Phytochemical Analysis and Antifungal Effect of *Erythrophelum suaveolens* Seeds Essential Oil on *Aspergillus flavus* Isolated from Locally Purchased Maize (*Zea mays*) Seeds In Abuja, Nigeria

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ABSTRACT

Aspergillus flavus is ubiquitous and it is responsible for attacking and contamination of maize and the use of organic substances to arrest the proficiency of *A. flavus* is quiet limited, this study therefore aimed at evaluating the phytochemical composition and antifungal effect of *Erythrophelum suaveolens* seed essential oil on *A. flavus* isolated from locally purchased maize seeds in Abuja. Extraction of an essential oil of the grinded seeds of *E. suaveolens* isolation through hydro-distillation method and petroleum ether as solvent utilizing cleverger apparatus. The qualitative phytochemical analysis of *E. suaveolens* seed oil was determined. Swabbing using sterile cotton swabs in a potato dextrose Agar (PDA) plates with 8hrs old broth culture of the *A. flavus* and a sterile cork borer used in making wells at the center of the culture plate at room temperature for 2hrs for diffusion to take place, this was done in triplicate to determine antifungal effect of the seed oil. The plates were incubated at 28°C for 48hrs to measure the diameter of the inhibition zones. The results show that tannins, steroids, terpenoids, balsams, and cardiac glycosides were present in the plant seeds oil after 48 hrs, the triplicate shows an inhibitory diameter zone of 1.8, 1.7 and 1.4cm respectively. The plant seed essential oil had higher antifungal potency towards *A. flavus* isolated from maize seeds and presence of some bioactive compound.

Keywords: Antifungal, *Aspergillus flavus*, *Erythrophelum suaveolens*, essential oil, Phytochemical

Citation

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INTRODUCTION

There has been an increase in consumers' demand and interest in the use of plants for culinary, medicinal, and other anthropogenic applications which have added to the attraction of medicinal and aromatic plant grown by farmers (Dhifi *et al.*, 2016). The organic constituents found in plants such as tannins, saponins, alkaloids, flavonoids and essential oils are based on the efficacy of medicinal plants for therapeutic purposes. (Akanji and Adewumi, 2020). It has been reported by Ragno *et al.*, (2020), Butnariu and Sarac (2018) and Abd El-Hack *et al.*, (2016) that the naturally occurring plant extracts are the essential oils (EOs) from seeds, leaves, roots, stems and petals. The essential oils are alcohol soluble and water insoluble and volatile. These volatile oils are colorless at room temperature and generally liquid in nature (Dhifi *et al.*, 2016). With 3000 known EOs only 300 are used for commercial purposes and the most commonly ones used in industrial sectors are the lemon, peppermint, citronella, eucalyptus, mint, orange while the domestic use ones are the tea tree, chamomile, lavender, rosemary, orange, lemon, rose, eucalyptus, jasmine, geranium, sandalwood (Barbieri and Borsotto, 2018). Ghabraie *et al.*, (2016) reported that frankincense oil is the most popular of essential oil.

As reported by Butnariu and Sarac, (2018) that the EOs are highly volatile substances with some containing greater than 300 different compounds, this includes aldehydes, amides, esters, alcohols, ethers, terpenes, heterocycle and ketones phenols which belong to the numerous class of chemicals in plants. Dhifi *et al.*, (2016) and Buckle, (2015) reported that the most common classes of compound in EOs are terpenes that are either, cyclic or acyclic which includes monoterpenes, sesquiterpenes and diterpenes. Butnariu and Sarac, (2018) found out that plant essential oils has a highly complex composition which are synthetic on replication. The multiple roles of plants EOs is the attraction of pollinators of pest, predator and disease protection (Ragno *et al.*, 2020; Nazzaro *et al.*, 2017). The common complementary treatment use of EOs is the aromatherapy that has been used for over 6000 years by the Indians, Egyptians and Chinese as reported by Lee *et al.*, (2012) and Ali *et al.*, (2015). In the study by Lin *et al.*, (2019), Reis and Jones, (2017) and Muzzarelli *et al.*,

(2006), it was found that there has been anxiety reduction, cancer cure and improvement of sleep in humans with the use of EOs.

