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 KMnO₄ scavenging effects of the plant parts show them to displayed effective potentials as anti – oxidants as the % KMnO₄ 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
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, KMnO₄, ascorbic acid, H₂O₂, 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
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**

**0.011mol/dm³ of ascorbic acid**

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

**0.0012mol/dm³ of KMnO₄**

0.02g of potassium permanganate was also weighed and dissolve in 100mL of distilled water.

**KMnO₄ acidification**

KMnO₄ solution was acidified by adding 10mL of 2M H₂SO₄ solution

The % KMnO₄ scavenging effect was determined using

\[
\% \text{ KMnO}_4 \text{ scavenging effect} = \frac{Ac - At}{Ac} \times 100/
\]

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 H₂SO₄ test; 1g of the extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to
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 KMnO₄ solution and 0.5mL of hydrogen peroxide (H₂O₂) was developed as the pro-antioxidant. Absorbance of the KMnO₄/ascorbic acid and KMnO₄/sample extracts of *Citrus aurantifolia* (i.e. leaves and roots taken spectrophotometrically at a wave
length of 520 nm after 30-minute incubation period were plotted.

The \( \% \text{ KMnO}_4 \) scavenging effect was determined using

\[
\frac{\% \text{ KMnO}_4 \text{ scavenging effect} = A_c - A_t}{A_c} \times 100\%
\]

Where: \( A_c \) = Absorbance of control; \( A_t \) = Absorbance of the tested sample (Isaac et al., 2016).

**Results and Discussion**

Table 1: Phytochemical screening Result

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Test performed</th>
<th>Observation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Ficric acid test</td>
<td>Reddish colour was formed</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Benedicts test</td>
<td>Orange precipitate was formed</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Conc. ( \text{H}_2\text{SO}_4 ) test</td>
<td>Pink to red colour was formed</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>Persistent foam formation for 10min</td>
<td>+</td>
</tr>
<tr>
<td>Phenols.</td>
<td>Ferric chloride test</td>
<td>Bluish black colour was formed</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterol</td>
<td>Salkowski’s test</td>
<td>Golden yellow colour was formed in the leave and absent in the root</td>
<td>-</td>
</tr>
<tr>
<td>Tannins.</td>
<td>Braymer’s test</td>
<td>White precipitate was formed</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids.</td>
<td>Alkaline test</td>
<td>Yellow color was formed on addition of ( \text{NaOH} ) solution which turn colourless on addition of ( \text{HCl} )</td>
<td>+</td>
</tr>
<tr>
<td>Protein &amp; amino acid</td>
<td>Xanthoproteic test</td>
<td>Yellow precipitate was formed</td>
<td>+</td>
</tr>
</tbody>
</table>

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

Figure 1. Antioxidant activity of the root of *citrus aurantifolia*
Fig. 2. A graph of antioxidant activity of the leaves of *Citrus aurantifolia*

Key: blue = Standard; Orange = sample

**Antioxidant activities of the root of Citrus aurantifolia**
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 KMnO₄ scavenging potency of the root and leaves of *Citrus aurantifolia*. The % KMnO₄ 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 KMnO₄ scavenging result in Fig 1 and Fig 2 shows that *Citrus 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
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 *Citrus 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 *Citrus 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.

**References**


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