Trop J Nat Prod Res, November 2020; 4(11):976-980 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antifungal Activity of *Telfaira occidentalis* Extracts on some Phytopathogenic Fungi Isolated from *Carica papaya*

Oniha I. Margaret, Eni O. Angela, Akinnola O. Olayemi, Omonigbehin A. Emmanuel, Olorunshola J. Folashade

Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria

ARTICLE INFO

Article history:
Received 28 August 2020
Revised 24 October 2020
Accepted 24 November 2020
Published online 30 November 2020

ABSTRACT

Infused within plants are potent phytochemicals with beneficial activities to mankind which includes the management and treatment of microbial infections. Fungal diseases result in significant economic losses and hindrance of global papaya production. Phytochemicals have made medicinal plants become sources of environmentally friendly alternative antimicrobials. This study aimed at evaluating the antifungal activity of leaf extracts of *Telfeira occidentalis* against phytopathogenic fungi isolated from *Carica papaya*. Ethyl acetate, n-Hexane, ethanol, methanol and aqueous extracts of *Telfeira occidentalis* leaves were evaluated for their antimicrobial properties. Agar-well diffusion method was employed for *in vitro* screening, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extract types against test fungal species of *Aspergillus*, *Penicillium*, *Rhizopus* and *Trichoderma*. All the extracts evaluated inhibited fungal growth to some degree, with the aqueous extract exhibiting more inhibitory activities than the organic extracts. MIC and MFC values of the extracts ranged between 15.625 to 31.25 and 62.5 to 125 mg/mL, respectively. The antifungal activity of *T. occidentalis* was found to be equal or higher than commercially available fungicide, ketoconazole. The results of this study indicate that leaf extracts of *T. occidentalis* has potential for use as bio-fungicides for plant protection against fungal diseases.

Keywords: Antifungal, *Telfaira occidentalis*, Fungi, *Carica papaya*, Phytochemicals.

Introduction

Plant kingdom has since existence provided man with diverse resources including a variety of compounds with therapeutic properties required for survival by all living beings.[1,2] Numerous phytochemicals, also known as secondary metabolites constituting major sources of microbicides, pesticides and many pharmaceutical drugs abound in plants which retains its role as the main source of pharmaceutics employed in traditional medicine.[3-6] Many studies have broadly shown that medicinal plants are considered as bounteous provenances of antimicrobial media [7, 3, 4, 8, 9] with consequent screening for their potential exploitations as substitute remedy in the therapeutics of microbial infections[10] and reports have shown different potent effects against both plant and human pathogenic microorganisms.[11,12] Extensive studies have been conducted on numerous medicinal plants with the aim of discovering more potent and less toxic compounds from these natural substances.[13,12] Pathogenic fungi are the premier transmissible media in plants responsible for variations during developmental stages including the after harvest period.[14,15] Studies have shown that in fruits and herbs, an extensive range of different fungal species are responsible for characteristic problems that include features, nutritional merit, organoleptic traits and deficient shelf life with some cases of allergic or toxic disorders among consumers caused indirectly by fungi from the production of allergens or mycotoxins.[16-18]

*Corresponding author E mail: margaret.oniha@covenantuniversity.edu.ng
Tel: +2348066596932

Citation: Oniha MI, Eni AO, Akinnola OO, Omonigbehin EA and Olorunshola JF. Antifungal Activity of *Telfaira occidentalis* Extracts on some Phytopathogenic Fungi Isolated from *Carica papaya*. Trop J Nat Prod Res. 2020; 4(11):976-980. doi.org/10.26538/tjnpr/v4i11.22