It has also been reported by Narayanasamy *et al.*, (2019) that an EOs has widely been utilized in treatment like skin sores, wounds, inflammation, rheumatic joints, burns, syphilis, fungal infections, cystitis and pharyngitis. The antimicrobial activity of EOs is attributed to its composition, configuration, volume, pathogens interactions and their mode of action on single or multiple targets in a pathogen (Dhifi *et al.*, 2016). It has been reported by Chraibi *et al.*, (2020) and Chouhan *et al.*, (2017) that the primary constituents of EOs drive its biological activity making up greater than 85% of the oil and Swamy *et al.*, (2016) has reported that EO's mechanism of action against pathogens are controlled by the antimicrobial chemical class. It was also reported by Bassolé and Juliani (2012) that the high proportion of phenols and aldehydes greatest activity can also occurs in contrast with the activity of esters, ketones, or terpene hydrocarbons. It was also reported that the impacts of hydrophobicity activity of EOs increase in permeability and results in the leakage of cells (Dhifi *et al.*, 2016). Modification in bacterial cell wall or cell membrane of most EOs causes the release of lipopolysaccharides and thereby changes the balance in ATP internally and externally, pH fluctuation, protein synthesis, and internal cytoplasmic changes resulting in coagulation of cytoplasmic material, DNA disruption, and inhibition of quorum sensing (Lopez-Romero *et al.*, 2015; Faleiro, 2011). These changes also show effects in the growth regulation, nutritional balances and energy conversion in phenolic components, such as carvacrol, eugenol, and thymol having an effect on the cytoplasmic membrane, electron flow, proton forces, active transportation, and cell content coagulation (Dhifi *et al.*, 2016; Swamy *et al.*, 2016).

Erythrophleum suaveolens is used traditionally for a variety of diseases treatment and also cultivated for ornamental purposes (Akanji and Adewumi, 2020). *E. suaveolens* Brenan is one of an important medicinal plant belonging to the family of Fabaceae. It has an average height growth of 30m, with a spreading branch, forming a rounded crown. It produces fruits of an oval flat shape in wood pod. Its bark is rough and blackish in color and

the leaves are green in color and have an ovate shape (Ziba *et al.*, 2017).

In Nigeria, *E. suaveolens* is cultivated in the southeastern states and the Igbo tribes eat the seeds mainly as a soup thickener for various dishes as they presumed that the seed of *E. suaveolens* are relatively cheaper to purchase compared to melon" egusi" seeds in preparing their staple vegetable soup (Adekunle *et al.*, 2006). *E. suaveolens* seed can be said to be edible, because the qualitative properties of the oil fit the description of edible oil described by Kirk (1991) and International Seed Testing Association (Ngounou *et al.*, 2005 and Lmganau, 1984) reported the antimicrobial activity of diterpenoid and alkaloids isolate from *E. suaveolens* seeds essential oil which is rich in monoterpenes and triterpene and the major compound in the seeds oil is (62.37%) citral which is an oxygenated compound followed by (20.07%) squalene, and triterpene. The isomeric citral in *E. suaveolens* composition is 59.3% α -citral and 40.7% β -citral. Okhale *et al.*, (2018) reported the antimicrobial activity of an essential oil against *E. coli* to be high in its citral content.

Aspergillus is the most described fungal genera because of its highly toxic secondary metabolites negative impact on agricultural production (Amaike and Keller, 2011). Aflatoxin contamination is highly susceptible with maize crops colonized with *Aspergillus flavus* and served as the main causes of aflatoxin contamination (Fountain *et al.*, 2015; Wu and Khlangwiset, 2010).

There is limited literature review on organic material inhibitory effect on *A. Flavus* that attack maize, therefore, this study aims at evaluating the phytochemical composition of *Erythrophelum suaveolens* seeds essential oil and its antifungal effect on *A. flavus* isolated from locally purchased maize seeds in Abuja, Nigeria.

