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Efficacy of Methanolic Leaf Extract of *Hyptis suaveolens* and *Moringa oleifera* in the Control of Soil-Borne Pathogens

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Authors' contributions

This work was carried out in collaboration between both authors. Author OCA designed the study, managed the analyses of the study and wrote the first draft of the manuscript. Author OCN managed the literature searches and edited the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Soil-borne diseases are difficult to control because they are caused by pathogens that can survive for long periods in the absence of the normal crop host and often have a wide host range including weeds. This present study was design to assess the antifungal assay dependent effect of methanolic extracts of *Hyptis suaveolens* and *Moringa oleifera* on *Phytophthora colocasiae* and *Fusarium oxysporum.*
Methods: The presence of biologically active ingredients (alkaloid, saponin, tannins, flavonoid, terpenoids, tannins, steroids, hydrogen cyanides, phenols and glycoside) in the leaf extracts was investigated using standard procedures. The antifungal activities of the plant extract were tested against *Phytophthora colocasiae* and *Fusarium oxysporum* using disc and well diffusion assays.
Results: The results of the phytochemical evaluated showed that *H. suaveolens,* contained significantly higher alkaloids, saponins, hydrogen cyanide, flavonoids and phenols than *M. oleifera,* while on the other hand, *M. oleifera* contained significantly higher terpenoids, tannins, steroids and glycosides. Consequently, *H. suaveolens* extract similarly recorded significantly ($P < 0.05$) higher inhibition on the organisms as compared to *M. oleifera.* The disc diffusion assay method was more

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sensitive than the well diffusion assay. *H. suaveolens* at 100 mg/ml using disc diffusion assay method showed higher inhibition on both *P. colocasiae* and *F. oxysporum.* While *M. oleifera* recorded higher inhibition on *F. oxysporum* at 50 mg/ml and *P. colocasiae* at 100 mg/ml using the disc diffusion assay method. MIC was lowest with *H. suaveolens* (12.5 mg/ml) against *F. oxysporum*.

Conclusion: These results promote the identification of actives substances from these plants for use as lead molecules in the development of new fungicides for the control of *Phytophthora colocasiae* and *Fusarium oxysporum.*

Keywords: Biological control; biological efficacy; fungicides; Fusarium oxysporum; Phytophthora colocasiae.

1. INTRODUCTION

Fusarium is a genus of filamentous fungi that includes many toxin-producing plant pathogens of agricultural importance. Collectively, *Fusarium* diseases include wilts, blights, rots, and cankers of many horticultural, ornamental and forest crops in both agricultural and natural ecosystems. *Fusarium* also produces a diverse array of toxic secondary metabolites (mycotoxins) [1].

The soil-borne fungus, *Fusarium oxysporum* Schlecht is the causal agent of vascular wilt, a disease that affects a large variety of economically important crops worldwide [2]. Fungal and oomyceteous plant pathogens of taro have been reported to cause losses in taro fields [3]. The Oomycetes water mould, *Phytophthora colocasiae* Raciborski is a significant pathogen as it causes taro leaf blight [4]. Taro leaf blight disease is a major limiting factor in taro production worldwide, causing up to 100% leaf and corm losses [3,5]. The effect of fungi on a plant can be devastating, in that, the cellular structure can be destroyed, physiological functions of the plant impaired and rates of metabolism and metabolic pathways can be altered [6].

Dellavalle et al. [7] reported that fungi are indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens. The main toxic effects of these metabolites are carcinogenicity, genotoxicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immunosuppression [8]. In addition to this, Bajpai et al. [9] reported that the soil-borne pathogens focused on this study, *Phytophthora colocasiae* and *Fusarium oxysporum*are more frequently reported as responsible for opportunistic plant fungal diseases associated with marked industrial and economic losses.

Soil-borne diseases are difficult to control because they are caused by pathogens that can survive for long periods in the absence of the normal crop host, and often have a wide host range including weeds [10]. Generally, phytopathogenic fungi are controlled by synthetic fungicides. However, these chemical fungicides are not readily biodegradable, they tend to persist for years in the environment and a few fungi have developed resistance to them [11]. Mahdizadehnaraghi et al. [12] reported that continuous and long-term use of chemical pesticides in agriculture has led to environmental contaminations and negative impacts on non-target organisms including humans. The increased awareness of the environmental problems associated with fungicides has led to the search for non-conventional chemicals of biological origin for the management of fungal diseases [13].

