Trop J Nat Prod Res, March 2020; 4(3):63-68

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 Ethyl-acetate Leaf Extract and Terpenoid-Rich Fraction from *Hyptis spicigera* Lam

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ARTICLE INFO

Article history:
Received 06 December 2019
Revised 06 March 2020
Accepted 18 March 2020
Published online 30 March 2020

ABSTRACT

Plant extracts act as contact fungicides by disrupting cell membrane integrity at different stages of fungal development, while others inactivate key enzymes. The present study is aimed at evaluating the phytochemical constituents and antifungal activity of the ethyl-acetate extract and terpenoid-rich fraction from *Hyptis spicigera*. Qualitative phytochemical analysis was done using standard methods and quantitative analysis was done spectrophotometrically. Antifungal activity was done using Agar well diffusion method. Qualitative phytochemical analysis revealed the presence of anthraquinones, sterols, triterpenes, cardiac glycosides, flavonoids, tannins and alkaloids. The quantitative phytochemical screening revealed high concentrations of saponins (920 mg/g) and phenolics (200 mg/g). Sensitivity test of the extract and terpenoid-rich fraction on the test organisms revealed highest diameter zone of inhibition against *Aspergillus parasiticus* (24.67 ± 0.88 mm) and the least inhibition zone against *Fusarium oxysporum* (18.00 ± 0.58 mm). Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the ethyl-acetate extract was observed at concentrations ranging between 6.25 and 12.5 mg/mL. The MIC of the terpenoid-rich fraction was observed at concentrations ranging between 6.25 mg/mL and 12.5 mg/mL while the MFC were between 12.5 mg/mL to 25 mg/mL. The result of the antifungal activity revealed that there was a significant difference between the ethyl-acetate extract, terpenoid-rich fraction and the control fungicide (mancozeb). The result obtained from this research suggest that the leaf extract of *Hyptis spicigera* and its terpenoid-rich fraction could be used as bio-fungicide for the control of fungal diseases caused by species of the genus *Aspergillus* and *Fusarium.*

Keywords: Antifungal, Phytochemical, Ethyl-acetate, Terpenoids-rich fraction.

Introduction

Plant extracts act as contact fungicides by disrupting cell membrane integrity at different stages of fungal development, while others inactivate key enzymes and interfere with metabolic process.[1] Crops treated with plant extract produce and accumulate elevated levels of specialized protein and other compound which inhibit the development of fungal diseases.[2]

The use of most synthetic fungicides has been restricted because of the long degradation period, pathogen resistance, toxicity on human, plants, animals and the environment.[3] Due to high usage of chemical fungicides on farm land near water bodies, there is an increase release of toxin in water bodies which will invariable enter into the food chain. Hyptis (*Hyptis spicigera*) is a genus of flowering plant in the Lamiaceae family. *Hyptis spicigera* is an erect, aromatic, annual herb growing up to one meter tall, the plant is frequently grown as a food crop for its seeds in parts of tropical Africa. The plant is commonly known as bushmint (due to their aromatic nature of their leaves), they are widespread in tropical North and South America, as well as parts of West Africa.[4]

However, studies have shown that the genus of this plant contain some major bioactive compounds among which are saponin glycosides, alkaloids and flavonoids.[5] Also, the antibacterial activity of the essential oil of *Hyptis suoevulens* was reported on *S. aureus*, *P. aeruginosa*, *E. coli* and *P. multocida.*[6]

Crops are naturally infested with fungi during drying, processing, transportation and subsequent storage and it may be difficult to completely prevent mycotoxins formation in contaminated commodities, particularly those that are produced in tropical and subtropical climates, in countries where high temperature and humidity promote the growth and proliferation of fungi.[7] Thus, they are often colonized by fungi, including species from the genus *Aspergillus*, *Penicillium* and *Fusarium*, which cause significant reductions in crop yield, quality and safety due to their ability to produce mycotoxins.[8]

