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Comparative Phytochemical Analysis of *Citrus medica* fruit juice and peels

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For the correct use of plant extracts, the relative phytochemical composition of different plants should be examined to ensure effective plant use. This study aims to compare the phytochemical composition of *Citrus medica* juice and peels. The phytochemical compositions of the juice extracts and peel extracts was evaluated. The acute toxicity study of juice was carried out by the modified Lorke's method. No deaths were recorded 24 hours after administration of different doses of grape juice extract (10, 100, 200 and 400 ml/kg body weight). In the second stage, 4 doses of injection were administered: 500, 1000, 1500 and 2000 ml/kg body weight, and no death recorded after 24 hours. Acute toxicity studies indicate that the oral LD_{50} of the juice is ≤ 2000 mg/kg. Phytochemical analysis of the juice revealed the presence of secondary metabolites such as flavonoids, phenols, tannins, terpenoids, steroids, carbohydrates and alkaloids. The peels contain same phytochemicals as the juice except for alkaloids which was not detected. Additionally, citrus juice extracts have higher phenolic, total flavonoids and total tannins content compared to the peels. The results indicated that the juice has higher phytochemical constituents than the peels, and are toxicologically safe for consumption.

Keywords: *Citrus medica,* Fruit juice, Peels, Rat, Phytochemical constituents.

1. Introduction

Phytochemicals are compounds produced by plants that often help them fight fungi, bacteria, and other plant pathogens and are also used by insects and other animals. Some phytochemicals are used as poisons, while others are used as medicines. Phytochemicals are used as a term to describe plant compounds that are frequently researched, whose effects on health have not yet been determined, and which are not essential nutrients. Food labelling regulators in Europe and the United States have advised businesses to limit or avoid health related phytochemicals in foods or foods (Arash et al., 2010). The phytochemical category includes compounds that occur in plants and are essential for physical activity and are therefore considered essential nutrients for human diet (Zhishen et al., 1999). Some phytochemicals are plant toxins known to be toxic to humans; For example, aristolochic acid is carcinogenic in low doses. Some phytochemicals are anti-inflammatory and may affect nutrient absorption (Bores et al., 1996). Other chemicals, such as polyphenols and flavonoids, can be pro-oxidants when consumed in large amounts. Indigestible fibers from plant foods, generally considered phytochemicals, are now considered as food with health promoting effects in reducing the risk of some diseases such as cancer and heart disease (Buckingham and Nemesis 2010). Many plants with different properties are used without side effects (Chenet al., 2006).

Citrus medica Linn.cv Diamante (CD) (Plate 1) is a native to India, found in streams in the eastern foothills of the Himalaya (Oyepata and Opeyemi 2024). Mature fruits are large, thin, smooth skinned, lemon yellow in colour and have less juice in the flesh. It is one of the first citrus fruits, all other citrus fruits were created by natural hybrid speciation or artificial hybridization. Citrus medica fruits are similar to some lemons (oblong or oval to obovate), but the fruit has more seeds, is larger and yellow. Its colour and flesh are hard, very thick, rough and itchy after aging (Okafor and Akabuike 2024). It has been cultivated in India since ancient times and is considered to be indigenous to India. Citrus is a perennial or small tree, usually reaching a height of 5 to 10 meters. It has a round face, beautiful green leaves and thorns (Gu et al., 2011). The leaves consist of oval to elliptical leaflets with aroma glands on the upper surface of the leaves. The flowers are fragrant, single or clustered, five-petaled, white or pale pink. The fruit of *Citrus medica* L. has a unique shape that resembles a large, segmented, fingerlike orange. *Citrus medica* L. has many uses in cooking and medicine. In traditional cuisine, grapefruit is used as an ingredient that gives a special flavour to many dishes, desserts and drinks (Mechqoq et al.,

2011). Additionally, the fruit juice and the peels can be used in medicinal preparations as they are rich in bioactive compounds such as flavonoids and limonoids. Vitamins etc. In medicine, citrus fruits are used in the treatment of many ailments. Its extracts have been shown to act as antioxidant, anti-inflammatory and antibacterial agents. The citron plant is also valued for its medicinal properties and has traditionally been used in aromatherapy to reduce pain, inflammation, stress, anxiety and digestive discomfort (Mills et al., 2019). Historically, plants are very beneficial and have been widely used to maintain health in many cultures. The present study was designed to compare the phytochemical constituents of *Citrus medica* fruit juice and peels.