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Synthetic fungicides are the most efficacious method of the pest and fungal disease curb in spite of precarious consequences of chemical pesticide implementation including problems of public health, territorial pollution, toxic repercussions on non-target organisms and causing impediment in pest and infection instruments.[19-21] There is essential need to produce antifungal schemes based on novel groups of molecules, that function via new routes and/or mechanisms.[22,23,18] Fluted pumpkin (*Telfairia occidentalis* HOOK F.), belonging to the family Cucurbitaceae, majorly grows in the greenwood territory of West and Central Africa and they are originated furthermore in Benin, Nigeria and Cameroon.[24-26] Fluted pumpkin, a tropical vine plant found in lush green rain forest of West Africa (Okoye and Orakwue, 2019),[27] is one of the most immeasurably and popularly propagated leafy greens all over Nigeria distinctly in Southeastern part (Bassey and Opara, 2016; Umeoka, 2018)[28-29] where it is of ethnobotanical significance in the mythology, nutrition and harvest regimen of the people.[30-31] The phytochemical screening of *Telfaira occidentalis* leaf extract published the presence of phenol; that possess curative properties, phytate, HCN, terpenoids, cardiac glycosides and phenolics while alkaloid, flavonoid, tannin, saponin, steroids, glycosides, triterpenoids, anthraquinones and reducing sugars were present in the leaflets, trunk and rootstock extracts.[32-35, 29, 27] A study by Umeoka [29] showed that aqueous and ethanol leaf extracts of *T. occidentalis* inhibited the growth of the four fungal pathogens employed which include *Geotrichum candidum*, *Aspergillus niger*, *Fusarium oxysporum* and *Rhizopus stolonifer*. The current pronounced obstacle in agriculture is the suppression of plant infections caused by phytopathogenic bacteria and fungi with the consequent increase in the advent of antifungal/antibacterial-resistant breeds thus emphasizing the alacritous exigency for the generation of novel antifungal media with assets and mechanisms of action dissimilar from the obtainable ones.[36-37] The screening of more plant extracts for their antifungal properties can help to proffer effective controls on microbial attack of plants. However, there is little on the antifungal activity of the leaf extract as compared to studies from its antibacterial

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

activity. This study was aimed at identifying the phytochemical integrants of the different leaf extracts of *T. occidentalis* and to determine the antifungal activity of *T. occidentalis* leaf extracts employing *in vitro* antifungal screening techniques.

Materials and Methods

Plant collection and identification

Leaves of *Telfaira occidentalis* were garnered from the Covenant University Farm in Ota, Ogun State, Nigeria between July and December, 2017. Authentication of the already identified plant species (voucher specimen number: To/Bio/H821) was conducted at the Herbarium Section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria and allocated forestry herbarium identification number of *T. occidentalis*-FHI No: 112776.

Preparation of aqueous and organic extracts

Mature disease-free leaves were rinsed to eliminate dust and other foreign particles, air-dried in a sheltered area at ambient temperature for 3 weeks.[38] Dried materials were ground into powder using a blender and preserved in airtight bottles at room temperature (25-30°C) until use. Weighed quantity of 300 g of the dried powder leaf was extracted by soaking and macerating in 1.5 L of n-hexane, ethyl acetate, ethanol, methanol and water at ambient temperature for 72 h for each solvent used. The five different solvent mixtures were vigorously agitated and subsequently filtered through cheese cloth and Whatman No.1 filter paper. The resultant filtrates were subsequently concentrated utilizing a rotary evaporator and the solvents used were recovered under pressure until slurried/dry extracts were obtained. The five different crude extracts obtained were then kept at 4°C for subsequent use. The concentrated extracts were later dissolved in appropriate volumes of dimethyl-sulphoxide (DMSO) to make the various crude concentrations (1000 mg/mL, 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL and 15.625 mg/mL where 1000 mg/mL was used for the antifungal assay) for antifungal screening. All the stock solutions were stored in sterile capped bottles, labelled accordingly and stored at 4°C for analysis. Each antifungal test was carried out in three replicates against each fungal isolate.

Phytochemical screening of plants extract

Tests were conducted on the five crude extracts (n-hexane, ethyl acetate, ethanol, methanol and aqueous extracts) to detect the presence of phytochemicals according to protocols previously described by Sofowora and Trease and Evans.[39,40]

Fungal isolates and inoculum quantification

Fungi were obtained from pawpaw samples from the Pawpaw research demonstration farm, Covenant University, Ota. *Aspergillus niger, A. fumigatus, A. flavus, Rhizopus* spp, *Penicillium* spp and *Trichoderma* spp were identified, maintained and later stored in cryovials at -20°C at the Microbiology Laboratory, Covenant University, Ota. Fresh fungal isolates of 72 h were prepared on PDA by subculturing and fungal spore suspensions were obtained after filtration. The spore suspension for the different fungal isolates was estimated utilizing the haemocytometer cell-counting chamber and final inoculum was modified to obtain 0.5 McFarland's standard (10^6 spores/mL).