Chemical control of most of fungal diseases of plants may be available and could extensively reduce the impact of plant diseases, but field application of synthetic fungicides may not always be desirable. During the last decades, there has been a global awareness that excessive and improper use of chemical fungicides is hazardous to the health of humans and animals. Therefore, an extensive research for environmentally safe and easily biodegradable bio-fungicides is being carried out. Furthermore, these compounds are natural in origin, have minimum adverse effects on the physiological processes of plants and are easily converted into common eco-friendly organic materials.[9] Plant extracts, essential oils, gums, resins, etc. have been shown to exert biological activity against plant fungal pathogens *in vitro* and *in*

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Citation: Adamu K, Musa H, Musa AO, Mikailu AS. Antifungal activity of Ethyl-acetate Leaf Extract and Terpenoid-Rich Fraction from *Hyptis spicigera* Lam. Trop J Nat Prod Res. 2020; 4(3):63-68. doi.org/10.26538/tjnpr/v4i3.1

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

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

vivo and can be used as bio-fungicidal products.[10-12] These products are generally assumed to be more acceptable and less hazardous for the ecosystems and could be used as alternative remedies for the treatment of plant diseases.[13]

Materials and Methods

Collection of Plant Material
Fresh leave samples of *Hyptis spicigera* Lam. was collected within Zaria Environs. The plant material was identified at the herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria. Herbarium specimen was deposited with voucher number ABU2050.

Preparation and Extraction of Plant Material
The fresh leave was dried at room temperature for 21 days, the dried leaves were grounded into fine powder.
The powdered plant material (250 g) was extracted with ethyl-acetate by cold maceration at room temperature for 24 hours. The extract was left to air-dry at room temperature.[14]

Isolation of Terpenoid-rich Fraction and Thin Layer chromatography
Terpenes was extracted from the ethyl-acetate extract by acid base extraction.[15] The extract (5 g) was dissolved in 100 mL Ethyl-acetate and poured into a separatory funnel, the extract was basified using 200 mL of 5% Potassium Hydroxide (w/v) and the funnel was swirled gently to allow complete mixing of the base and the extract. The aqueous phase was collected and the ethyl-acetate phase was re-extracted (3x) with 200 mL of 5% potassium hydroxide. The ethyl-acetate phase was further extracted three times with 5% hydrochloric acid (200 mL). The aqueous layer was collected leaving the ethyl-acetate phase in the separatory funnel. The organic fraction (ethyl-acetate phase) was washed with 50 mL of water and then concentrated in a rotary evaporator to 30 mL, it was then centrifuged min at 6000 rpm for 10 minutes to remove the suspended particles. The solvent was evaporated to dryness, leaving a residue which is the terpenoid-rich fraction. The fraction was spotted on a silica gel coated aluminium plate. The plate was developed with n-hexane-ethyl acetate (7:3). Terpenoid test was done using Lieberman-bucchard reagent for both the chemical test and by spraying.[15, 16]

Qualitative Phytochemical Screening
The leaves extract was tested for anthraquinone, saponins, cardiac glycoside, flavonoids, tannins, unsaturated sterols and triterpenes and alkaloids using standard methods.[17]

Quantitative Phytochemical Screening
Total phenolic content was measured spectrophotometrically by Folin Ciocalteu method, using Gallic acid as standard, and the result was expressed as Gallic acid equivalent (GAE) per gram of sample.[18]
Total flavonoids contents was determined by Aluminium chloride Colourimetric method using Quercetin as standard.[19] The Total flavonoid content was expressed in mg quercetin equivalent/g extract.
Total alkaloids content was determined spectrophotometrically, this method was based on the reaction between alkaloid and bromocresol green (BCG), the total alkaloids contents was expressed in mg atropine equivalent/g extract.[20, 21]
Total tannin content was determined spectrophotometrically by Folin Ciocalteu method. The tannin content was expressed in terms of mg gallic acid equivalent/g extract.[22-24]
Total saponin content was determined according to the method described by Makkar *et al*.[25] The standard calibration curve was obtained from suitable aliquots of Diosgenin (0.5 mg/mL). The total saponins concentration was expressed as mg Diosgenin equivalent/g extract.

