
8. Squire SA, Ryan U. Cryptosporidium and Giardia in Africa: Current and future challenges. Parasites Vectors. 2017; 10(1):195.
9. Yadav NP, Dixit VK. Recent approaches in herbal drug standardization. Int J Integr Biol. 2008; 2(3):195-203.
10. Balandrin MF, Kinghorn AD, Farnsworth NR. Plant-derived natural products in drug discovery and development: An overview. ACS Symposium Series. 2018;2-12. DOI: 10.1021/bk-1993-0534.ch001
11. Mba OJ, Aloh GS, Nwachukwu KC, Okoh MP. Phytochemical characterization, acute toxicity studies of the methanol extract of *Napoleonae imperialis* leaves. J Genet CellBiol. 2020; 3(2):169-173.
12. Ajaiyeoba E, Ashidi J, Abiodun O, Okpako L, Ogbole O. Antimalarial ethnobotany: *In vitro* antiplasmodial activity of seven plants identified in the Nigerian middle belt. Pharmaceutical Biology. 2004;42:588-591.
13. Idu M, Asowata I, Erhabor JO. Preliminary antimicrobial and phytochemical study of the aqueous, ethanol, methanol and chloroform extract of the leaves of Napoleonae imperialisp.beauv. (lecythidiaceae). J Plants Dev Sc. 2011; 3:3-4.
14. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, CarmeliY. Multidrug resistant Pseudomonas aeruginosa: Risk factors and clinical impact. Antimicrobial agents and chemotherapy. 2006; 50(1):43-8.
15. Enye JC, Chineke HN, Onubeze DPM. wound healing effect of methanol leaf extract of *Napoleona vogelii* family (Lecythidaceae) 3, Nweke Journal of Dental and Medical Sciences. 2013; 8(6):31-34.
16. Srinivas KS, Grierson DS, Afolayan AJ. Ethnobotanical information of medicinal plants used for treatment of cancer in the Eastern Cape Province, South Africa, Current Science. 2007;92(7):345-351.
17. Lorke, D. A new approach to tropical acute toxicity testing. Arch. Toxicol. 1983;53:275-287.
18. Trease GE, Evans WC. Pharmacognosy. 15th Ed. London: Saunders Publishers. 2002;42–44. 221–229, 246–249, 304–306, 331–332, 391–393.
19. Orieke D, Ohaeri OC, Ijeh II, Ijioma SN, Achi NK. Acute and sub-acute evaluations

of methanolic leaf extract of Corchorus olitorius in experimental animal models. Asian Journal of Research in Animal and Veterinary Sciences. 2019;2(4): 1-12.

20. Ijioma SN, Osim, EE, Nwankwo AA, Kanu K, Orieke D, Ezike M. Antimotility effect of a southeast Nigerian polyherbal formulation (Ajumbise): An *In vitro* and *In vivo* evaluation. Animal Research International. 2019;16(3):3494-3502.
21. Ijioma SN, Nwosu CO, Emelike CU, Okafor AI, Nwankwo AA. Antinociceptive property of Costus afer Ker stem juice and ethanol leaf extract in albino rats. Comprehensive Journal of Medical Sciences. 2014;2(2): 14–19.3:84–98.
22. Ehlert FJ. Contractile role of M2 and M3 in gastrointestinal, airway and urinary bladder smooth muscles. Life Sciences. 2003;74(2-3):355-66.
23. Thiam S, Diène AN, Fuhrimann S, Winkler MS, Ndione JA, Schindler C, Vounatsou P, Utzinger J, Faye O, Cissé g. Prevalence of diarrhoea and risk factors among children under five years old in Mbour, Senegal: A cross-sectional study. Infectious Diseases of Poverty. 2017;6(1).

Available:https://doi.org/10.1186/s40249-017-0323-1

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/100658