2. Materials and Methods

2.1 Study area

This study was conducted at Federal University, Oye–Ekiti (FUOYE), Ekiti State, Nigeria between 21st December 2023 and 30th January 2024.

2.2 Sample collection and Identification

Fresh *Citrus medica* fruits were bought from Oja Oba Market, Ado Ekiti, Ekiti State, Nigeria. They were transported to Federal University Oye-Ekiti in Ekiti State, Nigeria. The plant was certified at the Department of Botany, Federal University Oye-Ekiti, Ekiti State, Nigeria, and was given a voucher specimen OYE/2023/4232 for future reference.

2.3 Preparation of *Citrus medica* Fruit Extract

The fruit thoroughly washed and rinsed with distilled water. The juice was extracted manually by cutting the fruit into half and then squeezed. The juice was filtered through 4x muslin cloth and the free juice was collected in a clean container and used for the experiment (Maurer et al., 2016; Simeon et al., 2024).

2.4 Preparation of *Citrus medica* peel Extract

Fresh fruit of *Citrus medica* were washed using distilled water and then peeled, and the edible portions were carefully separated. The peels were air dried in a ventilated oven at 40°C for 48 h and grounded to a fine powder and passed through a 24-mesh sieve. The powdered sample (100g) was extracted with ethanol at room temperature by Soxhelet extraction method for 8 h. The mixture was them filtered through a Whatman No. 2 filter paper to peel particles, and the filtrate was evaporated to dryness under reduced pressure at 60°C by a rotary evaporator (Joseph et al., 2024).

2.5 Experimental Animals

Male and female Wister rats (120-150 g) for acute toxicity study were obtained from the Animal House of Federal University Oye-Ekiti. They were fed with standard Grand Cereals Limited animal pellets and given unlimited amounts of water. The FUOYE University College of Health Sciences Animal Ethics Committee granted approval and consent were obtained for animal experiments (FUOYE/2023/113). Rats (n=6) were randomly assigned to different treatment groups.

Animal experiments were carried out under the same conditions. The care and handling of animals followed established public health guidelines in the Guide for the Care and Use of Laboratory Animals (Mbeunkeua et al., 2018).

2.6 Determination of LD_{50} of the Fruit juice

The LD_{50} of *Citrus medica* juice was determined by the modified Lorke method (1983). Sixteen (16) mice were used to determine LD_{50} and were divided into seven groups; In the first stage, four groups were used, three mice per group, 10, 100, 200 and 400 ml/kg body weight were administered respectively. Then, in the second stage, four groups of mice were used, one mouse per group, and 500, 1000, 1500 and 2000 ml/kg body weight were given. The animals were monitored for behavioural changes and mortality at 2 hours, 24 hours, and 14 days after administration of the extract. The LD_{50} value is calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where: D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

2.7 Phytochemical screening

2.7.1 Qualitative phytochemical screening

The peel was separated from the fruit and dried under laboratory temperature and pressure and grounded to powder with the aid of a pestle and mortar. The powder was used for the extraction using a Soxhlet extraction method in the Department of Pharmacognosy FUOYE, Ekiti state. The extract was sieved (Whatman paper) and concentrated to aridity under low compression in a revolving evaporator at 45-56°C yielding 12.1% (w/w) peel extract. The extract was kept at -20°C until usage. Peel extracts and juice of *Citrus medica* were analysed for presence of resins, carbohydrates, alkaloids, proteins, fats and oil, flavonoids, phenols, tannins, saponins, steroids, terpenoids, cardiac glycosides by standard procedures (Modupe et al., 2023).

