Phytochemical Profile, Antioxidant Activity of Leaf of *Hoslundia opposita* Vahl and Docking of its Phytochemical Components with Uropathogenic Strain F11(ID:3NRP) and NFeoB from *Escherichia coli* BL21(ID:5FH9)

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**Authors' contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**ABSTRACT**

*Hoslundia opposita* Vahl is a multi-purpose medicinal plant used traditionally to treat gonorrhea, cystitis, cough, fever, snake bites, convulsion in many parts of Africa. The objectives of this research are to evaluate the phytochemical profile, antioxidant activity of the leaves of *Hoslundia opposita* Vahl and to dock its phytochemical components with uropathogenic *Escherichia coli* strain.

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1. INTRODUCTION

Secondary metabolites that are discovered from plants are constantly increasing. Plants synthesize these compounds as part of their survival strategies but most of these compounds have been shown to be beneficial to man and animals due to their biological and pharmacological activities. Secondary metabolites such as flavonoids possess multiple biological functions such as antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic among others [1-3]. Despite the immense contribution of modern medicine to healthcare, medicinal plants also contribute a huge quota and was most preferred for the improvement and maintenance of health. Although most medicinal plants toxicity profile has not been examined, it is generally believed to be safer than the synthetic drugs. This has inspired researchers as they are showing greater interest in plant materials in order to identify and explore plants with potential safe, effective and cheap drugs. Especially drugs that can combat accumulated free radicals are important in the treatment of certain diseases such as ischemia, arthritis, asthma, inflammation, etc. However the antioxidant effects of medicinal plants are attributed to the presence of phytochemicals such as flavonoids, polyphenolics and tannins [4].

Among those plants with diversified pharmacological activities is *Hoslundia opposita* an herbaceous perennial plant of the family of Lamiaceae. It is found both in tropical and subtropical regions of Africa [5]. In Nigeria, it is commonly known as Efirin odan by the Yorubas and oke ota by the Igbos and is considered to be a multipurpose plant due to its application in the treatment of many arrays of diseases which include malaria, wounds, gonorrhea, cough, chest pain, stomach disorder, convulsion, rheumatism, inflammation among others [6-8]. The plant has been reported to contain some phytochemicals which might be responsible for the various biological activities of the plant [9]. Most researchers have evaluated the biological and pharmacological activities of the plant [10-13]. The objective of this study was to determine the phytochemical profile, antioxidant activity, total phenolic and flavonoid contents of the leaves of *Hoslundia opposita* Vahl. Also, to identify the active phytochemicals that can exhibit inhibitory activity against *Escherichia coli* by in silico methods to understand the nature of interactions involving binding of these phytochemicals with uropathogenic *Escherichia coli* strain F11(ID: 3NRP) and NFoeB from *Escherichia coli* BL21 (ID: 5FH9). Likewise, binding affinity of these active phytochemicals were compared with some commercial drugs in
the market such as ciprofloxacin, levofloxacin and tetracyclin.

2. MATERIALS AND METHODS

2.1 Chemicals and Equipment

All solvents used were of analytical grade and were purchased from Sigma Chemicals Co (St Louis, MO, USA). UV/visible absorption measurements were carried out on a Genesys 10Svl.200217H311008 spectrophotometer.

2.2 Preparation of Plant Material and Extract

*Hoslundia opposita* leaves were collected from a herbal farm in Omu aran, Kwara State, Nigeria. The plant was identified by Prof Ogunkunle A. T. J at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria by matching the Yoruba name of the plant with what was written in the book, “Vernacular of Nigerian plants” by Gbile and Soladoye, [14] and the plant specimen with a voucher number LHO 567 was deposited in the University Herbarium. The leaves were air dried for two weeks. The dried leaves were pulverized into fine powder and stored in clean and sterilized plastic container.

2.2.1 Extraction

Five hundred grammes (500 g) of the pulverized leaves were extracted with methanol using Soxhlet extractor in a water bath. The extract was concentrated by distillation using a rotary evaporator and finally evaporated to dryness under vacuum and stored below room temperature for further analysis.

2.3 Qualitative Phytochemical Screening

The plant leaf extract was screened for presence of flavonoids, steroids, tannins, saponins, alkaloids, glycosides, terpenoids and anthraquinones based on the method described by Harborne and Ngbede [15,16].