Antifungal activity of crude extracts of Telfaira occidentalis

Potato dextrose agar medium (Oxoid) was prepared according to the manufacturer's instructions. Antifungal activity of the crude extracts was conducted using the agar-well diffusion method as illustrated by Cheesbrough.[41] Standard dose was prepared by dissolving 1000 mg of crude extract in 1 mL of DMSO (1:1) for all the extracts (stock concentration for the antifungal test). The antifungal agent Ketoconazole (100 mg/mL) was used as the positive control while DMSO was used as the negative control. Surface of medium was streaked with the standardized fungal spore suspension for even distribution of the inoculum on the agar. The seeded plates were permitted to dry. Wells were then bored into agar media employing a sterile cork borer of 10 mm and the wells were supplied with 0.2 mL of the various extracts. Succeeding the diffusion of the extracts into the agar at ambient temperature, the bored agar plates were incubated at room temperature for 3-5 days and observations made at 24 -72 h. Antifungal assay of the leaf extracts was deduced by measuring zone of inhibition surrounding the agar wells. The sensitivity tests were carried out in triplicates and the average diameter of the zones of inhibition were recorded accordingly.

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of plant extracts

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the plant extracts were deduced utilizing agar well diffusion as elucidated by Samie and Mashau [42] and agar dilution method by Sule *et al.*[43] Different concentrations (125 mg/mL, 62.5 mg/mL, 31.25 mg/mL and 15.625 mg/mL) of the extracts were prepared from which the MIC and MFC of the plant extracts were determined. Potato dextrose agar was prepared according to the manufacturer's instructions and dispensed into petri plates. Agar surfaces were seeded with 0.1 mL of standardized fungal spores and allowed to dry. Sterile 10 mm diameter cork borer was utilized to perforate two equidistant holes in the center of the inoculated agar plates. These holes were then loaded with 0.2 mL of the various extract concentrations. Following the diffusion of the extracts into the agar at ambient temperature, the bored agar plates were incubated at room temperature for 3-5 days and observations established at 24-72 h. The antifungal activity of the leaf extracts was determined by evaluating the zone of inhibition bordering the agar well. Negative and positive control plates were without any plant extracts but Ketokonazole (positive control) and DMSO (negative control) were added. The sensitivity tests were carried out in triplicates and the mean diameter of the zones of inhibition were recorded accordingly.

Statistical analysis

The diameters of zones of inhibition for the *in vitro* antifungal activity were expressed as means of three replicates. Significant differences between and within the averages of treatments and controls were analyzed employing Anova at $p \leq 0.05$ and post hoc tests. Statistical analyses were computed using SPSS version 20 software package.

Results and Discussion

The release of pollutants and biohazards from consumption of chemicals via pesticides and fungicides has greatly increased consumer demand for natural preservatives as alternatives. Numerous medicinal and aromatic flora profuse in phytochemical combinations that include polyphenols, flavonoids, saponins, alkaloids and others in their dissimilar segments (leaflets, bark, florets, ovules, greenwood and offshoots) possess extensive implementations as antioxidants and antimicrobials and are notable for their pharmaceutical and biopesticide potentials.[44-46, 37] In this study, the antifungal activity of the crude leaf extracts of *Telfeira occidentalis* was assayed *in vitro* against *Aspergillus niger, A. fumigatus, A. flavus, Rhizopus, Penicillium* and *Trichoderma*. This medicinal plant was selected based on it ethnobotanical and pharmacological usage suggestive of its antimicrobial properties. [47-49, 24, 50-51, 29, 27] The phytochemical screening revealed saponins, tannins, anthocyanin and betacyanin, cardiac glycosides, phenols, alkaloids, quinones, flavonoid, carbohydrates, coumarins, phenol, steroids and terpenoids in *Telfaira occidentalis* leaf extracts as presented in table 1. The presence of alkaloids, saponins, tannins, carbohydrates, cardiac glycosides, anthocyanins, betacyanins, terpenoids, triterpenoids, phenols, coumarins and steroids in the extracts is supported by previous reports[32-35, 29, 27] where alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, steroids, anthraquinones, cardiac glycosides, reducing sugars amongst others were found present in the leaf and stem extracts. The results obtained showed that the five crude leaf extract types (n-hexane, ethyl acetate, ethanol, methanol and aqueous) at 1000 mg/mL concentration of *Telfaira occidentalis* evaluated had

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

antifungal activity against all the fungal genera tested as shown in Figure 1.