Source of Fungal Isolates
Four different species each of *Aspergillus* and *Fusarium* known to affect cereals and had been identified by Innovative Medicine Initiative (IMI) was used for the study. Already cultured isolates were collected from the Department of Crop Protection, Ahmadu Bello University Zaria. The four species of *Aspergillus* used are: *Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus* and *Aspergillus fumigatus*. The four species of *Fusarium* used are: *Fusarium verticilloides, Fusarium graminerum, Fusarium oxysporum* and *Fusarium proliferatum*

Media Preparation
Potatoe Dextrose Agar (PDA) was prepared according to the manufacturer instruction, autoclaved at 121°C for 15 minutes. The prepared medium (20 mL each) was dispensed in universal bottle with caps covered and kept in the refrigerator prior to usage.

Preparation of Fungal Inoculum
The spores from the surface of the plates were collected with inoculating needle and suspended in 10 mL of normal saline solution, the mixture was homogenized, 2 mL of 10% tween 20 was added and heavy particles were allowed to settle down and gradually decanted into a sterile tube. The suspension of the inoculum was adjusted to 0.5 McFarland standard equivalent to the turbidity of the suspension by a spectrophotometer at a wavelength of 530 nm to obtain a final concentration that will match 0.5 McFarland standard for mould based on the optical density of the solution ($0.4\text{-}0.5 \times 10^6$) CFU/mL. [26, 27]

Sensitivity Test
Agar well diffusion method was used to screen the extract against the test organism. The prepared PDA (20 mL) was dispensed into the petri dish and allowed to cool, 0.1 mL of the test inoculum was smeared across the petri dish containing the PDA. The extract (0.5 g) was dissolved in 10 mL of 20% DMSO to obtain a concentration 50 mg/mL, then 0.1 mL of the prepared extract was introduced into the well and incubated at 30°C for 48 hours after which the plates was observed for zone of inhibition, the inhibition zone was measured with a meter rule and documented excluding the diameter of the well.[27] A positive control (Mancozeb) and a negative controls (Normal Saline and 20% DMSO) were used.

Minimum Inhibitory Concentration (MIC)
The MIC was determined using the broth dilution method, Potatoe Dextrose Broth was prepared as prescribed by the manufacturer, two-fold serial dilution of the extract was done in the sterile broth to obtain a concentration of 50-1.67 mg/mL. The already prepared standard inoculum (0.1 mL) was introduced into each of the test tube containing varied concentration of the extracts dissolved in the Potatoe Dextrose broth and incubation was carried out for 48 hours at 30°C. The MIC was read as the test tube having the least concentration of the extract with no sign of sporulation of fungi. [27]

Minimum Fungicidal Concentration (MFC)
The already prepared PDA was dispensed into sterilized petri dishes, test-tubes containing the cultured fungi species (starting with the tube where MIC was recorded and those with increasing concentration of the extract in serial dilution) were sub-cultured onto the prepared medium, and incubation was done at 30°C for 48 hours after which the plates were observed for colony growth. The petri dish containing the least concentration of the extract without colony growth is referred to as the minimum fungicidal concentration.[27]

Data Analysis
Data were subjected to two way analyses of variance (ANOVA) and comparison between means where done using Duncans Multiple Range Test. Significant difference was taken at $P < 0.05$.

Results and Discussion

Qualitative Phytochemical Screening
The qualitative phytochemical screening of ethyl-acetate leaves extract of *Hyptis spicigera* Lam (Table 1) indicates the presence of anthraquinones, sterols, triterpenes, cardiac glycosides, flavonoids, tannins, alkaloids and saponins. The result of the phytochemical

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

screening of this plant is in agreement with the findings of Sharma and Tripathi who reported similar phytochemicals in *Hyptis suaveolens*.[28]

Quantitative phytochemical screening

The quantitative phytochemical screening of ethyl-acetate leaves extract of *Hyptis spicigera* was carried out to determine the quantity of the major classes of secondary metabolites which are phenolics, tannins, flavonoids, alkaloids and saponins and was expressed in mg standard equivalent/g extract. Saponins had the highest concentration with a quantity of 920 ± 0.33 mg/g while tannins had the least concentration of 30 ± 0.03 mg/g (Table 2).