2.3.1 GC-MS screening

A gas chromatograph (7890A) from Agilent USA hyphenated to a mass spectrometer (5975C) with triple axis detector equipped with an auto inject (10 µl syringe) was used. Helium gas was employed as the carrier gas. All chromatographic separations were performed on capillary column treated with phenyl methyl siloxane having the following specifications: length - 30m; internal diameter 2.2 µm; thickness 250 µm; ion source temperature (EI) - 250°C; interface temperature - 300°C. Other GC-MS conditions for operation include pressure - 16.2 psia; out time - 1.8 min, 1µl aliquot of sample was injected automatically using 1 µl injector in split mode, split mode ratio-1: 50; injection temperature - 300°C. The column temperature was started at 35°C and held for 5 min. The total elution time was 47.5 min. MS solution software provided by the supplier was used to control the system and to acquire the data.

2.4 Determination of Total Flavonoid Content

Total flavonoid content of the plant extract was determined by the aluminum chloride colorimetric method of Chang et al., [17]. A 50 µg of the extract was dissolved in 4 mL of distilled water in a test tube and 1mL of methanol added. Later, 0.3 mL of 5% NaNO₂ solution was added and incubated for 5 mins. Then 0.3 mL of 10% AlCl₃ solution was added to the mixture and was left undisturbed for 15 mins and the absorbance measured at 510 nm using a uv/visible spectrophotometer (752N) procured from United Kingdom. The total flavonoid content was calculated from a calibration curve using quercetin as the standard.

2.5 Determination of Total Phenolic Content

Total phenolic content of the extract was estimated using the Folin-Ciocalteau assay [18]. About 3.8 mL of the extract was dissolved in 3 mL of distilled water and mixed with 0.5 mL of Folin-Ciocalteau reagent and stirred for 3 mins, 2 mL of 20% NaCO₃ was later added to the solution and allowed to stand in the dark for 1 hr. Later, the absorbance was measured at 650 nm. The total phenolic content was calculated from a standard curve using gallic acid as the standard.

2.6 Antioxidant Activity by DPPH Assay

DPPH scavenging potential of the extract was determined using the method of Mensor et al., [19]. About 3.8 mL of 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution was added to 200µL of the extract (100-500µg/mL) and incubated in the dark at room temperature for 1 hr. The absorbance was later measured at 517
nm [20]. Ascorbic acid was used as the positive control and the free radical scavenging ability of the extract was determined from the equation:

\[
\% \text{ inhibition of free radical DPPH} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

Where \( A_{\text{control}} \) = absorbance of the control  
\( A_{\text{sample}} \) = absorbance of the extract

2.7 Computational Details

The metal transporters from *Escherichia coli* strain F11 (ID: 3NRP) and NFeoB from *Escherichia coli* BL21 (ID: 5FH9) which are of importance to iron metabolism of many bacteria [21,22] were downloaded from protein data bank. The molecular docking simulation was used to investigate the interactions of the phytochemicals with the *Escherichia coli* receptors. The software used for the docking studies are Discovery Studio (version v17.2.0.16349), Auto Dock Tool (version 1.5.6) and autodockvina1.1.2win32.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Studies

Identification of certain class of phytochemicals is an important tool in the screening of plants that possess medicinal properties. This screening will help to eliminate steps that are not necessary in the search for active metabolites. In this research work the plant leaf extracts were screened for presence of eight classes of phytochemicals as presented in Table 1.

It was observed that only four of these classes of phytochemicals were present; saponins, flavonoids, terpenoids and cardiac glycosides. This result is in contrast to the report of Ojo et al., [23]. They reported presence of alkaloids and tannins in their work. Okach et al., [24] reported presence of sterols and absence of alkaloids and glycosides. This difference could be as a result of soil, climate and environmental factors. In the GC-MS analysis of the methanolic leaf extract of *Hoslundia opposita*, the identification of the compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from NIST library incorporated into the operating system. The retention time, peak area, molecular weight and molecular formula of the identified compounds are shown in Table 2 and the chromatogram of the separated compounds is given in Fig 1. A total of seventeen compounds were separated while fourteen were identified. The compound 4-cyclopentene-1,3-dione was the first to emerge with a retention time of 8.64 min while trans-3-(trifluoromethyl) cinnamic acid, octadecyl ester was the last to emerge with retention time of 41.67 min. The compound with the highest quantity in the extract is 1,2,3-benzenetriol (38.11%). Other compounds with appreciable amount in the extract are n-hexadecanoic acid (13.52%) and catechol (9.98%). The least was 4-cyclopentene-1,3-dione (1.22%). The compounds identified belong to the class of phytochemicals known as phenolics, alcohols and fatty acid esters.

