JOURNAL OF AGRICULTURAL SCIENCES & TECHNOLOGY
https://www.jast.com.ng
Volume 1 Issue 1 (2022)

DOI: 10.36108/jast/2202.10.0110

Bioactive components of *Erythrophleum suaveolens* (Brenan) leaves extracts and their antifungal effect on *Aspergillus flavus* isolated from maize seeds

**** YUSUF, Hajara Oyiza [1], OLU, Joshua [2], SOLEBO-AJENIFUJAH, Shakirat O.[3], PETU-IBIKUNLE, Abiona Michael[4], ALU, Joy Adaeze [5]***

[1] Environmental Biotechnology and Bio conservation Department, National Biotechnology Development Agency, Along Musa Yar'adua Expressway, Airport Road, Lugbe, Abuja, Nigeria
[2] Faculty of Environmental Science, Nasarawa State University, Keffi, Nigeria
[3] Plant Biotechnology Lab, Agricultural Biotechnology Department, National Biotechnology Development Agency along Musa Yar'adua Expressway, Airport Road, Lugbe –Abuja
[4]Faculty of Agricultural Science, National Open University of Nigeria, Abuja
[5]Crop Science Laboratory, Faculty of Agriculture, University of Abuja

*Corresponding Author- Email: haj4yusuf@gmail.com Orcid No: https://orcid,org/0000-0003-4296-6674

ABSTRACT

This study set out to evaluate the phytochemical composition of *Erythrophleum suaveolens* leaves extract and its fungicidal attributes on *Aspergillus flavus* isolated from maize seeds. The leaves of *E. suaveolens* were collected from Gwagalada, Abuja, Nigeria, and subjected to soxhlet extraction with methanol and chloroform as solvents to determine the bioactive compounds in the leaves compared to water crude extract. Different crude concentrations of the plant extracts were added for the antifungal effect into wells within *Aspergillus flavus* swabbed in a potato dextrose agar plate to diffuse at room temperature for 2hrs and the plates were incubated at 28^{o}C for 48hrs before measuring the diameter of the inhibition zones. Tannins, alkaloid, phenols, Triterpenoids, saponin, and flavonoid were present in the plant with tannin, flavonoid, saponin, alkaloid, and phenol having a concentration of 2.49, 1.448, 2.69, 0.505 and 0.366 mg/g respectively. The water, chloroform, and methanol extracts had an inhibitory diameter zone of 10 ± 0.58, 3 ± 0.58, and 0 mm, respectively at $p<0.05$. This study showed the presence of some bioactive compounds in *E. suaveolens*. Furthermore, water extract of the plant had higher antifungal potency on A. *flavus* isolated from maize seeds.

Keywords: Antifungal, *Aspergillus flavus,* Bioactive, *Erythrophleum suaveolens,* Phytochemicals

How to Cite: Yusuf, H.O., Olu, J., Solebo-Ajenifujah, S.O., Petu-Ibikunle, A.M., & Alu, J. A. (2022). Bioactive components of *Erythrophleum suaveolens* (Brenan) leaves extracts and their antifungal effect on *Aspergillus flavus* isolated from maize seeds. *J. Agric. Sci. Tech*. 1(1), 1-7. https://doi.org/10.36108/jast/2202.10.0110

Publisher: Lujosh Ventures Limited

INTRODUCTION

Maize (*Zea mays* L.) is one of the major staple foods in Nigeria and it is also used for industrial purposes. It serves as a food and nutritional supplement for humans and animals and its fungal infection is a major concern (Nithiyaa *et al*., 2012). Fungi are eukaryotic organisms that are associated with various substrates, like soil, plants, and food (Sukmawati and Miarsyah, 2017; Sukmawati, 2016).

