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Phytochemicals, acute toxicities and actual median lethal doses (actual LD50) of *Zingiber officinale* and *Allium sativum* given singly and in combination via mice models

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Abstract

Phytochemicals are substances produced mainly by plants which are very important due to their biological and pharmacological activities. The pharmacological effects of these components range from the treatment of bacterial and fungal infections to the treatment of chronic-degenerative diseases such as diabetes and cancer. Mice were used for assessing the acute toxicities; studies usually conducted to evaluate the effects of a single substance. Each animal receives a single dose of the test substance but repeated doses may be administered provide all doses are administered within 24 hours. This study is aimed at assessing the phytochemical components, acute toxicities and actual Mean Lethal Doses (actual LD_{50}) of *Zingiber officinale* and *Allium sativum* when administered separately and in combination. Both the phytochemical analysis and acute toxicity testing were done using standard methods with slight modification of the acute toxicity method which led to obtaining the actual LD_{50} for the tested samples and minimizing the number of study animals used. The phytoconstituents of *Zingiber officinale* were alkaloids, tannins, flavonoids, steroids and terpenoids and those of *Allium sativum* were alkaloids, saponins, flavonoids, and glycosides. The actual lethal doses of *Zingiber officinale*, *Allium sativum* and combination of the two were 8,660, 4,472, and 5,477 mg/kg body weight respectively. In conclusion, *Zingiber officinale* was practically none toxic while *Allium sativum* had slight toxicity which was ameliorated when the two herbs were administered together. Thus, rendering the combination of the two herbs safe, as evident in the actual LD_{50} being raised to 5,477 mg/kg body weight.

Keywords: Acute toxicities; *Allium sativum*; Phytochemicals; *Zingiber officinale*

1. Introduction

Phytochemicals are substances produced mainly by plants, and these constituents are very important due to their biological and pharmacological activities. Plants have been shown to be the source of various active ingredients. The pharmacological effects of these components range from the treatment of bacterial and fungal infections to the treatment of chronic-degenerative diseases such as diabetes and cancer [1]. Various studies have also shown that many plants are rich sources of antioxidants. For instance, vitamins A, C, E, and phenolic compounds such as flavonoids, tannins, and lignin, found in plants, all act as antioxidants [2]. There has been a great increase in the use of herbal medicines globally. In developing countries, many patronize them largely due to cultural tolerability, availability and low cost. In developed countries, they are used because they are natural and therefore assumed to be safer than synthetic medicines [3]. In recent times, however, there has been a growing concern about the safety of herbal medicines

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and this has diverted attention towards disputes regarding their use. It therefore becomes pertinent to investigate the safety of herbal drugs before employing them for pharmacological use. This is achieved by evaluating the acute toxicities of the herbal drugs as an intuition to their overall safety profile. This study is therefore aimed at assessing the phytochemical components, actual Mean Lethal Doses (LD_{50}) and other acute toxicities of *Zingiber officinale* and *Allium sativum* when administered separately and in combination.

1.1. Acute toxicities

Rodents such as mice are usually used for assessing the acute toxicities. Acute toxicity studies are conducted to evaluate the effects of a single substance. Usually each animal receives a single dose of the test substance but repeated doses may be administered provide all doses are administered within 24 hours or less. Acute toxicity study was primarily aimed at determining LD_{50} dose which is the dose of the administered substance that would be lethal to 50% of the animals treated. Acute toxicity testing also determines the most important clinical signs of toxicities that can be associated with high doses of the test substance, time of onset and reduction of those signs. In the case of death of animal(s), the sequence and timing of effects leading to death or recovery is also observed. In a certain study, the researchers stated that the assessment of the lethal dose (LD_{50}) (the dose that kills 50% of test animals population) has now been used as a major parameter in measuring acute toxicity and also as an initial procedure for general screening of chemical and pharmacological agents for toxicity [4]. From the result of an acute toxicity test and the LD_{50} obtained, a conclusion can be made on the toxicity status of the test substance. LD_{50} < 5 mg/kg body weight is classified as extreme toxic; 5 – 50 mg/kg body weight as highly toxic; 50 – 500 mg/kg body weight as moderately toxic; 500 – 5,000 mg/kg body weight; 5,000 – 15,000 mg/kg body weight as practically none toxic; and >15,000 mg/kg body weight as relatively harmless [5].

1.2. Actual median lethal dose (actual LD_{50})

In most cases, acute toxicity testing were ended after a high dose of 5,000 mg/kg body weight failed to cause mortality to the test animals; the test substance at this point would be classified as practically none toxic. However, considering the words of Paracelsus, the renowned Toxicologist, which he wrote 500 years ago, that all things are poison and nothing is without poison; solely the dose determines that a thing is not a poison [6]; it may be necessary sometimes to evaluate the actual mean lethal doses (actual LD_{50}) of substances and more importantly herbal drugs. This will help to avert unforeseen danger inherent in overdosing of herbal products by virtue of being confirmed to have LD_{50} greater than 5,000 mg/kg body weight. In this study therefore, we determined the actual LD50s of *Zingiber officinale* and *Allium sativum* when administered separately and in combination. This will give an insight to possible formulation of these two herbs for clinical uses. More so, most herbal medicines are formulated with more than one herb without taking into cognizance possible herb-herb interactions. Example of such poly-herbal medicines here in Nigeria are: Female Correctives, 7Keys to power, Super7 [7].