3.2 Phenolic and Flavonoid Contents

In the spectrophotometric determination of the phenolic and flavonoid contents, Folin-Ciocalteau method was used for phenolic content assay while aluminum colorimetry assay was used for the determination of flavonoid content. The results obtained are as presented in Tables 3 and 4. The total phenolic content is expressed as mg/gallic acid equivalent/mg extract since gallic acid was used as the standard. A standard curve was calibrated using gallic acid and the total phenolic content obtained from the curve (\( y = 0.023x + 0.292; R^2 = 0.946 \)) while the total flavonoid content is expressed in mg/quercetin equivalent/mg extract. A calibration curve of

| Secondary metabolite | Inference |
|----------------------|-----------|
| Saponins             | +         |
| Steroids             | -         |
| Flavonoids           | +         |
| Terpenoids           | +         |
| Tannins              | -         |
| Alkaloids            | -         |
| Glycosides           | +         |
| Anthraquinone        | -         |

*Note: + = present - = absent*
Table 2. GC-MS Report on Methanolic Leaf Extract of *Hoslundia opposita*

| Peak number | Retention Time | Name of compound | % of total extract | Molecular mass | Molecular formula |
|-------------|----------------|------------------|--------------------|----------------|------------------|
| 1           | 8.64           | 4-cyclopentene-1, 3-dione | 1.22               | 96             | C_5H_4O_2        |
| 2           | 13.07          | Phenol           | 2.23               | 94             | C_6H_5O        |
| 3           | 17.42          | Unidentified     | 1.95               | -              | -                |
| 4           | 17.84          | Phenylethyl alcohol | 1.44              | 122            | C_8H_10O        |
| 5           | 21.32          | Catechol         | 9.98               | 110            | C_6H_6O_2      |
| 6           | 21.89          | Benzofuran, 2, 3-dihydro- | 3.08              | 120            | C_6H_6O        |
| 7           | 23.19          | 3-methoxy-1, 2-benzenediol | 6.82            | 140            | C_7H_8O_2      |
| 8           | 23.45          | Unidentified     | 4.80               | -              | -                |
| 9           | 27.49          | 1, 2, 3-benzenetriol | 38.11            | 294            | C_20H_36O      |
| 10          | 38.65          | Unidentified     | 2.51               | -              | -                |
| 11          | 39.05          | 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol | 1.34            | 294            | C_20H_36O      |
| 12          | 39.42          | Hexadecanoic acid, methyl ester | 2.68            | 270            | C_{17}H_{28}O_2 |
| 13          | 39.74          | n-hexadecanoic acid | 13.52            | 256            | C_{16}H_{22}O_2 |
| 14          | 40.24          | Coumarin         | 1.31               | 146            | C_9H_6O_2      |
| 15          | 40.78          | Oleic acid       | 4.32               | 282            | C_{18}H_{36}O_2 |
| 16          | 40.90          | Octadecanoic acid | 2.48             | 284            | C_{18}H_{36}O_2 |
| 17          | 41.67          | Trans-3- (trifluoromethyl)cinnamic acid, octadecyl ester | 2.21            | 468            | C_{28}H_{48}F_3O_2 |

Fig. 1. Total ion chromatogram of the methanolic leaf extract of *Hoslundia opposita*

quercetin was plotted and the total flavonoid content obtained from the curve \( y = 0.013 + 0.008, R^2 = 0.981 \). The polyphenolic compounds possess redox properties; in that the molybdtungstate in the Folin-Ciocalteau reagent reacts with the phenolics in the extract to produce a coloured compound which can absorb UV radiation around 745-750 nm. Polyphenols have been shown to possess both pharmacological and biological properties. They can protect pancreatic B-cells against glucose toxicity, inhibit \( \alpha \)-amylase or \( \alpha \)-glucosidases thereby causing a decrease in starch digestion. They also possess antimicrobial, antioxidant and anti-inflammatory activities [23]. These activities could be due to the presence of hydroxyl groups on the aromatic rings [24]. Most plant flavonoids exhibit antioxidant, anti-inflammatory,
antimicrobial, etc., properties. Their activity is also dependent on the presence of free hydroxyl groups, especially the 3-OH on the benzene ring. Researchers have shown that flavonoids have the ability to scavenge free radicals such as singlet oxygen and other free radicals implicated in several diseases. They can also suppress the formation of reactive oxygen species, up-regulate and protect antioxidant defenses [25]. The phenolic content of plants has been shown to be dependent on both intrinsic (genetic, extraction solvent) and extrinsic (environmental, handling and development stages) factors [26].

