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BIOCHEMICAL CONSTITUENT AND ENZYME INHIBITORY ACTIVITY OF *Thaumatococcus daniellii* METHANOLIC EXTRACT AGAINST FUNGI ISOLATES OF MAIZE

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ABSTRACT

The pulverized dried leaves of *Thaumatococcus daniellii* was extracted with methanol. The extract was examined for its antioxidant potential using 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity. This was measured against that of butylated hydroxytoluene (BHT) which serves as the standard. The results show that the radical scavenging property of BHT was concentration-dependent whereas, that of *T. daniellii* varied until the highest concentration. The result of the antimicrobial assay of the two fungal isolates of maize- *Aspergillus niger* and *Penicillium notatum* indicate that *T. daniellii* extract has inhibitory activity against the organisms. At minimum inhibitory concentration of 200 mg/ml, the plant extract showed a higher activity on the organisms than the standard, itraconazole. The molecular docking was done using the target enzyme, lanosterol-14α-demethylase. Daidzein, a constituent of the plant extract has a higher binding affinity (-11.5 kcal/mol) in comparison to the standard ligand, itraconazole (-11.2 kcal/mol). The outcome of this study show that Daidzein, a phytoconstituent in the *T. daniellii* methanolic extract have the ability to inhibit key enzyme involved in microbial growth. This indicates that

the plant has a great potential for use in post-harvest management of maize molds.

Keywords: Antioxidant, Lanosterol-14α-demethylase, Molecular docking, Post-harvest, *T. daniellii*

INTRODUCTION

Post-harvest losses represent a recurrent problem affecting food security, especially in developing countries. One primary cause of such losses is microbial infestation. Postharvest losses in maize are significantly associated with reduced food utilization (Johnson et al. (2021, Beyu et al. 2023). On a global average, a third of the total food produced is lost post-harvest annually (Kumar et al. 2017). Infestation by insects (weevils) and mold causing organisms like Aspergillus and Penicillium species have contributed significantly to this loss. The menace of mycotoxins caused by harmful toxins from these microorganisms is a cause for concern regarding public health worldwide. The issue of pesticide poisoning from pesticide residue is another problem requiring timely intervention.

According to Bello et al. (2016), microorganisms have been largely identified to be responsible for spoilage of food crops. Food-borne pathogens are also risk factor in terms of public health in both developed and developing countries. Thaumatococcus is a significant African genera of Marantaceae plant with antimicrobial activity which belong to a family of perennial, under-utilized herbs from the Zingiberales order of flowering plants (Chinedu et al. 2018). *Thaumatococcus daniellii* (Benth.), or the sweet prayers plant is a monocotyledonous herb known as "ewe eran" by the Yoruba people of Southwest Nigeria or "Katemfe" by natives of Sierra Leone. There is widespread distribution of *T. daniellii* (Benn.) in the rainforest and coastal areas of West and Central Africa. The economic, nutritional and medicinal potentials of the plant are yet to be fully exploited (Ajayi and Ojelere 2013). Locals in Southern part of Nigeria use the leaves to wrap foods and stalks to thatch houses. The antibacterial, antioxidant and insecticidal activities of essential oil of the Thaumatococcus plant have been reported (Anthony et al. 2013, Adeyemi et al. 2014, Adeola et al. 2015,). The antioxidant and antimicrobial activity of *T. daniellii* have been investigated (Hamid et al. 2017). Adebayo and Kolawole (2010) have also reported on the antimicrobial properties of *T. daniellii* plant.

This research work seeks to explore safe and green alternatives to harmful pesticides in post-harvest management of stored products against fungi and mold infestation. With this background in mind, this work aims to study the inhibition of Daidzein, a constituent from the GCMS analysis of *T. daniellii* methanolic extract against drug target of microorganisms of interest by molecular docking method.

EXPERIMENTAL

The materials used include plant extract of *T. daniellii*, test tubes, petri dishes, methanol, distilled water, measuring cylinder, micro-pipette, phytochemical and antioxidant tests reagents.

Collection and Identification of Plant

Fresh mature leaves of *T. daniellii* were collected from bush located in Oje, Ibadan, Oyo State, Nigeria. The authentication of the plant was carried out in the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria. *T. daniellii* was given the voucher number UILH/001/1077.

