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Efficacy of Aqueous Leaf Extract of *Tectona grandis* (Teak) in Growth Inhibition of *Rhizopus stolonifer* and *Aspergillus niger*

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ABSTRACT

The phytochemical properties of aqueous leaf extract of *Tectona grandis* (Teak) was evaluated and the inhibitory efficacy of the leaf extract against test fungi, *Rhizopus stolonifer* and *Aspergillus niger*, was evaluated. The efficacy or antifungal activities of the crude aqueous extract of the teak plant leaves were evaluated using the agar well/disc diffusion method using various concentrations (100%, 75%, 50% and 25%) of the leaf extract and measuring the diametric zones of inhibition in millimeters (mm); against the use of chemical antifungal agent, Ketoconazole as the control and distilled water. The result of phytochemical constituent of the aqueous leaf extract of *Tectona grandis* showed that, Tannin, Saponin and Steroid were present and were much more than Terpenoid, while Alkaloid, Flavonoid and Glycoside were completely absent in the leaf extract of *Tectona grandis*. The 25% and 50% of the extract recorded inhibition on the fungal mycelia in 24h, and the inhibition at higher concentration of 75% and 100% recorded significantly higher inhibition. The higher the concentration of the aqueous leaf extracts the higher the inhibitory efficacy. The leaf extract of *T. grandis* can be employed as an antifungal agent to the selected fungi and may be to many more fungi.

Keywords: Teak **(***Tectona grandis*), phytochemicals, terpenoids, ketoconazole, antifungal agent, *Aspergillus niger*, inhibition.

Introduction

Plants naturally produce a wide range of molecules, especially secondary metabolites acknowledged for their role in protecting against pathogens due to inherent biological properties (Hancock *et al*., 2015). These metabolites, referred to as phytochemicals, are non-nutritive (Prakash *et al*., 2020) and demonstrate robust antimicrobial properties, impeding the growth of bacteria, fungi, and even viruses. As a result of these phytochemicals, extracts from diverse plants have proven effective in inhibiting growth of various pathogenic microorganisms (Budianto *et al.*, 2023).

Fungi are extremely versatile in the environment, exhibiting traits that are beneficial as well as adverse. Fungi play important roles in ecosystems by forming advantageous relationships such as mycorrhizal partnerships, which improve plants' ability to absorb nutrients. But fungi's adaptability also extends to negative effects; they can function as pathogens, harming both plants and animals (Fones *et al.*, 2017).

The action of fungi as pathogens of plants is a well-documented phenomenon with significant implications for agriculture. These fungal pathogens have been identified as causative agents of various plant diseases, impacting crop yields, food security, and overall plant health. The intricate interactions between fungi and plants often result in diseases that range from superficial infections to more severe and widespread afflictions.

In the pursuit of optimal fungal disease management, researchers have directed their efforts towards investigating biological approaches, particularly botanical products, to protect crops from the penetration and spread of pathogens (Gwinn, 2018). This avenue is increasingly prominent due to its perceived lack of environmental risks and the absence of microbial resistance issues commonly associated with synthetic chemicals.

Teak (*Tectona grandis* Linn), commonly known as African teak or Sagwan, belongs to the Lamiaceae family.

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Renowned as one of the world's most valuable timber, Teak is prized for its exquisite surface and remarkable resistance to both termites and fungal damage. The key active compounds contributing to these properties include tectoquinone, lapachol and deoxylapachol (Vyas *et al*., 2019). Teak is rich in various metabolites, with naphthoquinones, anthraquinones, and isoprenoid quinones being particularly abundant.

In addition to these compounds, teak also harbours a wide range of phytochemicals, including triterpenoids, flavonoids, steroids, lignans, fatty esters, tannins, proteins, resins, anthraquinone-naphthoquinone pigments, diterpenes, phenolic compounds, and dye (Kolli *et al*., 2022) This diverse array of botanical components in teak enriches its multifaceted properties and also contributes to its potential applications as an antimicrobial agent.

In fact, research has indicated that teak extracts possess antimicrobial activity. Compounds extracted from Teak leaves and bark has demonstrated inhibitory effects against certain bacteria and fungi. These findings open avenues for exploring teak-derived compounds in the development of natural antimicrobial agents for various applications in agriculture and medicine, contributing ultimately to the improvement of both plant and human health.

