ACUTE TOXICITY, PRELIMINARY PHYTOCHEMISTRY AND ANTI-PEPTIC ULCER STUDY OF THE BI-HERBAL MIXTURE OF *Persea americana* (Lauraceae) SEED and *Citrus sinesis* (Rutaceae) PEEL USING SWISS ALBINO MICE

Obaro-Onezeyi, O. E. and Obaro, P. O.
Department of Department of Science Laboratory Technology (Pharmacology/Physiology and Biological Science Techniques), Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria
Email: osasere.obaro-onezeyi@uniben.edu; Tel: +234 9044068330
Received: 9th May, 2024 **Accepted**: 18th October, 2024 **Published**: 31st December, 2024

ABSTRACT
Background: The first known type of medicine is the usage of herbs and has been practiced in every culture. In various systems of traditional medicine as well as in current "western" medicine, plants and the constituents of their secondary metabolites have a long history of use and are the source of significant medications.
Aim: This study was aimed to investigate the acute toxicity, phytochemical composition and anti-peptic ulcer effect of the Bi-herbal mixture of the methanol extract of avocado (*Persea americana*) pear seed and orange (*Citrus sinesis*) peel on peptic ulcer induced in albino Swiss mice.
Methods: Phytochemical constituents of the bi-herbal formulation, evaluation of the acute toxicity effect of the bi-herbal formulation and anti-peptic ulcer studies using Hydrochloric acid mixed with ethanol and Indomethacin induced peptic ulcers in albino Swiss mice were carried out using standard procedures.
Results: Results obtained from the phytochemical study showed that Cardiac Glycoside, Flavonoids, Eugenols and Alkaloids where highly present, Saponins, Phenolics and Terpenoids where present, while Tannins was absent. The acute toxicity study revealed that the LD_{50} was more than 2000 mg/kg as none of the animals died. While the anti-ulcer study revealed dose dependent gastro-protective activities of the extract in both indomethacin and Ethanol/HCl induced peptic ulcers.
Conclusion: It is concluded that the methanol extract of the Bi-herbal formulation of pear seed and orange peel, possess anti-ulcer activity and clinical trials can be extended on peptic ulcer patients.
Keyword: Anti-Ulcer, Biherbal Mixture, *Persea Americana* (Lauraceae) Seed, *Citrus Sinesis* (Rutaceae) Peel, Swiss Albino Mice

INTRODUCTION
The first known type of medicine is the usage of herbs and has been practiced in every culture. In various systems of traditional medicine as well as in current "western" medicine, plants and the constituents of their secondary metabolites have a long history of use and are the source of significant medications like atropine and codeine and also serve as natural dye (Calixto, 2002; Omorodion and Achukwu, 2017). Avocado pear (*Persea americana*) is a dicotyledonous plant belonging to the Lauraceae family and the Ranales order. Traditional medicines have utilized various avocado pear parts for a variety of functions, including as an antibacterial (Deepti, 2012). It is an underutilized, inedible portion of the fruit that is often dumped as waste (Tassew *et al*., 2019).

Citation: Obaro-Onezeyi, O. E. and Obaro, P. O. (2024): Acute Toxicity, Preliminary Phytochemisrty and Anti-Peptic Ulcer Study of the Bi-Herbal Mixture of *Persea americana* (Lauraceae) Seed and *Citrus sinesis* (Rutaceae) Peel Using Swiss Albino Mice *BJMLS 9(2): 65 - 73*

The seeds in an avocado fruit make up roughly 16% of its weight. Fruit matures in shades of green, black, purple, or reddish, and depending on the variety, the thickness and texture of the skin change (Deepti, 2012).