In an attempt to modify this condition, some alternative methods of control have been adopted. Within this context is the utilization of plant extracts which are natural sources of antimicrobial substances, regarded as safe and degraded by natural soil microbes; they do not pose any health, residual or environmental problems at any concentration used [14]. Consequently, this present study was designed to assess the antifungal assay dependent effect of methanolic extracts of *Hyptis suaveolens* and *Moringa oleifera* on *Phytophthora colocasiae* and *Fusarium oxysporum.*

2. MATERIALS AND METHODS

2.1 Collection of Samples

The leaves of *Hyptis suaveolens* and *Moringa oleifera* were collected in the month of September from the Botanic Garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka and confirmed at

the Herbarium of the Department of Plant Science and Biotechnology. The study was carried out in the Plant Pathology Laboratory Department in the same Department.

2.2 Preparation of Sample

Fresh leaves of the plants collected were washed with tap water and rinsed with sterile distilled water. Then allowed to dry under room temperature for two weeks after which they were ground into fine powder using a Porcelain mortar and pestle (EISCO, 150 mL) and kept in sealed containers for extraction.

2.3 Extraction and Phytochemical Analysis Procedure

The extraction was carried out using modified soaking methods as described by Doherty *et al*. [15]. The methanolic extract of the plants was prepared by soaking 100 g of the ground plant samples in 1,000 ml of absolute methanol. The suspension was left overnight for 24 hours before filtering with No. 1 Whatman filter paper and concentrated using a rotary evaporator leaving behind the crude extracts. The crude extracts were collected in a sterile 1000 ml round bottom flask and stored in a refrigerator at 12°C until required.

The presence of biologically active ingredients (alkaloid, saponin, tannins, flavonoid, terpenoids, tannins, steroids, hydrogen cyanides, phenols and glycoside) in the leaf extracts was investigated using different standard methods as described by Anukworji et al. [16] and Doherty et al. [15].

2.4 Reconstitution of the Extract

The method described by Eze and Ezejiofor [17] was used to reconstitute the Extract by dissolving the extract in 20% concentration of Dimethyl Sulphoxide (DMSO) (JHD, China) in the ratio of 1:10 (1 g of crude extract dissolved in 10 ml of DMSO) to give a concentration of 100 mg/ml. Other concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml were made from the stock concentration (100 mg/ml) while fluconazole served as the control.

2.5 Preparation of Culture Media

Potato Dextrose Agar (PDA) (Titam biotech, India) was used for both culturing and sub-culturing fungi to obtain pure cultures while Muller Hinton Agar ((MHA) Titam biotech, India) was used for Disc diffusion, Agar well diffusion and Dilution assay. Following the modified method of Kalpana et al. [18], MHA was prepared by dissolving 39 g of the commercially available Muller Hinton Agar in 1000 ml of distilled water. The dissolved powder was autoclaved at 103 KNM^{-2} for 15 minutes at 121°C. The autoclaved medium was mixed thoroughly and poured into sterile Petri dishes approximately 25 ml and allowed to sit at ambient temperature until when required. Potato Dextrose Agar (PDA) was dissolved in 500 ml of water by boiling at the same time. The filtrate of potato broth was poured into the agar and dextrose was added and the volume restored to 1000ml with sterile distilled water. The medium was poured into two 500 ml conical flasks and test tubes plugged with cotton wool and sterilized by autoclaving at 103 KNM^{-2} pressures for 20 minutes at 121°C. The medium was allowed to cool and 200 µg of Chloramphenicol was added. The medium was poured aseptically into sterile Petri dishes and allowed to solidify.

2.6 Sources and Isolation of Test Organisms

Diseased taro leaves for the isolation of *Phytophthora colocasiae* was collected and confirmed at the herbarium unit of the Federal University of Agriculture, Umudike, while *Fusarium oxysporum* was collected from Plant Pathology Laboratory, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The organisms were maintained on Potato Dextrose Agar (PDA) at 12°C and constantly revived on fresh PDA plates.