In recent times, there has been a heightened emphasis on the study of traditional plants due to their increased utilization. Among these plants, *Tectona grandis* (teak) has garnered attention for its phytochemical and pharmacological attributes. Teak is found to contain various secondary metabolites, a diverse group of compounds that likely contribute to its range of antimicrobial properties (Budianto *et al.*, 2023).

Explorations into the phytochemical composition of *Tectona grandis* leaves have revealed some insights into the diverse range of bioactive compounds present in the plant. In a study conducted by Daramola (2022), the phytochemical analysis of Teak leaves uncovered the presence of various bioactive compounds. Specifically, flavonoid, saponin, phenol, alkaloids, and tannins were identified, with notable concentrations of saponin (55.55±20.83 mg/g), phenol (44.87±4.41 mg/g), and tannin (10.50±0.56 mg/g). These compounds are known for their antioxidant and antimicrobial properties.

Another study by Balogun and Mariam (2020) on the phytochemical analysis of *Tectona grandis* revealed the presence of specific bioactive compounds, including flavonoids, tannins, and saponin.

Additionally, Muhammed *et al*. (2021) conducted a phytochemical analysis on both aqueous and methanol extracts of *T. grandis* leaves. The results revealed a diverse array of compounds in the methanol extract, such as steroids, tannins, saponin, coumarin, protein, carbohydrates, alkaloids, diterpenes, phytosterol, and phlobatannin. The aqueous extract contained all the compounds found in the methanol extract except steroids. These comprehensive findings provide valuable insights into the chemical composition of Teak leaves, indicating the potential presence of various bioactive substances. Such compounds, renowned for their diverse antimicrobial activities, suggest the promising utility of teak plant extracts in controlling pathogenic microorganisms.

Fungal pathogens pose a substantial threat to crops, causing extensive damage; both before and after harvest and leading to reduced yields, financial losses for farmers, and increased food insecurity. To prevent infestation by these phytopathogens, farmers commonly resort to synthetic chemicals, considering them cost-effective and efficient for crop protection. However, the overuse and misuse of these chemicals present significant challenges, including the presence of toxic residues in treated products, the development of resistance among pathogenic microorganisms, and environmental pollution due to slow biodegradation. Consequently, there is a crucial need to explore natural and environmental friendly alternatives to synthetic ingredients.

This study therefore aims to analyze the phytochemical properties of the crude aqueous leaf extract of *Tectona grandis* investigate the efficacy of *Tectona grandis* (Teak) leaf extract in the growth inhibition of pathogenic fungi; *Rhizopus stolonifer* and *Aspergillus niger in vitro*. This study also aims to investigate the dose-dependent response of pathogenic fungi to Teak leaf extract, identifying the minimum effective concentration for optimal growth inhibition; and to compare the antifungal efficacy of Teak leaf extract with that of commonly used synthetic fungicides; to determine its relative effectiveness.

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Materials and Methods

Test organisms

Pure cultures of test fungi were obtained from mycological stock collections in the Department of Botany, University of Ibadan, Nigeria.

Sample Collection of Leaves of *Tectona grandis*

Leaves of *Tectona grandis* (teak) was obtained from Federal University of Lokoja permanent site.

The Teak leaf collection was washed under running water, air dried for three weeks, and then pounded with mortar and pestle until fine powder was obtained.

Solvent extraction

50g of the powdered sample was mixed in 500ml of sterilized distilled water and kept at room temperature for 72h. The filtrate was obtained by filtering the mixture using two folds of muslin cloth. Subsequently the extract was concentrated using a rotary evaporator, and stored in the fridge at 4^{o}C.

Phytochemical screening

The crude aqueous extract of the teak plant leaves were evaluated for phytochemical composition using established protocols as described by Trease and Evans (1989), Sofowora (1993), and Harborne (1998).

Analysis of antimicrobial activity

The antifungal activities of the crude aqueous extract of the teak plant leaves were evaluated using agar well diffusion method as described by Yusuf *et al*. (2021). The antifungal activity was the mean zone of inhibition diameters (mm) produced by the various concentrations of the plant leaf extract.