1.2.1. Zingiber officinale

Zingiber officinale is a perennial herb belonging to the family zingiberaceae. It has been in use since ancient times both as a spice and as herbal medicine. As herbal medicine, *Zingiber officinale* has been used to treat a variety of ailments including gastrointestinal disorders. Its numerous components have anti-oxidative, anti-inflammatory, anticancer, anti-diabetic, antiemetic, cardiovascular, antitussive effect among others [8]. *Zingiber officinale* is used in numerous forms including fresh, dried, preserved, pickled, crystallized, candied, and powdered forms. The rhizome of *Zingiber officinale* contains a wide variety of biologically active compounds such as phenolic and terpene compounds. The phenolic compounds in *Zingiber officinale* are mainly gingerols, shogaols, and paradols. In newly harvested *Zingiber officinale*, gingerols are the major polyphenols, such as 6-gingerol, 8-gingerol, and 10-gingerol. With heat treatment or long-time storage, gingerols can be transformed into corresponding shogaols. After hydrogenation, shogaols can be transformed into paradols. Other phenolic compounds in *Zingiber officinale* include quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione while terpene components include β-bisabolene, α-curcumene, zingiberene, α-farnesene, and β-sesquiphellandrene, which are considered to be the main constituents of *Zingiber officinale* essential oils. Besides these, polysaccharides, lipids, organic acids, volatile oils such as zingiberol, and raw fibers are also present in *Zingiber officinale* [9]. According to phytochemical screening done in a previous study, both the aqueous and petroleum ether extract of ginger contain alkaloids, saponins, flavornoids, polyphenols, cardiac glycosides and reducing sugars. The researchers also showed that the mineral elements contained in ginger include iron (Fe), chromium (Cr), copper (Cu), nickel (Ni), zinc (Zn), and cadmium (Cd). The mean concentrations (mg/kg) were ranged from 4.63 to 5.43 for Cd, 2.17 to 4.44 for Cr, 62.52 to 65.14 for Cu, 77.71 to 81.12 for Fe, 6.49–7.58 for Ni and 16.74–19.31 for Zn [10]. In this present study, we confirmed by qualitative analysis the phytochemicals present in ethanol extract of *Zingiber officinale* which conferred to the herb it's anticancer among other potentials.

1.2.2. Allium sativum

Allium sativum has been used since ancient times for its medicinal properties. Its bulbs are found in many traditional medicines. In India, a juice or paste prepared from bulbs of *Allium sativum* has been used traditionally to relieve coughs, fevers, and ear aches, as well as improve skin conditions. In Ayurvedic and Siddha medicine, *Allium sativum* juice has been used to alleviate sinus problems. Extracts from dried *Allium sativum* bulbs have been used in Unani medicine to regulate menstruation and treat digestive problems and fevers. Hot water extracts from *Allium sativum* bulbs mixed with honey were a folk remedy for whooping cough and intestinal worms. Some studies have shown that sulphur-containing compounds in *Allium sativum*, like allicin, may have anti-bacterial, anti-fungal, anti-viral, and antioxidant properties [11]. *Allium sativum* contains about 0.1 percent essential oil, the principal components of which are diallyl disulfide, diallyl trisulfide, and allyl propyl disulfide [12]. It is also bestowed with an array of organosulphur compounds rich in phytochemicals which act as antioxidant agents. In another study which was undertaken for qualitative as well as quantitative analysis of phytochemical constituents of *Allium sativum*, the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of methanol and chloroform extracts of garlic was found to be better than other extracts used in the study. These extracts showed good antibacterial activity against selected pathogenic bacterial cultures including Pseudomonas, Bacillus, Shigella and Salmonella. Silver nanoparticles were also synthesized with the aid of *Allium sativum* extract and were characterized by X-Ray diffraction. Synthesized silver nanoparticles showed good antibacterial activity against all Salmonella followed by Bacillus, Shigella and Pseudomonas [13]. Due to its biologically active components that contribute to its pharmacological properties, *Allium sativum* is used in the drug development for various human diseases. To obtain crucial data and scientific knowledge about the therapeutic uses of *Allium sativum*, systematic literature searches were conducted and *Allium sativum* was found to have fundamental nutritional components notably carbohydrates, protein, fat, minerals, water, and vitamins are all found in abundance in this plant. According to another study, *Allium sativum* is effective as anti-inflammatory, rheumatological, ulcer inhibiting, anticholinergic, analgesic, antimicrobial, anti-stress, anti-diabetes, and anticancer, among other ailments. The researchers concluded that the nutritional content of the plant is significant, and it has incredible therapeutic potential [14].