The highest antifungal activity of *T. occidentalis* was in the aqueous extract against *Rhizopus oryzae* (31.33 mm) while the least activity was observed in the methanol leaf extract against *Trichoderma* sp. with an inhibition zone of 12 mm as shown in figure 1. For the antifungal activity of control antifungal agents, the zones of inhibition measured between 11-13 mm and 0 mm for the positive and negative controls, respectively.

Umeoka [29] conducted a study on the ethanol and aqueous crude leaf extracts of *T. occidentalis* with both extracts showing inhibitory activity against the growth of *Geotrichum candidum*, *Aspergillus niger*, *Fusarium oxysporum* and *Rhizopus stolonifer*. The report further stated that the ethanol extract can inhibit the growth of the afore listed common fungi but the aqueous extract was specifically observed to inhibit the growth of *Rhizopus stolonifer* as compared to the other common fungi tested. Morcos and Sarkis,[52] found that the extracts of fluted pumpkin leaf inhibited, paralysed and destroyed worms based on level of extract concentrations. In furtherance, research by Morcos and Sarkis,[52] showed that the aqueous extract had higher inhibitory and destructive activities compared to the methanol extract. However, the report of Oboh *et al.*[50] stated that the aqueous and ethanol extracts of *T. occidentalis* had no inhibitory activity against fungal isolates tested which include *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium italicum* and *Geotrichum albidum*. There was significant difference at p value ≤ 0.05 for all the extracts tested against *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus oryzae*, *Penicillium* sp. *Trichoderma* sp. and *Aspergillus fumigatus* using a 2-way Anova with treatment (test extracts and control) as the predictor and fungal growth as the response variable followed by post hoc tests for comparison. The aqueous concentrate of *T. occidentalis* was most significant ($p < 0.05$).

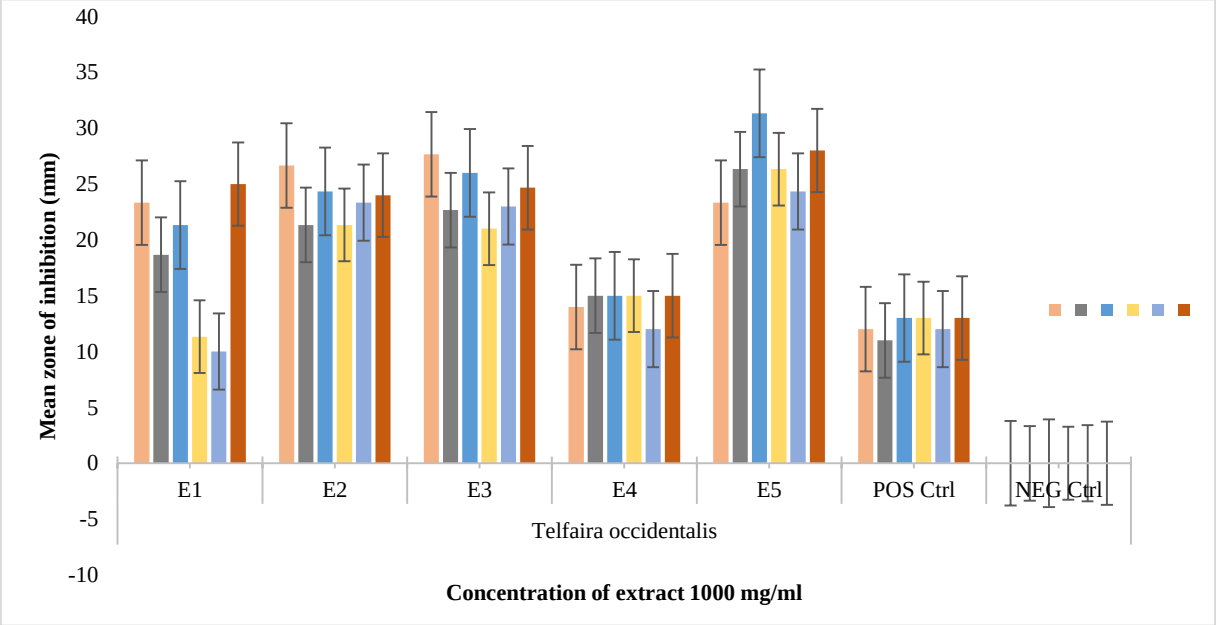

Figure 1: Mean zones of inhibition for antifungal activity of *Telfaira occidentalis* leaf extracts against fungal isolates from pawpaw (*Carica papaya*) samples.