Methanolic Extraction of Plant Materials

The fresh plant was washed thoroughly with tap water followed by distilled water to remove dirt or filth and was air-dried under shaded conditions at room temperature for 14 days. The dried plant was grinded using electronic blender into powdered form. Methanol (2000 mL) was added to 200 g powdered plant sample and run through soxhlet extraction for 8h. The extract was concentrated on a water bath at a temperature of 50 ^{o}C for five days to remove the solvent. The extract was refrigerated and kept for further usage. Preliminary phytochemical screening of leaf methanolic extract was carried out (Ajayi et al. 2024).

Phytochemical Screening

The extracts were tested for the presence of bioactive metabolites using standard methods. The result of the phytochemical screening is presented in Table 1.

Qualitative Phytochemical Screening

Phytochemical analysis of extracts was carried out using the methods from literature (Evans 2009, Aguru et al. 2017).

Quantitative Phytochemical Determination

Phytochemical analysis of extracts were carried out using the method described by Evans (2009) and Aguru *et al*. (2017) for the detection of phytochemicals. The total phenolic content of the samples was estimated (Makkar et al. 2009) Total flavonoids content was measured by aluminium chloride colorimetric assay (Talari et al. 2012). The spectrophotometric method of Brunner (1984) was used for analysis of saponins. Determination of alkaloids, steroids, glycosides and triterpenes were carried out (Aguru et al. 2017). The quantity of tannins was determined (Ademoye et al. 2018).

Antimicrobial Assay

The result of the antimicrobial assay of the plant is shown in Table 2.

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of the plant samples was determined using DPPH (1,1 diphenyl-2-picrylhydrazyl radical) with some modifications (Aguru et al. 2017). DPPH has a 517 nm dissolving band that disappears after the anti-radical compound has been reduced. In brief, 0.1M of DPPH was prepared in methanol. 500 µL of the DPPH reagent solution was added to 1.0 mL of the plant extracts of varied concentrations in clean test tubes. This solution was incubated in the dark at room temperature for 30 minutes after which the absorbance was measured at 518 nm. The scavenging ability of the samples was calculated as follows:

% antioxidant activity = Absorbance of control - Absorbance of test/Absorbance of control x 100

The result of the DPPH scavenging activity is shown in Table 3.

RESULTS AND DISCUSSION

Phytochemical Screening

Table 1 shows the results of quantitative phytochemical analysis of the plant. The analysis shows the amount of each secondary metabolites present in mg/100g of the plant sample. From the results, phenolics were the largest metabolite compared to other secondary metabolites, followed by flavonoids. Glycosides were the least metabolites (Ajayi et. al 2024).

Table 1: Quantitative Phytochemical Analysis *T. daniellii*

Phytochemicals	Quantity (mg/100mg)
Glycosides	7.06 ± 0.03^{a}
Alkaloids	36.91 ± 0.06^{a}
Triterpenes	165.85 ± 0.23^{a}
Tannins	12.17 ± 0.01^{a}
Saponins	23.50 ± 0.02^{a}
Steroids	183.27 ± 0.35^{a}
Flavonoids	387.49 ± 0.73^{a}
Phenolics	591.83 ± 0.20^{a}
Terpenoids	42.49 ± 0.06^{a}

Values represent the mean of duplicate determinations ± standard deviation (SD). *Values with different letters are significantly different at $p < 0.05$.*

The phytochemical results obtained for the plant extract in this study tally with what has been documented for *T. daniellii* plant reporting the presence of saponins, flavonoids, as well as tannins and alkaloids (Ukwubile et al. 2017). *T. daniellii* leaves contain flavonoids, polyphenols, alkaloids and saponins (Ayodeji et al. 2016). Phyto-compounds are currently being harnessed by food industry as a source of important food condiments or nutraceuticals (Yadav 2012). The phytochemical properties recorded for *T. daniellii* plant in this study also agree with the findings of Anthony et al. (2013) and Aguru et al. (2017). The availability of these phyto-compounds in the studied specie indicates a good phytochemical profile of the plant.