2. Materials and methods

2.1. Materials

2.1.1. Animals

Swiss female Albino mice (30.4 – 38.6 g) were used for the study. All the animals were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria. The animals were housed in standard laboratory conditions of 12 hours light, room temperature, and 40 - 60% relative humidity and fed with rodent feed (Guinea Feeds Nigeria Ltd). They were allowed free access to food and water. Maintenance and care of all animals were carried out in accordance with EU Directive 2010/63/EU for animal experiments. Guide for the care and use of Laboratory Animals, DHHS Publ. # (NIH 86-123) were strictly adhered to. Ethical approval was obtained from the Animal Ethical Committee of the Enugu State University of Science and Technology. There was additional approval by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes.

2.1.2. Chemicals and Reagents

Hydrochloric acid (Prime laboratories, India); Dragendoff reagent (Sigma Aldrich, United States of America); Ammonia (Shackti Industrial Gases, India), sodium hydroxide (Treveni Chemical Pvt., India); Ferric chloride (AkashPurochem. Pvt., India); Fehling's solution (Lab care Diagnostics, India); Million reagent (Interlab Chemical Pvt., India): Ethanol (TAJ Pharmaceutical Ltd., India); Acetic anhydride (Ashok Organics Industries, India); Concentrated sulfuric acid (Navin Chemical Pvt., India), Acetic acid (Kayla Africa Suppliers, South Africa); Molisch reagent (Interlab Chemical Pvt., India); alcoholic alpha naphatol (Prat Industry Corcopation, India).

2.1.3. Equipment

Glass column, flasks, beakers, test tubes, measuring cylinders, surgical blade, forceps, scissors, graph paper, white transparent paper, rotary evaporator, Analytical Weighing Balance (Metler H30, Switzerland), Electric Oven (Gallenkamp, England), Water Bath (Techmel & Techmel, Texas, USA), National Blender (Japan), Micropippete (Finnipipette® Labsystems, Finland), Plethysmometer (Biodevices, New Delhi, India) and Intubation tubes. Precision pipettes (25, 50, 100 and 300 µl, 1,000 µL) (Labcompare USA); Disposable pipette tips (Labcompare USA); Distilled or

deionized water (SnowPure Water Technologies USA); Stop watch (Avi Scientific India); disposable hand gloves (Supermax Malaysia).

2.2. Plant materials

Fresh *Zingiber officinale* and *Allium sativum* were procured from the market in Enugu State of Nigeria.

2.2.1. Extraction of plant material

The *Zingiber officinale* and *Allium sativum* were scraped, sliced, washed and dried away from sunlight at room temperature for 48 hours. The dried materials were pulverized to powder using an electronic blender and kept in clean airtight amber colored bottles separately. Then, 750 g of each of the powdered plant material was cold macerated in 95% ethanol. The mixture was allowed to stand for two days (48 hours) with intermittent agitation. It was filtered and the filtrate concentrated to dryness using water bath at 40^0C for 72 hours. The extract was stored in a refrigerator until used.

2.3. Methods

2.3.1. Phytochemical analysis of Zingiber officinale and Alliun sativum separately

The qualitative phytochemical analysis of the extract and fractions were carried out using standard methods described by Odoh [15].

2.3.2. Test for alkaloids

The plant extract and fractions (0.2 g) was heat in 20 mL of 2% acid solution (HCL) individually in a water bath for about 2 min. The resulting solutions were allowed to cool and then filtered then 5 mL of the filtrates used for the following tests:

- **Dragendorff's test**: To each labeled test tube, 5 mL of the sample was added, followed by 1 mL of Dragendorff's reagent. Formation of orange or red precipitates indicates the presence of alkaloids.
- **Hager's test**: The samples (5 mL) were placed in labeled test tubes and a few drops of Hager's reagent (saturated picric acid solution) were added. Formation of yellow precipitate indicates the presence of alkaloids.
- **Wagner's test**: The samples (5 mL) were placed in labeled test tubes and a few drops of Wagner's reagent (solution of iodine and potassium iodide) were added. A reddish brown precipitate indicates the presence of alkaloids.
- **Mayer's test**: A quantity of 5 mL of each of the samples was placed in labeled test tubes and a few drops of the Mayer's reagent (potassium mercuric iodide solution) were added. Formation of cream color precipitate indicates the presence of alkaloids.

2.3.3. Test for glycosides

The samples were extract with 1% H 2 SO 4 solution in hot water bath for about 2 minutes. The resulting solution was filtered and made distinctly alkaline by adding 4 drops of 20% KOH (confirmed with litmus paper). One milliliter of Fehling's solution (equal volume of A and B) was added to the filtrates and heat on hot water bath for 2 minutes. Brick red precipitate indicates the presence of glycosides.