2.7.2 Quantitative phytochemical analysis

(a). Estimation of total phenolic contents

The total phenolic content was determined by the spectrophotometric method (Panara et al., 2012; Simeon et al., 2024) with slight modification. 1 ml of

the ethanol extract and juice were mixed separately with 1 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 10 ml of a 7% Na_2CO_3 solution was added to the mixture followed by the addition of 13 ml of distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min, after which the absorbance was read at 750 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (20 - 100 µg/ GA and expressed as (µg GA/g) of dried extract.

(b). Estimation of total flavonoids contents

The total flavonoids contents of Citrus medica peel and juice were estimated by method described by Zhishen (1999) with slight modification. 1ml of the ethanol extract and juice were mixed separately with 4ml of distilled water and subsequently with 0.30ml of a $NaNO_2$ solution (10 %). After 5min, 0.30 ml AlCl3 solution (10 %) was added fallowed by 2.0 ml of NaOH solution (5 %) to the mixture. The mixture was thoroughly mixed and absorbance was then determined at 510 nm versus the blank. Standard curve of quercetin was prepared (20 - 100 µg/ml) and the results expressed as quercetin equivalents (µg quercetin/g dried extract).

(c). Estimation of total tannins contents

The tannin contents was determined using Folin-Ciocalteu assay Tamilselvi *et al*. (2003). with slight modification. 100 µL of the ethanol extract and juice were added to 750 µL of distilled water, 500 µLFolin-Ciocateu reagent and 1000 µL of 35 % sodium carbonate. The mixture was shaken vigorously after diluting to 10 mL of distilled water, then incubated for 30 min at room temperature and read at 725 nm using Genway 6305 UV-Vis spectrophotometer. Distilled water was used as blank. The total tannins content was calculated from the prepared standard curve with 20 - 100 µg/ GA and expressed as (µg GA/ g) dry extract.

3. Results and Discussion

3.1 Results

3.1.1 Acute toxicity study

No mortality was recorded after administration of different doses of *Citrus medica* fruit juice extract (10, 100, 200 and 400 ml/kg body weight) for 24 hours. Similar result was recorded in the second stage of the administration (500, 1000, 1500 and 2000 ml/kg body weight) were administered and no one died after 24 hours, suggesting that The LD_{50} is approximately LD50 ≥ 2000 ml/kg body weight. in mice. The result is indicated in Table 1.

Table 1: Acute toxicity studies

Phases	Dose (mL/kg)	Mortality
1	10	0/3
	100	0/3
	200	0/3
	400	0/3
2	500	0/1
	1000	0/1
	1500	0/1
	2000	0/1

3.1.2 Phytochemical screening

The result of phytochemical screening of the juice extracts and peel of Citrus medica studied is shown in Table 2 and 3 and Fig 1, the result indicated the presence of flavonoids, phenols, tannins, terpenoids, steroids, carbohydrates, cardiac glycosides in juice extracts and peel, Alkaloids, phenols, terpenoids, tannins, resins, steroids, terpenoids of Citrus medica. Peel extract had almost same constituent as the juice except for absence of alkaloids. The result also showed that juice extract had 19.1% of total phenolic contents while peel had 12.7%; total flavonoids content of juice was 15.9% compared to the 13.7% of the peels. The total tannins content for juice is 9.8% as against 7.5% for the peels.