Table 2: Antimicrobial Activity of *T. daniellii* (Mean Zone of Inhibition (mm))

Conc. of the Extract (mg/ml)	*A. niger*	*P.chrysogenum*
200	19	17
100	16	13
50	14	10
25	10	4
12.5	-	-
6.25	-	-
-ve	-	-
+ve	18	16

Key: A. niger =Aspergillus niger; P. chrysogenum = Penicillium chrysogenum; +ve =Itraconazole; -ve =Solvent of dilution

Table 3: DPPH Radical Scavenging properties of *T. daniellii*

Concentration (mg/ml)	*T. daniellii*	BHT
10	23.58 ± 0.13^{a}	58.52 ± 0.17^{c}
20	23.40 ± 0.13^{a}	68.83 ± 0.55^{c}
30	22.36 ± 0.17^{a}	74.60 ± 0.34^{b}
40	22.77 ± 0.17^{a}	77.82 ± 0.60^{c}
50	44.32 ± 0.20^{a}	92.02 ± 0.62^{c}

Values represent the mean of duplicate determinations ± standard deviation (SD). *Values with different letters are significantly different at $p < 0.05$.*

The percentage inhibition or scavenging is expressed in mg/mL of the plant extract. The concentration of the plant studied ranged from 10 mg/mL to 50 mg/mL. The antioxidant activity of the plant extract was measured against Butylated hydroxytoluene (BHT) used as control.

Molecular Docking Study

The crystal structure of lanosterol14α-demethylase with PDB ID, 4LXJ was obtained from protein databank (www.rcsb.org). The existing ligands and water molecules were removed and hydrogen molecules were added. SDF structures of Itraconazole and Daidzein were obtained from the PubChem database. The molecules were converted to mol2 chemical format using Open babel (O'Boyle et al. 2011). The protein and ligand molecules were further converted to the dockable pdbqt format using AutoDock tools. Docking of the ligands to various protein targets and determination of binding affinities was carried out using Vina (Trott & Olson 2010). Molecular interactions between the protein and the ligand was compared to the reference drug and viewed with Discovery Studio 2020.

Table 4: Binding Affinity of the Ligand to Lanosterol 14α-demethylase

S/No		Binding Affinity (kcal/mol)
	Compounds	Lanosterol-14α-demethylase
1	Itraconazole	-11.2
2	Daidzein	**-11.5**

Figure 1: 3D view of the interaction between Itraconazole and lanosterol 14α-demethylase (Trott & Olson 2010).

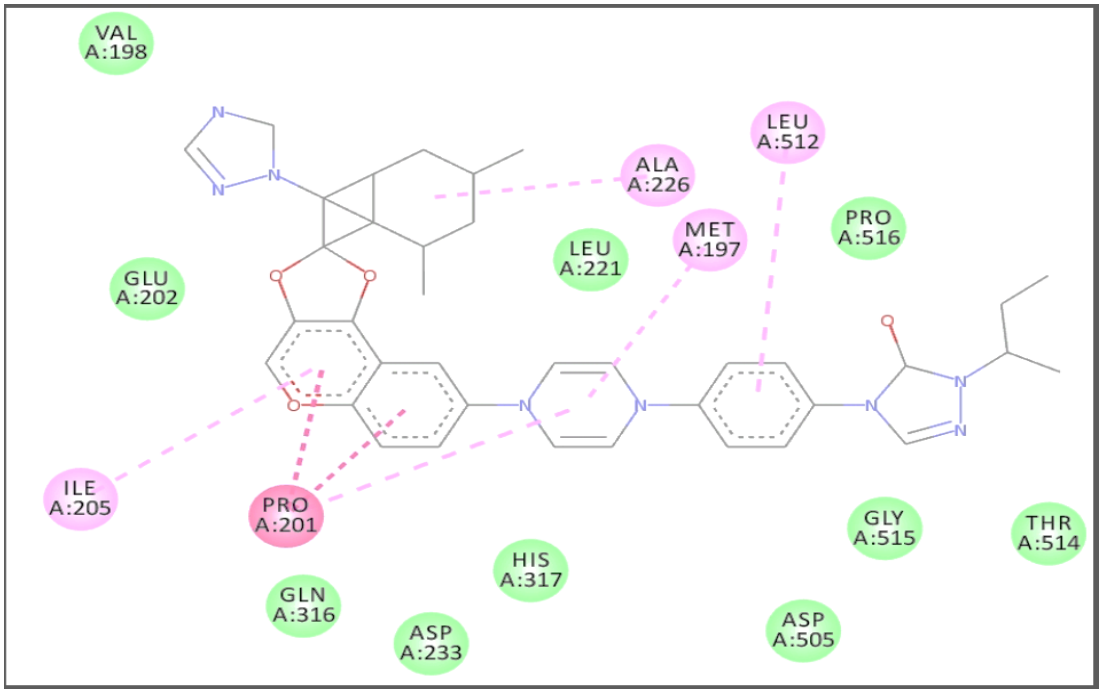

Figure 2: 2D view of the interaction between Itraconazole and amino acids in the binding site of lanosterol 14α-demethylase (Trott & Olson 2010).