2.3.4. Test for saponins

The plant extracts and fractions (0.2 g) were dissolved in methanol individually and the resulting solutions were used for the following test:

Frothing test: The samples (5 mL) were placed in labeled test tubes and 5 mL of distilled water was added and the mixtures and shaken vigorously. The test tubes were observed for the presence of persistent froth.

2.3.5. Test for tannins

The plant extracts and fractions (0.2 g) were dissolved in methanol individually and the resulting solutions were used for the test. To 3 mL of each of the samples a few drops of 1% Ferric chloride was added and observed for brownish green or a blue-black coloration.

2.3.6. Test for flavonoids

Using methanol, 0.2 g of the plant extracts and fractions were dissolved individually and resulting solutions were used for the following test:

- **Ammonium hydroxide test**: A quantity of 2 mL of 10% ammonia solution was added to a portion of each of the samples and allowed to stand for 2 minutes. Yellow coloration at the lower ammoniacal layer indicates the presence of flavonoid.
- **Sodium hydroxide solution test**: A quantity of 10 mL of 10% sodium hydroxide solution was\ added to a portion of each of the samples and observed for color changes in the lower alkaline layer. Yellow color (flavones), Blue to violet color (anthocyanins), yellow to orange color (flavonones).
- **Concentrated sulphuric acid test**: A portion of each of the samples were mixed gently with conc. Sulphuric acid and observed for color change, yellowish orange color (anthocyanins), yellow to orange color (flavones), orange to crimson (flavonones).

2.3.7. Test for steroids and terpenoids

- **Salkowski test**: The plant extracts and fractions were dissolved in methanol individually and the resulting solutions were used for the test. A 5 mL of each of the samples was mixed in 2 mL of chloroform and concentrated H 2 SO 4 was carefully added to form a layer. A reddish brown coloration at the interface indicates a positive test.
- **Liebermann-Burchard test**: Acetic anhydride (2 mL) was added to 0.5 g of each of the fractions and methanol extracts. Concentrated H 2 SO 4 (2 mL) was carefully added to the resulting mixture and observed for color change from violet to blue or green.

2.3.8. Actual (LD_{50}) and acute toxicity of Zingiber officinale ethanol extract

The actual median lethal dose (LD_{50}) estimation of the *Zingiber officinale* ethanol extracts was conducted with the method described by Lorke [16], with modifications. The tests was done in phases; in the first phase three groups of rats (n = 3) were given oral administration of 1,000, 2,000 and 3,000 mg/kg body weight of *Zingiber officinale* ethanol extracts. The animals were observed for 24 hours for number of deaths and for any sign of toxicity. In the second phase, new sets of three groups of rats (n = 3) were orally administered 3,500, 4,000, and 5,000 mg/kg body weight of *Zingiber officinale* ethanol extracts and were again observed for 24 hours for deaths and for sings of toxicity. Because no death was detected so far, two more groups of rats (n = 3) were used for phase three and were given 7,500 and 10,000 mg/kg body weight of *Zingiber officinale* ethanol extract respectively. These mice were observed for mortality and signs of toxicity within 24 hours. Actual LD_{50} was then determined using the formula:

$LD_{50} = (H \ X \ L)^{1/2}$

H = Highest dose that resulted to no mortality

L = Lowest dose that resulted to mortality

2.3.9. Actual (LD_{50}) and acute toxicity of Allium sativum ethanol extract

The median lethal dose (LD_{50}) estimation of the *Allium sativum* ethanol extract was similarly conducted with the method described by Lorke, [16], with modifications. The tests was also done in phases; in the first phase three groups of rats (n = 3) were given oral administration of 1,000, 2,000 and 3,000 mg/kg body weight of *Allium sativum* ethanol extracts. The animals were observed for 24 hours for mortality and for any sign of toxicity. In the second phase, new set of three groups of rats (n = 3) were orally administered 3,500, 4,000 and 5,000 mg/kg body weight of *Allium sativum* ethanol extracts and were also observed for 24 hours for mortality and for sings of toxicity.

The actual LD_{50} was then determined using the formula:

$LD_{50} = (H \ X \ L)^{1/2}$

H = Highest dose that resulted to no mortality

L = Lowest dose that resulted to mortality

2.3.10. Actual (LD_{50}) of combined Zingiber officinale and Allium sativum ethanol extract

The median lethal dose (LD_{50}) estimation of the combined *Zingiber officinale* and *Allium sativum* ethanol extract was similarly conducted with the method described by Lorke, [16], with modifications. The tests were done with six groups of 3 mice each given oral administration of *Zingiber officinale*:*Allium sativum* in the ratio of 1:1 (1,000:1,000; 2,000:2,000; 3,000:3,000; 4,000:4,000; 5,000:5,000 and 6,000:6,000 mg/kg body weight). The animals were observed for 24 hours for mortality and for any sign of toxicity. The actual LD_{50} was then determined using the formula:

$LD_{50} = (H \ X \ L)^{1/2}$

H = Highest dose that resulted to no mortality

L = Lowest dose that resulted to mortality

3. Results

Table 1 Results of phytochemical analysis of *Zingiber officinale* and *Allium sativum* ethanol leaf extracts