The higher concentration of Saponins in this extract may be responsible for it great potential antimicrobial effect to a broad range of microorganisms. Research has shown that Saponins from plants has chemotherapeutic effect against pathogenic microbes causing mycotic infections in plant.[5] Similarly, the antifungal potential against *Candida* species of saponins from the juice extract of *Gmelina arborea* has been reported where it was observed to cause cell lysing and membrane disruption.[29]

The phenolics content of the extract was 200 mg Gallic acid equivalent/g extract and it had been reported that phenolic compound at a very little concentration inhibits the growth and sporulation of several fungi species leading to fungistatic or fungicidal effect against *Fusarium* species.[30] Alkaloids were also detected to be present in the extract at a concentration of 70 mg Atropine equivalent/g extract. Alkaloids has been shown to disrupt fungal cell wall by intercalating into the cell wall component of the cells.[31]

The quantity of flavonoids detected was 80 mg Quercetin equivalent/g extract. In plants, flavonoids play an important role in biological processes. Beside their function as pigments in flowers and fruits, flavonoids are involved in UV-scavenging, fertility and disease resistance especially to fungal infection.[32] Tannins with the least concentration of 30 mg Gallic acid equivalent/g extract was also detected, the least concentration of tannins could be as a result of the extraction solvent used and possibly the tannin content of the plant is un-hydrolyzed which makes it more soluble in polar solvents.

Table 1: Phytochemical constituents of ethyl-acetate leave extracts of *Hyptis spicigera* Lam.

Phytochemicals	Inference
Carbohydrates	+
Anthraquinone	+
Unsaturated sterols and triterpenes	+
Cardiac Glycoside	+
Saponins	+
Flavonoids	+
Tannins	+
Alkaloids	+

Key: + = Positive, - = Negative.

Table 2: Quantitative phytochemical of ethyle-acetate leave extracts of *Hyptis spicigera* Lam.

Phytochemicals	Quantity (mg Standard Equivalent/g extract)
Phenols	200 ± 0.00^{b}
Tannins	30 ± 0.03^{e}
Flavonoids	80 ± 0.33^{c}
Alkaloids	70 ± 0.13^{d}
Saponins	920 ± 0.33^{a}

Means with different superscript letter are significantly different at $P < 0.05$.

Liebermann-burchard test was used to confirm the terpenoid-rich fraction. The fraction was mixed with few drops of acetic anhydride, boiled and cooled, concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration was observed at the upper layer and there was formation of deep red color in the lower layer which indicate a positive test for steroids and triterpenoids, respectively.

The TLC chromatogram of the terpenoid fraction was run on a silica gel coated plate using Lieberman-bucchard spraying reagents and 11 different spots were observed with Rf values of 0.97, 0.78, 0.74, 0.55, 0.46, 0.43, 0.32, 0.28, 0.22, 0.18 and 0.11. The mechanism of action of terpenoids on fungi is related to the hydrophobic nature of terpenoids which makes it bind to the Calcium ion on the cell wall of fungi there by producing a hydro-oxyle complex which had been associated with membrane expansion and fluidity of cell leading to cell disruption and consequently cell death.[33]

Sensitivity of Fungal species

Five fungal species were sensitive to both the extract and fraction. The inhibitory zone of fungi sensitive to the extract ranges from 18.00 ± 0.58 to 24.67 ± 0.88 and that of the terpenoid-rich fraction ranges from 19.00 ± 0.00 to 21.00 ± 0.58 (Table 3). It was observed that the ethyl-acetate extract of *Hyptis suaveolens* shows wider inhibition zone on *A. flavus* than the control fungicides however there was no significant difference between the inhibition zone of the ethyl-acetate extract and the terpenoid-rich fraction on *A. flavus;* this could be as a result of the membrane disruptive ability of the cell wall of the fungal spores since the terpenoid was extracted from the ethyl-acetate extract. However, the ethyl-acetate extract shows greater inhibition zone when compared to the control fungicide. This could be as a result of the combined effects of both terpenes, saponins and phenolic in the inactivation of key enzymes and also the toxic nature of Saponins to microorganism.[5, 34]