The literature on *Citrus sinesis* peel is extensive and encompasses a wide range of topics, including phytochemistry, pharmacology, food science, agriculture, and environmental sustainability. The botanical characteristics, including tree structure, leaf morphology, flower structure, and fruit development, have been extensively documented (Nicolosi *et al*., 2000). Industrial fruit processing is becoming more and more popular around the world, and the leftovers of these operations are typically thrown away (Janice e*t al*., 2012). But these byproducts may result in ecological issues like a rise in insects and rodents (Tassew *et al*., 2019). Therefore, research is required to determine whether these byproducts are useful as sources for dietary supplements or pharmaceuticals.

This study was aimed to study the effect of Bi-herbal formulation of the mixture of Methanol extract of avocado (*Persea americana*) pear seed and orange (*Citrus sinesis*) peel in the treatment of peptic ulcer induced in albino Swiss mice.

MATERIALS AND METHODS

Collection of plant samples, identification and authentication

Avocado (*Persea americana*) pear and Orange (*Citrus sinesis*) plant samples were collected from fruit vendors in Uselu market in Egor local government area of Benin City, Edo state, Nigeria. The plant samples were authenticated by Prof. H.A. Akinnibosun in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. After being collected, the seeds of Avocado (*Persea americana*) pear and Orange (*Citrus sinensis*) peels pieces were first separated and left to air dry for two weeks at room temperature. An electric mill was used to grind the dried seeds and peels into a fine powder, which was then placed in airtight containers for later usage as reported by (Obaro-Onezeyi and Oshomoh, 2019).

Preparation of extract

Each powder sample weighed 500 grams (g) and was extracted using an absolute methanol solvent. The weighed powered sample was continuously shaken and stirred for 72 hours while macerating with 2.5 liters of methanol. Cheese cloth, a conical flask, and a funnel were used in the filtering process to separate the filtrate from the residue. To make the concentrate, which was subsequently dried to powder in an oven, the filtrate was concentrated to paste level using a crucible and water bath at 400 °C to produce a crude extract of 35.8 g that was kept in a sample bottle inside a refrigerator according to Oshomoh and Obaro (2019).

Drugs and solvents: Drugs and reagents were of pharmaceutical standards. Omeprazole (Glaxosmith Kline Nigeria Plc), Absolute methanol solvent, hydrochloric acid and chloroform (supplied by Fharmatrends Nigeria Ltd), all of analytical standards.

Experimental animals

Male and female *Swiss* albino mice use for this study, weighing between 25 – 30 g and were purchased for the Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria. The mice were housed in plastic cages under natural lighting and temperature conditions. They were fed with standard feed (Top Feeds, Nigeria Plc) and water *ad libitum*.

Experimental Design

Acute Toxicity Study

Acute toxicity research was conducted using Organization for Economic Co-operation and Development (OECD). 2000 mg/kg of the bi-herbal product's methanol extract was given to 3 male and 3 female mice, and the mice were then monitored for 24 hours for any potential indicators of toxicity, death, or morbidity. The experiment was over since no animals died.

Phytochemical Studies

Qualitative Phytochemical Screening

Standard phytochemical techniques were used to analyze the ethanol and aqueous extracts of orange (*Citrus sinesis*) and avocado (*Persea americana*) seeds for terpenoids, glycosides, flavonoids, alkaloids, tannins, eugenols, and phenolic compounds according to the methods of Obaro-Onezeyi and Obaro (2023).

Anti-ulcer studies

A total of sixty (60) albino mice were used for this experiment. In the Ethanol/HCI and Indomethacin - induced ulceration models, thirty (30) mice were used and divided into 5 groups of six mice each respectively.

Experimental Design

Ethanol/HCl -induced Ulcer

Before oral injection of 1 ml of ethanol/hydrochloric acid (0.4 ml HCl in 60% ethanol, 0.6 ml) to induce ulcerations, mice were primed with extract for seven days and denied food for 24 hours following the final dosage. Omeprazole (20 mg/kg) and distilled water (2 ml/kg) were given 30 minutes after the extract. One hour after the ethanol/HCl treatment, the animals were slaughtered. Animals' stomachs and abdominal cavities were torn out thereafter, and gastric ulcers were checked for.