Data analysis

All analysis were conducted in triplicate and analyzed using statistical package for social science (SPSS) version 16 and presented as mean values.

Results

The result of the phytochemical properties of the extract of the leaves of *Tectona grandis* is presented in Table 1. The leaves of *T. grandis* lack alkaloid, flavonoid, and glycosides. The leaves strongly contain tannin, saponin and steroid, while the terpenoid is only moderately present.

Table 1: Presence of phytochemicals of aqueous leaf extract of *Tectona grandis*

Phytochemicals	Level of Presence/Absence
Alkaloid	-
Tannin	+++
Flavonoid	-
Saponin	+++
Steroid	+++
Terpenoid	++
Glycosides	-

Key: + (Present); - (Absent); ++ (Moderately Present); +++ (Strongly Present)

The result of the Inhibitory efficacy of the crude aqueous leaf extract of *T. grandis* on *Rhizopus stolonifer* is presented in Figure 1 while the Inhibitory efficacy of the crude aqueous leaf extract of *T. grandis* on *Aspergillus niger* is presented in Figure 2. The crude aqueous leaf extract of *T. grandis* is inhibitory on cultures of *R. stolonifer* and *Aspergillus niger* in Petri dish. The zone of inhibition in the culture of the fungus increased with concentrations. The zones of inhibition with 75mg/ml and 100mg/ml were by far higher than were obtained with 50mg/ml and 25mg/ml. Distilled water as a control had no effect on the growth of the fungi. But, Ketoconazole was significantly more effective on the growth of the two fungi, indicating the relative effectiveness of Teak leaf extract in inhibiting the growth of the fungi compared to commonly used synthetic fungicides.

Table 2 presents the diametric zone of inhibition (mean±SD) (mm) of aqueous leaf extract of *T. grandis* and controls on the test fungi. Dose-response relationship is established, with varying concentrations of Teak leaf extract demonstrating different degrees of growth inhibition.

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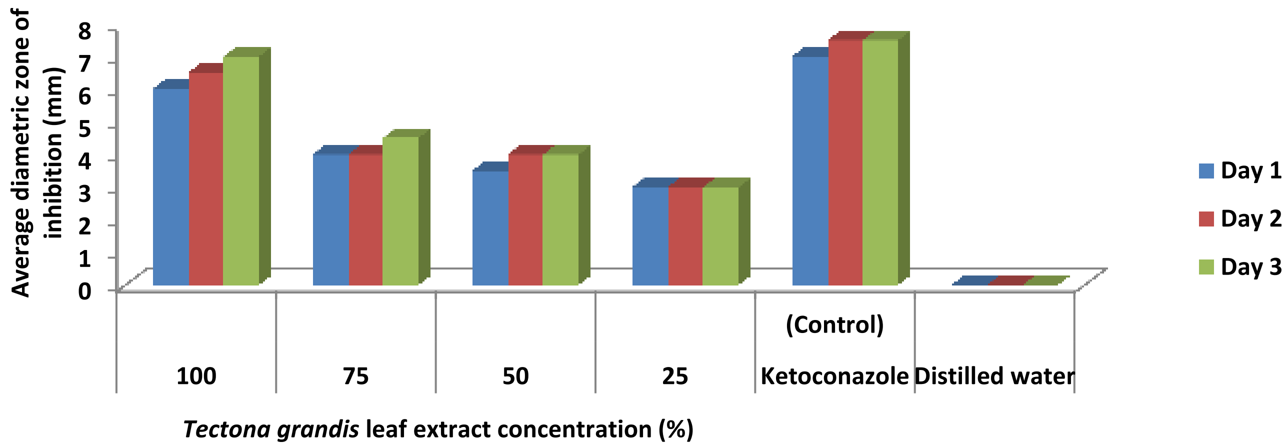

Figure 1: Diametric Zone Of Inhibition (mm) of Aqueous Extract of the Leaves of *Tectona grandis* and Chemical Control against *Rhizopus stolonifer*