Key: E1 = n-hexane, E4 = methanol, POS Control = Ketoconazole, E2 = ethyl acetate, NEG Control = DMSO, E3 = ethanol, E5 = aqueous

Table 1: Phytochemical screening of n-Hexane, ethyl acetate, ethanol, methanol and aqueous extracts of *Telfaira occidentalis*

Tests	n-Hexane	Ethyl acetate	Ethanol	Methanol	Water
Alkaloids	-	-	+	-	-
Quinones	-	-	+	-	+
Glycosides	-	-	-	-	-
Flavonoids	-	-	-	-	-
Saponins	-	+	-	-	-
Tannins	-	-	+	+	-
Carbohydrates	-	-	-	-	+
Anthocyanin and Betacyanin	-	+	+	-	+
Cardiac glycosides	+	+	+	+	+
Terpenoids	-	+	+	+	-
Triterpenoids	-	-	-	+	-
Phenols	-	-	-	+	-
Coumarins	-	+	-	+	-
Steroids	-	+	-	-	+
Acids	-	-	-	-	-

Key: + = Present; - = Absent

There was significant difference across all the extract types (p-value = 0.000) as compared to the controls but no significant difference was detected for the isolates (p-value > 0.05). Minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were established for the leaf extracts of the plant. The minimum inhibitory concentration (MFC) values for the extract types ranged from 15.625 to 31.25 mg/mL while the MFC values ranged from 31.25 to 125 mg/mL.

Conclusion

T. occidentalis leaf extracts have significant antifungal activity against common fungal isolates cultured from samples of *C. papaya*. This shows that its extracts can serve as an alternative and ecofriendly means of controlling and treating fungal diseases in plants.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors want to acknowledge the financial support offered by Covenant University in accomplishment of this research work for publication.