Moreover, the control fungicide did not show any sign of inhibition on *A. parasiticus* and *F. oxysporum* while the extract and its fraction showed activity against these fungi species with mean diameter of inhibition ranging from 18.00 ± 0.58 mm to 24.67 ± 0.88 mm, this implies that the extract and its fraction had better activity against these fungi species than the control fungicides. Also the terpenoid-rich fraction showed inhibition zone against *F. proliferatum* while the ethyl-acetate extract did not inhibit the growth of the fungi species, this could be that the activity of the terpenoid might have been masked by the presence of other phytochemicals in the crude extract.

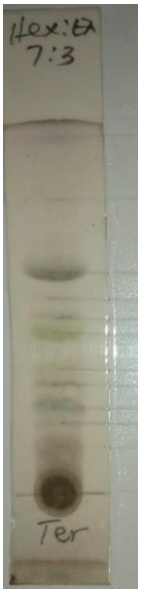

Plate 1: TLC chromatogram of terpenoid-rich fraction

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

The higher activity of the terpenoid-rich fractions may be attributed to its more refined nature and its activity was similar to what was observed with the control fungicide (mancozeb) suggesting that both may contains similar chlorinated hydrocarbons.[35]

Minimum Inhibitory Concentration (MIC) of ethyl-acetate leaves extract of Hyptis spicigera

For the fungi sensitive to the ethyl-acetate leaves extract, the MIC for *A. flavus*, *A. fumigatus* and *A. parasiticus* were observed to be 6.25 mg/L while that of *F. oxysporum* and *F. graminearum* were observed to be 12.5 mg/mL, all the fungi species does not show any sign of fungal sporulation and as such the MIC was recorded at that concentration (Table 4). The MIC for *A. flavus*, *A. fumigatus* and *A. parasiticus* were observed to be 6.25 mg/mL while that of *F. oxysporum* was observed to be 12.5 mg/mL. This implies that at this concentration of the extracts in a test tube containing a known inoculum, there was no sign of fungal sporulation and as such the MIC was recorded at that concentration and it implies that the extract is fungistatic as this phase.

Minimum fungicidal concentration of ethyl-acetate leaves extract of Hyptis spicigera Lam.

The minimum fungicidal concentration of ethyl-acetate extract indicated that the MFC is same as the MIC for *A. flavus*, *A. fumigatus* and *A. parasiticus* where they were observed at a concentration of 6.25 mg/mL while that of *F. oxysporum* and *F. graminearum* were observed at a concentration of 12.5 mg/mL. At these concentrations all the fungi species did not show any sign of growth, therefore, the MFC was recorded at that concentration. The outstanding activity observed by this extract may be due to the high concentration of Saponins detected in the extract which might have been toxic to the fungal spores, hence the low MIC and MFC for *A. flavus*, *A. fumigatus* and *A. parasiticus* (MIC ≤ 6.25 mg/mL, MFC = 6.25mg/mL. The MFC of *F. oxysporum* was at a concentration of 12.5 mg/mL.

Minimum Inhibitory Concentration (MIC) of the Terpenoid-rich fraction

For the terpenoid-rich fraction, MIC was recorded at 6.25 mg/mL for *A. flavus* and *A. fumigatus* while *A. parasiticus*, *F. proliferatum* and *F. oxysporum* showed their MICs at a concentration of 12.5 mg/mL. (Table 6). This suggests that fraction may be fungistatic to the fungal spores by hindering their ability to multiply.

Minimum Fungicidal Concentration of the Terpenoid-rich fraction

Evaluation of the minimum fungicidal concentration of the terpenoid-rich fraction showed MIC of 12.5 mg/mL for *A. fumigatus* and *A. parasiticus* while for *A. flavus*, *F. proliferatum* and *F. oxysporum* MICs of 25 mg/mL was observed (Table 7).