Figure 3: 3D view of the interaction between Daidzein and lanosterol 14α-demethylase (Trott & Olson 2010).

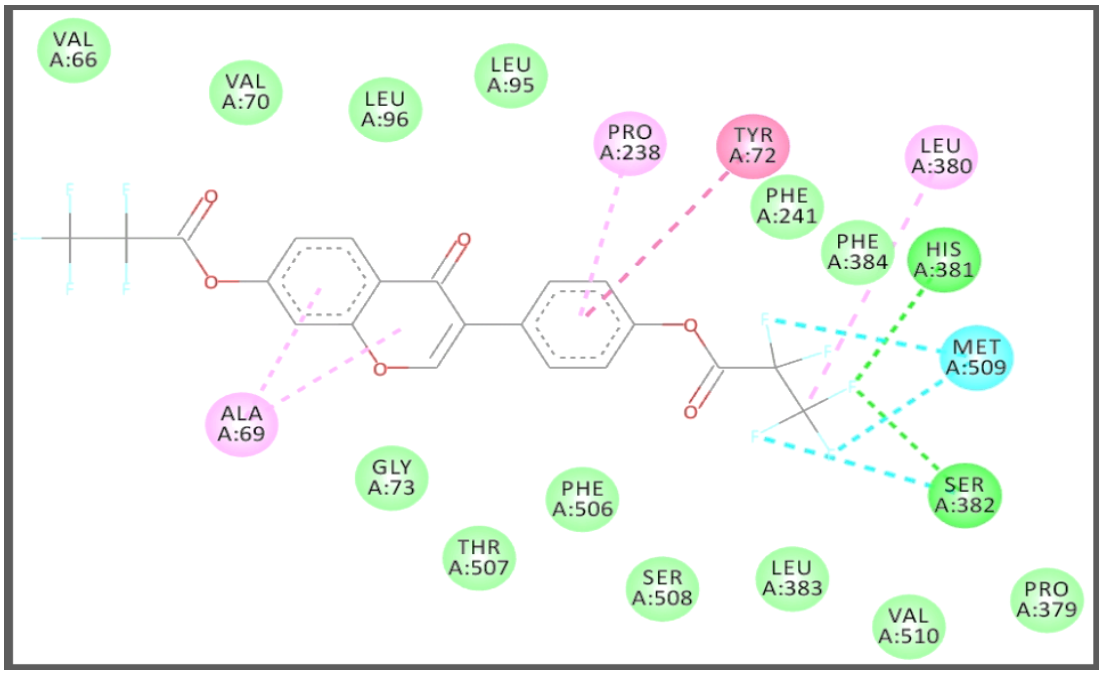

Figure 4: 2D view of the interaction between Daidzein and amino acids in the binding site of lanosterol 14α-demethylase (Trott & Olson 2010).

DPPH Radical Scavenging Activity of Ethanolic Extracts of *T. daniellii*

The DPPH antioxidant activity of the plant is given in Table 3. The % inhibition or % scavenging is expressed in mg/mL of the plant extract. The concentration of the plant extract studied range from 10 mg/mL to 50 mg/mL. The antioxidant activity of *T. daniellii* extract was measured against that of butylated hydroxytoluene (BHT) which serves as the standard. The result shows that the percentage scavenging ability of the standard, BHT increases with increase in the concentration. For *Thaumatococcus daniellii*, the highest inhibition is noted at the concentration of 50 mg/mL, followed by the concentration at 10 mg/mL. The DPPH radical scavenging property of BHT was higher ($p < 0.05$) than the extract at the concentration considered (10-50 mg/mL). At 50 mg/mL (highest concentration considered), the radical scavenging property of BHT was $92.02 \pm 0.62\%$. A lower radical scavenging property was observed in *T. daniellii* extract with $44.32 \pm 0.20\%$. The radical scavenging property of BHT was concentration-dependent; whereas, that of *T. daniellii* varied until the highest concentration.