The isolation technique described by Chiejina [19] was used for the fungal isolation while identification of fungal isolates was based on their macroscopic and microscopic features then confirmed with the aid of standard mycological identification texts by Barnett and Hunter [20], Watanabe [21] and Agrios [22]. Inoculum preparation was performed according to the method described by Ohikhena et al. [23].

2.7 Determination of *F. oxysporum* and *P. colocasiae* Sensitivity to Methanolic Extracts of the Plants Using Disc and Well Diffusion Methods

The Disc diffusion assay and Agar well diffusion assay were used to measure the inhibition zone diameter of the extracts [15,24,25,26]. The minimum inhibitory concentration (MIC) was determined using agar dilution assay according

to the methods of Ohikhena et al. [23]. The minimum extract concentration that inhibited the growth of the pathogens will be taken to be the (MIC).

Data collected from the study were subjected to univariate analysis using IBM SPSS Statistics software version 23 and significant means were separated using least significant difference (LSD) at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Plants contain thousands of constituents which are valuable sources of new and biologically active molecules possessing antimicrobial property [27]. The phytochemicals evaluated were observed to be present in varying proportions in the leaf extract of the two studied plants. As presented in Table 1, *H. suaveolens,* contained significantly higher alkaloids, saponins, hydrogen cyanide, flavonoids and phenols than *M. oleifera,* while on the other hand, *M. oleifera* contained significantly higher terpenoids, tannins, steroids and glycosides (Table 1). Undeniably, all the different bioactive compounds notice in the samples (terpenoids, tannins, alkaloids saponins, steroids, flavonoids, phenols) are well known for their significant inhibitory effects against bacteria and fungi [28,29].

Table 1. Phytochemical analysis of the different plant extracts

Extract	*H. suaveolens* (mg/100 g)	*M. oleifera* (mg/100 g)
Terpenoids	935.80 ± 0.14	1410.13 ± 0.01*
Tannins	2678.82 ± 0.00	2745.35 ± 0.00*
Alkaloids	292.41 ±0.01*	210.17 ± 0.01
Saponins	2.07 ± 0.01*	1.97 ± 0.00
Steroids	1.98 ± 0.00	2.19 ± 0.00*
HCN	2.25 ± 0.00*	2.11 ± 0.00
Flavonoids	637.03 ± 0.00*	590.13 ± 0.00
Phenols	2394.55 ± 0.00*	1766.12 ± 0.00
Glycosides	238.87 ± 0.01	254.23 ± 0.01*

**-represents significantly higher means at P < 0.05 using independent sample T-test; HCN- hydrogen cyanide*

Table 2. Inhibition zone diameter for the effect of plant extract exposed with different assay methods at different concentrations against soil-borne pathogens

	Plants	*H. suaveolens*		*M. oleifera*	
Assay Method	**Concentration**	***P. colocasiae***	***F. oxysporum***	***P. colocasiae***	***F. oxysporum***
Disc diffusion	Control	30.00 ± 1.15^{a}	30.00 ± 0.58^{a}	29.67 ± 0.33^{a}	30.00 ± 1.15^{a}
	100 mg/ml	12.67 ± 0.67^{c}	12.00 ± 0.58^{cd}	11.00 ± 0.58^{ef}	11.00 ± 0.58^{ef}
	50 mg/ml	10.33 ± 0.33^{fg}	7.33 ± 0.33^{ijk}	9.33 ± 0.88^{h}	12.67 ± 0.88^{c}
	25 mg/ml	10.67 ± 0.33^{f}	10.67 ± 0.67^{f}	7.67 ± 0.33^{ij}	12.00 ± 0.58^{cd}
	12.5 mg/ml	9.67 ± 0.33^{gh}	7.67 ± 0.33^{ij}	6.83 ± 0.12^{klm}	11.67 ± 0.88^{de}
	6.25 mg/ml	12.33 ± 0.33^{cd}	9.67 ± 0.88^{gh}	6.50 ± 0.12^{klm}	10.33 ± 0.33^{fg}
Well diffusion	Control	29.67 ± 0.33^{a}	28.67 ± 0.67^{b}	29.67 ± 0.88^{a}	29.67 ± 0.88^{a}
	100 mg/ml	9.00 ± 1.15^{h}	6.60 ± 0.31^{klm}	6.13 ± 0.03^{lm}	6.17 ± 0.03^{lm}
	50 mg/ml	12.00 ± 1.53^{cd}	8.00 ± 0.58^{i}	6.10 ± 0.00^{m}	6.13 ± 0.03^{lm}
	25 mg/ml	6.73 ± 0.64^{klm}	6.83 ± 0.60^{klm}	6.00 ± 0.00^{m}	6.10 ± 0.06^{m}
	12.5 mg/ml	6.78 ± 0.15^{klm}	7.67 ± 0.33^{ij}	6.00 ± 0.00^{m}	6.13 ± 0.03^{lm}
	6.25 mg/ml	6.95 ± 0.55^{jkl}	6.17 ± 0.17^{lm}	6.00 ± 0.00^{m}	6.00 ± 0.00^{m}