Phytocompounds	***Zingiber officinale***	***Allium sativum***
Alkaloids	+	+
Saponins	-	+
Tannins	+	-
Flavonoids	+	+
Steroids and terpenoids	+	-
Glycosides	-	+

Key: + = present; - = absent

Table 2 Phase 1 results of acute toxicity studies for *Zingiber officinale*

Phase 1 for Zingiber officinale			
Group	Number of mice	Dose of extract (mg/kg body weight)	Observation
1	3	1,000	No mortality, no convulsion, normal fur, skin, respiration and faecal consistency
2	3	2,000	No mortality, no convulsion nor other signs of toxicities
3	3	3,000	No mortality, no convulsion, normal fur, skin, respiration and faecal consistency

Table 3 Phase 2 results of acute toxicity studies for *Zingiber officinale*

Phase 2 for Zingiber officinale			
Group	Number of mice	Dose of extract (mg/kg body weight)	Observation
1	3	3,500	No mortality, no obvious sign of toxicity
2	3	4,000	No mortality, no obvious sign of toxicity
3	3	5,000	Diarrhoea observed, no mortality or other obvious signs of toxicity

Table 4 Phase 3 results of acute toxicity studies for *Zingiber officinale*

Phase 3 for Zingiber officinale			
Group	Number of mice	Dose of extract (mg/kg bw)	Observation
1	3	7,500	Hypoventilation in all the mice which was normalized 6 hours post extract administration
2	3	10,000	Spastic paralysis and hypoventilation in the three mice which led to death of one mouse.

Actual LD_{50} of *Zingiber officinale* ethanol extract was calculated as follows:

- Actual LD50 = $(7,500 \times 10,000)^{1/2}$
- Actual LD50 = 8,660 mg/kg body weight

Table 5 Phase 1 results of acute toxicity studies for *Allium sativum*

Phase 1 for Allium sativum			
Group	Number of mice	Dose of extract (mg/kg Bw)	Observation
1	3	1,000	No mortality, no obvious sign of toxicity
2	3	2,000	No mortality, loss of appetite for two hours with calmness
3	3	3,000	No mortality, diarrhoea, loss of appetite for4 hours

Bw – body weight

Table 6 Phase 2 results of acute toxicity studies for *Allium sativum*

Phase 2 for Allium sativum			
Group	**Number of mice**	**Dose of extract (mg/kg Bw)**	**Observation**
1	3	3,500	Loss of appetite, loss of mechanical activity, diarrhoea, no mortality
2	3	4,000	Loss of appetite, fast respiration, diarrhoea, partial paralysis, no mortality
3	3	5,000	Loss of appetite, fast respiration, tachycardia, paralysis and death of one mouse after 6 hours

Bw = body weight

Actual LD_{50} of *Allium sativum* ethanol extract was calculated as follows:

- Actual LD_{50} = $(4,000 \times 5,000)^{1/2}$
- Actual LD_{50} = 4,472 mg/kg body weight

Table 7 Results of the acute toxicity studies of combined administration of *Zingiber officinale* and *Allium sativum* ethanol extracts in equal proportion.

Groups	**Dose of *Z. officinale* + *A. sativum* (mg/kg Bw)**	**Number of mice**	**Number of death**
1	1,000 + 1,000	3	Nil
2	2,000 + 2,000	3	Nil

3	3,000 + 3,000	3	Nil
4	4,000 + 4,000	3	Nil
5	5,000 + 5,000	3	Nil
6	6,000 + 6,000	3	1

Bw = body weight

Actual LD_{50} of *Zingiber officinale* and *Allium sativum* respectively when given in combination was calculated as follows:

- Actual $LD_{50} = (5{,}000 \times 6{,}000)^{1/2}$
- Actual LD_{50} = 5,477 mg/kg body weight

