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Phytochemical Screening and Anti – oxidant Activities of the Roots and Leaves of *Citrus Aurantifolia* (Lime orange) grown in Imane, Olamaboro, Kogi, Nigeria

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Abstract

Traditional doctors have been utilizing the advantages of the potency of natural plants in the treatment and prevention of several ailments for decades without them or their patience knowing the active ingredients responsible for the potency. Hence the need to evaluate and understand the medicinal bio – active substances present in plants. This work analyses the phytochemical substances and the anti – oxidant activities of the roots and leaves of *citrus aurantifolia.* The plant parts were collected, dried, pulverized and extracted in absolute ethanol. The extracts collected were analyzed for bio – active substances and antioxidant activities. The results show the presence of alkaloids, carbohydrates, glycosides, saponins, phenols, tannins, flavonoids, protein and amino acid in both the roots and leaves of *citrus aurantifolia*. The $KMnO_4$ scavenging effects of the plant parts show them to displayed effective potentials as anti – oxidants as the % $KMnO_4$ radical scavenging competes favourably; (root – 85.60% and leaves – 89.88%) with the reference ascorbic acid (83.82%). This indicate that the plant parts can

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scavenge free radical from the body. These finding denotes that the root and leave of *citrus aurantifolia* can be a good source of therapeutic for health management.

Keywords: Phytochemicals, Antioxidant, lime orange, *Citrus Aurantifolia.*

Introduction

Citrus aurantifolia belong to the family Rutaceae. It is commonly known as lime orange. It is a small densely irregular branched green tree which is 5 m tall, twig armed with short stiff sharp spines. The leaves are alternate, elliptic to oblong ovate. The petioles are narrowly winged. The flowers are small, white in bud; calyx cup sharp with 4 – 6 lobed. The fruit is ovoid berry, 3 – 6cm in diameter, sometime with apical papillae, greenish – yellow thin peel; very densely glandular; segments with yellow - green pulp – vesicles; very acidic, juicy and fragrant. The seeds are small, plump, ovoid, smooth with white embryos. (Micheal, 2016).

Citrus aurantifolia leaves are widely use as raw material for cosmetics, food flouring, flour enhancer in beverages and as ingredient in traditional medicine (Swanding *et al*., 2021). It contains active substances that are responsible for their medicinal potential. These substances are secondary metabolites known as phytochemicals; they include alkaloids, coumarins, flavonoids, terpenes, phenols etc. (Al Namani *et al*., 2018; Kzeem *et al*., 2020).

The part of *Citrus aurantifolia* possesses various bioactive substances which have unique medicinal value attributed to it. The leave was reported by Ujung *et al*., (2023) to contain flavonoids, saponins, terpenoids and steroids. Bukola *et al*., (2016) reported flavonoids, phenol, and terpenes in the juice. The juice was also analyzed by Ehigboni *et al*., (2019) to contain flavonoids, steroids, terpenoids, saponins, cardiac glycosides and reducing sugar. Alkaloids, oxalate, phytate, tannin and glycosides were investigated to be present in the peel by Moji *et al*., (2019). The root showed alkaloids, steroid, tripenes, flavonoids, saponins, tannins, carbohydrate, reducing sugar coumarins as bioactive substances in the root by Nazer *et al*., (2018). The stem was evidence in the work of

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Mohammad *et al*., (2018) were they reveled alkaloids, flavonoids, steroids, anthraquinone and carbohydrate as the bioactive components.

Citrus aurantifolia partshas the ability to scavenge free radical from the body making them medically effective in the prevention and treatment of several ailments. Ujung *et al*., (2023) assert that *citrus aurantifolia*, juice has a strong antioxidant activity IC50 of 32.59 mg/mL while the IC50 of ascorbic acid is 8.57 mg/mL. also, in an analysis conducted by Maha *et al*., (2018) the oil obtained from *Citrus aurantifolia* was found to exhibit antioxidant capacity at IC50 of 21.57 ug/mL analyzed using DPPH method. Also, Ehigboni *et al.*, (2015) affirms the oxidant activity of the juice as their results show the DPPH radical scavenging to be highly significant in ferric - reducing oxidant potential in the juice than the reference ascorbic acid.

The antioxidant potential and the presence of bioactive substances have made the parts of *citrus aurantifolia* medicinally active and could be the reason of its potency in the treatment, prevention and health management traditionally. The need to analyze this plant part in our locality is pertinent as they traditional doctors and the patients have no knowledge of what is exactly responsible for their medicinal potency. In this light, the root and leaves of *citrus aurantifolia* were evaluated for its bioactive substances and antioxidant activities as they are used in therapeutics for health management in our locality.