In storage, fungi infection can cause deterioration of grains or seeds or the fungi can simply remain viable within the seeds for infecting the germinating seedlings in the next generation. The *Aspergillus, Penicillium, Fusarium* are fungi genera typically found in stored grains and some are xerophytic species in nature and most often capable of producing toxins (Castlellarie *et al*., 2010). Fungi infection can be encouraged/favoured by the high moisture content of the product (Gtorni *et al*., 2009) with factors such as storage time, temperature, and degree of contamination prior to storage. Insect and mite activity also facilitates fungal spread as well as mycotoxin production (Suleiman and Omafe, 2013). An increase in consumption of contaminated grain with mycotoxins can result in different health problems including death (Lerda *et al*., 2005; Voss *et al*., 2007).

Aspergillus flavus are commonly seen in seeds and nuts (Sukmawati *et al*., 2018). Also in poultry, contamination in feed rations such as local maize is associated with *A. flavus* (Rahmawati, 2005). Toxins found in grains occur at 4 to 40˚C with an optimum at 25 to 32˚C with a water content of 18% and relative humidity of 85% (Suparto, 2004). Different types of *Aspergillus* species are reported as major fungi associated with plant-based drugs during different steps of their preparation (Gautam and Bhadauria, 2010; Sareen *et al*., 2010). Aflatoxins, sterigmatocystin, and ochratoxins are mycotoxins produced by *Aspergillus* species that cause several immunosuppressives, hepatogenic and carcinogenic effects (Milićević *et al*., 2010).

Bio-controls activities are determined by the natural substances which have been declared to be the vital sources of novel chemical structures with relevance in the emergence of molecules used in agronomy, pharmacology, and other areas that are important to humans. Bioactive components are present naturally in plants (Banu and Cathrine, 2015). They are organic compounds present in plants and products of physiological activity in the human body. These include alkaloids, flavonoids, steroids and terpenoids, tannins, and carbohydrates (Edoga *et al*., 2005). Phytochemicals are also known as bioactive component in plant with various chemical classes that shows inhibitory effects in varying degree on several types of microorganisms *in-vitro* (Cowan, 1999).

Erythrophleum suaveolens (Guill. & Perr.) Brenan is a perennial tree in the Caesalpiniaceae family. It has a dense spreading crown that is slightly buttressed and low-branching and can grow in height to 30m. *E. suaveolens* is seen as an ornamental plant in Tropical Asia (Olorunishola and Akintunde, 2003). It is prevalent in Mozambique as an herbaceous perennial plant associated with a characteristic odor (Akinpeh *et al.,* 2012). The leaves of *E. suaveolens* are said to be poisonous to cattle, goats, and horses. The leaves of *E. suaveolens* when air dried are used in controlling pest as an insect repellent; The Tanzanians use the leaves as a fish bait whereas in Nigeria it is used as an emetic, anesthetic, disinfectant and purgative The Jukuns in the Benue state of Nigeria uses it as a charm against witchcraft (Burkill, 1995 in Okhale *et al*., 2018).

This study, therefore, aims at assessing the bioactive components of *Erythrophleum suaveolens* Brenan leaf extracts and to evaluate its antifungal effect on *Aspergillus flavus* isolated from maize seeds

MATERIALS AND METHODS

Plant Materials Collection, Preparation and Extraction

The *E. suaveolens* fresh leaves obtained from Adadu village in Kwali Area Council, Abuja – FCT, was air dried, finely powdered, and used for extraction.

Fig. 1: *E. suaveolens* leaves

Publisher: Lujosh Ventures Limited

Water Extraction

A 5g portion of powdered leaves was taken into a beaker with 200ml distilled water added and heated to 30-40ºC for 20 minutes and continuously stirred. After then a filtrate from the water extract was obtained by the use of filter paper for the phytochemical analysis and preserved in the refrigerator at 4 ºC prior to the period of usage for the study.

Solvent (Methanol and Chloroform) Extraction

E. suaveolens crude leaves extract was prepared by using the Soxhlet extraction principle. A 20g leaf powder portion was uniformly and separately packed into thimbles with 250ml of solvents, methanol, and chloroform for extraction. The process continued for 24 hours till the solution in the siphon tube turned colorless. It was kept in a beaker on a hot plate and heated at 30-40ºC to evaporate the solvent. The dried extract obtained was stored for 48 hours in the refrigerator at 4ºC for further phytochemical analysis.