3.3 Antioxidant Activity

Several methods have been developed for the screening of plant materials for antioxidant activity. One of such methods is the use of DPPH free radical. This method is based on the ability of the phytochemical to donate electron to DPPH free radical thereby converting it to its non-radical form. The in-vitro antioxidant assay of the leaf extract using DPPH resulted in the reduction of DPPH radical into its non-radical form. The free radical scavenging activity of the extract was compared with that of ascorbic acid which was used as the standard. DPPH scavenging activity of the plant is presented in Table 4. The result of this study shows that the plant possess high phenolic and flavonoid contents (Tables 4 and 5). The antioxidant activity of the plant might be attributed to the presence of these phytochemicals [4]. The antioxidant capacity of flavonoids is attributed to the configuration, total number of hydroxyl groups and substitution of enzymes such as xanthine oxidase, lipoxygenase, protein kinase etc, they have the capacity to directly scavenge free radicals and chelate metal ions by donating hydrogen atom or by the transference of an electron to them thereby preventing them from being accessible for oxidation. These free radicals are generated during oxidative metabolism in humans. The accumulated oxygen molecule is biotransformed into reactive oxygen species such as O₂⁻, H₂O₂ and OH radicals. These reactive species can affect the DNA, proteins, lipids etc. resulting in different types of degenerative diseases like cancer, osteoarthritis, cardiovascular diseases

| Concentration (ug/ml) | Methanolic extract (%) | Ascorbic acid (%) |
|-----------------------|------------------------|-------------------|
| 100                   | 21.93 ± 0.11           | 42.18 ± 0.46      |
| 200                   | 32.42 ± 0.49           | 70.06 ± 0.31      |
| 300                   | 51.38 ± 0.52           | 79.98 ± 0.53      |
| 400                   | 63.36 ± 0.83           | 83.16 ± 0.32      |
| 500                   | 79.86 ± 0.54           | 90.85 ± 0.19      |

Table 4. Total Phenolic Content of Methanolic Extract of Hoslundia opposite

| Concentration (ug/ml) | Methanolic extract | Gallic acid |
|-----------------------|--------------------|-------------|
| 100                   | 102.39 ± 0.58      | 156.28 ± 0.78 |
| 200                   | 162.46 ± 0.36      | 195.42 ± 0.84 |
| 300                   | 195.84 ± 0.28      | 234.06 ± 0.94 |
| 400                   | 204.21 ± 0.54      | 342.14 ± 0.66 |
| 500                   | 262.54 ± 0.88      | 385.12 ± 0.79 |

Table 5. Total Flavonoid Content of Methanolic Extract of Hoslundia opposite

| Concentration | Methanolic extract | Quercetin |
|---------------|--------------------|-----------|
| 100           | 2.70 ± 0.02        | 7.03 ± 0.02 |
| 200           | 3.83 ± 0.07        | 7.42 ± 0.04 |
| 300           | 4.24 ± 0.08        | 8.94 ± 0.05 |
| 400           | 5.46 ± 0.05        | 10.54 ± 0.09 |
| 500           | 6.24 ± 0.03        | 12.46 ± 0.07 |

Mean value ± standard deviation of three replicates
Table 6. Binding affinity and binding interactions of some antibiotic drugs and Trans-3-(Trifluoromethyl)Cinnamic Acid, Octadecyl Ester against *E. coli*

| Ligands                        | 3nrp receptor | 5fh9 receptors |
|--------------------------------|---------------|----------------|
|                                | Binding Affinity AG (Kcal/mol) | Inhibition Constant Ki (μM) | H-bond distance (Å) | Hydrophobic interactions involved | Binding Affinity AG (Kcal/mol) | Inhibition Constant Ki (μM) | H-bond distance (Å) | Hydrophobic interactions involved |
| Trans-3-(Trifluoromethyl)Cinnamyl acid, Octadecyl Ester | -5.6 78.14 GLY'58 3.5 HIS'125, TRP'137, ASN'57, ARG'126 VAL'134, ET'124 PHE'56, VAL'91 PRO'96, TRP'64 PRO'66, GLY'58 THR'132, HIS'127 | -5.9 47.08 VAL'175, TYR'177 HIS'176, LEU'182 GLN'179, ARG'202 LEU'206, LYS'199 VAL'172, GLU'173 LEU'174, GLU'110 TRP'203, ALA'185 ASP'186 |
| Tetracycline                   | -7.2 5.24 GLU'18, 2.9,3.0, 3.0 ASP'48, 3.1,2.7, 3.3 ASP'94, 3.6 ASP'94, 2.8 GLY'62 3.0 GLU'46,ALA'51 2.9,3.0, 3.0 ASP'28, SER'93 PHE'3, GLU'25 LEU'24, ALA'92 HIS'97, ILE'27 VAL'91, MET'90 | -8.5 0.59 GLU'110 3.0 VAL'175, TYR'177 TRP'203, LYS'199 LEU'206, ALA'185 ARG'202, ASN'183 GLN'179 |
| Ciprofloxacin                  | -6.1 33.59 GLU'30 2.2 MET'29, GLU'30 2.2 ASP'28, SER'93 PHE'3, GLU'25 LEU'24, ALA'92 HIS'97, ILE'27 VAL'91, MET'90 | -7.0 7.35 ASN'183 3.5 ARG'202, TRP'203 LEU'206, LYS'199 LEU'174, VAL'175 TYR'177, GLU'110 GLN'179 |
| Levofloxacin                   | -6.6 14.43 ASN'101 3.1 LYS'103, GLN'83 3.1 LEU'36, ASN'101 ILE'102, ALA'100 GLY'85, GLY'35 MET'34 | -7.4 3.74 GLU'210 3.5 GLN'207, TYR'61 TYR'103 3.4 GLU'74, ILE'66 GLN'107 2.5 THR'64, THR'65 ARG'248 2.5 SER'62 |
(a) 3NRP + Ciprofloxacin