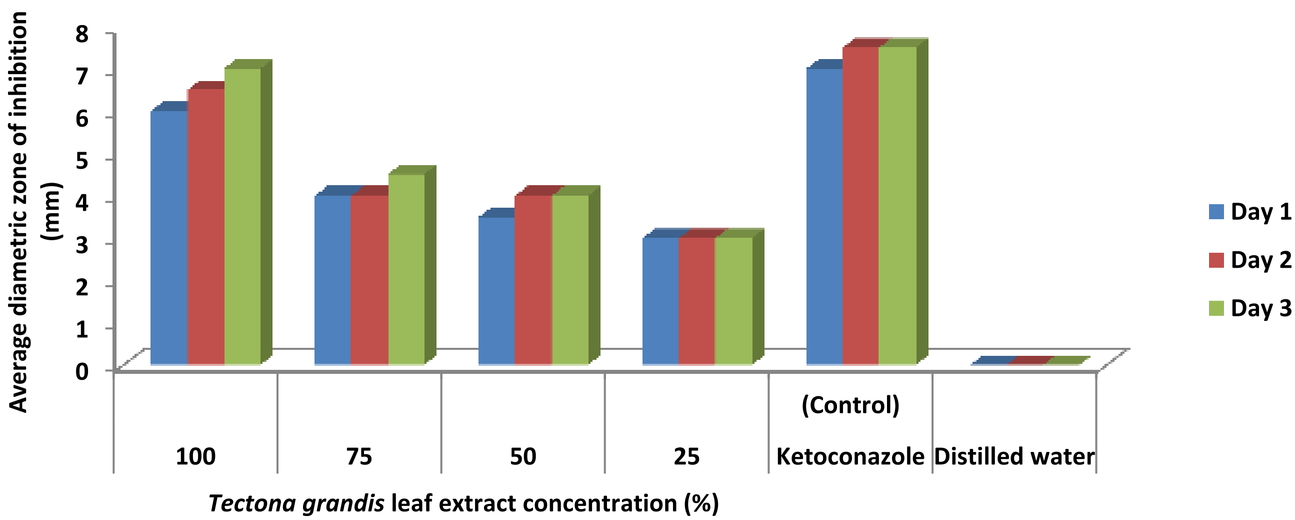

Figure 2: Diametric Zone of Inhibition (mm) of Aqueous Extract of the Leaves of *Tectona grandis* and Chemical Control against *Aspergillus niger*

Table 2: Diametric Zone Of Inhibition (mean±SD) (mm) of Aqueous Extract of the Leaves of *Tectona grandis* and Controls on the Test Fungi

Treatment	*Rhizopus stolonifer*	*Aspergillus niger*
100% concentration of *T. grandis* leaf extract	5.67±1.15[b]	4.33±1.55[b]
75% concentration of *T. grandis* leaf extract	3.33±0.58[c]	3.33±1.15[c]
50% concentration of *T. grandis* leaf extract	2.67±0.58[d]	2.33±0.58[d]
25% concentration of *T. grandis* leaf extract	2.00±1.00[d]	1.67±0.58[d]
Ketoconazole	12.33±2.52[a]	15.67±1.15[a]
Distilled water	-	-

Values with the same letters in a column are not significantly different at $p<0.05$

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Discussion

The phytochemicals of *Tectona grandis* investigated in this study confirms the presence of Saponin, and Tannin, according to Daramola (2022), while on the contrary flavonoid and alkaloid were absent. However, Steroid was copiously present and to a lesser extent Terpenoid. Phytochemicals are alternative management options for inhibiting toxigenic fungus.

The in vitro application of leaves of *T. grandis* has antimicrobial effects similar to Pradhan *et al.* (2013) with leaf of *Artocarpus altilis*(breadfruit), Ogumefun *et al.* (2017) with leaf of *T. grandis* and Habtamu *et al.*(2023) also with leaf of *T. grandis*.

It is however, not all leaf extracts that have antimicrobial activity (Purushothan *et al.*, 2010), who had worked with extracts of four (4) plants and only *T. grandis* had the bioefficacy against some studied microorganisms. Purushotham and Sankar(2013) had observed that extract of *T. grandis* had antimicrobial activity against *Staphylococcus aureus, Escherichia coli,* and *Salmonella typhii.*

The presence of Tannin in *T. grandis* and may be as tannic acid is confirmed to inhibitgrowth of *Apiospora arundinis* (Wenyan *et al.*, 2023). Saponin-rich leaf extracts has been found to be inhibitory to several fungi (Bishnu *et al.*, 2007). Steroid hormones are toxic to fungi (Bronislava and Marija, 2009), though could be inhibitory and to a lesser extent stimulation; the effect of Steroid hormones is strain specific.