References

1. Ahvazi M, Khalighi-Sigaroodib F, Charkhchiyanc MM, Mojabd F, Mozaffariane V-A, Zakerif H. Introduction of medicinal plants species with the most traditional usage in Alamut region. Iranian J Pharm Res. 2012; 11(1):185-194.
2. Abu-Darnish MS and Efferth T. Medicinal plants from near East for cancer therapy. Front Pharmacol. 2018; 9(56):1-17.
3. Mahesh B and Satish, S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J Agric Sci. 2008; 4:839-843.
4. Al-Taisan WA, Bahkali AH, Elgorban AM, El-Metwally MA. Effective influence of essential oils and microelements against *Sclerotinia sclerotiorum*. Int J Pharmacol. 2014; 10:275-281.
5. Al-Muniri RMS and Hossain MA. Evaluation of antioxidant and cytotoxic activities of different extracts of folk medicinal plant *Hapllophyllum tuberculatum.* Egypt J Basic Appl Sci. 2017; 4:101-106.
6. Kooti W and Daraei N. A review of the antioxidant activity of celery (*Apium graveoleus* L.). J Evid-Based Compl Altern Med. 2017; 22(4):1029-1034.
7. Ates DA and Erdogrul OT. Antimicrobial activities of various medicinal and commercial plant extracts. Turk J Biol. 2003; 27:157-162.
8. Zare-Marzouni H, Daraei N, Sharafi-Ahvazi N, Kalani N, Kooti W. The effects of aqueous extract of celery leaves (*Apium graveveoleus*) on fertility in female rats. World J Pharm Pharm Sci. 2016; 5:1710-1714.
9. Anyanwu MU and Okoye RC. Antimicrobial activity of Nigerian medicinal plants. J Intercult Ethnopharmacol. 2017; 6(2):240-259.
10. Elgorban AM, Bahkali AH, El- Metwally MA, Elsheshtawi M, Abdel- Wahab MA. *In vitro* antifungal activity of some plant essential oils. Inter J Pharmacol. 2015; 11:56-61.
11. Ramezani H, Singh HP, Batish DR Kohli, Dargan JS. Fungicidal effect of volatile oils from *Eucalyptus citriodora* and its major constituent citronetial. New Zealand Plant Protect. 2002; 55:327-330.
12. Nakamura CV, Ishida K, Faccin LC, Filho BPD, Cortez DAG, Rozental S, de Souza W, Ueda-Nakamura T. *In vitro* activity of essential oil from *Ocimum gratissimum* L. against four *Candida* species. Res Microbiol. 2004; 155:579-586.
13. Senguttuvan J, Paulsamy S, Krishnamoorthy K. *In vitro* antifungal activity of leaf and root extracts of the medicinal plant, *Hypochaeris radicata* L. Inter J Pharm Pharm Sci. 2013; 5(3):758-761.
14. Shuping DSS and Eloff JN. The use of plants to protect plants and food against fungal pathogens: A review. Afr J Trad Compl Altern Med. 2017; 14(4):120-127.
15. Patel R, Pandya J, Purohit N. Antifungal activity of *Tithonia rotundifolia* Blake and *Tagetes erecta* L. against *Aspergillus niger*. Int J Creat Res Thou. 2020; 8(7):2320-2882.
16. Agrios GN. Losses caused by plant diseases. Plant Patho. United Kingdom Elsevier, Oxford; 2004. 45 p.
17. Dellavalle PD, Cabrera A, Alem D, Larrañaga P, Ferreira F, Dalla Rizza M. Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria* spp. Chilean J Agric Res. 2011; 71(2):231-239.
18. Joshi S, Kumar P, Pant P, Sati SC. A comparative evaluation of *Kumaun himalayan* gymnosperms for their antifungal potential against plant pathogenic fungi. The J Phytopharmacol. 2018; 7(3):230-241.
19. Rai MK and Carpinella C. Naturally occurring bioactive compounds: a new and safe alternative for control of pests and microbial diseases (3rd ed.). Netherlands: Elsevier Pub; 2006. 514 p.
20. Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. Physio and Mol Plant Pathol. 2004; 65:91-100.
21. Milan P, Hassler SC, Baysal-Gurel F. Methods for management of soilborne diseases in crop production. Agric. 2020; 10(16):1-21.
22. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004; 39:309-317.
23. Venkatesan P, Perfect JR, Myers S. Evaluation and management of fungal infections in immune compromised patients. Dermatol Ther. 2005; 18:44-57.
24. Kayode AAA and Kayode OT. Some medicinal values of *Telfairia occidentalis:* A Review. Am J Biochem Mol Biol. 2011; 1:30-38.
25. Opara EU and Uduak R. Effect of agro effluents on the control of bacterial spot disease of fluted pumpkin (*Telfaira occidentalis* Hook f.) in South East Nigeria. Nigerian Agric J. 2019; 50(2):123-129.
26. Orole RT, Orole OO, Aisoni JE, Isyaku J, Mohammed YS. Comparative study of the physicochemical properties of male and female flutted pumpkin (*Telfaira occidentalis*). The J Med Res. 2020; 6(2):55-61.
27. Okoye EI and Orakwue FC. The chemical evaluation and anti-microbial screening of extracts from seeds and leaves of *Telfairia occidentalis* (Fluted pumpkin). Chem Res J. 2019; 4(3):98-104.
28. Bassey IN and Opara EU. Potency of plant ashes as organic fertilizers in the performance and control of leaf spot disease of *Telfairia occidentalis* in South Eastern Nigeria. J Agric Sustain. 2016; 9(2):210-227.