Phytochemicals Qualitative Analysis of *E. suaveolens* Leaves

The methods described by Harborne (1973), Trease and Evans (1989), Sofowora, (1993), and James *et al.* (1996) were used in testing for the presence of bioactive compounds from the methanol extracts as follows:

Carbohydrates testing (*Fehling's test*)

Mixture of equal volume of Fehling A and Fehling B reagents and 2ml was added to the crude extract and gently heated to a boiling point. A precipitate of brick red appeared at the bottom of the test tube to indicate the presence of reducing sugars.

Saponins test

Crude extract and 5ml of distilled water were mixed thoroughly in a test tube and the formation of stable foam indicated the presence of saponins.

Phenols and tannins test

A 2ml sample of 2% solution of $FeCl_3$ was mixed with the crude extract. A blue-green colour indicated the presence of phenols and tannins.

Alkaline reagent test for Flavonoids

Crude extracts were mixed with 2ml of 2% solution of NaOH. An intense yellow colour that turned colourless on the addition of a few drops of diluted sulphuric acid indicated the presence of flavonoids

Steroid test

A 2ml portion of chloroform was mixed with each crude extract with the addition of a 2ml concentrated H_2SO_4. Production of red colour in the lower chloroform layer indicated the presence of steroids.

Terpenoids test

Crude extracts were dissolved in 2ml of chloroform and evaporated to dryness with the addition of 2ml of concentrated H_2SO_4 and heated for 2 minutes. A resulting grayish colour indicated the presence of terpenoids.

Alkaloids test

Crude extracts were mixed with 2ml of 1% HCl and heated gently, then Mayer's & Wagner's reagents were added to the mixture. The formation of turbid precipitate indicated the presence of alkaloids.

~~V~~olatile oil test

The powdered material of 50mg was subjected to hydro-distillation. The distillate was collected in a well assembly graduate tube. The aqueous portion formed was automatically separated from the volatile oil.

Cardenolides test

To 1ml of the crude extract, 2ml of benzene was added. The formation of a turbid brown colour indicated cardenolides presence.

Cardiac Glycosides test

The crude extract (0.5g) was dissolved in 2ml glacial acetic acid containing 1 drop of ferric chloride solution and downplayed with 2ml of concentrated H_2SO_4 forming a brown ring at the interphase. The reaction indicated the presence of deoxy sugar characteristics of Cardiac glycosides.

Phlobatannins test

A drop of 1% HCl was added to 1ml of crude extract and brought to boiling point. Reddish precipitation indicated phlobatannins presence.

Antifungal Activity Determination

An eight hours old broth culture of fungus *Aspergillus flavus* was introduced on a potato dextrose Agar (PDA)

Publisher: Lujosh Ventures Limited

plate prepared as directed by the manufacturer using a sterile cotton swab stick. A sterile cork borer was used in making wells at the center of the culture plate. To prepare the inert organic solvent, that will serve as a negative control, 2ml of Dimethyl Sulfoxide (DMSO) was pipette into a conical flask, and Fluconazole (10µg, antifungal drug) was used as a positive control. The Plant extracts (water, chloroform, and methanol) at crude concentration, DMSO, and fluconazole were added into their labeled wells respectively using sterilized micropipettes and allowed for diffusion with the plate medium for 2hrs at room temperature, this was done in triplicate and then incubated at 28ºC for 48hrs.

Data Analysis

The zone of inhibition on some of the plates were measured in millimeters (mm) and the mean and standard error of the mean (SEM) were determined at $p<0,05$ using SPSS version 22.