METHODOLOGY

Essential oil isolation

Air-dried seeds of *E. suaveolens* was grinded and 500g of the grinded seed was put in a 2L round bottom-flask with 1000 ml of ether as a solvent. EO isolation was by hydro-distillation method and petroleum ether was used as the solvent in a Clevenger type apparatus for 4hrs. The essential oil isolated was filtered through 0.22micron

filter and dried with anhydrous sodium sulphate, within 30 minutes for analysis.

Qualitative phytochemical analysis of *E. suaveolens* seeds essential oil

Qualitative analysis was used in testing for the presence of bioactive compounds in the essential oil.

Fehling's test

Crude extract and 2ml Fehling A and Fehling B reagents were mixed thoroughly and gently heated to a boiling point. A red brick precipitate settled at the bottom of the test tube which indicates the presence of sugars.

Saponins test

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

Phenols and tannins test

Crude extract and 2ml FeCl_3 of 2% solution was mixed thoroughly and a blue-green color formed indicating the presence of phenols and tannins.

Flavonoids test

Crude extract and 2ml NaOH of 2% solution was mixed to give an intense yellow color and later turned colorless with a few drops of diluted acid indicating the presence of flavonoids

Steroid test

2ml chloroform and crude extract were thoroughly mixed and 2ml concentrated H_2SO_4 was added to give red color indicating the presence of steroids.

Terpenoids test

Crude extract was dissolved in 2ml chloroform and evaporated to dryness and 2ml concentrated H_2SO_4 was added and brought to a heating stage for 2 minutes and a grayish color formed indicating the presence of terpenoids.

Alkaloids test

Crude extract and 2ml of 1% HCl was heated gently and added to Mayer's & Wagner's reagents and a turbidity precipitate formed indicating the presence of alkaloids.

Volatile oil test

Crude extract of 50mg was subjected to hydro-distillation. The distillate collected in a well assembly graduate tube and an aqueous portion formed separated from the volatile oil (James *et al.*, 1996).

Cardenolides test

Crude extract and 2ml benzene thoroughly mixed to give a turbid brown color indicating the presence of cardenolides.

Cardiac Glycosides test

Crude extract dissolved in 2ml glacial acetic acid with a drop of ferric chloride solution to downplay the 2ml of concentrated sulphuric acid to form a brown ring indicating the presence of deoxy sugar characteristics of Cardiac glycosides.

Phlobatannins testing

Crude extract of 1ml with a drop of 1% HCl was brought to a boiling point and a reddish precipitation formed indicating the presence of phlobatannins.

Essential oil antifungal activity

Swabbing using sterile cotton swabs in a potato dextrose Agar (PDA) plates with 8hrs old broth culture of the fungus *A. flavus* and a sterile cork borer used in making wells at the center of the culture plate. The essential oil of *E. suaveolens* (100µl) was added into the well using a sterilized micropipette and allowed to diffuse for 2hrs at room temperature, this was done in triplicate and then incubated at 28°C for 48hrs and measured the inhibition zones in diameter.

RESULTS AND DISCUSSION

The use of chemicals as antimicrobial agents to combat various microbial attack and infections is on the rise in the endogenous microorganisms resistant and the hydro-alcoholic extracts of an essential oil derived from plant plays a fundamental role in the use of synthetic antimicrobials (Nazzaro *et al.*, 2017). The treatment of fungal infections using essential oil from plant has increased over time and this can be attributed to the extensive use of antifungal agents and Olu *et al.*, (2021) reported the use of aloe vera extract in disinfecting coliform infected water. It was also reported by (AbdRashed *et al.*, 2021) that the insight for nonconventional approaches required for an effective antifungal treatment strategy for EOs key components are phenolic compounds, monoterpenes, and sesquiterpenes. The phenolic compounds are majorly responsible for the antimicrobial properties in EOs as

reported by Chanthaphon *et al.*, (2008). In this study from Table 1, the essential oil of *E. suaveolens* contains steroids, triterpenoids, tannins, phenols, terpenoids, balsams and Cardiac Glycosides.