Each animal's ulcer score was calculated, where 0 represents no lesions, 1 hyperemia, 2 one or two minor lesions, 3 very serious lesions, and 4 mucosal full of lesions. Ulcer index were calculated as mean ulcer scores according to the methods of Fouad *et al*. (2011).

Grouping of experimental animals in Ethanol/HCI - induced ulceration model

Group 1: Received distilled water (2 ml/kg)

Group 2 (negative control): Received Ethanol/HCI only

Group 3: (positive control): Received omeprazole 20 mg/kg + Ethanol/HCI

Group 4: Received 50 mg/ml of the extract + Ethanol/HCI

Group 5: Received 100 mg/ml of extract + Ethanol/HCI

Indomethacin-induced Ulcer

Before receiving medication treatment, food and water were discontinued for 24 hours. 30 minutes after the administration of the extract (50 and 100 mg/kg), omeprazole (20 mg/kg), and distilled water (2 ml/kg), indomethacin 10 mg/kg (dissolved in 5% sodium bicarbonate solution) was given (orally). After 15 hours, indomethacin administration was repeated. One hour after the last dose of indomethacin, all the mice were killed, and the stomachs were removed to assess the degree of mucosal injury. In cases of ulcer caused by ethanol-acid, the ulcer index was calculated as previously mentioned.

The ulcer index (UI) was obtained from the sum of the scores of all lesions for each stomach, and the mean ulcer index (UIMEAN) was calculated for each group according to the method of Fouad *et al*. (2011).

Percent ulcer inhibition of the samples was determined using the following equation:

$$\% \text{ ulcer inhibition} = \frac{(\text{UIMEAN control} - \text{UIMEAN sample})}{\text{UIMEAN control}} \times 100\%$$

Grouping of experimental animals in Indomethacin - induced ulceration model

Group 1: Received distilled water (2 ml/kg)

Group 2: (negative control): Received + Indomethacin

Group 3: (positive control): Received omeprazole 20 mg/kg + Indomethacin

Group 4: Received 50 mg/ml of the extract + Indomethacin

Group 5: Received 100 mg/ml of extract + Indomethacin

Statistical Analysis

The results from the studies were expressed as the mean ± SEM. Statistical analysis was carried out using graph pad prism 8 version software (UK). Comparisms between the control and treated groups were analysed using one way ANOVA by, Dunnett's multiple comparisms test.

P ≤0.05 was regarded as indicating significant differences

RESULTS

Acute Toxicity study

Table 1: **Acute toxicity study of methanol extract of the bi-herbal formulation of peer seed and orange peel (MEBFPSOP) on albino Swiss mice after 24 hours administration of single dose (2000 mg/kg) of extract**

Group(s)	Dose (mg/kg)	Cognition	Agility	Signs of Toxicity such as: Grooming, nausea, writhing,	Mortality after 24 hours of administration
Control	2 ml/kg	Normal	Normal	None	0/6
MEBFPSOP	2000 mg/kg	Normal	Normal	None	0/6

Phytochemical analysis

Table 2: Qualitative Phytochemical compound of the methanol extract of the bi-herbal formulation of peer seed and orange peel (MEBFPSOP)

S/N	Phytochemicals	MEBFPSOP
1.	Cardiac Glycoside	++
2.	Saponins	+
3.	Flavonoids	++
4.	Phenolics	+
5.	Tannins	-
6.	Eugenols	+ +
7.	Terpenoids	+
8.	Alkaloids	++

Key: += present, ++ = highly present, - = absent

Anti- Ulcer Studies

Table 3: Gastro-protective activity of methanol extract of the bi-herbal formulation of peer seed and orange peel (MEBFPSOP) on indomethacin -induced ulcer in mice