Data are presented as mean ± standard error and means with different alphabets representing significant differences using Least Significant Difference (LSD = 0.83) at P < 0.05

Table 3. The MIC (mg/ml) of plant extracts on soil-borne pathogens using agar dilution assay

Plant species	*P. colocasiae*	*F. oxysporum*
Hyptis suaveolens	50	12.5
Moringa oleifera	50	50

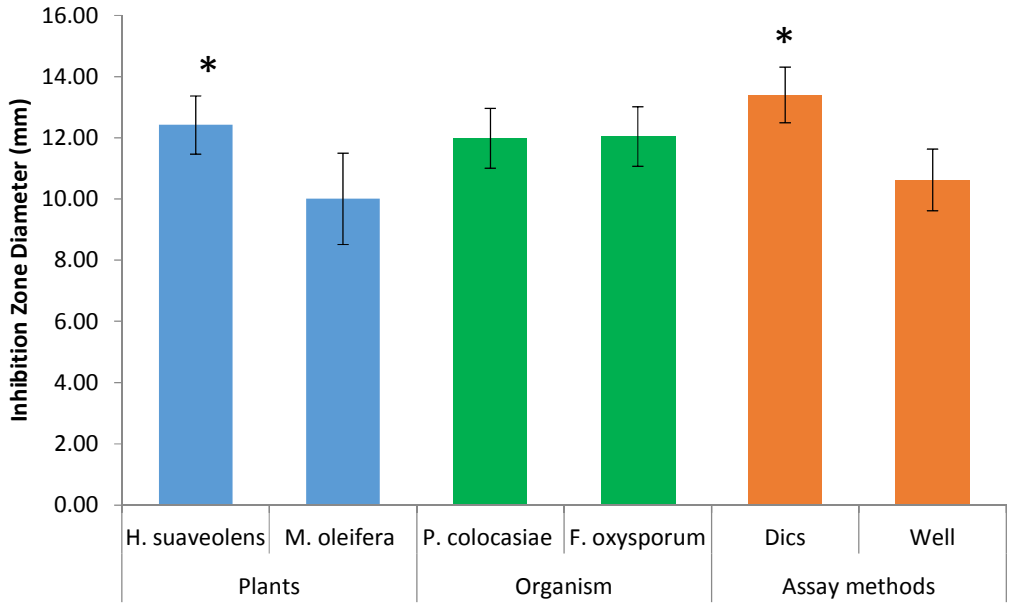

Fig. 1. Graphical representation of inhibition zone diameter against plants, organisms and assay methods