Table 1: chemical constituents of *Erythrophleum suaveolens* essential oil

Parameter	<i>Erythrophleum suaveolens</i> oil
Tannins	+
Steroids	+
Triterpenoids	+
Phenols	+
Alkaloids	-
Terpenoids	+
Cardenolids	-
Carbohydrate	-
Saponin	-
Flavonoid	-
Balsams	+
Volatile oils	-
Phlobatannins	-
Cardiac Glycosides	+
Cardenolids	-

It was reported by (Hu *et al.*, 2007) that plant EOs is one of the natural products found in fungal inhibition and as seen from literature review that many of the EOs from different plants or herbs has inhibition intense of an antifungal property (Ghalem *et al.*, 2016; Lang *et al.*, 2012; Prakash *et al.*, 2012). Therefore, the phytochemicals in EOs has effects in the microbial growth and biofilm development (Hyldgaard *et al.*, 2012) and the in-vitro indicated that terpenes/terpenoids shows an ineffective antimicrobial activity when used in a compounds compared to the overall of EOs (Tian *et al.*, 2012; Bajpai *et al.*, 2011).

According to Yusuf *et al.*, (2021), *Aspergillus flavus* has been reported to be isolated from some stored maize seeds. In this study as shown in Fig.1, the essential oil of *E. suaveolens* seeds inhibits the growth of *A. flavus* in the three replicate. The inhibition zone diameters for the triplicate antifungal test were 18, 17 and 14 mm as illustrated in Table 2.

The inhibitory characteristics displayed by the essential oil of *E. suaveolens* seeds against *A. flavus* has been

reported to be displayed by some plants essential oil. In a study reported by Da Silva *et al.*, (2012), indicated that the EO of thyme leaves shows a better antifungal effect against *A. flavus* and Freire *et al.*, (2011) also reported the antifungal activity of fennel and mint leaves EOs. The *Ocimum gratissimum* and *Plectranthus glandulosus* essential oil was also found to inhibit the growth of *A. flavus* (Tatsadjieu *et al.*, 2008). Also in another study by Achar *et al.*, (2020), *A. flavus* growth was found to be inhibited by essential oil of clove, thyme, lemongrass, and cinnamon.

The Ostrosky *et al.*, (2008) reported that the diffusion method is limited to a fast-growing microorganism, aerobic or facultative aerobic and also reported that the agar diffusion method of EO antimicrobial activity can be influenced by the culture medium of an agar concentration, pH, oxygen availability, amount of inoculum and conditions of the incubation.

Table 2: Diameter of the zone of inhibition

Seed oil extract	Inhibition zone measurement (mm)
R1	18
R2	17
R3	14
Mean $\pm$ SEM	16.33 $\pm$ 1.20

Key: R = replicate

Fig 1: Evaluation of antifungal effect of *Erythrophleum suaveolens* essential oil on *Aspergillus flavus*

CONCLUSION

From the findings in this study, some bioactive compounds such as steroids, triterpenoids, tannins, phenols, terpenoids, balsams and cardiac glycosides are present in *E. suaveolens*. In addition, the plant seed essential oil had antifungal potency towards *A. flavus* isolated from maize seeds.

Author's Contribution:

YUSUF, Hajara O. carried out the experimental design, performed the experiments and proof-read submitted manuscript,

OLU, J. drafted the manuscript, contributed to data analysis, study design and proof-reading of manuscript, OJO, E.O performed the phytochemical analysis findings of the *E. suaveolens* of the study.