4. Discussion

The phytochemical compounds present in *Zingiber officinale* include: alkaloids, tannins, flavonoids, steroids and terpenoids while saponins and glycosides are absent. These present phytochemicals are responsible for the profound pharmacological potentials of *zingiber officinale* which include but not limited to its antioxidant, antiinflammatory, anticancer activities. In a particular study, alkaloids were recognized to be a vast class of natural occurring organic molecules, which contain nitrogen atom or amino or amido group in their structures. Alkaloids are present not only in human daily life, in food and drinks but also as stimulant drugs, medicines, narcotics, insecticides and in many physiological activities. They showed strong biological effects on animal and human organisms even in very small doses. According to the researchers, alkaloids show several pharmacological activities on human health such as anti-cancer, anti-inflammatory, Anti-malarial, Anti-microbial, Anti-hypertensive, Anti-diabetic, Anti-oxidant among others. Alkaloids directly act on the central nervous system in the human body and also affect nucleic acid, DNA (Deoxy Ribonucleic acid), RNA (Ribonucleic acid), membrane permeability and proteins [17]. Another group of researchers' defined tannins are a heterogeneous group of high molecular weight, water-soluble, polyphenolic compounds, naturally present in plants where they provide protection against a wide range of biotic and abiotic stressors. They also noted that tannins exert several pharmacological effects, including antioxidant and free radical scavenging activity as well as antimicrobial, anti-cancer, anti-nutritional and cardio-protective properties. Tannins also seem to exert beneficial effects on metabolic disorders and prevent the onset of several oxidative stress-related diseases [18]. Furthermore, an earlier study confirmed that flavonoids which are phytochemical compounds present in many plants, possess a number of medicinal benefits, including anticancer, antioxidant, anti-inflammatory, and antiviral properties. They also have neuroprotective and cardio-protective effects. However, these biological activities were stated by the workers to depend upon the type of flavonoid, its (possible) mode of action, and its bioavailability [19]. Since their identification in 1935, steroids have served a wide range of uses. In accordance to one study done on this subject matter, many of the clinical roles of steroids are related to their potent antiinflammatory and immune-modulating properties. The antiinflammatory properties of steroids have been attributed to their inhibitory effects on the action of phospholipase A2, an enzyme critical to the production of inflammatory compounds. Steroids were shown to be active in affecting gene expression, translation, and enzyme activity. Their physiologic effects stems from a multitude of biochemical pathways such as their induction of the production of proteins called lipocortins. Glucocorticoids stop the production of inflammatory mediators such as leukotrienes and prostaglandins and effectively halt the inflammatory cascade [20]. A recent study defined essential oils as volatile and concentrated liquids extracted from different parts of plants. According to the researchers, bioactive compounds found in essential oils, especially terpenes and terpenoids possess a wide range of biological activities including anticancer, antimicrobial, anti-inflammatory, antioxidant, and antiallergic [21].

The phytochemical compounds present in *Allium sativum* include: alkaloids, saponins, flavonoids, and glycosides while tannins, steroids and terpenoids are absent. In addition to the components discussed above, saponins and glycosides also play some roles in the Pharmacological activities of herbs, *Allium sativum* inclusive. A study reported that saponins are amphiphilic molecules consisting of carbohydrate and either triterpenoid or steroid aglycone moieties and are noted for their multiple biological activities including fungicidal, antimicrobial, antiviral, anti-inflammatory, anticancer, antioxidant and immunomodulatory effects. According to the study, saponins from natural sources have long been used in herbal and traditional medicines [22]. An article aimed at reviewing the current state of knowledge on the use of glycosides from medicinal plants to induce analgesia and anti-inflammatory effect utilixed various databases and search engines, including PubMed, ScienceDirect, Scopus, Web of Science and Google Scholar to search and collect relevant studies on glycosides with antinociceptive activities. According to the researchers, as pain represents an unpleasant sensation linked to actual or potential tissue damage attributed to inflammatory mediators and as current medicines

used to treat inflammation and pain despite being effective, cause severe side effects, such as ulcer, anemia, osteoporosis, and endocrine disruption, increased attention is recently being focused on the examination of the analgesic potential of phytoconstituents, such as glycosides of traditional medicinal plants, because they often have suitable biological activities with fewer side effects as compared to synthetic drugs. These glycosides were found to induce most of the analgesic effects through cyclooxygenase and lipoxygenase pathways [23].