**-significantly higher mean and P < 0.05*

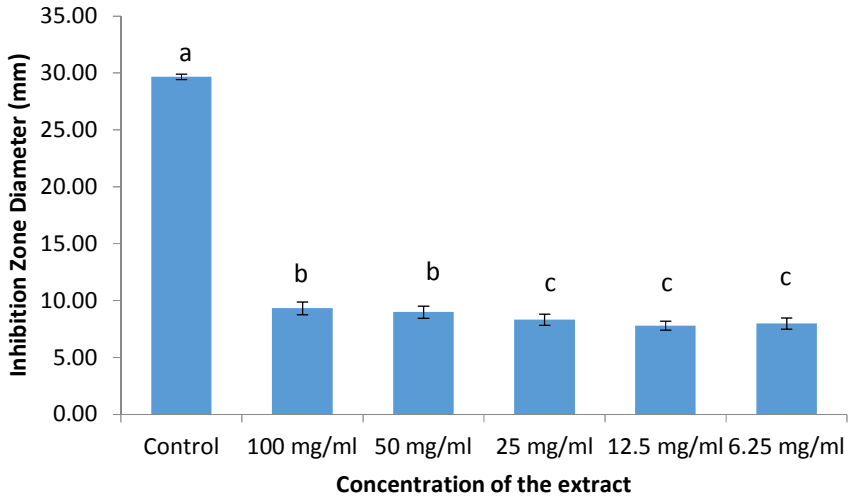

Fig. 2. Graphical representation of inhibition zone with extract concentration

Bars with different alphabet represent significant differences (P < 0.05)

The result in Fig. 1 showed that on the average, *H. suaveolens* extract recorded significantly higher inhibition on the organisms as compared to *M. oleifera.* The disparities observed in the action of the plant on the inhibition could be as a result of the quantity of phytochemicals present in the leaf extract. The higher alkaloids, saponins, hydrogen cyanide, flavonoids and phenols observed in *H. suaveolens* would be responsible for the inhibitory effect of the plant. More so, *H. suaveolens* was observed to have a higher concentration of phenols than other compounds. Phenolic compounds had been described to be part of the many phytochemicals found in plants [30]. These compounds are strongly related in defending plants against any

invading enemy, such as pests or pathogenic microorganisms, working as a molecular response mechanism to intense stress conditions [31]. Similarly, disc diffusion assay method was more effective than the well diffusion assay method, which is in agreement with the work of King and Dykes [32] who revealed that the well diffusion assay hindered diffusion of each of their test agents used to a larger extent, probably because in the well diffusion assay the agent is not initially in direct contact with the organism and must first diffuse into the agar to exert an antimicrobial effect [32]. However, the response of the organism to the treatments was similar (Fig. 1).

There was a significant increase in the inhibition of the extracts with increased concentration (Fig. 2). However, fluconazole (32 mg/ml) used as the positive control recorded significantly higher inhibition. DMSO which was used as the negative control because it was used as a solvent in dissolving the extracts showed no significant effect on the growth; hence inhibition zone diameter was not measured. The increase in inhibition with increasing concentration is an indication that if higher concentrations are used, higher inhibition rates would be observed. Generally, the results of this research equally revealed that *H. suaveolens* and *M. oleifera* contain fungi toxic compounds since they were able to inhibit the growth diameter of the pathogens. This agrees with earlier reports of several workers on different fungal organisms [24,33,34,35,36]. However, the efficacy of the extracts differed with the assay method and concentration. As observed in Table 2, *H. suaveolens* at 100 mg/ml using disc diffusion assay method showed higher inhibition on both *P. colocasiae* and *F. oxysporum. M. oleifera* recorded higher inhibition on *F. oxysporum* at 50 mg/ml using the disc diffusion assay method and at 100 mg/ml using the disc diffusion assay method it recorded higher inhibition on *P. colocasiae* (Table 2). The lower MIC value of *H. suaveolens* (12.5 mg/ml) is indicated that it is more effective against *F. oxysporum* as it inhibited the growth of the pathogen even at lower concentrations (Table 3).

