

ORIGINAL RESEARCH ARTICLE

Comparative Phytochemical Screening and Acute Toxicity Study of Two Varieties of Ginger, *Zingiber officinale*

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ABSTRACT

Phytochemicals are a wide range of compounds that exist naturally in plants, enhancing them with a defensive role that prevents the advancement of chronic diseases. The present study was conducted to determine and compare the presence of phytochemicals and acute toxicity of the two varieties of *Zingiber officinale*. Samples of *Z. officinale* were extracted with methanol by maceration, and the extracts were screened for phytochemicals by conventional techniques, while extracts were evaluated for acute toxicity to estimate the LD₅₀ through oral administration in albino mice. Results showed the presence of alkaloids, flavonoids, saponins, steroids, tannins, terpenoids, cardiac glycosides, carbohydrates and the absence of anthraquinone in all the two varieties of the methanolic extracts for both the leaves and the rhizomes. The acute toxicity study showed that the local variety (LV) had oral LD₅₀ values of 2154.1mg/kgBW (leaves) and 3807.8 mg/kgBW (rhizomes). In comparison, the improved variety (IV) had LD₅₀ values of 3807.8 mg/kgBW (leaves) and > 5000 mg/kgBW (rhizomes) in the mice. Findings from the preliminary phytochemical screening implies that both two varieties of *Z. officinale* are rich in phytochemicals and that both varieties are less toxic with the local variety having higher toxic effect with respect to the LD₅₀ values.

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INTRODUCTION

Ginger, *Zingiber officinale* is a flowering plant, belongs to the family *Zingiberaceae* that are widely used as spices throughout the world (Jean *et al.*, 2020). In Nigeria, ginger is widely grown in Northern part of the country, including Kaduna state (Haruna *et al.*, 2020). There are two varieties that are usually planted in Nigeria, local variety (LV) and improved variety (IV). The local (“yatsun biri”) has a dull-grey coloured rhizome, whereas the improved (“Tafin giwa”) has a bold yellow rhizome flesh, stout with short internodes (Kure, 2007). Ginger’s leaves and flowers are used for a lot of purposes, it serves as medicine, spice and its root is applied in attenuating and treating other common diseases, like headaches, colds, nausea, and emesis (Osabor *et al.*, 2015; Qian *et al.*, 2019). The rhizome of *Z. officinale* has several biological activities comprising of antimicrobial, cytotoxic and anti-tumor (Naiyl and Ahmed, 2018). Chemical analysis of ginger reveals the presence of over 400 different compounds (Sahdeo and Amit, 2015). Certain properties in ginger that include anticancer,

antiviral and antihypertensive are due to the availability of compounds like polyphenols and flavonoids (Shoaib *et al.*, 2016). Many studies have pointed out the role of ginger in prevention and management of certain diseases such as neurodegenerative diseases, *Diabetes mellitus* (Ho *et al.*, 2013; Wei *et al.*, 2017). Phytochemicals are a wide range of compounds that exist naturally in plants enhancing them with a defensive role that prevents the advancement of chronic diseases and also carries out several biological functions such as reducing oxidative stress and degenerative ailments (Haruna *et al.*, 2020; Manju and Pushpa, 2020). Common Phytochemicals constitute of flavonoids, tannins, terpenoids, steroids, saponins, alkaloids among others (Barbosa *et al.*, 2013). Unprecedented phytochemical and biological properties are attributed to *Z. officinale*, it contains active ingredients that are often useful in the management of many diseases and almost all parts of the plant are medicinal (Ahmad and Beg, 2001).

The use of *Z. officinale* ethno medicinally in the treatment

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of certain bacterial infections namely typhoid, cholera makes it extremely important and therefore the need to establish the phytochemical and acute toxicity potential of its two varieties (LV and IV). Therefore, the objective of this study was to determine and compare the presence of phytochemicals and acute toxicity of the two varieties of *Zingiber officinale*.

MATERIALS AND METHODS

Ethical Statement

The following research methodology and its ethics have been fully reviewed and approved, in the 2018/2019 academic session, by the Departmental academic board, Department of Biological Sciences, Kaduna State University, Nigeria.

Plant Collection and Identification

Both two varieties of *Z. officinale* (LV and IV) were collected from Kachia Local Government Area, Kaduna State. The two varieties of the plant were identified and certified by a taxonomist at the herbarium section of the Department of Biological Sciences, Kaduna State University, Kaduna. Voucher specimen number 1612 was assigned to both the LV and IV respectively and then stored in the herbarium section for future reference.

Preparation of Plant Materials

The collected leaves and rhizomes of both varieties of ginger were prepared according to (Assam *et al.*, 2020). They were washed with distilled water, cut and air dried at room temperature, grounded into powdered form using pestle and mortar, in the animal ware house of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria. The powdered form of both plants were stored in clean air tight 400ml containers and extracted with methanol.