From the results of the acute toxicity studies, *Zingiber officinale* had an actual LD_{50} of 8,660 mg/kg body weight because it took a very high dose of 10,000 mg/kg body weight to cause mortality of mouse. On the other hand, *Allium sativum* had actual LD_{50} of 4,472 mg/kg body weight. Its ethanol extract kills one mouse at the dose of 5,000 mg/kg body weight. However, when an equal amount of *Zingiber officinale* and *Allium sativum* were administered in combination, the LD_{50} of *Zingiber officinale* reduced to 5,477 mg/kg body weight while that of Allium sativum increased to 5,477 mg/kg body weight. These facts implied that *Zingiber officinale* had more safety profile than *Allium sativum*. Some of the reasons for this discrepancy might be as a result of the following: firstly, steroids presence in *Zingiber officinale* and its absence in *Allium sativum*. Steroids are reknowned for their antiinflammatory, analgesic and antipyretic among other activities. Therefore, absence of steroids in *Allium sativum* implied that these activities were devoid and the protection conferred to the mice by steroids was not enjoyed by the mice treated with *Allium sativum* alone and this might have contributed to the various signs of toxicities observed and a consequent lower LD_{50}. In a certain study, corticosteroids have been shown to exert beneficial effects in the treatment of acute myocardial infarction through a novel mechanism involving the rapid, non-transcriptional activation of endothelial nitric oxide synthase (eNOS). Binding of corticosteroids to the glucocorticoid receptor (GR) stimulated phosphatidylinositol 3-kinase and protein kinase Akt, leading to eNOS activation and nitric oxide–dependent vasorelaxation. Acute administration of pharmacological concentrations of corticosteroids in mice led to decreased vascular inflammation and reduced myocardial infarct size following ischemia and reperfusion injury [24]. Secondly, glycosides present in *Allium sativum* but absent in *Zingiber officinale*. Glycosides are noted to have narrow therapeutic window and despite their therapeutic effects might have contributed to the adverse reactions and signs of toxicities which might have also led to mortality of a mice at lower dose (5,000 mg/kg body weight) than *Zingiber officinale* (10,000 mg/kg body weight). As an insight to this fact, a certain study reported that despite the fact that digitalis glycosides are generally the most valuable drugs available for the treatment of heart failure, a relatively high incidence of toxic manifestations has accompanied the widespread employment and beneficial positive contractile action of these agents. Thus, digitalis intoxication appears to be among the most common adverse drug reactions and has been reported to occur in as many as 20% of patients receiving the glycosides. Although the most common and earliest side effects are related to the gastrointestinal tract owing to glycoside action on the central nervous system rather than local gastric irritation, disorders of cardiac rhythm are the first manifestations in one-third of patients. It has been estimated that arrhythmias and conduction disturbances are provoked in up to 80% of patients in whom toxic effects are observed [25]. These was evident in the loss of appetite and diarrhea that were observed when the dose of *Allium sativum* extract was increased to 3,000 mg/kg body weight as well as tachycardia and mortality observed at dose of 5,000 mg/kg body weight. Nevertheless, when the two herbs were given concomitantly, there were antagonistic interactions both ways whereby *Zingiber officinale* inhibited the toxicity of *Allium sativum* thus increasing its LD_{50} to 5,477 mg/kg body weight. On the other hand, *Allium sativum* antagonizes the safty of *Zingiber officinale* thus reducing its LD_{50} to 5,477 mg/kg body weight. However, despite these antagonisms, the combination of *Zingiber officinale* and *Allium sativum* have high safety range since the LD50 of the combination was > 5,000 mg/kg body weight. The combination was advantageous because the safety of *Allium sativum* which had LD_{50} of 4,472 mg/kg body weight during monotherapy was enhanced. Although there have not been researches reported on herb-herb interactions, a certain review stated that herb-herb interactions are common type of drug interaction that occurs when more herbal medicine are administered together. One herb may increase activity of other herb or inhibit its activity or may cause side effects [26]. This is in accordance with our observation in this present study when Zingiber officinale and *Allium sativum* influenced each other's safety profile leading to overall more safety combination.

5. Conclusion

At the end of the experiments, we concluded that the phytochemicals in *Zingiber officinale* and *Allium sativum* were responsible for their overall pharmacological activities including their safety profile. Also, the actual LD_{50} of *Zingiber officinale* is 8,660 mg/kg body weight indicating high safety profile. Meanwhile, interaction between the two herbs ameliorated the toxicity of *Allium sativum* and increased its median lethal dose (LD_{50}) from 4,472 – 5,477 mg/kg body weight.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

Maintenance and care of all animals were carried out in accordance with EU Directive 2010/63/EU for animal experiments. Guide for the care and use of Laboratory Animals, DHHS Publ. # (NIH 86-123) were strictly adhered to. Ethical approval was obtained from the Animal Ethical Committee of the Enugu State University of Science and Technology. There was additional approval by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes.

References

[1] Mendoza, N., & Silva, E. M. E. Introduction to Phytochemicals: Secondary Metabolites from Plants with Active Principles for Pharmacological Importance. InTech, 2018. doi: 10.5772/intechopen.78226

[2] Ammar A*, Naoufal L, Azam B, Dennis G. W, and David A. L. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants (Basel). 2017; 6(4): 42. doi: 10.3390/plants6040042.

[3] Mensah L.K., Komlaga M., Forkuo G., Firempong D., A., Anning C., K., A., & Dickson A., R. (). Toxicity and Safety Implications of Herbal Medicines Used in Africa. IntechOpen, 2019. doi: 10.5772/intechopen.72437.

[4] Enegide C*, David A, and Fidelis S A. A New Method for Determining Acute Toxicity in Animal Models. Toxicol Int. 2013; 20(3): 224–226. doi: 10.4103/0971-6580.121674.

[5] Erhirhie E O, Ihekwereme C P, and Ilodigwe E E. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdiscip Toxicol; 2018; 11(1): 5–12. doi: 10.2478/intox-2018-0001.

[6] Philippe G. Paracelsus Revisited: The Dose Concept in a Complex World. Basic Clin Pharmacol Toxicol. 2016; 119(2): 126–132. doi: 10.1111/bcpt.12622.

[7] Oraekei DI, Ihekwereme CP. Agbata CA, Ike JC. *Effects of Super7 – a poly-herbal antimalarial drug – on female Wister rats Gonadotropin hormones.* Global Scientific Journal, 2020; Volume 8 Issue 3; pages 1296-1320.