29. Umeoka N. Antifungal effect and phytochemical screening of *Telfeira occidentalis* (hook f.) leaf extracts. J Plant Biotech Microbiol. 2018; 1(1):21-24.
30. Olaniyi JO and Oyerele TA. Growth, nield and Nutritional compositions of fluted pumpkin (*Telfairia occidentalis*) as affected by fertilizer types in Ogbomoso, Southwest Nigeria. Bull Environ Pharmacol Life Sci. 2012; 1(9):81-88.
31. Osai EO, Udo SE, Okoli CE, Bemoh BE. Comparative efficacy of three plant extracts for the control of leaf spot disease in fluted pumpkin (*Telfairia occidentalis* Hook F.). J Bio Agric and Healthc. 2017; 7(12):56-62.
32. Sofowora A. Medicinal plant and traditional medicine in Africa. (2nd Ed.). Ibadan, Nigeria: Nigeria Spectrum Books; 1996. 12 p.
33. Akalazu JN, Onyike NB, Offong AU, Nwachukwu CU. Investigation of the role of calcium in prolonging the shelf life of fluted pumpkin (*Telfairia occidentalis* Hook F.). J Biotechnol and Bioinform. 2011; 1(1):1-9.
34. Inuwa HM, Aimola IA, Muhammad A, Habila N, Okibe P, Latayo M, Ahmed Z. Isolation and determination of omega-9 fatty acids from *Telfairia occidentalis*. Inter J Food Nutr Saf. 2012; 1(1):9-14.
35. Oyewole OA and Abalaka ME. Antimicrobial activities of *Telfairia occidentalis* (fluted pumpkins) leaf extract against selected intestinal pathogens. J Health Sci. 2012; 2:1-4.
36. Al-Huqail AA, Behiry SI, Salem MZM, Ali HM, Siddiqui MH, Salem AZM. Antifungal, antibacterial, and antioxidant activities of *Acacia saligna* (Labill.) H. L. Wendl. Flower Extract: HPLC analysis of phenolic and flavonoid compounds. Mole. 2019; 24 (700):1-14.
37. Meyer V. A small protein that fights fungi: AFP as a new promising antifungal agent of biotechnological value. Applied Microbiol and Biotechnol. 2008; 78:17–28.
38. Vinoth B, Manivasagaperumal R, Rajaravindran M. Phytochemical analysis and antibacterial activity of *Azadirachta indica* Juss. Inter J Res Plant Sci. 2012; 2(3):50-55.
39. Sofowora A. Medicinal plants and traditional medicinal in Africa. In: Screening plants for bioactive agents. (2nd ed.). Ibadan, Nigeria: Spectrum Books Ltd Sunshine House; 1993. 134-156 p.
40. Trease GE and Evans WC. Pharmacognosy: a physician guide to herbal medicine. (13th ed.). London: ELBS/Bailliere, Tindall; 1989. 176-180 p.
41. Cheesbrough M. District laboratory practice in tropical countries. (2nd ed.). Cambridge: Cambridge University Press; 2000; 47-54.
42. Samie A and Mashau F. Antifungal activities of fifteen Southern African medicinal plants against five *Fusarium* species. J Med Plants Res. 2013; 7(25):1839-1848.
43. Sule WF, Okonko IO, Omo-Ogun S, Nwanze JC, Ojezele MO, Ojezele OJ, Alli JA, Soyemi ET, Olaonipekun TO. Phytochemical properties and *in-vitro* antifungal activity of *Senna alata* Linn. crude stem bark extract. J Med Plants Res. 2011; 5(2):176-183.
44. Wu CY, Chen R, Wang XS, Shen B, Yue W, Wu Q. Antioxidant and anti-fatigue activities of phenolic extract from the seed coat of *Euryale ferox* Salisb. and identification of three phenolic compounds by LC-ESI-MS/MS. Mole. 2013; 18:11003-11021.
45. Dai J and Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Mole. 2010; 15:7313-7352.
46. Salem MZM, El-Hefny M, Ali HM, Elansary HO, Nasser RA, El-Settawy AAA, El Shanhorey N, Ashmawy NA, Salem AZM. Antibacterial activity of extracted bioactive molecules of *Schinus terebinthifolius* ripened fruits against some pathogenic bacteria. Microbiol Pathol. 2018; 120:119-127.
47. Alada ARA. The haematological effects of *Telfairia occidentalis* diets preparation. Afr J Biomed. 2000; 3:185-186.
48. Dina OA, Adedapo AA, Oyinloye OP, Saba AB. Effects of *Telfairia occidentalis* extract on experimentally induced anaemia in domestic. Afr J Biomed Res. 2006; 3:181-183.
49. Eseyin O, Ebong P, Eyong E, Awofisayo O, Agboke A. Effect of *Telfairia occidentalis* on oral glucose tolerance in rats. Afr J Pharm Pharmacol. 2010; 4:368-372.
50. Oboh G, Nwanna E, Elusiyan C. Antioxidant and antimicrobial Properties of *Telfairia occidentalis* (Fluted pumpkin) leaf extracts. J Pharmacol Toxicol. 2006; 5(8):539-547.
51. Eseyin OA, Sattar MA, Rathore HA. A review of the pharmacological and biological activities of the aerial parts of *Telfairia occidentalis* Hook.f. (Cucurbitaceae). Trop J Pharm Res. 2014; 13(10):1761-1769.
52. Morcos A and Sarkis T. Histo-pathological alterations of the stomach of guinea pigs following administration of root aqueous extract of *Telfairia occidentalis*. Eur J Med Sci. 2013; 11(2):1-5.