Treatments	Ulcer index (Mean ±SEM)	Ulcer Inhibition (%)
Normal Control	0.00± 0.00	-
Indomethacin only	6.00.± 0.58	0
Indomethacin+50	2.80± 0.54*	53
Indomethacin± 90	2.65±0.72*	56
Indomethacin+Omeprazole	2.70±0.65*	55

Results was significant at*= *p≤0.05* when compared with control

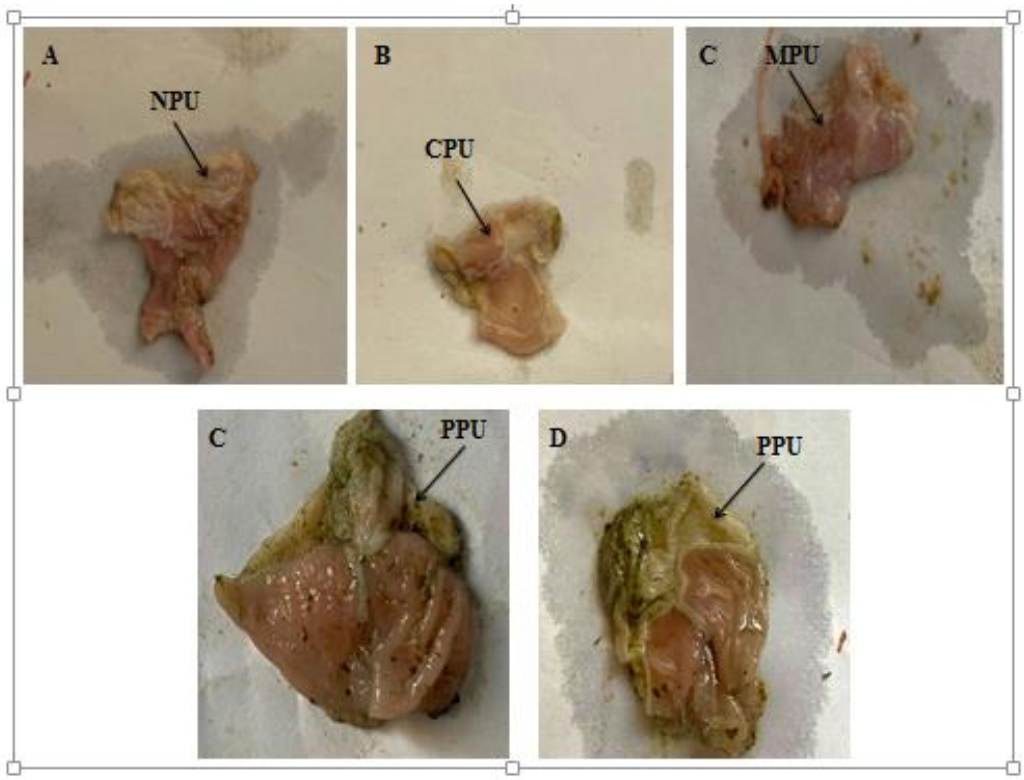

Plate 1: Anti-ulcer effect of MEBFPSOP 50 and 100 mg/kg, negative control and positive control of indomethacin -induced ulcer in mice

Key: A = Normal Control (stomach appeared firm with no peptic ulceration (NPU))
B = Negative Control (untreated): (stomach appeared very thin with chronic peptic ulcerations (CPU)
C = Positive control (omeprazole 20 mg/kg) (stomach appeared moderately firm with mild peptic ulcerations (MPU)
D = 50 mg/kg of MEBFPSOP (stomach appeared firm with protection against peptic ulceration (PPU)
E = 100 mg/Kg of MEBFPSOP (stomach appeared firm with protection against peptic ulceration (PPU)

Table 4: Gastro-protective activity of methanol extract of the bi-herbal formulation of peer seed and orange peel (MEBFPSOP) on ethanol/HCL-induced ulcer in mice

Treatments	Ulcer index (Mean ±SEM)	Ulcer Inhibition (%)
Normal Control	0.00± 0.00	-
Ethanol/HCL only	6.00± 0.58	
HCL/Eth.+ 50	2.75± 0.65*	54
HCL/Eth.+ 100	1.85±0.49*	69
HCL/Eth.+ omeprazole	2.40±0.71*	60

Results are expressed as mean ± SEM (n=6) which were significant at *=$p \leq 0.05$ when compared with control.