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REFERENCES

- Abd El-Hack, M.E., Alagawany, M., Farag, M.R., Tiwari, R., Karthik, K., Dhama, K., Zorriehzahra, J. and Adel, M. (2016). Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: a review. *J. Essent. Oil Res.* 28, 365–382.
- AbdRashed, A., Rath, D.N.G., Ahmad Nasir, N.A.H. and Abd Rahman, A.Z. (2021). Antifungal Properties of Essential Oils and Their Compounds for Application in Skin Fungal Infections: Conventional and Nonconventional Approaches. *Molecules* 26, 1093. <https://doi.org/10.3390/molecules26041093>
- Achar, P.N., Quyen, P., Adukwu, E.C., Sharma, A., Msimanga, H.Z., Nagaraja, H. and Sreenivasa, M.Y. (2020). Investigation of the Antifungal and Anti-Aflatoxigenic Potential of Plant-Based Essential Oils against *Aspergillus flavus* in Peanuts. *J. Fungi*, 6, 383; <http://doi.org/10.3390/jof6040383>
- Adekunle A. A., Aya E. L. and Dabiri O. O. (2006). The Composition and Antifungal Properties of the *Erythrophleumsuaveolens* Guill and Perr. (Leguminosae) Seeds and Oil. *Science Journal*. 3(4), 61-64.
- Akanji, O. C. and Adewumi B. (2020). Phytochemical, Mineral Contents and Proximate Compositions of the Stem Bark of *Erythrophleum suaveolens* Brenan (Guill. & Perr.). *IRE Journals* 3(10), 89-97.
- Ali, B., Al-Wabel, N.A., Shams, S., Ahamad, A., Khan, S.A. and Anwar, F., (2015). Essential oils used in aromatherapy: A systemic review. *Asian. Pac. J. Trop. Biomed.* 5, 601–611. <https://doi.org/10.1016/j.apjtb.2015.05.007>.
- Amaike, S. and Keller, N.P. (2011). *Aspergillus flavus*. *Annu. Rev. Phytopathol.*, 49, 107–133
- Bajpai, V.K., Kang, S., Xu, H., Lee, S.G., Baek, K.H. and Kang, S.-C. (2011). Potential roles of essential oils on controlling plant pathogenic bacteria *Xanthomonas* species: A review. *Plant Pathol. J.*, 27, 207–224.
- Barbieri, C. and Borsotto, P., (2018). Essential oils: market and legislation. In: H.A., El-Shemy (Ed), Potential of Essential Oils. IntechOpen, (pp. 107-128). <http://dx.doi.org/10.5772/intechopen.77725>.
- Bassolé, I.H. and Juliani, H.R., (2012). Essential oils in combination and their antimicrobial properties. *Molecules* 17, 3989–4006. <https://doi.org/10.3390/molecules17043989>.
- Buckle, J., (2015). Basic plant taxonomy, basic essential oil chemistry, extraction, biosynthesis, and analysis. In: J. Buckle, (Ed.), *Clinical Aromatherapy*. Churchill Livingstone, Missouri, (pp. 37–72). <http://dx.doi.org/10.1016/B978-0-7020-5440-2.00003-6>.
- Butnariu, M. and Sarac, I. (2018). Essential oils from plants. *J. Biotechnol. Biomed. Sci.* 1, 35–43. <https://doi.org/10.14302/issn.2576-6694.jbbs-18-2489>.
- Chanthaphon, S.; Chanthachum, S. and Hongpattarakere, T. (2008). Antimicrobial activities of essential oils and crude extracts from tropical Citrus spp. against food-related microorganisms. *Songklanakarin J. Sci. Technol.*, 30, 125–131.
- Chouhan, S., Sharma, K. and Guleria, S., (2017). Antimicrobial activity of some essential oils—Present status and future perspectives. *Medicines*. 4, 58. <https://doi.org/10.3390/medicines4030058>.
- Chraïbi, M., Farah, A., Elamin, O., Iraqui, H.M. and Fikri-Benbrahim, K., (2020). Characterization, antioxidant, antimycobacterial, antimicrobial effects of Moroccan rosemary essential oil, and its synergistic antimicrobial potential with carvacrol. *J. Adv. Pharm. Technol. Res.* 11, 25–29. https://doi.org/10.4103/japtr.JAPTR_74_19.
- Da Silva, F.C., Chalfoun, S.M., de Siqueira, V.M., Botelho, D.M., Lima, N., and Batista, L.R. (2012). Evaluation of antifungal activity of essential oils against potentially mycotoxigenic *Aspergillus flavus* and *Aspergillus parasiticus*. *Brazilian Journal of Pharmacognosy* 22(5), 1002–1010
- Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., and Mnif, W. (2016). Essential Oils' Chemical Characterization