Table 3: Zones of inhibitions of ethyl-acetate leave extract and terpenoid-rich fraction from *Hyptis spicigera* Lam.

Fungal species	Ethyl-acetate extract	Terpenoid-rich fraction	Control fungicides (Mancozeb)
A. flavus	22.67 ± 0.88^{a}	21.00 ± 0.58^{ab}	20.00 ± 1.16^{b}
A. fumigatus	21.33 ± 0.67^{a}	19.00 ± 0.58^{b}	22.67 ± 0.88^{a}
A. parasiticus	24.67 ± 0.88^{a}	21.33 ± 0.33^{b}	ND
A. niger	ND	ND	10.33 ± 0.88^{a}
F. graminearum	20.23 ± 0.02^{a}	ND	18.33 ± 1.45^{b}
F. proliferatum	ND	19.00 ± 0.00^{b}	21.00 ± 0.57^{a}
F. oxysporum	18.00 ± 0.58^{a}	19.33 ± 0.33^{a}	ND
F. verticilloides	ND	ND	22.00 ± 0.58^{a}

ND: Not detected. Means with different superscript letter are significantly different at $P < 0.05$.

Table 4: Minimum Inhibitory Concentration (MIC) of ethyl-acetate leave extract of *Hyptis spicigera* Lam.

	Concentrations in mg/mL					
Test organism	**50**	**25**	**12.5**	**6.25**	**3.13**	**1.57**
A. flavus	-	-	-	*	+	++
A. fumigatus	-	-	-	*	+	++
A. parasiticus	-	-	-	*	+	++
F. graminearum	-	-	*	+	++	+++
F. oxysporum	-	-	*	+	++	+++

-- = No fungal sporulation, * = MIC, + = presence of fungal growth.

Table 5: Minimum Fungicidal Concentration (MFC) of ethyl-acetate leave extract of *Hyptis spicigera* Lam.

	Concentrations in mg/mL					
Test organism	**50**	**25**	**12.5**	**6.25**	**3.13**	**1.57**
A. flavus	-	-	-	*	+	++
A. fumigatus	-	-	-	*	+	++
A. parasiticus	-	-	-	*	+	++
F. graminearum	-	*	+	++	+++	++++
F. oxysorum	-	-	*	+	++	+++

-- = No growth, * = MFC, + = Slow growth

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

Table 6: Minimum Inhibitory Concentration (MIC) of Terpenoid-rich fraction from leave extract of *Hyptis spicigera* Lam.

	Concentrations in mg/ml					
Test organism	**50**	**25**	**12.5**	**6.25**	**3.13**	**1.57**
A. flavus	-	-	-	*	+	++
A. fumigatus	-	-	-	*	+	++
A. parasiticus	-	-	*	+	++	+++
F. proliferatum	-	-	*	+	++	+++
F. oxysporum	-	-	*	+	++	+++

-- = No fungal sporulation, * = MIC, + = presence of fungal growth

Table 7: Minimum Fungicidal Concentration (MFC) of Terpenoid-rich fraction from leave extract of *Hyptis spicigera* Lam.

	Concentrations in mg/ml					
Test organism	**50**	**25**	**12.5**	**6.25**	**3.13**	**1.57**
A. flavus	-	*	+	++	+++	++++
A. fumigatus	-	-	*	+	++	+++
A. parasiticus	-	-	*	+	++	+++
F. proliferatum	-	*	+	++	+++	++++
F. oxysporum	-	*	+	++	+++	++++

-- = No growth, * = MFC, + = Slow growth

Conclusion

The finding from this research revealed that the leaves extract of *Hyptis spicigera* contains anthraquinones, sterols and triterpenoids, cardiac glycosides, flavonoids, tannins and alkaloids. The TLC analyses of the terpenoid-rich fraction from this extract revealed 7 major spots. The antifungal activities of the ethyl-acetate extract and its terpenoid fraction showed significant antifungal activity against *A. flavus, A. fumigatus, A. parasiticus* and *F. oxysporum.* The terpenoid-rich fraction alone also exerts inhibitory action against *F. proliferatum.* These observations could suggest the potential for the use and development of *Hyptis spicigera* as bio-fungicides against these species of fungi.