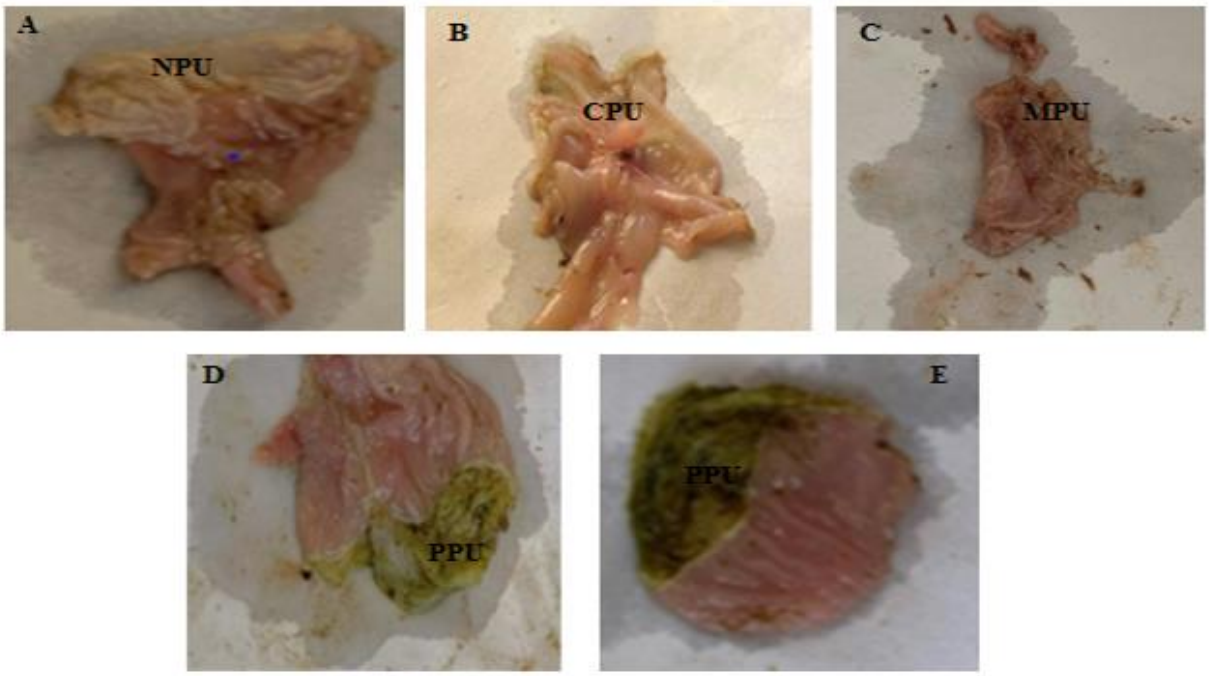

Plate 2: Anti-ulcer effect of MEBFPSOP 50 and 100 mg/Kg, negative control and positive control of ethanol/HCL -induced ulcer in mice

Key: A = Normal Control (stomach appeared firm with no peptic ulceration (NPU))
B = Negative Control (untreated): (stomach appeared very thin with chronic peptic ulcerations (CPU)
C = Positive control (omeprazole 20 mg/kg) (stomach appeared moderately firm with mild peptic ulcerations (MPU)
D = 50 mg/kg of MEBFPSOP (stomach appeared firm with protection against peptic ulceration (PPU)
E = 100 mg/kg of MEBFPSOP (stomach appeared firm with protection against peptic ulceration (PPU)

DISCUSSION

Acute toxicity is the term used to describe the negative consequences that accompany the oral or topical administration of a single dose, repeated doses given within 24 hours, or a 4-hour inhalation exposure. No mortality was noted in the mice who received 2000 mg/kg of the bi-herbal extract, corroborating with the finds of Valentine *et al*. (2014).