Material and methods

The materials used are leaves and root of *citrus aurantifolia*, Hager reagent, Benedict reagent, ferric chloride solution, pyridine, gelatin, sodiumnitropruside, Chloroform, concentrated tetraoxosulphate (vi) acid, Sodium chloride, Sodium hydroxide (NaOH), Nitric acid, ethanol, hydrochloric acid, $KMnO_4$, ascorbic acid, H_20_2, UV Spectrometer and ethanol for extraction.

Methods

Plant collection and sample preparation

The leaves and root of *Citrus aurantifolia* were collected from Imane. The plant was identified and authenticated by a botanist in Kogi State University, Anyigba and was deposited in our institution department of Biology. The plants were washed and cut into

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tiny pieces, tried at room temperature and were pulverized into fine powder. 100g each was weighed and added 300mL absolute ethanol and allowed to stand for 2 weeks for effective extraction. The mixtures were then filtered and the solutes were sacrificially obtained and stored at room temperature (Leonard *et al*., 2022).

Preparation of solution

0.011mo1/dm^3 of ascorbic acid

0.1g of ascorbic acid was weighed and dissolved in 100mL of distilled water

0.0012mol/dm^3 of $KMnO_4$

0. 02g of potassium permanganate was also weighed and dissolve in 100mL of distilled wate***r.***

$KMnO_4$ acidification

$KMnO_4$ solution was acidified by adding 10mL of 2M H_2SO_4 solution

The % $KMnO_4$ scavenging effect was determined using

% $KMnO_4$ scavenging effect = Ac – At / Ac * 100/1

Where: Ac = Absorbance of control; At = Absorbance of the tested sample (Isaac et al., 2016).

Phytochemical screenings

The phytochemical screening of the plant was conducted according to Prashant *et al.*, (2011); Junaid, R. S., and Patil, M. K. (2020).

Detection of alkaloids

Ficric acid test; 1g of the extracts separately was dissolved in dilute hydrochloric (HCl) and filtered, the filtrate was treated with Hager reagent and the formation of brown/ reddish colour precipitate would indicate the presence alkaloids.

Detection of carbohydrates

Benedicts test; 1g of the extract was dissolved in 5 mL distilled water and filtered. The filtrate was treated with Benedict reagent and heated gently; the formation of orange precipitate Indicates the presence of carbohydrates.

Detection of Glycosides

Concentrated H_2SO_4 test; 1g of the extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to

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blood red color will indicate the presence of glycosides

Detection of Saponins

Foam test; 1g of the extract was dissolved in 2 mL of water. The solution was shaking and was allowed to stand for ten minutes. Persistent foam formation for this period of time shows the presence of saponins.

Detection of phytosterols

Salkowskis test; 1g of the extract was treated with Chloroform and filtered. The filtrate was treated with few drops of concentrated sulphuric acid, it was shaken and allowed to stand. Golden yellow color formation will indicate the presence of phytosterols.

Detection of phenols

Ferric chloride test; 1g of the extract was treated with few drops of ferric chloride solution. The mixture was observed for a bluish black formation which will indicate the presence of phenol.

Detection of tannins

Braymers test; to the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate will indicate the presence of tannins.

Detection of flavonoids

Alkaline reagent test; 1g of the extract was treated with few drops of sodium hydroxide solution, yellow color formation which turns colorless on addition of dilute hydrochloric acid will indicate the presence of flavonoids.

Detection of protein and amino acids

Xanthoproteic test; 1g of the extracts was treated with few drops of concentrated nitric acid and formation of a yellow color will indicate the presence of protein and amino acids

Antioxidant activities

Radical scavenging method using acidified potassium permanganate is used in this method, Ascorbic acid at concentration 2.0mg/mL (i.e. 2.0, 1.5, 1.0, 0.5, and 0.25mL) was used as antioxidant. 0.5mL of acidified $KMnO_4$ solution and 0.5mL of hydrogen peroxide (H_2O_2) was developed as the pro-antioxidant. Absorbance of the $KMnO_4$/ascorbic acid and $KMnO_4$/sample extracts of *Citrus aurantifolia* (i.e. leaves and roots taken spectrophotometrically at a wave

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length of 520 nm after 30-minute incubation period were plotted.