RESULTS AND DISCUSSION

The qualitative phytochemical analysis carried out on the extracts of leaves of *Erythrophleum suaveolens* shows the presence of alkaloids, tannins, saponins, and others (Table 1). These constituents are known to have medicinal and physiological activities (Sofowra, 1993). The phenolic compounds were detected in the leaves extract as indicated in Table 1, These compounds are said to be a group of bioactive components that are omnipresent in plants (Singh *et al.*, 2007), and with biological properties that have been linked to anti-aging, anti-atherosclerosis anti-apoptosis, anti-carcinogen, cardiovascular protection, anti-inflammation and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al.*, 2007). The phenol compounds had a concentration of 0.366 mg/g as indicated in Table 2. Adebayo *et al.* (2015) had earlier ascertained the presence of phenolic compounds and flavonoids in *Erythrophleum* plant. Flavonoids which are hydroxylated phenol substances were also present in the *E. suaveolens* under investigation in this study (Table 1). It has been reported that flavonoids exhibit an antimicrobial effect against some microorganisms which could be attributed to the complexity of extracellular and soluble proteins (Marjorie, 1996). The flavonoid detected in this study had a concentration of 1.448mg/g as indicated in Table 2.

Table 1: Results of qualitative analysis for presence or absence of Phytochemicals and other Bioactive substances in *Erythrophleum suaveolens*

Compound	*Erythrophleum suaveolens* leaf
Tannins	+
Steroids	-
Triterpenoids	+
Phenols	+
Alkaloids	+
Terpenoids	-
Cardenolids	-
Carbohydrate	+
Saponin	+
Flavonoid	+
Volatile oils	-
Phlobatannins	-
Cardiac Glycosides	-

+: Presence, -: Absence

Publisher: Lujosh Ventures Limited

The *E. suaveolens* leaves extract was found to contain saponins as indicated in Table 1. This is a substance that had been reported with an inhibitory effect on inflammation (Just *et al* 1998). The saponins had a concentration of 2.690 mg/g as indicated in Table 2. Alkaloids were also found in the *E. suaveolens* leaves extract (Table 1) with a concentration of 0.505 mg/g (Table 2). From previous studies, alkaloids have been reported to have cytotoxicity biological properties (Nobori *et al.,* 1994).

Table 2: Quantities of Phytochemicals in extracts of *Erythrophleum suaveolens*

Parameter	*Erythrophleum suaveolens* leave (mg/g)
Tannins	2.490
Flavonoids	1.448
Saponin	2.690
Phenols	0.366
Alkaloids	0.505

Tannin was also found in the *E. suaveolens* leaves extract (Table 1) with a concentration of 2.49 mg/g as indicated in Table 2. Triterpenoids, were also detected in the *E. suaveolens* leaves extract

Fig. 2 shows the antifungal effect of the extracts of *E. suaveolens* on the *A. flavus*. In the antifungal studies as shown in Fig. 2, fluconazole the positive control had an inhibitory zone diameter of 19mm. The water extract of *E. suaveolens* leaves showed intense inhibitory potency against *A. flavus* after 48 hrs with an inhibitory zone of 10 ± 0.58 mm which was significantly higher at $p<0.05$ when compared with methanol and chloroform extracts (Table 3).

Fig 2: Antifungal effect of *Erythrophleum suaveolens* extracts on mycelial growth of *Aspergillus flavus*

Table 3: antifungal effects of *Erythrophleum suaveolens* on *Aspergillus flavus* as shown by inhibition zone after incubation at 28 ^{0}C for 48hrs.

Extracts	Inhibition zone (mm) (Mean ± SEM)	T values	Sig.
Water	10 ± 0.58	20.785	0.002
Chloroform	3 ± 0. .58	5.196	0.035
Methanol	0	-	-
Negative Control (DMSO)	0	-	-
Positive control (fluconazole)	19 ± 1.15	16.454	0.004

$P<0.05$, df=2

The methanol extract of *E. suaveolens* leaves and the DMSO (negative control) induced no noticeable inhibitory effects against *A. flavus* while the chloroform *E. suaveolens* leaves extract showed mild effect with an inhibitory zone of 3 ± 0.58 mm in diameter at $p<0.05$ (Fig. 2 and Table 3). Nielsen *et al*. (2012) reported that methanol leaf and stem extracts of *E. lasianthum* exhibit antifungal effect against *Candida albicans* and *Microsporum audouinii*.

