Antifungal Potential of *Tetrapleura tetraptera, Xylopia aethiopica, Citrus aurantifolia* and *Cymbopogon citratus* on Different Fungal Isolates

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ABSTRACT

study on the antifungal potentials of various plants extracts on mycoflora of groundnut pod was carried out in the Department of Plant Science and Biotechnology, Rivers State. Cultural laboratory methods was used to evaluate the mycoflora. Powdered plants extracts used were extracted by air drying and blending the leaves of the plants. The antifungal activity on the various fungal isolates was done at different concentrations (100, 50, 25, and 12.5mg/ml). For antifungal activity of C. citratus at 100, 50 and 25mg/ml revealed higher inhibition of Scopulariopsis sp compared to other isolates, although at 12.5mg/ml there was complete inhibition across all fungal isolates. X. aethiopica showed more inhibition for all isolates at the different concentrations while T. tetraptera and C. aurantifolia revealed total inhibition of these isolates at 12.5mg/ml with variations across other concentrations respectively. Generally all plants extract showed complete inhibition at 12.5mg/ml.

keywords: *Antifungal, Tetrapleura tetraptera, Xylopia aethiopica, Citrus aurantifolia and Cymbopogon citratus*

INTRODUCTION

Tetrapleura tetraptera is a west African known flowering plant with common name Aridan tree (Uyoh *et al.,* 2013) belonging to the Fabaceae plant family. It is found coonly in Ghana as it is used in preparation of soup because of its fragrance and medicinal properties (Aladesanmi, 2007) and as a high vitamin supplement (Osei-tutu *et al.,* 2010).

Negro pepper known botanically as *Xylopia aethiopica* belonging to the annonaceae plant family. It is an evergreen, tall and slim aromatic tree growing naturally in the savanna region of Africa (Fetse *et al.,* 2016), growing mostly in Nigeria, Ghana, Ethiopia, Senegal and Cameroun (Yin *et. al.,* 2019). The plant is used in managing ailments like cough, fever, candidias (Ogbonna *et al.,*

2013), rheumatism, headache, asthma, epilepsy, wounds and sores (Erhirhie and moke 2014) and has anticancer, antidiabetic, antibacterial and antimicrobial properties (Mohaed and Islam, 2017).

Citrus aurantifolia is a blooming plant, native to southern Asia known commonly as lime (Gruenwald *et al.*, 2000). The oil extracted from this plant has antibacterial (Aibinu *et al.*, 2007) and antifungal properties (Barrera-Necha *et al.*, 2009). It also serves as a good candidate for food products preservation (Jafari *et al.*, 2011). Kandpal *et al.*, 2012 also reported that leaves extract of lime revealed significant activity against *Pseudomonas spp, Mucor spp, Aspergillius fumigatus* and *A. niger.*

Lemon known botanically as *Cymbopogon citratus* is a well cultivated medicinal plant having anticancer and antibacterial properties (Dev and Nidhi, 2016). It is also used as a relief for constipation, aids in good digestion, treatment of scurvy, piles, gout, gums and respiratory disorders (Mohanapriya *et al.*, 2013).

Pathogenic fungi are fungi that causes diseases in humans and other organisms, although fungi are eukaryotic. In 2022, the world health organization released a list of fungal pathogens which are critical priority (*Cryptococcus neoformas, Candidia auris, Aspergillius fumigatus*), high priority (*Candidia glabrata, Histoplasma spp, Fusarium spp*) medium priority (*Scedosporium spp, Lomentospora prolificans, Coccidioles spp*) (WHO, 2022). The most common pathogenic species which are *A. fumigatus* and *A. flavus* produce aflatoxin and carcinogen, which has ability to contaminate foods such as nuts (San-Blas *et al.*, 2008). These pathogenic fungi damage plants by killing cells and causing plant stress, as they enter the plants through natural openings or wounds caused by pruning, insects, mechanical damages, etc.

Pathogenic fungi can be controlled by using disease-free seed, use of resistant varieties, use of chemical and biological fungicides and by the use of natural plant extracts.

Natural products are made of various chemical compounds and hence they contain great potentials. Researchers had turned to the use of natural products from plants as a source of pharmeuticals in treating various ailments (Harvey, 2008). Reports also show that most antifungal drugs in use are linked to natural products from plants (Butler 2005). Siva *et al.*, 2008 stated that plants possess bioactives which are used in controlling diseases and also active against fungi.