Extraction of Plant Material

This was done with the aim of obtaining the various solvent extracts of the two varieties by successive solvent maceration using the modified method of (Anas *et al.*, 2010; Alemu *et al.*, 2020). The powdered leaves and rhizomes (160g) each of the two varieties were successively macerated with methanol (2.5 litres each) for three days and extracts were filtered. The filtrate of each solvent extract of the two varieties was evaporated to dryness on water bath and the yield was calculated in percentage. Methanolic extracts of the two varieties were subsequently used for phytochemical screening and acute toxicity studies.

Preliminary Phytochemical Screening of both Varieties of *Z. officinale*

Methanolic extracts of the two leaves and rhizomes of both LV and IV were subjected to phytochemical screening in order to determine the presence or absence of phytochemical constituents in each of them (Evans, 2002; Musa, 2005; Sofowora, 2008).

Test for Alkaloids (Dragendorff's reagent): each of the ginger extracts (5g) and 5ml of honey were stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath. About 1ml of the filtrate was treated with few drops of

Dragendorff's reagent. Blue black turbidity serves as preliminary evidence of alkaloids (Evans, 2002).

Test for Saponins (Frothing test): each of the extracts (5g) and 5ml of honey was shaken with distilled water in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins (Sofowora, 2008).

Test for Tannins (Ferric chloride test): each of the extracts (5g) and 5ml of honey was stirred with 10ml distilled water and filtered. Ferric chloride reagent was added to the filtrate and blue-black or blue green precipitate determines the presence of Tannins (Musa, 2005).

Test for Flavonoids (Shibata's test): Diluted ammonia solution was added to aqueous filtrate of the test samples followed by the addition of concentrated H₂SO₄. A yellow coloration observation determines the presence of flavonoids (Evans, 2002).

Test for Cardiac glycosides (Keller-Killiani's test): Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of Ferric chloride solution. The mixture was then poured into another test tube containing 2ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of cardiac glycosides (Sofowora, 2008).

Test for Steroids (Liebermann-Burchard's test): To a small portion of the extract, 2-3 drops of concentrated sulphuric acid was added at the side of the test tube. At the interface a brown or reddish brown ring was formed between the two liquids, the upper layer being bluish green indicated the presence of steroids (Musa, 2005).

Test for Terpenoids (Salkowski's test): To 0.5g of the extract, 2ml of chloroform was added to form solution concentrated H₂SO₄ (3ml) was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoid (Evans, 2002).

Test for Carbohydrates (Molisch's test): To a small portion of the extract in a test tube, few drops of Molisch reagent was added and concentrated sulphuric acid was added down the side of the test tube to form a lower layer, a reddish colour ring at the interphase which indicated the presence of carbohydrate (Sofowora, 2008).

Test for Anthraquinones (Borntrager's test): To a portion of the extract in a dry tube, 5ml of chloroform was added and was shaken, for at least 5 minutes. This was filtered and the filtrate shaken with 10% ammonia solution, bright pink colour in the aqueous (upper) layer indicated the presence of free anthraquinones (Musa, 2005).

Experimental Animals

A total of fifty-three (53) albino mice of weight 20g to 24g constituting different sexes collected from Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, were used for each variety of the plant. All the mice were acclimatized for seven days under standard hygienic conditions and kept at a temperature of 24-26 °C. They were given access to mice pellets and water ad libitum. (CIOMS guidelines, 1985; Adesola *et al.*, 2021).

Acute Toxicity Study

The toxicity study was performed using albino mice of both sexes according to (Lorke, 1983; Ayawa *et al.*, 2021). In the first phase, nine mice were divided into three groups of three mice each. The groups received 10, 100 and 1000 mg/kg of standard methanol extract. Oral administration was performed and monitored for signs and symptoms of toxicity and death for a period of 24 hours. In the second phase, four groups of one mouse were treated, the groups received dosages of 1200, 1600, 2900 and 5000 mg/kg of methanolic extract via oral route. Symptoms and signs of toxicity such as rolling, distress, diarrhoea and death were observed for a period of 24 hours. LD₅₀ was obtained using the OECD method (OECD, 2001)

RESULTS

Percentage Yield from Extraction of the Two Varieties of *Z. officinale*

The percentage yield of the methanolic extracts were (4.47% and 4.85%) for the leaves and (6.26% and 5.30%) for the rhizomes of the LV and IV respectively.

Preliminary Phytochemical Screening of the two Varieties of *Z. officinale*

The phytochemical constituents detected were alkaloids, flavonoids, saponins, steroids, tannins, terpenoids, cardiac glycosides, and carbohydrates, while anthraquinones were not detected in all the four extracts of the two varieties as shown in Table 1.