Cognition refers to the animal's ability to solve an elementary logic task which is presented to it for the first time, i.e., ability to recognize food, and move towards it (Olga and Inga, 2022). In contrast to Kun-Ruey and Shu-Chuan, 2019, the animals remained agile, even after the administration of the bi-herbal mixture

Phytochemicals are substances derived from plants. The Greek word "plant" is where the term "phyto" originates. It is a term that describes the secondary metabolites that plants contain (Chuwkwuebuka *et al*., 2018) Plants are shielded against harm and illness by phytochemicals, which also affect the color, flavor, and aroma of the plant (Mamta *et al*., 2016). Plants with advantageous phytochemicals may act as natural antioxidants to augment the body's requirements (Ammar *et al*., 2017).

Heart glycosides are steroids that can have a focused, potent effect on the cardiac muscle. Natural sources of modest amounts of cardiac glycosides can be found in the seeds, leaves, stems, roots, or bark of widely dispersed plant species (Yousef, 2013).

Saponins are naturally occurring glycosides with surface activity that have a particular foaming property. The soapwort plant (Saponaria), whose roots were once used as soap, gave them their name despite the fact that they are mostly produced by plants, lower marine creatures, and some bacteria (Latin sapo means soap). According to published research, saponins have biological functions and therapeutic qualities such hemolytic factor, cytotoxicity, anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal, and anticancer effects (Maher *et al*., 2019).

Flavonoids are a significant family of natural products that are commonly present in fruits, vegetables, and some drinks. They are secondary plant metabolites with a polyphenolic structure (Panche *et al*., 2016). They serve a variety of crucial roles in plants, like protecting them from harmful UV rays or producing plant pigment. They also possess antibacterial, antiviral, and antioxidant effects. They also control enzymatic activity and gene expression (Aleksandra *et al*., 2016). They are soluble in water and give flowers, fruits, and occasionally leaves their color (Kursinszki, 2010). The secondary metabolites that are most prevalent in the plant kingdom are phenolic chemicals (Vincenzo, 2010). Due to their antibacterial and anti-fungicidal properties, they are involved in chemical interactions between organisms and play a significant role in the metabolism of plant cells (Babenko *et al*., 2019). Clove essential oil's volatile phenolic component eugol ($C_{10}H_{12}O_2$ or $CH_3C_6H_3$) is extracted from the buds and leaves of the Eugenia caryophyllata plant, which is primarily gathered in Indonesia, India, and Madagascar (Solmaz *et al*., 2019). Along with substantial analgesic and local anesthetic efficacy, eugenol also has significant cardiovascular, anti-inflammatory, and antioxidant characteristics. Human studies have been done on the compound's pharmacokinetics and metabolism. Eugenols have additionally been employed as a penetration booster (Kannissery *et al*., 2010).

Terpenoids are flammable compounds that give flowers and plant their scent. They are widely distributed in the leaves and fruits of higher plants such as eucalyptus, citrus, and conifers. The largest and most common class of secondary metabolites, found primarily in plants and lower invertebrates, are terpenoids. Several of them have been utilized for medical purposes for ages (Heras *et al*., 2014).

Alkaloids are one of the most diverse classes of secondary metabolites, with more than 20,000 distinct compounds spread across 20% of all known vascular plants. They are among the largest categories of secondary metabolites (Hélio and Arthur, 2015).