MATERIALS AND METHODS

Collection of Plant Samples

Plant samples such as Guinea pepper (*Xylopia aethiopica*), Adridan leaf (*Tetrapluera tetraptera*), lemon grass (*Cymbopogon citratus*) and lime leaf (*Citrus aurantifolia*) were used in the present study. The aerial parts of lime, lemon grass and adridan leaves were collected from the respective plants while Guinea pepper was bought from vendors in the Mile One market. All samples were sent to the Department of Plant Science and Biotechnology for identification before they were processed for further analysis according to the method of Gul *et al.* (2017).

Isolation of Fungi from Groundnut Samples

The tissue segment method of Vasumathi and Ahila (2020) was adopted in cultivation and isolation of fungi. In this method, the infected parts were cut into small pieces immediately after washing the tissues thoroughly with sterile distilled water and in 0.1% mercuric chloride solution used for surface sterilization of plant tissues. The cut parts of the pods were transferred to freshly prepared Sabouraud Dextrose Agar (SDA) plates supplemented with 50mg tetracycline antibiotics (to inhibit bacterial growth) and incubated for 5-7 days at 22℃ (Douglas and Robinson, 2018; 2019). The control were pods showing no rots or observable disease.

Purification of Fungal Isolates

The SDA plates were observed for fungal growth after incubation. The fungal isolates were isolated and purified using the hyphal tips technique (Thilagam *et al.*,2018) on freshly prepared SDA plates and incubated for 2-5 days. After incubation, the fungal isolates were subcultured on SDA slant and preserved in the refrigerator for further studies.

Identification of Isolates

The fungal isolates were identified using their morphological features such as colony color, shape, texture and size of colony followed by microscopic examination (conidial shape, arrangement of hyphae and type of spore) of their wet mounts prepared with lactophenol cotton blue (Robinson *et al.,* 2020) and reference made to fungal identification manual (Sarah *et al.,* 2016).

Extraction of Plant Parts for Antifungal Susceptibility

Methanol Extraction of the Various Plant Parts

The powdered form of the respective plant part under study was extracted using the method of Robinson *et al.* (2020). with slight modification**.** In this method, 50g of the powdered plant parts were transferred into well labelled 250ml conical flasks each. After which 200ml of methanol was added, swirled carefully for proper homogenization and allowed to stand for forty-eight hours (48 hours). After forty-eight hours of extraction, the supernatant was filtered with sterile filter paper into sterile 250ml beakers and were labelled according to the type of extract. The filtrate was evaporated to dryness in the hot air oven at 45℃. The resulting oily residue was weighed and stored in sterile containers which were preserved in the refrigerator for further analysis.

Preparation of Extracts for Antifungal Assay

Stock solution of the methanol extract was prepared by dissolving 100mg (0.1g) of the oily residue in 1ml of Dimethyl sulfoxide (DMSO) which gave rise to 100mg/ml stock of the methanol extract. Further two-fold serial dilution was carried out by diluting 1ml of the stock solution into three different test tubes containing 1ml of sterile distilled water to achieve the concentrations of 50mg/ml, 25mg/ml and 12.5mg/ml.

Antifungal Activity of Extracts

The antifungal activity of the extracts was carried out using the well in agar diffusion method as described by Robinson *et al.* (2020). In this method, 48 hours old fungal isolates which was standardized to 1.5×10^8 CFU/ml was inoculated on well dried and labelled accordingly in duplicates. The plates were allowed to dry for 3 minutes before five holes (wells) were bored using sterile 6 cork borer. The different concentrations (100, 50, 25, and 12.5mg/ml) of the methanol extracts were transferred into the wells using sterile pipettes. The positive control was 10mg/ml fluconazole antifungal agent. The plates were incubated at 22℃ for forty-eight hours. Zone diameter were measured using graduated rule and the result was recorded. Sterility of the extract was confirmed by streaking the different extracts on Nutrient and Sabouraud Dextrose Agar plates. Absence of growth after 24-48 hours of incubation was interpreted as sterility.