CONCLUSION

The *E. suaveolens* leaves extracts have been shown in this study to contain some bioactive components such as tannin, alkaloid, saponins, flavonoids, Triterpenoids, and phenols. The water extracts of *E. suaveolens* leaves exhibit intense antifungal potency, chloroform extracts had mild potency, while methanol extracts had no noticeable potency against *A. flavus*.

Author's Contribution:

YUSUF, H.O.: Designed and carried out the experimental work, wrote the Manuscript.
OLU, J.: Was involved in the data analysis, involved in manuscript writing and interpretation of results.
SOLEBO-AJENIFUJAH, S.O.: Edited the manuscript
PETU-IBIKUNLE, A.M.: Read and edit the manuscript
ALU, J. A.: Monitored the extraction process of the leaves in the laboratory

ACKNOWLEDGEMENT:

Expression of gratitude goes to the staff of Crop Protection and Crop Science Laboratory, Faculty of Agriculture, University of Abuja for their support in the course of this research.

FUNDING: This research had no external financial support apart from what each of the author contributed to carry it out and also get it published.

CONFLICT OF INTEREST: The authors declare that there is no personal benefit from actions or decisions made in their official capacity with respect to this study.

REFERENCES

Adebayo, S.A., Dzoyem, J.P., Shai, L.J. and Eloff, J.N. (2015). The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. *BMC Compl. Alt. Med.* 15, 159.

Akinpeh, B.A., Dare, C.A., Adebesin, F.I/, Iwalewa, E.O. and Oyedapo, O.O. (2012). Effects of stem bark of *Erythrophleum suaveolens* (Gull and Perri) saponin on fresh water snail (Lanistesiybicus) tissue. *African Journal of Environmental Science and Technology*, *6*(11), 446-451

Banu, K.S and Cathrine, L. (2015). General Techniques Involved in Phytochemical Analysis. *International Journal of Advanced Research in Chemical Science (IJARCS) 2*(4), 25-32

Castlellarie, C., Marcos, F.V., Mutti, J., Cardoso, L. and Bartosik, R (2010). *Toxigenic fungi in Corn (Maize) stored in hermetic plastic bags*. (pp.115-297), National institute of agricultural Technologies Mardel Plata University Argentina.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev. 12*(4), 564-582. https://doi.org/10.1128/CMR.12.4.564

Edoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemicals constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol., 4*(7), 685-688.

Gautam, A.K., and Bhadauria, R. (2010). Mycoflora and mycotoxins contamination of some common stored herbal fruit samples. *J. Ind. Bot. Soc. 89*,74-79.

Gtorni, P., Battilani, P., and Magan, N. (2009). Effect of solute and matric potential on in- vitro growth and sporulation of strains from anew population of *Aspergillus flavus* in Italy. *Fungi Ecology 1*, 101-106.

Han, X., Shen, T., and Lou, H. (2007). Dietary polyphenols and their biological significance. *Int. J. Mol. Sci*., *8*(9), 950-988

James, E, K., Speedie, R.M. and Tyler, E.V. (1996). *Pharmacognosy and pharmaco Biotechnology*. Williams and Willkins, New York, U.S.A.

Just, M.J., Recio, M.C., Giner, R.M., Cueller, M.U., Manez, S., Billia, A.R. and Rios, J.L. (1998). Anti-inflammatory activity of unusual lupine saponins from Bupleurum fruticescens, *Planta med. 64*, 404-407.

Lerda P., Blaggi, B.M., Peralta, N,, Ychari, S., Vazquez, M., and Bosio, G (2005). Fumonisins in foods from Cordoba (Argentina), presence and genotoxicity. *Food and Chemical Toxicology 43*, 691-698.

Marjorie, C. (1996). Plant products as antimicrobial agents. *Clinical Microbiol. Rev., 12*, 564-582.

Milićević, D.R., Skrinjar, M. and Baltić, T. (2010). Real and perceived risks for mycotoxin contamination in foods and feeds: challenges for food safety control.