Cyto-protectors, antioxidants, immune-regulatory, anti-secretory, and anti-Helicobacter pylori are just a few of the many mechanisms of action that extracts containing key phytochemicals like flavonoids can use to protect the gastric and/or duodenal mucosa from various induction models that mimic the ulcer in men (ethanol, NSAIDs, stress, and pyloric As a result, they may be used as dietary supplements, preventive medications, or both to help with the conventional treatment of ulcerative lesions as well as to stop the development of peptic ulcers and their episodes of recurrence. The bi-herbal extract dramatically decreased ulcer score and ulcer index, demonstrating the combination of plants possess anti-ulcerogenic action since the extract had no inhibiting effect on acid secretion. This suggests that the bi-herbal formulation may be acting through cyto-protection as a potential mechanism of antiulcer action which is in line with the finds of Fouad *et al*. (2011).

It is likely that the presence of bioactive substances like flavonoids and saponins is what causes the antiulcer activity. These protect the stomach from damage most likely by acting through anti-secretory and antioxidant processes corroborating with the report of Neelapu *et al*. (2012).

CONCLUSION

In conclusion the methanol extract of the Bi-herbal formulation of pear seed and orange peel, possess anti-ulcer activity and should be tried clinically on peptic ulcer patients.

Contending interests

Authors declare that they have no contending interests.

Acknowledgments

Authors want to thank the Department of Science Laboratory Technology, Faculty of Life Sciences University of Benin, Nigeria for the laboratory support.

REFERENCES

Aleksandra, K., and Dorota, S. (2016). FLAVONOIDS - Food Sources and Health Benefits. *Journal of Food Medicine*. **5**:1-2

Ammar, A., Naoufal, L., Azam B., Dennis G. W. and David A.L. (2017). Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Journal of Plant Science*. **2**-3

Babenko, L. M., Smirnov, O. E., Romanenko, K. O., Trunova, O. K., Kosakivska, I. V. (2019). Phenolic compounds in plants: biogenesis and functions. *Journal of Ukraine Biochemistry*. **3**:91

Calixto, J. (2002). Plants Metabolites. *Journal of Plants History*. **2**:90-91

Chuwkwuebuka, E., Minaksi, M., Jonathan, C. I., and Hameed, S. (2018). Introduction to Phytochemistry. *Journal of Fundamentals, Modern techniques and Applications of Phytochemistry*, **10**-11

Deepti, D. (2012). A Colored Avocado seed extract with Antioxidant, Anti-Carcinogenic and Anti-inflammatory effects. *Journal of Food Science*, **30**:22-23

Elhadi, M. Y. (2018). Avocado. *Journal of Autonomous University of Queretaro*. **10**:1-2

Evans, W.C. (2002). Trease and Evans Pharmacognosy, 15th Edition, Bailiere, W.B. Sanders company Ltd. London. Pp. 321-345.

Fouad, A., Mahamood, A. and Poya D. (2011). Evaluation of hyaluronate anti-ulcer activity against gastric mucosal injury. *African Journal of Pharmacy and Pharmacology*, **5**(1):23-30,

Hélio, N. M. and Arthur, G. F. (2015). Plant Alkaloids: Main Features, Toxicity, and Mechanisms of Action. *Journal of Plant Toxins.* **2**-4

Heras, B., Rodríguez, B., Boscá, L. and Villar, A. M. (2014). Terpenoids: Sources, Structure Elucidation and Therapeutic Potential in Inflammation. *Journal of Medicinal Chemistry* **3**:53-67

Janice, D. A., John, A. and Jemmy, F. T. (2012). Morphological characteristics of avocado (Persea americana Mill.). *Journal of Plant Science.* **12**(4):88-97

Kannissery, P., Shahid, H. A. and Javed, A. (2010). Eugenol: A Natural Compound with Versatile Pharmacological Actions. *Journal of Phytochemicals.* **5**:2-7

Kun-Ruey S. and Shu-Chuan Y. (2019). Exploratory and agile behaviors with central dopaminergic activities in open field tests in Formosan wood mice (Apodemus semotus). *Journal of Experimental Biology*. **2**-22