4. CONCLUSION

The results of this study have shown that *H. suaveolens* and *M. oleifera* had the potential to inhibit *P. colocasiae* and *F. oxysporum,* however*, H. suaveolens* showed higher inhibitory effects*.* The higher inhibitory effect of *H. suaveolens* can be linked to the presence of alkaloids, saponins, hydrogen cyanide, flavonoids and phenols in the extract. The next phase of this research is focused on isolating and identifying active compounds in the extracts which could be utilized as leads molecule(s) in the development of new fungicides for the control of *P. colocasiae* and *F. oxysporum*. More so, the assay method is also a contributing factor in the efficacy of the extract. The disc diffusion assay method proved to be more sensitive than well diffusion assay in this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ma LJ, Geiser DM, Proctor RH, Rooney AJ, O'Donnell K, Trail F, Gardiner DM, Manners JM, Kazan K. *Fusarium* pathogenomics. Annual Review of Microbiology. 2013;67:399-416.
2. Joshi M, Srivastava R, Sharma AK, Prakash A. Isolation and characterization of *Fusarium oxysporum*, a wilt causing fungus, for its pathogenic and non-pathogenic nature in tomato (*Solanum lycopersicum*). Journal of Applied and Natural Science. 2013;5:108-117.
3. Dervis S, Soylu S, Serce CU. Corm and root rot of *Colocasia esculenta* caused by *Ovatisporangium vexans* and *Rhizoctonia solani.* Romanian Biotechnological Letters. 2014;19:9868–9874.
4. Deo PC, Anand PT, Taylor M, Douglas KB, Harding RM. Improving taro (*Colocasia esculenta* var. *esculenta*) production using biotechnological approaches. South Pacific Journal of Natural Science. 2009;27:6-13.
5. Tsopmbeng GR, Fontem DA, Yamde KF. Evaluation of culture media for growth and sporulation of *Phytophthora colocasiae* Racib., causal agent of taro leaf blight. International Journal of Biology and Chemistry Sciences. 2012;6:1566–1573.

6. Yulia E. Antifungal activity of plant extracts and oils against fungal pathogens of pepper, cinnamon and tumeric. M.Sc Thesis (Unpublished). School of Topical Biology and the School of Veterinary and Biomedical Sciences, James Cook University, Australia. 2005;140.
7. Dellavalle D, Cabrera A, Alem D, Larrañaga P, Ferreira F, Rizza MD. Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria* spp. Chilean Journal of Agricultural Research. 2011;7:231–239.
8. Mohana DC, Raveesha KA. Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. Journal of Agricultural Technology. 2007;4(1):119-137.
9. Bajpai VK, Kim HR, Hou CT, Kang SC. Microbial conversion and *in vitro* and *in vivo* antifungal assessment of bioconverted docosahexaenoic acid (bDHA) used against agricultural plant pathogenic fungi. Journal of Industrial Microbiology and Biotechnology. 2009;36:695–704.
10. Rani VD, Sudini H. Management of soil-borne diseases in crop plants: An overview. International Journal of Plant, Animal and Environmental Sciences. 2013;3:156–164.
11. Ramaiah AK, Garampalli RKH. *In vitro* antifungal activity of some plant extracts against *Fusarium oxysporum* f. sp. *Lycopersici*. Asian Journal of Plant Science and Research. 2015;5(1):22–27.
12. Mahdizadehnaraghi R, Heydari A, Zamanizadeh HR, Rezaee S, Nikan J. Biological control of garlic (*Allium*) white rot disease using antagonistic fungi-based bioformulations. Journal of Plant Protection Research. 2015;55:136–141.
13. Neela FA, Sonia IA, Shamsi S. Antifungal activity of selected medicinal plant extract on *Fusarium oxysporum* Schlecht the causal agent of *Fusarium* wilt disease in tomato. American Journal of Plant Sciences. 2014;5:2665-2671.
14. Al-Rahmah ANI, Mostafa AA, Abdel-Megeed A, Yakout SM, Hussein SA. Fungicidal activities of certain methanolic plant extracts against tomato phytopathogenic fungi. African Journal of Microbiology Research. 2013;7:517-524.
15. Doherty VF, Olaniran OO, Kanife UC. Antimicrobial activities of *Aframomum melegueta* (Alligator pepper). International Journal of Biology. 2010;2(2):126-131.
16. Anukworji CA, Ramesh RU, Okigbo RN. Isolation of fungi causing rot of cocoyam (*Colocasia esculenta* (L.) Schot) and control with plant extracts: *Allium sativum*, *Garcinia kola,* Hekel, *Azadirachta indica* (L.) and *Carica papaya* (L.). Global Advanced Research Journal of Agricultural Science. 2012;1:33-47.
17. Eze CS, Ezejiofor AJ. Phytochemial screening of *Acanthus montanus* (Nees) T. Anderson and *Crinium jagus* (Thomps) extracts and their potential for controlling rot fungi of stored cocoyam (*Colocasia esculenta* (L.) Schott. International Journal of Engineering Sciences and Research Technology. 2014;3:659-665.
18. Kalpana S, Moorthi S, Kumara S. Antimicrobial activity of different extracts of leaf of *Moringa oleifera* (Lam.) against gram positive and gram negative bacteria. International Journal of Current Microbiology and Applied Sciences. 2013;2(12):514–518.
19. Chiejina NV. Mycoflora of some salad vegetables. Bio-Research. 2008;6(2):392-395.
20. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi (4th Ed.). The American Phytopathological Society, St. Paul, Minnessota, USA. 1999;218.
21. Watanabe T. Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species (2nd Edition). CRC Press LLC. Boca Raton, Florida USA. 2000;235.
22. Agrios GN. Plant pathology. Academic Press, New York. 2005;922.
23. Ohikhena FU, Wintola OA, Afolayan AJ. Evaluation of the antibacterial and antifungal properties of *Phragmanthera capitata* (Sprengel) Balle (Loranthaceae), a mistletoe growing on rubber tree, using the dilution techniques. The Scientific World Journal. 2017;1–8.
24. Bukar A, Uba A, Oyeyi TI. Antimicrobial profile of *Moringa oleifera* Lam. extracts against some food-borne microorganisms. Bayero Journal of Pure and Applied Sciences. 2010;3(1):43-48.
25. Pachkore GL, Dhale DA, Dharasurkar AN. Antimicrobial and phytochemical screening of *Hyptis suaveolens* (L.) Poit. (Lamiaceae). International Multidisciplinary Research Journal. 2011;1:01-03.
26. Mbajiuka CC, Obeagu EI, Chude CN, Ihezie OE. Antimicrobial effects of *Chromolaena odorata* on some human