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 wish to appreciate the contribution of Prof. M. Ilyas, Dr. A. A. Babando, Mallam Kabir, Zainab Sulaiman, Ibrahim Rahmat and Fatima Adamu Uzairu.

References

1. Rey P, Henhamou E, Tirilly Y. *Phythium oligandrum* Biological control. Pak J Sci. 2018; 10: 22-29.
2. Gebore J, Benhamou N, Vallance J, Le-floch G, Grizard D, Regnault-roger C, Rey P. Biological control of plant pathogen; Advantage and Limitation seen through the case study of *Pythium oligandrum.* Env Sci Poll Res. 2013; 21(7):1-14.
3. Wuyep PA, Musa HD, Ezemokwe GC, Nyam DD, Silagtang MD. Phytochemicals from *Agerantum conyzoids* L. extracts and their antifungal activities against virulent *Aspergillus* Spp. J Acad Ind Res. 2017; 6:32-39
4. Patricia CE, Aldo FC, Mayela YR, Elvira G, Laura A, Maria RC. Volatile constituents identified in hexane extract of *Citrus sinensis* peel and anti-mycobacterium tuberculosis activity of some of its constituents. J Mex Chem Soc. 2014; 58(4):431-434.
5. Ladan Z, Anupitan JO, Oyele AO, Okonkwo EM, Ladan E O, Odjobo B, Habila N. Chemical composition and biological activity of the volatile oil of *Hyptis spicigera* against *Trypanosome brucei brucei* found in Northern-Nigeria. Afr J Pure Appl Chem. 2014; 5 (4):53-58.
6. Witayapan N, Sombat C, Sinporn O. Antioxidant and antimicrobial activities of *Hyptis suaveolens* essential oil, Scientia Pharm. 2007; 75:35-46.
7. Kumar KV, Basu S, Rajendran TP. Mycotoxin Research and Mycoflora in Some Commercially Important Agricultural Commodities. Crop Proc. 2008; 27:891-905.
8. Alkenz S, Sassi AD, Abugnah YS, Alryani MB. Isolation and Identification of Fungi Associated with Some Libyan Food. Afr J Food Sci. 2015; 9 (7):406-416.
9. Gnanamanickam SS. Biological control of crop diseases. Marcel Dekker Inc., New York, USA, 2002. 468 p.
10. Fawzi EM, Khalil AA, Afifi AF. Antifungal effect of some plant extracts on *Alternaria alternata* and *Fusarium oxysporum*. Afr J Biotech. 2009; 8(11):2590–2597.
11. Jalili-Marandi R, Hassani A, Ghosta Y, Abdollahi A, Pirzad A, Sefidkon F. *Thymus kotschyanus* and *Carum copticum* essential oils as botanical preservatives for table grape. J Med Plants Res. 2010; 4(22):2424-2430.
12. Romanazzi G, Lichter A, Gabler FM, Smilanick JL. Recent advances on the use of natural and safe alternatives to conventional methods to control postharvest gray mold of table grapes. Postharvest Biol Tech. 2012; 63:141–147.