Table 2: Phytochemical screening of Juice and peel of Citrus medica

Phytoconstituents	Juice	Peels
Phenols	+	+
Tannins	+	+
Cardiac glycosides	+	+
Saponins	-	-
Terpenoids	+	+
Steroids	+	+
Fats and oil	-	-
Resins	+	_-
Carbohydrate	+	+
Proteins	+	+
Alkaloids	+	_-

Table 3: Total phenolic, flavonoids and tannins content of the juice and peel of Citrus medica

Phytoconstituents	Juice	Peels
Total phenolic content (mg/g of dry extract)	19.12	12.76
Total flavonoid content (mg/g of dry extract)	15.94	13.74
Total tannin content (mg/g of dry extract)	9.81	7.52

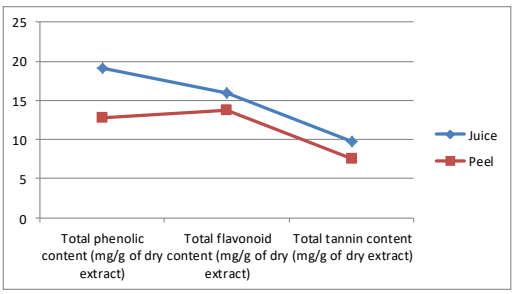

Figure 1: Percentage phytochemical constituents of the fruit juice and peel

3.2 Discussion

3.2.1 Acute toxicity studies

The LD_{50} of citrus juice was determined to be over 2000 ml/kg. The results also showed no significant side effects in mice. No mortality was recorded within 24 hours. LD_{50} is usually expressed as the mass of substance per unit of the test, usually milligrams of substance per kilogram of body weight. This approach allows the relative toxicity of different substances to be compared and normalized to changes in body size of exposed animals (although toxicity does not always equal body weight). For substances in the environment, such as toxic vapors or chemicals toxic to fish in the water, the concentration in the environment (one cubic meter or one liter) is used to give the LC50 value. However, in this case the exposure time is important. This agrees with previous study done by Humaira et al (2024) which noted that citrus medica fruit has LD_{50} > 4000 mg/kg.

3.2.2 Phytochemical constituents

Phytochemicals are non-food bioactive substances responsible for the elimination of toxic free radicals after oxidative stress through the production of antioxidants. Analysis of phytochemicals in the juice showed that compounds including flavonoids, phenols, tannins, terpenes, steroids, carbohydrates, cardiac glycosides and other substances were present in the juice and peels. These molecules may be responsible for many of the medicinal and health benefits reported for this plant. Secondary metabolites have been reported to be important allergens. (Humaira et al., 2019). Chemicals found in plants are divided into primary and secondary metabolites according to their chemical structure and biosynthetic derivation. Secondary metabolites exhibit various pharmacological activities and can be further classified according to their chemical structure and functional groups present in them. The most important metabolites include terpenoids, phenols, flavonoids, alkaloids and glycosides, which are important bioactive components in nutraceuticals and modern pharmaceuticals (Sofowora 1993). Secondary metabolites have excellent antioxidant properties and can be used as natural antioxidants in nutraceuticals (Tiwari et al., 2011). Many of the secondary metabolites have broad therapeutic effects and interact directly with receptors, cell membranes and nucleic acids. Juice extracts contain more important secondary metabolites than the peel. This is an important observation because it provides clear guidelines for drug development and research.

The peels extract of the fruit was found to contain no alkaloids, which is the main difference compared to the juice extract. Alkaloids are one of the most important and largest compounds produced by plants and are metabolic byproducts derived from amino acids (Kabera et al., 2014). Alkaloids are known to have anti-inflammatory, antiviral, antiviral and antifungal properties (Kim et al., 2003).

4. Conclusion

The study revealed that fruit juice extract of *Citrus medica* is safe for consumption. It was also shown that both fruit juice and peel extract of *Citrus medica* contain pharmacologically significant phytochemical constituent. Although, fruit juice extract possesses higher quantity than the peels.

Conflict of Interest

The authors declare no conflict of interest.

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Ethics approval

Ethical approval for this study was obtained from FUOYE University College of Health Sciences Animal Ethics Committee (FUOYE/2023/1 13).

Availability of data and materials

Available on request

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