The % $KMnO_4$ scavenging effect was determined using

% $KMnO_4$ scavenging effect = Ac – At / Ac * 100/1

Where: Ac = Absorbance of control; At = Absorbance of the tested sample (Isaac *et al*., 2016).

Results and Discussion

Table 1: Phytochemical screening Result

Parameters	Test performed	Observation	Results	
			Root	Leave
Alkaloids	Ficric acid test	Reddish colour was formed	+	+
Carbohydrates	Benedicts test	Orange precipitate was formed	+	+
Glycosides	Conc. H_2SO_4 test	Pink to red colour was formed	+	+
Saponins	Foam test	Persistent foam formation for 10min	+	+
Phenols.	Ferric chloride test	Bluish black colour was formed	+	+
Phytosterol	Salkowski's test	Golden yellow colour was formed in the leave and absent in the root	-	+
Tannins.	Braymer's test	White precipitate was formed	+	+
Flavonoids.	Alkaline test	Yellow color was formed on addition of NaOH solution which turn colourless on addition of HCI	+	+
Protein & amino acid	Xanthoproteic test	Yellow precipitate was formed	+	+

Key: (+) = present and (-) = absent

Figure 1. Antioxidant activity of the root of *citrus aurantifolia*

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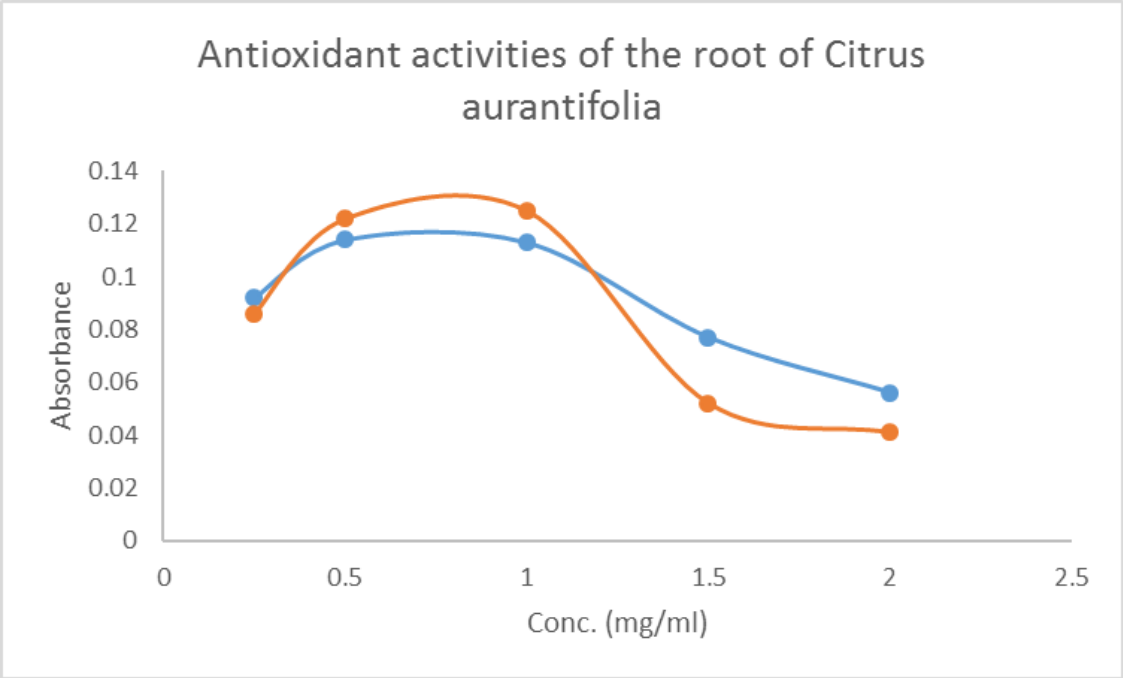