[8] Bera, K., Nosalova, G., Sivova, V., & Ray, B. Structural Elements and Cough Suppressing Activity of Polysaccharides from Zingiber officinale Rhizome. *Phytotherapy research: PTR*, 2016; 30(1), 105–111. https://doi.org/10.1002/ptr.5508.

[9] Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., & Li, H. B. (). Bioactive Compounds and Bioactivities of Ginger (*Zingiber officinale* Roscoe). *Foods (Basel, Switzerland)*, 2019; 8(6), 185. https://doi.org/10.3390/foods8060185.

[10] Aschalew G, Atnafu G, and Molla T∗. Levels of heavy metals in ginger (Zingiber officinale Roscoe) from selected districts of Central Gondar Zone, Ethiopia and associated health risk. Heliyon; 2021; 7(4): e06924. Doi: 10.1016/j.heliyon.2021.e06924.

[11] Sushma B B, Rajesh M, Suresh B N K, Jamila K A, Patrick G, and Rajaneesh A*. Antibacterial Properties of Organosulfur Compounds of Garlic (Allium sativum). Front Microbiol. 2021; 12: 613077. doi: 10.3389/fmicb.2021.613077.

[12] Britannica, T. Editors of Encyclopaedia (2023, May 5). *Garlic*. *Encyclopedia Britannica*. https://www.britannica.com/plant/garlic.

[13] Manpreet K, Priyanshi G, Reena G. Phytochemical analysis, antioxidant and antimicrobial activity assessment of Allium sativum (garlic) extract. Research Journal of Biotechnology, 2021; 16(3):134-140.

[14] Tesfaye A. Revealing the Therapeutic Uses of Garlic (*Allium sativum*) and Its Potential for Drug Discovery. *The Scientific World Journal*, 2021; 8817288. https://doi.org/10.1155/2021/8817288.

[15] Odoh, U. E., Obi, P. E., Ezea, C. C., Anwuchaepe, A. U. Phytochemical methods in plant analysis. 1st Ed. Pascal Communications, Nsukka, Enugu, Nigeria. 2019; 47 p.

[16] Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983; 54(4):275-87.

[17] Ayushi R, Renu S, Ruchi B. Pharmacological activities and toxicities of alkaloids on human health, Materials Today: Proceedings. 2022; Volume 48, Part 5; Pages 1407-1415, https://doi.org/10.1016/j.matpr.2021.09.189.

[18] Antonella S, Davide B, Ersilia B, Domenico T. Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. British Journal of Pharmacology. 2016; Volume 174, Issue 11 p. 1244-1262 https://doi.org/10.1111/bph.13630.

[19] Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., Emwas, A. H., & Jaremko, M. Important Flavonoids and Their Role as a Therapeutic Agent. *Molecules (Basel, Switzerland)*, 2020; 25(22), 5243. https://doi.org/10.3390/molecules25225243.

[20] Ericson-Neilsen, W., & Kaye, A. D. Steroids: pharmacology, complications, and practice delivery issues. *The Ochsner journal*, 2014; 14(2), 203–207.

[21] Masyita, A., Mustika Sari, R., Dwi Astuti, A., Yasir, B., Rahma Rumata, N., Emran, T. B., Nainu, F., & Simal-Gandara, J. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food chemistry:* 2022; *X*, 13, 100217. https://doi.org/10.1016/j.fochx.2022.100217.

[22] Juang, Y. P., & Liang, P. H. Biological and Pharmacological Effects of Synthetic Saponins. *Molecules (Basel, Switzerland)*, 2020; *25*(21), 4974. https://doi.org/10.3390/molecules25214974.

[23] Khan, H., Pervaiz, A., Intagliata, S., Das, N., Nagulapalli Venkata, K. C., Atanasov, A. G., Najda, A., Nabavi, S. M., Wang, D., Pittalà, V., & Bishayee, A. The analgesic potential of glycosides derived from medicinal plants. *Daru: journal of Faculty of Pharmacy, Tehran University of Medical Sciences*, 2020; 28(1), 387–401. https://doi.org/10.1007/s40199-019-00319-7.

[24] Hafezi-Moghadam, A., Simoncini, T., Yang, Z., Limbourg, F. P., Plumier, J. C., Rebsamen, M. C., Hsieh, C. M., Chui, D. S., Thomas, K. L., Prorock, A. J., Laubach, V. E., Moskowitz, M. A., French, B. A., Ley, K., & Liao, J. K. Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase. *Nature medicine*, 2002; 8(5), 473–479. https://doi.org/10.1038/nm0502-473.

[25] Mason, D.T., Foerster, J.M. Side Effects and Intoxication of Cardiac Glycosides: Manifestations and Treatment. In: Greeff, K. (eds) Cardiac Glycosides. Handbook of Experimental Pharmacology, 1981; vol 56 / 2. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-68166-0_13.

[26] Ranjeet K B and Rehana P. HERBAL DRUG INTERACTION: AN OVERVIEW. World Journal of Pharmaceutical and Life Sciences WJPLS; 2020; Vol. 6, Issue 5, 62-68.