Kursinszki, L. (2010). Flavonoids. *Journal of Pharmacognosy*. **4**:1-2

Maher, M. A. , Aziza, S. A., Al Sadek G. (2019). A review on saponins from medicinal plants: chemistry, isolation, and determination. *Journal of Nanomedicine Research* **7**:4-5

Mamta, S., Jyoti, S., Rajeev, N., Dharmendra, S. and Abhishek, G. (2016). Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry.* **1**:6

Gastroprotective effect of Phyllanthus niruri leaf extract against ethanol-induced gastric mucosal injury in rats. African Journal of Pharmacy and Pharmacology 4(5)

Neelapu, N., Muvvala, S. , Mrityunjaya, B. P., Lakshmi, B. (2012). Anti-ulcer Activity and Analysis of Mangifera indica Leaves. *International Journal of Pharmaceutical and Phytopharmacological Research.* 1(**4**): 146-155

Nicolosi, E., Deng, Z. N., Gentile, A., La Malfa, S., Continella, G., and Tribulato, E. (2000). Citrus phylogeny and genetic origin of important species as investigated by molecular markers. Theoretical and Applied Genetics, **85:** 1155-1166.

Obaro-Onezeyi, O.E. and Obaro, P.O. (2023). Phytochemical screening, acute toxicity study and evaluation of the n-Hexane extract of brassica oleracea Linn. on the stages of pregnancy and parturition in experimental animals. *Nigerian Journal of Applied Sciences,* **41**(2): 158-168.

Obaro-Onezeyi, O.E. and Oshomoh, E. O. (2019). Assessment of Several Concentrations of Aqueous Extract of *Croton lobactus* (Linn) on Reproductive Parameters of Wistar Rats. *Journal of Science and Technology Research* **1**(1): 72-80

OECD (Organization for Economic Co-operation and Development) (2008a). Guidance Document on Acute Oral Toxicity Testing 420; Organization for Economic Co-operation and Development: Paris, France. 349p.

Olga V. P. and Inga I. P. 2022. Selection of Mice for Object Permanence Cognitive Task Solution. *International Journal of Neurology*. **14**:2-6

Omorodion, N. T., and, Achukwu, P.U. (2017). Investigation of Hibiscus Sabdariffa (Roselle) as Histological Stain and in Assessment of Bar Bodies. America Journal of Biomedical Sciences. 9(1), 15-19;

Oshomoh, E.O and Obaro, P. O. (2019). Contraceptive and Abortificent Effects of Aqueous Crude Extract of Sclerotia of *Pleurotus tuberregium* on Non pregnant and Pregnant Wistar Rats. *NIPES Journal of Science and technology research,* 1(2): 100-108.

Panche, A. N., Diwan, A. D., and Chandra, S. R. 2016. Flavonoids: an overview. *Journal of Nutritional Science*. **5**:1-15

Sofowora, A. (2008). Medical Plant and Traditional Medicine in Africa (3rd Ed.). Spectrum Books Limited Ibadan. 289p.

Solmaz, M. N., Hilal, Ö., Nurşen, B. (2017). Pharmacological and Toxicological Properties of Eugenols. *Journal of Pharmaceutical Science.* **14**(2):201-206

Tassew, B. B., Zinaba, H. T. and Eyasu, G. A. (2019). A Review on Avocado Seed: Functionality, Composition, Antioxidant and Antimicrobial Properties. *Journal of International Chemical Science.* **10**:9-12

Valentine U. O., Thelma, E. I., Gabriel I. E., Iheanyi, K. P., Samuel, E. O. (2014). Evaluation of the acute toxicity, phytochemical constituents and anti - ulcer properties of methanolic leaf extract of Annona muricata in mice. *Journal of Intercultural Ethnopharmacology.* **3**(1):37-43

Vincenzo, L. (2010). Phenolic Compounds: Introduction. *Journal of Biochemistry*. **2**(2):3-5

Yousef, A. (2013). Cardiac Glycosides. *Journal of Pharmacognosy & Phytochemistry*. **10**-11