Table 1: Phytochemical screening of methanolic extracts of the two varieties of *Z. officinale*

| S/N | Constituents | Test | Leaves | | Rhizomes | |
|-----|--------------------|---------------------|--------|----------|----------|----------|
| | | | Local | Improved | Local | Improved |
| 1 | Carbohydrates | Molich’s | + | + | + | + |
| 2 | Anthraquinones | Bontrager’s | - | - | - | - |
| 3 | Steroids | LiebermanBucehard’s | + | + | + | + |
| 4 | Terpenoids | Salkowski’s | + | + | + | + |
| 5 | Cardiac Glycosides | Killer-kilant’s | + | + | + | + |
| 6 | Saponins | Frothing’s | + | + | + | + |
| 7 | Tannins | Ferric chloride | + | + | + | + |
| 8 | Alkaloids | Dragendorff’s | + | + | + | + |
| 9 | Flavonoids | Shibata’s | + | + | + | + |

Key: (+) = Present, (-) = Absent.

Acute Toxicity Studies of the Two Varieties of *Z. officinale* in Albino Mice

The oral LD₅₀ values from the acute toxicity study of the methanolic leaf extracts of LV and IV of *Z. officinale* were 2154.1mg/kgBW and 3807.8mg/kgBW in the mice respectively. In contrast, the oral LD₅₀ values from the acute toxicity study of the methanolic rhizome extracts of the LV and IV of the plant were 3807.8mg/kgBW and 5000mg/kgBW in the mice respectively.

DISCUSSION

According to [Tambunan *et al.*, \(2017\)](#), extract yield is influenced by temperature, type of solvent used, extraction time as well as digestion method. High extractive values of any plant would serve as a useful tool or gauge in standardization of the plant material and isolation of medicinal plants ([Ajazuddin and Shaidender, 2010](#)). In the present study, the rhizome of the LV yielded higher value (6.25%) compared to the rhizomes of the IV (5.30%). Results obtained is similar with the findings of [Bashir *et al.*, \(2015\)](#) who reported a value of (6.878%) percentage yield from methanolic extract of *Zingiber officinale*.

However, same number of phytochemicals were obtained in both extracts, but the phytochemicals reported in this study are contrary to those reported in previous studies. Ibukun and [Oluwadare, \(2021\)](#) reported the presence of flavonoids, tannins, phenolic compounds, saponinis, glycosides, steroids and absence of alkaloids in a study conducted in Ondo State, Southwest Nigeria. [Adesola *et*](#)

[al., \(2021\)](#) also found the presence of saponnins, steroids, cardiac glycosides, alkaloids and absence of Tannins, flavonoids and anthraquinones in a research that was carried out in Vom, Plateau State, North Central Nigeria. In Another study performed in Assam, India by [Biswas *et al.*, \(2019\)](#), alkaloid, flavonoid, saponin and glycosides were reported positive while Carbohydrates, Phenol, tannins and proteins were absent. Similarly, the work of Yusuf *et al.*, (2018) done in Niger State, North Central Nigeria revealed that methanolic extracts of *Zingiber officinale* had phenols, tannins, alkaloids, saponins, glycoside, terpenoids, anthraquinone, flavonoids but phlobatannins were not found.

These observations could be attributed to the fact that phytoconstituents are related to the geographical origin of the plant. *Zingiber officinale* used in this study was gotten from Kachia, Kaduna State, Northwest Nigeria compared to those used in mentioned studies from various locations as listed above. Another factor that could contribute to differences in phytoconstituents is polarity of the solvent used as reported by Yusuf *et al.*, (2018). In addition, organ/part of plant used is also known to affect phytochemical composition of plants as reported by [Lawal *et al.*, \(2014\)](#).

Findings from acute toxicity studies showed that methanolic extracts of both the LV and IV had LD₅₀ values that were not lethal to the mice, as physical observations revealed no significant changes in the well-being of the mice or deaths and thus, methanolic extracts

of both varieties were considered safe. This agrees with the work of Benny *et al.*, (2021) in research conducted in Kerala, India and that of Bekkouch *et al.*, (2019) from Errachidia, Morocco. It is also in conformity with the work of Yusuf *et al.*, (2018) in a study conducted in Niger State, North Central, Nigeria. Similarly, the safety of methanolic extract of *Zingiber officinale* was reported by Kobo *et al.*, (2014) in their research carried out in Zaria, Northwest Nigeria.

CONCLUSIONS

Methanolic extracts of the two varieties of *Z. officinale* (LV and IV) contained Carbohydrates, Steroids, terpenoids, cardiac glycosides, saponins, Tannins, alkaloids, flavonoids and absence of anthraquinones suggesting that both varieties have some medicinal and physiological properties. Acute toxicity studies showed that the LV had lower LD₅₀ values 2154.1 mg/kgBW(leaves) and 3807.8mg/kgBW(rhizome) than the IV 3807.8mg/kgBW (leaves) and > 5000mg/kgBW(rhizome) per orally in mice. This implies that both varieties are safe but the improved variety is safer because it has higher LD₅₀ value.

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