The DPPH assay is a rapid, economical and widely used method of evaluating the antioxidant capacity of bioactive compounds. Although, it is often quoted that the mechanism of radical scavenging is the hydrogen-donating ability of bioactive compounds to DPPH radical. However, it has been observed that this is a rather slow process and considered to be a minor pathway. Hence, the primary mechanism of scavenging DPPH radicals has been attributed to the electron transfer underlying the deprotonation of antioxidants (Banothou et.al. 2017). The radical scavenging property of BHT was concentration-dependent; whereas that of *T. daniellii* varied till the highest concentration. In this study, BHT had a higher DPPH radical scavenging activity than the plant extract considered. This implies the ability of BHT to donate hydrogen and electron to DPPH radical was higher than that of the plant extract. A study carried out by Rahman et al. 2015, asserted a radical scavenging activity ($96.45 \pm 0.41\%$) for BHT similar to what was observed in this study, howbeit, at a much lower concentration (100 µg/mL). The plant extract yielded a considerable DPPH radical scavenging ability. The radical scavenging ability of the plant extract may be attributable to its high phenolic content. Indeed, polyphenols have been known to scavenge DPPH radicals (Huang 2005). Therefore, there is a high correlation between the total polyphenol content of the plant extract and its radical scavenging antioxidant activity.

Docking analysis of Daidzein against lanosterol-14α-demethylase

Table 4 shows the binding energies obtained from the docking of the standard ligand and the GC-MS-derived compound, daidzein against lanosterol-14α-demethylase. The standard ligand, itraconazole had the binding energy of -11.2 Kcal/mol. The binding energy of daidzein was -11.5 Kcal/mol. The two- and three-dimensional structures of the interactions of the standard ligand, itraconazole and the hit compound with the target protein, lanosterol-14α-demethylase are shown in Figures 1, 2, 3 and 4. Lanosterol-14α-demethylase (CYP51A1) an enzyme that is often the target of novel antimicrobials is the main enzyme involved in the synthesis of ergosterol, an essential component of the fungal cell membrane (Becher and Wirsel 2012). The inhibition of lanosterol -14α-demethylase leads to the accumulation of 14α-methylsterols on the fungal surface and the alteration of plasma membranes' permeability and rigidity, which results in the arrest of fungal growth (Stana *et al*. 2017). Daidzein, a constituent of *T. daniellii* inhibited CYP51A1 better than itraconazole. Figures 1 and 2 show the molecular interactions of the standard ligand, itraconazole, with the target protein, lanosterol 14α-demethylase. The chemical interactions at the protein's active site included: 7 hydrophobic interactions involving A:197, A: 201, A: 205, A: 226 and A: 512 residues. Figures 3 and 4 show the molecular interactions of daidzein with the target protein, lanosterol 14α-demethylase. The chemical interactions at the protein's active site included: 2 conventional hydrogen bonds involving A: 381 and A: 382 residues; 5 hydrophobic interactions involving: A: 69, A: 72, A: 238 and A: 380 residues; and 3 halogen bonds involving A: 382 and A: 509 residues. In this study Daidzein had binding energies higher than that of the standard ligand, itraconazole. This implies that the compound inhibits the enzyme to greater degree than the standard drug. The chemical interactions at the protein's active site might be responsible for this observation. The hit ligand with a total of 10 interactions (3 variants); all in contrast to the standard ligand with 7 interactions (1 variant). By such overriding attributes, the observed outcome of higher binding affinity of the hit compound compared to the standard ligand is thus expected. The most abundant bond variant in the interactions of the hit compound with the target protein were hydrophobic interactions. Shi et al. (2020) had asserted clearly the role of hydrophobic interactions in the binding of triazoles (a most effective class of inhibitors) to the active site of lanosterol 14α-demethylase. The authors pointed out clearly that hydrophobic residues at the active site of the enzyme are responsible for anchoring inhibitors binding to

the enzyme. Thus, it is crystal clear that the higher binding affinity of the hit compound over the standard ligand is due to the increased presence of hydrophobic interactions at the active site of the enzyme when bound to the former than the later.

CONCLUSION

The results obtained in this study revealed that daidzein, a phytoconstituent of *Thaumatococcus daniellii* possess inhibitory properties against lanosterol-14α-demethylase, an important fungal enzyme crucial to the fungi cell membrane. This implies that the plant has the ability to prevent fungal growth. This biological activity of *T. daniellii* can be further exploited in the post-harvest management of stored products against fungi and mold infestation.

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