(b) 3NRP + Levofloxacin

(C) 3NRP+Tetra 3NRP+Tetracycline
(d) 3NRP + Trans-3-(Trifluoromethyl)Cinnamic Acid, Octadecyl Ester

Fig. 2. 2D and 3D docked complexes of the ligands with 3NRP receptor

(a) 5FH9 + CIPROFLOXACIN

(b) 5FH9 + LEVOFLOXACIN
etc. Oxygen molecules become detrimental to human health when there is a critical imbalance between the reactive oxygen species production and endogenous antioxidant defense mechanism.

### 3.4 Insilico Assessment of Trans-3-(trifluoromethyl) Cinnamic Acid, Octadecyl Ester

GC-MS analysis showed that methanolic leaf extract of *Hoslundia opposita* contain trans-3-(trifluoromethyl) cinnamic acid, octadecyl ester(2.21%). This compound has been reported to be active against *Escherichia coli*, therefore it was docked with uropathogenic *Escherichia coli* strain F11(ID:3NRP)and NFeoB from *Escherichia coli* BL21 (ID:5FH9). Both are metal transporters especially ferrous; thus, are very important factors in the iron metabolism of many bacteria [27,28] and were downloaded from protein data bank. The molecular docking was performed to investigate its binding mode and compared with those of three antibiotic drugs that are commercially available vis-à-vis ciprofloxacin, levofloxacin and tetracyclin using Discovery Studio (version 17.2.0.16349), Auto Dock Tool (version 1.5.6) and AutoDockVina1.1.2win32. The docking simulation was carried out with AutoDockVina for the calculation of binding affinity using various scoring functions. Molecular docking gives information on the binding mode of the ligands and the docked poses for each ligand were viewed, the highest dock score was chosen as the final conformation. The binding affinities of trans-3-(trifluoromethyl) cinnamic acid, octadecyl ester, ciprofloxacin, levofloxacin and tetracycline and) with 3NRP and 5FH9 are presented in Table 6. The docked results for 5FH9 with the ligands were -8.5, -7.4, -7.0 and -5.9 Kcal/mol for tetracycline, levofloxacin, ciprofloxacin and trans-3-(trifluoromethyl) cinnamic acid, octadecyl ester
respectively. Also, binding affinities for 3NRP with tetracycline, levofloxacin, ciprofloxacin and trans-3-(trifluoromethyl)cinnamic acid, octadecyl ester were -7.2, -6.6, -6.1 and -5.6 Kcal/mol respectively (Table 6). This shows that trans-3-(trifluoromethyl)cinnamic acid, octadecyl ester possess inhibitory potential although, lower than the commercial drugs. The 2D and 3D hydrogen bond interactions as well as hydrophilic interactions are displayed in Figs. 2 and 3.

4. CONCLUSION

In conclusion, the plant leaves exhibit antioxidant activity because they contain phytocomponents which are polyphenols and could be a potential source of natural antioxidants. Also, trans-3-(trifluoromethyl)cinnamic acid, octadecyl ester one of the phytochemicals reported to have exhibited an inhibition against E. coli were docked with E. coli strain F11 (ID: 3NRP)and E. coli BL21 (ID: 5FH9). The results showed trans-3-(trifluoromethyl)cinnamic acid, octadecyl ester actually interacted with the receptors and could serve as inhibitor, although not as potent as drugs that are available in the market. However, further research work such as isolation and characterization of the identified compounds and examination of their pharmacological and biological potentials will aid in the determination of phytocompounds that possess both pharmacological and biological activities into details.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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