13. Chuang PH, Lee CW, Chou JY, Murugan M, Sheik BJ, Chen HM. Antifungal Activity of the Crude Extract and Essential oil of *Moringa oliefera* Lam. Biores Tech. 2007; 98:232-236.
14. Kokate CK, Purohit AP, Gokhale SB. Textbook of pharmacognosy, Nirali prakasan, Pune; 2002. 1-4 p.
15. Cordeiro PJ, Vilegusj H, Lancasj FM. Chemical and biological investigations of *Dillenia indica* Linn. J Braz Chem Soc. 1999; 10:523-526.
16. Abubakar HMG, Karumi Y, Usman H. Assessment of Microbially Active Phytochemicals from the Partitioned Portions of the Stem Bark Extract of *Diospyros mespiliformis* Hochst. ex. A. DC. Nig J Pharm Sci. 2015; in press ABU Zaria
17. Trease GE and Evans WC. A textbook of Pharmacognosy, 13, London Bacilliere Tinall Ltd ; 2009. 234 p.
18. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, invitro antioxidant and anti-inflammatory activity of flower of *Morinag oleifera*. Asian Pac J Trop Biomed. 2013; 3 (8):623-627.
19. Zhisen J, Mengcheng T, Jiaming WU. The Determination of Flavonoids Content in Mulberry and their Scavenging Effect on Superoxide Radical. Chem. 1999; 64:555-559.
20. Shamsa F, Hamidreza M, Rouhollah G, Mohammadreza V. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J Pharm Sci. 2008; 32:17-20.
21. Sharief MD, Srinvasulu A, Uma MR. Estimation of alkaloids and total phenol in roots of *Derris trifoliate* L. and Evaluation for antibacterial and antioxidant activity. Ind J App Res. 2014; 4(5):1-11.
22. Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J Univ Chem Tech. Metallurgy 2005; 40(3):255–260.
23. Rajeev S, Pawan KV, Gagandeep S. Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*. J Compl Med Res. 2012; 1(2):101–104.
24. AfifyAel M, El-Beltagi HS, El-Salam SM. A Biochemical change in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties. Asian Pac J Trop Biomed. 2012; 2(3):203–209.
25. Makkar HP, Siddhuraju P, Becker K. Methods of molecular biology*:* Plant secondary metabolites, Totowa, Human Press; 2007. 93-100 p.
26. EUCAST. European committee on antimicrobial susceptibility testing of isavuconazolw susceptibility in *Aspergillus*: comparison of results for inoculum standardization using counting versus optical density. Antimicrob Agents Chemother. 2014; 58(11):6432-6436.
27. Clinical and Laboratory Standard Institutes (CLSI). Document M39-A3. Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data. Approved Guideline, Third Edition. CLSI, 940 West Valley Road, Suite 1400; Wayne, Pennsylvania. USA. 2009. 1898-1987 p.
28. Sharma N and Tripathi A. Integrated management of postharvest *Fusarium* rot of gladiolus corms using hot water, UV-C and *Hyptis suaveolens* (L.) Poit. essential oil. Postharvest Biol Tech. 2008; 47:246-254.
29. Adamu K, Musa H, Adelanwa MA, Ibrahim R, Rabilu SA. Effect of the crude juice extract of *Gmelina arborea* on four *Candida* species. Nig J Mycol. 2019; 11: 204-209.
30. Beatriz P, Luisa M, Calvarro1 DP, Alberto E, Maria L, Tello EP, Arancha GG. Antifungal effects of phenolic extract from industrial residues of *Aloe vera*. Spanish J Agric Res. 2018; 16(4):1010-1018.
31. Cowan MM. Plant Products as Antimicrobial Agents. Clin Microbiol Rev. 1999; 12:564-582.
32. Schijlen EG, Ric D, Vos CH, Tunen AJ, Bovy AG. Modification of flavonoid biosynthesis in crop plants. Phytochem. 2004; 65:2631-2648.
33. Rao A, Zhang Y, Muend S, Rao R. Mechanism of antifungal activity of terpenoid phenols resembles calcium stress and inhibition of the TOR pathway. Antimicrob Agents Chemother. 2010; 54(12):5062-5069.
34. Sodipo OA, Akanji MA, Kolawole FB, Adetuga OO. Saponin is the active antifungal in *Garcinia kola*. Heckle seed. Biol Sci Res Comm. 1991; 3:171-178.
35. Abhishek W, Preeti M, Shiwani G, Anjali C, Shirkot CK. Impact of fungicides Mancozeb at different application rates on soil microbial population, soil biological process and enzyme activities in soil. The Sci World J. 2014; 10:702909-702918.