Key: blue = Standard; Orange = sample

Fig. 2. A graph of antioxidant activity of the leaves of *citrus aurantifolia*

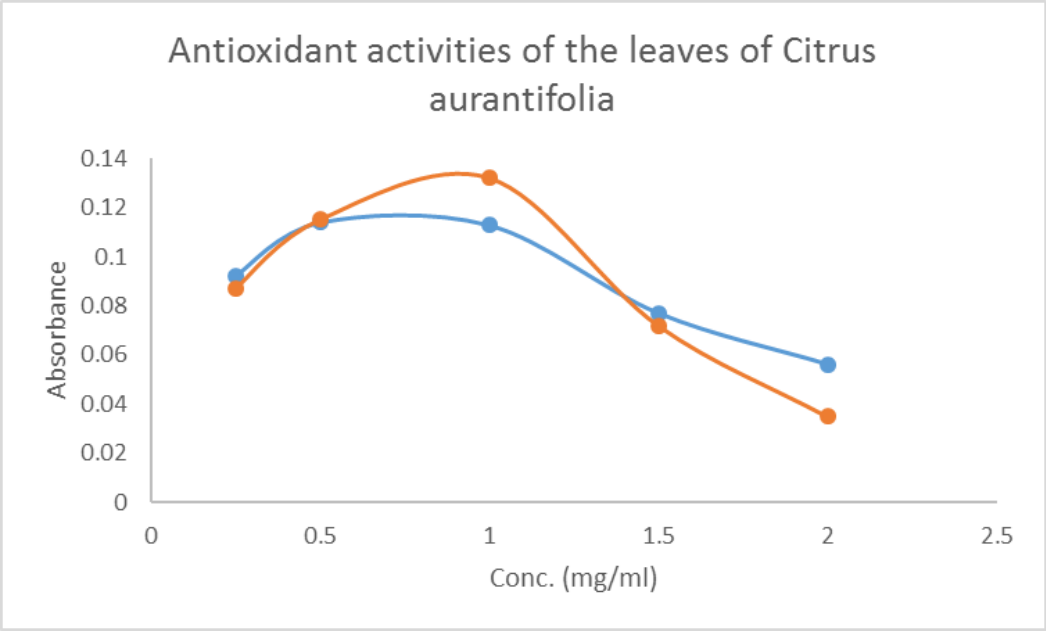

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Table 1 above shows the root of the plant to contain alkaloids, carbohydrates, glycoside, saponins, phenols, tannins, flavonoids, protein and amino acid with the absent of phytosterols. The leaves indicate the presence of alkaloids, carbohydrates, glycoside, saponins, phenols, tannins, flavonoids, protein and amino as well as phytosterols.

Fig 1 and Fig 2 shows the $KMnO_4$ scavenging potency of the root and leaves of *citrus aurantifolia*. The % $KMnO_4$ radical scavenging effect shows the root to be 85.60% and the leaves to be 89.88% as compared to the reference ascorbic acid which is 83.82%.

The phytochemicals result of this study shows it possesses therapeutic potency in both the root and the leave of *Citrus aurantifolia*. Research previously conducted have attested to the justification of this findings as their findings shows the presence of bioactive substances in the plant parts, although with slight variation in the results. The leaves of *Citrus aurantifolia* were analyzed for phytochemicals in Nakha and Nizwa in Oman by Jokha *et al*., (2018) and their findings shows that tannin, steroids, flavonoids, alkaloids and carbohydrate were the bio active substances present in the leaves but our finding show protein and amino acid, phenol as well as glycosides. In Sudan the root of *Citrus aurantifolia* were sampled and phytochemical analysis conducted by Nazer *et al*., (2018) reveals alkaloids, steroids, flavonoids, tannins, carbohydrates, coumarins and reducing sugar while our findings show the absent of phytosteroids in the root. These variations could be attributed to geographical origin of the plant as different geographical environment are endowed with different nutritional values. Solvent of extraction could also be responsible as different solvent have different polarity.

The anti – oxidant activities as displayed by the $KMnO_4$ scavenging result in Fig 1 and Fig 2 shows that C*itrus aurantifolia* root and leave possesses great potential to scavenge free radical. These is in agreement with the findings of Jokha *et al*., (2018) on the leaves sampled in Nakha and Nizwa of Oman, as well as the findings of Nazer *et al*., (2018) on the root of *citrus aurantifolia* analyzed in Sudan.

Conclusion

Citrus aurantifolia has been a plant used traditionally alone as well as in combination

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with other plants to treat several ailments within and around our localities without the traditional doctors or their patients having knowledge of the active ingredients responsible for their medicinal potencies. This study revealed alkaloids, carbohydrate, glycosides, saponins, phenols, phytosteroids, flavonoids, protein and amino acid as the bioactive substances present in the root and leave of C*itrus aurantifolia*. The presence of these bioactive substances and the antioxidant potentials displayed shows that these plant parts have the potential to inhibit free radicals, this ability denotes that the root and leave of C*itrus aurantifolia* could be of good therapeutics in managing varied health conditions.

Recommendation

The root and leave of *Citrus aurantifolia* have proven to be of medicinal importance, however for efficiency and standardization, isolation into pure forms is highly recommended.

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