The botanicals inhibit spore germination at the highest concentrations (Hamid, 2011) and loose efficacy with reducing concentration of the plant leaf extracts. This is similar to the result obtained with *T. grandis* obtained in this study.

Phytochemical profiling of *T. grandis* shows it that the leaves are rich. These botanicals are applicable as antifungal. The lowest concentration caused clearing of the mycelia of the test fungal cultures, which progressively increased with higher concentrations.

In conclusion, the findings of this study has revealed that, the aqueous leaf extract of *Tectona gradis* have antifungal efficacy. Higher concentration of the extract would measure up to the efficacy of the chemically synthesized ketoconazole fungicide.

References

Abd-Elkader, D.Y., Salem, M.Z., Komeil, D.A., Al-Huqail, A.A., Ali, H.M., Salah, A.H., Akrami, M. & Hassan, H.S. (2021). Post-harvest enhancing and *Botrytis cinerea* control of strawberry fruits using low cost and eco-friendly natural oils. *Agronomy*, *11(6)*,1246.

Adnan, M., Islam, W., Shabbir, A., Khan, K. A., Ghramh, H. A., Huang, Z. & Lu, G. D. (2019). Plant defense against fungal pathogens by antagonistic fungi with *Trichoderma* in focus. *Microbial pathogenesis*, *129*, 7-18.

Balogun, O. D. & Mariam, T. (2020). Antimicrobial and phytochemcial activities on (*Tectona grandis*) leaf extract on *Staphylococcus aureus, Escherichia coli*, *Bacillus subtilis* and *Proteus vulgaris. International Journal of Innovative Research and Advanced Studies*, *7(6)*, 150-153.

Bhardwaj, S. K. & Laura, J. S. (2018). Potential use of some plant extracts against foot rot of rice (*Oryza sativa* L.). *ORYZA-An International Journal on Rice*, *55(2)*, 353-356.

Bishnu, P. C., Zeer, W. & Leah, T. (Lahkim) (2007). In vitro study of antifungal activity of Saponin-rich extracts against prevalent phytopatogenic fungi. *Industrial Crops and Products*, *26(2)*, 109 – 115

Bronislava, C. & Marija, Z. (2009). Aspects of the Steroid response in fungi. *Chemico-Biological Interactions*, *178(1-3)*, 303 – 309.

Budianto, P., Suroto, S., Wasita, B. & Mirawati, D. K. (2023). *Tectona Grandis* Leaves: Determination of Total Flavonoid Content, Phenolic Content, Characterization of the Leaves, and Compound Identification in GC-MS. *Pharmacognosy Journal*, *15(1)*.

Daramola, O. T. (2022). Proximate composition and phytochemical screening of Teak (*Tectona grandis*) leaves as phytogenic feed additive in poultry diets. *Nigerian Journal of Animal Science*, *24(1)*, 121-127.

Fones, H. N., Fisher, M. C. & Gurr, S. J. (2017). Emerging fungal threats to plants and animals challenge agriculture and ecosystem resilience. *The fungal kingdom*, pp. 787-809.

Citation: Zubairu and Suberu (2024). Efficacy of aqueous leaf extract of *Tectona grandis* (teak) in growth inhibition of *Rhizopus stolonifer* and *Aspergillus niger*. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 66 – 71.

Habtamu, T., Getnet, Y. & Getachew, G. M. (2023). Phytochemicals reduce growth, sporulation and conidia dimensions of *Fusarium verticillioides*, cause of fumonisin contamination in maize grains. *Biotechnology Report (Science Direct)*, 40.

Hamid, W., Mohd, Y., Shauket, A. P. & Riyaz, M. (2011). *In vitro* inhibitory effect of fungicides and botanicals on mycelia growth and spore germination of *Fusarium oxysporum*. *Journal of Biopesticides*, *4(1),* 53 – 56.

Hancock, R. D., Hogenhout, S. & Foyer, C. H. (2015). Mechanisms of plant–insect interaction. *Journal of experimental botany*, *66(2),* 421-424.