- and Investigation of Some Biological Activities: A Critical Review. *Medicines* 3, 25-40. <http://doi.org/10.3390/medicines3040025>
- Faleiro, M., (2011). The mode of antibacterial action of essential oils. In: Méndez-Vilas, A. (Ed.), *Science against Microbial Pathogens: Communicating Current Research and Technological Advances*. (pp.1143–1156), Formatex Research Center, Badajoz.
- Fountain, J.C., Khera, P., Yang, L., Nayak, S.N., Scully, B.T., Lee, R.D., Chen, Z.Y., Kemerait, R.C., Varshney, R.V. and Guo, B. (2015). Resistance to *Aspergillus flavus* in maize and peanut: Molecular biology, breeding, environmental stress, and future perspectives. *The Crop Journal* 3, 229–237
- Freire, M.M., Jham, G.N., Dhingra, O.D., Jardim, C.M., Barcelos, R.C. and Valente, V.M.M. (2011). Composition, antifungal activity and main fungitoxic components of the essential oil of *Mentha piperita* L. *J Food Safety* 32, 29-36.
- Ghabraie, M., Vu, K.D., Tata, L., Salmieri, S. and Lacroix, M., (2016). Antimicrobial effect of essential oils in combinations against five bacteria and their effect on sensorial quality of ground meat. *LWT Food Sci. Technol.* 66, 332–339. <https://doi.org/10.1016/j.lwt.2015.10.055>.
- Ghalem, B.R. (2016). Essential oils as antimicrobial agents against some important plant pathogenic bacteria and fungi. In: D.K., Choudari, A., Varma, & N., Tuteja, Eds., *Plant-Microbe Interaction: An Approach to Sustainable Agriculture*; Springer Nature Singapore Pte Ltd.: Singapore.
- Hu, Y., Zhang, J., Kong, W., Zhao, G. and Yang, M. (2007). Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa* L.) on *Aspergillus flavus*. *Food Chem.*, 220, 1–8.
- Hylgaard, M., Mygind, T. and Rikke, L.M. (2012). Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Front. Microbiol.*, 3, 1–24
- James, E. K., Speedie, R.M. and Tyler, E.V. (1996). *Pharmacognosy and pharmaco Biotechnology*, Williams and Willkins, New York, U.S.A.
- Kirk, S.R. (1991). *Pearson's Composition and Analysis of Food*. (pp. 708), Longman Publishers, London.
- Lang, G. and Buchbauer, G. (2012). A review on recent research results (2008–2010) on essential oils as antimicrobials and antifungals. A review. *Flavour Fragr. J.*, 27, 13–39.
- Lee, M.S., Choi, J., Posadzki, P., and Ernst, E., (2012). Aromatherapy for health care: An overview of systematic reviews. *Maturitas*. 71, 257–260. <https://doi.org/10.1016/j.maturitas.2011.12.018>.
- Lin, P.C., Lee, P.H., Tseng, S.J., Lin, Y.M., Chen, S.R., and Hou, W.H., (2019). Effects of aromatherapy on sleep quality: A systematic review and meta-analysis. *Complement. Ther. Med.* 45, 156–166. <https://doi.org/10.1016/j.ctim.2019.06.006>.
- Lmganau, I.E.E. (1984). *The Essential Oil*. (Vol 2., pp.348) Krieger Publishing Co., New York.
- Lopez-Romero, J.C., González-Ríos, H., Borges, A. and Simões, M. (2015). Antibacterial effects and mode of action of selected essential oils components against *Escherichia coli* and *Staphylococcus aureus*. *Evid. Based Complementary Altern. Med.* 795435. <https://doi.org/10.1155/2015/795435>.
- Muzzarelli, L., Force, M. and Sebold, M., (2006). Aromatherapy and reducing preprocedural anxiety: A controlled prospective study. *Gastroenterol. Nurs.* 29, 466–471. <https://doi.org/10.1097/00001610-200611000-00005>.
- N'gouno, F.N., Manfouo, R.N., Tapndjuo, L.A., Lontsi, D., Kuete, V., Penlap, V.S., Etoa, F.X., Dubois, M. and Sondengam, B.L. (2005). Antimicrobial diterpenoid alkaloids from *Erythrophleum suaveolens* (Guill and Perr) Brenan. *Bulletin of Chemical Society of Ethiopia*, 19(2), 221–226.
- Narayanasamy, K., Elangovan, E., Keerthi, D., Jagadeeswari, S., Krithiga, B., Padmanabhan, V., and Periyasamy, S., (2019). Antimicrobial activity of selected essential oils against antibiotic resistant organisms. *Asian. J. Pharm. Pharmacol.* 5, 503–512. <https://doi.org/10.31024/ajpp.2019.5.3.11>.
- Nazzaro, F., Fratianni, F., Coppola, R. and De Feo, V., (2017). Essential oils and antifungal activity. *Pharmaceuticals*. 10, 86. <https://doi.org/10.3390/ph10040086>.