Antifungal Activity

The antifungal activity of the different botanicals used was assessed on the fungal pathogens of groundnut pod. Table 4.6 revealed Antifungal activity *of C. citratus* at different concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml)

RESULTS AND DISCUSSION

Table 1: Antifungal Activity *of C. citratus* on Fungal Pathogens of Groundnut Pod (mm)

Isolates	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
Rhizopus sp	**12**	**7**	**4**	**0**
Aspergillus sp	**22**	**19**	**10**	**0**
Gliocladium sp	**12**	**4**	**0**	**0**
Paecilomyces	**15**	**13**	**7**	**0**
Penicillium	**21**	**12**	**4**	**0**
Fusarium sp	**10**	**8**	**2**	**0**
Scopulariopsis sp	**5**	**0**	**0**	**0**

Table 2: Antifungal Activity of *X. aethiopica* on Fungal Pathogens of Groundnut Pod

Isolates	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
Rhizopus sp	**15**	**6**	**0**	**0**
Aspergillus sp	**18**	**10**	**2**	**0**
Gliocladium sp	**0**	**0**	**0**	**0**
Paecilomyces	**11**	**0**	**0**	**0**
Penicillium	**16**	**0**	**0**	**0**
Fusarium sp	**14**	**5**	**0**	**0**
Scopulariopsis sp	**12**	**4**	**0**	**0**

Table 3: Antifungal Activity of *T. tetraptera* on Fungal Pathogens of Groundnut Pod

Isolates	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
Rhizopus sp	**12**	**6**	**4**	**0**
Aspergillus sp	**22**	**17**	**10**	**0**
Gliocladium sp	**12**	**4**	**0**	**0**
Paecilomyces	**15**	**13**	**7**	**0**
Penicillium	**21**	**10**	**4**	**0**
Fusarium sp	**10**	**8**	**2**	**0**
Scopulariopsis sp	**5**	**0**	**0**	**0**

Table 4: Antifungal Activity of *C. aurantifolia*on Fungal Pathogens of Groundnut Pod

Isolates	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
Rhizopus sp	**15**	**10**	**4**	**0**
Aspergillus sp	**27**	**18**	**10**	**0**
Gliocladium sp	**18**	**9**	**0**	**0**
Paecilomyces	**18**	**10**	**7**	**0**
Penicillium	**20**	**15**	**9**	**0**
Fusarium sp	**19**	**6**	**2**	**0**
Scopulariopsis sp	**15**	**8**	**0**	**0**

Antifungal activity of plants was assessed and it was observed that these plant extracts inhibited the growth of a fungal pathogens isolated. Other researchers have also reported the inhibitive effect of plants extracts. (Naik *et al.,* 2010, Shi *et al.,* 2017) reported the inhibitive effect of *C.citratus* on the growth of *S. Aureus and Candidia sp.* (Petrasch *et al.,* 2019), also reported the high antifungal effect of plants extracts and found that *C. citratus strongly* inhibited the growth of *B. cineria* and therefore can be used as antifungicides to replace chemical fungicides, Syed *et al.,* 2018 also reported the antifungal effect of *C. citratus* on *Aspergillus niger*, which showed maximum inhibition zone as compared to the *Colletotrichum musae*, which indicates that *C. citratus* can be used as an antifungal agent or exploited as an ideal treatment for eliminating mycoflora growth and spread. Antifungal activity of leaves extracts of *C. citratus* was also noted by Bhavya and Padma (2014).

Also a research carried out by (Akwaji *et al.,* 2016) on antifungal activity of leaf extracts of *Corchorus olitorius* and *Gongronema latifolium* revealed that the ethanolic leaf extracts of these above plants completely inhibited the radia growth of *Penicillium sp, Fusarium sp., Rhizopus sp, Aspergillus sp* which is in line with current study. The difference in the fungitoxic potentials between the different plants extracts may be attributed to the susceptibility of these fungal pathogens to the different plants extracts used, which agrees with the results of (Amadioha 2000, Okigbo *et al.,* 2005 and Ilondu *et al.,* 2001) who reported that some plants contain phenolics, which inhibits growth of microorganisms. The presence of phytochemical compounds like saponins, tannins, flavonoids, alkaloids in extracts has been reported to be responsible for antifungal and inhibitory potency of these plant (Ahmed 1996) which is also in agreement with the work of (Chiejina 2013, Amadioha 1999 and Umana *et al.,* 2014) who revealed high potentials of extracts of plants containing the same bioactive compounds and can be used for controlling pathogens of plants.

CONCLUSION

All plants extracts possessed antifungal potentials on groundnut pod, they also inhibited the growth of fungal organisms and therefore can be used as natural fungicides to replace chemical fungicides or as a control for mycoflora spread.

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