Kolli, P. K., Obbalareddy, S., Yejella, R. P., Athili, L. D. & Ponnada, S. (2022). A Review on *Tectona Grandis*. *International Journal of Research in Pharmacy and Chemistry*, *12(3),* 359-394.

Lanka, S. (2017). Parimala, Antimicrobial activities of *Tectona grandis* leaf and bark extracts. *European journal of pharmaceutical and medical research*, *4(12),* 245-248.

Mittal, S., Rao, N. & Menghani, E. (2012). Phytochemical and antimicrobial activity of *Tectona grandis* L. *Research Journal of Science and Technology*, *4(5),* 111.

Muhammed, H., Abdullahi, L. H., Mayaki, F. G. & Boko, U. H. (2021). Phytochemical compositions, antimicrobial activities, and thin layer chromatography analysis of aqueous, and methanol extracts of *Tectona grandis* leaf. *AROC in Natural Product Research*, *1(01),* 044-051.

Ogunmefun, O. T., Ekundayo, E. A., Akharaiyi, F. C. & Ewhenodere, D. (2007). Phytochemical screaning and antibacterial activities of *Tectona grandis* L. f. (Teak) leaves on microorganisms isolated from decayed food sample. *Tropical Plant Research*, *4(3),* 376 – 382.

Persaud, R., Khan, A., Isaac, W. A., Ganpat, W. & Saravanakumar, D. (2019). Plant extracts, bioagents and new generation fungicides in the control of rice sheath blight in Guyana. *Crop Protection*, *119*, 30-37.

Pradhan, C., Mohanty, M. & Rout, A. (2013). Phytochemical screening and comparative bioefficacy assessment of *Artocarpus altilis* leaf extracts of antimicrobial activity. *Frontiers in Life Science*, *6(3-4),* 71 – 76.

Prakash, B., Kumar, A., Singh, P. P. & Songachan, L. S. (2020). Antimicrobial and antioxidant properties of phytochemicals: Current status and future perspective. *Functional and preservative properties of phytochemicals*, 1-45.

Salem, M.Z., Mervat, E.H., Ali, H.M., Abdel-Megeed, A., El-Settawy, A.A., Böhm, M., Mansour, M.M. & Salem, A.Z. (2021). Plants-derived bioactives: Novel utilization as antimicrobial, antioxidant and phytoreducing agents for the biosynthesis of metallic nanoparticles. *Microbial Pathogenesis*, *158*, 105-107.

Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa. Spectrum Books Limited*. Ibadan, Nigeria, Pp. 1-153.

Suwandee, S., Saelee, R., Koohakan, P. & Montri, N. (2023). Bioactivity of teak (*Tectona grandis* L.f.) and cajeput (*Melaleuca cajuputi* Powell) leaf extracted on inhibition fruit fungal pathogens. *International Journal of Agricultural Technology*, *19(5),* 2259-2280.

Trease, G. E., & Evans. W. C. (1989). *A textbook of pharmacognosy. 13th ed. London, Bailliere-Tyndall Ltd*. Pp. 345–356

Vyas, P., Yadav, D. K. & Khandelwal, P. (2019). *Tectona grandis* (teak)–A review on its phytochemical and therapeutic potential. *Natural product research*, *33(16),* 2338-2354.

Wenyan, J., Xuelian, L. Huiling, L., Leixing, M., Wei, C., Tianshun, W., Haijun, W., Yihao, X. & Jie, L. (2023). Inhibitory effect of Tannic acid on the growth of *Apiospora arundinis* and 3 Nitropropionic acid production. *Journal of Applied Microbiology*, 134 *(12),* December.

Yusuf, A.A., Lawal, B., Abubakar, A.N., Berinyuy, E. B., Omonije, Y.O., Umar, S. I., Shebe, M. N. & Alhaji, Y. M. (2018). In-vitro antioxidants, antimicrobial and toxicological evaluation of Nigerian *Zingiber officinale*. *Clinical Phytoscience*, *4,* 1-8.

Citation: Zubairu and Suberu (2024). Efficacy of aqueous leaf extract of *Tectona grandis* (teak) in growth inhibition of *Rhizopus stolonifer* and *Aspergillus niger*. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 66 – 71.