- 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). *IJMPPR* 2(4), 08-12
- Olu, J., Yusuf, H.O., Ikhide, C.E. and Okokoro, H. (2021). Evaluation of Biological Antimicrobial Effect of Aloe Vera on Coliform Isolates in Leachate from a Dumpsite. *Bacterial Empire* 10(10), e275. <https://doi.org/10.36547/be.275>
- Ostrosky, E.A., Mizumoto, M.K., Lima, M.E.L., Kaneko, T.M., Nishikawa, S.O. and Freitas, B.R. (2008). Métodos para avaliação da atividadeantimicrobiana e determinação da concentraçãominimainibitória (CMI) de plantasmedicinais. *Rev Bras Farmacogn* 18, 301-307.
- Prakash, B., Singh, P., Kedia, A. and Dubey, N.K. (2012). Assessment of some essential oils as food preservatives based on antifungal, antiaflatoxin, antioxidant, activities and in vivo efficacy in food system. *Food Res. Int.* 49, 201–208.
- Ragno, R., Papa, R., Patsilnakos, A., Vrenna, G., Garzoli, S., Tuccio, V., Fiscarelli, E., Selan, L., and Artini, M., (2020). Essential oils against bacterial isolates from cysticfbrosis patients by means of antimicrobial and unsupervised machine learning approaches. *Sci. Rep.* 10, 2653–2663. <https://doi.org/10.1038/s41598-020-59553-8>.
- Reis, D. and Jones, T., (2017). Aromatherapy: Using essential oils as a supportive therapy. *Clin. J. Oncol. Nurs.* 21, 16–19. <https://doi.org/10.1188/17.CJON.16-19>.
- Swamy, M.K., Akhtar, M.S., and Sinniah, U.R., (2016). Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review. *Evid. Based Complem. Altern Med.*, 3012462. <https://doi.org/10.1155/2016/3012462>.
- Tatsadjieu, N.L., Etoa, F.-X., Mbofung, C.M.F. and Ngassoum, M.B. (2008). Effect of *Plectranthus glandulosus* and *Ocimum gratissimum* Essential Oils on Growth of *Aspergillus flavus* and Aflatoxin B1 Production. *TROPICULTURA*, 26(2), 78-83
- Tian, J., Ban, B., Zeng, H., He, J., Bo, H. and Wang, Y. (2011). Chemical composition and antifungal activity of essential oil from *Cicutavirosa*L. Var. *latisecta*Celak. *Int. J. Food Microbiol.*, 145, 464–470.
- Wu, F. and Khlangwiset, P. (2010). Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: case studies in bio control and postharvest interventions, *Food Addit. Contam. A* 27, 496–509.
- Yusuf, H.O., Olu, J., Alu, A.J. and Anjorin, T.S (2021). Isolation and morphologically identification of *Aspergillus flavus* incidences from maize seeds in Abuja, Nigeria, *Fungal Territory Journal* 4(1), 5-8 <https://doi.org/10.36547/ft.276>
- Ziba, M., Munthali, C. and Missanjo, E. (2017). Seed Biology of *Erythrophleum suaveolens* (Guill. and Perr.) Brenan: A Threatened Medicinal Plant. *SDRP Journal of Plant Science* 2(1), 53-58.