pathogens. International Journal of Current Microbiology and Applied Sciences. 2014;3(3):1006–1012.
27. Gurjar MS, Ali S, Akhtar M, Singh KS. Efficacy of plant extracts in plant disease management. Journal of Agricultural Sciences. 2012;3:425-433.
28. Hayek SA, Gyawali R, Ibrahim SA. Antimicrobial natural products. In: Microbial Pathogens and Strategies for Combating them: Science, Technology and Education, ed A.Méndez-Vilas (Badajoz: FORMATEX), 2013;910–921.
29. Silva AP, Nascimento da Silva LC, Martins da Fonseca CS, Araújo JM, Correia MT, Cavalcanti MS, Lima VL. Antimicrobial activity and phytochemical analysis of organic extracts from *Cleome spinosa* Jaqc. Front. Microbiol. 2016;7:963.
30. Ajuziogu GC, Ugwu LU, Ojua EO. Termicidal properties of wood extractive partitions as a prospective eco-friendly wood preservatives. Journal of Indian Academy of Wood Science. 2019;16(1): 67–72.
31. Hounsome N, Hounsome B, Tomos D, Edwards-Jones D. Plant metabolites and nutritional quality of vegetables. Journal of Food Science. 2008;73:R48–R65.
32. King T, Dykes G. Comparative evaluation of methods commonly used to determine antimicrobial susceptibility to plant extracts and phenolic compounds. Journal of Association of Official Analytical Chemist International. 2008;91:1423–1429.
33. Mandal SM, Mondal KC, Dey S, Pati BR. Antimicrobial activity of the leaf extracts of *Hyptis suaveolens* (L.) Poit. Indian Journal of Pharmaceutical Sciences. 2007;69:568-569.
34. Witayapan NT, Sombat CP. Antioxidant and anti-microbial activities of *Hyptis suaveolens* essential oil. Scientia Pharmaceutica. 2007;75:35-46.
35. Ijato JY, Oyeyemi SD, Ijadunola JA, Ademuyiwa JA. Allelopathic effect of leaf extract of *Azardirachta indica* and *Chromolaena odorata* against post harvest and transit rot of tomato (*Lycopersicum esculentum* L). Journal of American Science. 2010;6:1595-1599.
36. Okigbo RN, Okorie RE, Ramesh P. *In vitro* effects of garlic (*Allium sativum* L.) and African basil (*O. gratissimum* L.) on pathogens isolated from rotten cassava roots. Intercienia Journal. 2009;4:742-747.

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