Publisher: Lujosh Ventures Limited

Toxins 2, 572-592. https://doi.org/10.3390/toxins2040572

Nielsen, T.R.H., Kuete, V., Jäger, A.K., Meyer, J.J.M. and Lall, N. (2012). Antimicrobial activity of selected South African medicinal plants *BMC Compl. Alt. Med.*, *12*, 74.

Nithiyaa, P., NurAinIzzati, M.Z., UmiKalsom, Y., and Salleh, B. (2012). Diversity and Morphological Characteristics of *Aspergillus* species and *Fusarium* species Isolated from Cornmeal in Malaysia. *Pertanika J. Trop. Agric. Sci. 35* (1), 103 –116.

Nobori, T., Miurak, K., Wu, D.J., Takabayashik, L.A. and Carson, D.A. (1994). Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature, 46*, 753-756

Okhale, S.E., Ugbabe, G.E, Abubakar, I.., Mohammed, S.B., Egharevba, H.O., Adamu, A., Ibrahim, J.A., and Oluyemisi, F.K. (2018). Chemical composition and antimicrobial activity of the leaf essential oil of *Erythrophleum suaveolens* Guill. and Perr. (Brenan) (Family: fabaceae/ caesalpinioideae). *IJMPR 2*(4), 08-12

Olorunishola, A.A.G. and Akintunde, M.A. (2003). Characterising *Erythrophleum suaveolens* charcoal as a viable Alternative Fuel to coke in iron melting in Nigeria. *Journal of mechanical and civil engineering (10SR-JMCE),10*(3), 06-11.

Rahmawati, S. (2005) AflatoksindalamPakanTernak di Indonesia: Persyaratan Kadar dan Pengembangan Teknik Deteksinya *Wartazoa15*(1), 26–37

Sareen, A., Ahirwar R., Gautam, A., and Bhadauria, R. (2010) Fungal contamination of some common medicinal plant samples of himachal pradesh. *Sci. Cult. 76*,118-120.

Singh, R., Singh, S.K. and Arora, S. (2007). Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. *Fod Chem. Toxicol., 45*, 1216-1223.

Sofowra, A. (1993). *Medicinal Plants And traditional Medicine In: Africa*. (pp. 191-289), Spectrum Books Ltd., Ibadan, Nigeria,

Sukmawati, D. (2016) Antagonism Mechanism of Fungal Contamination Animal Feed Using Phylloplane Yeasts Isolated from the Bintaro Plant (Cerberamanghas) Bekasi in Java Indonesia *Int. J. Curr. Microbiol. App. Sci. 5*(5), 63-74

Sukmawati, D. and Miarsyah, M. (2017) Pathogenic activity of *Fusarium equiseti*from plantation of citrus plants (*Citrus nobilis*) in the village Tegal Wangi, Jember Umbulsari, East Java, Indonesia *Asian J Agri& Biol. 5*(4), 202-213

Sukmawati, D., Setyaningsih, A., Handayani, T.K., Rahayu, S., Rustam1, Y., Moersilah, M., Wahyudi, P and Husna, S.N.A (2018). Isolation and characterization of aflatoxigenic *Aspergillus* spp. from maize of livestock feed from Bogor**.** *IOP Conf. Series: Materials Science and Engineering 434***,** 012105 https://doi.org/10.1088/1757-899X/434/1/012105

Suleiman, M.N. and Omafe, O.M. (2013). Activity of three medicinal plants on fungi isolated from stored maize seeds (*Zea mays* (L). *Global Journal of Medicinal Plant Research 1*, 77-81.

Suparto, D.A.H (2004). SituasiCemaranMikotoksinpadaPakan di Indonesia *Prosiding Seminar Parasitologi Dan Toksikologi pp.*121–126

Trease, G.E. and Evans, W.C. (1989). *Pharmacognosy*, 11th edn., (pp. 45-50), Bailliere Tindall, London.

Voss, K.A., Smith, G.W. and Haschek, W.M. (2007) Fumonisins: toxicokinetics, mechanism of action and toxicity. *Animal feed science and technology 137*, 299-325.

Publisher: Lujosh Ventures Limited