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Assessment of the Photochemical Properties and Post Harvest Fungi of Xylopia Aethiopica (Negro pepper)

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Abstract
Plants have shown to contain diverse organic components in them. The plant species Xylopia aethiopica wasbbo taken to the laboratory in Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State for the preparation of mycological medium, Isolation of fungi using Serial dilution method, determination of percentage Incidence of fungal occurrence.Based on the present study, this phytochemical content in the plant species indicates that the plant is of medicinal values as reported by other researchers,The qualitative screening for phytochemicals revealed that the plants had highest quantity in Phenol (34.51 – 0.72), Flavonoids (14.1 – 2.7), Terpernoid (11.4 – 3.40), Phylate (9.8 – 1.23), Alkanoids (9.65 – 0.75) , Tanin (2.87 – 0.52) and Oxalate (0.57-0.22) had the lowest quantity in the plant.

Introduction

The plant *Xylopia aethiopica* is an aromatic tree which grows up to 15–30 m high and about 60–70 cm in diameter. It is native to the lowland rainforest and moist fringe forest in the savanna zones of Africa, but largely found in West, Central and Southern Africa. These trees are widely distributed in the humid forest zones especially along rivers in the drier area of the region. Xylopia is a Greek word ('xylon pikron') for 'bitter wood', while aethiopica refers to its Ethiopian origin (Ethiopia). It is common names include; African pepper, Guinea pepper, spice tree, negro pepper, West African pepper and Senegal pepper. An attractive spicy flavour is obtained after Negro pepper is smoked during the drying process. *Xylopia aethiopica* are simple, alternate, oblong, and elliptic to ovate. Its flowers are bisexual, solitary or in 3-5 flowered fascicles or in strange, sinuous, branched spikes, or cymes, up to 5.5 by0.4 cm and creamy-green.
Fruits of Xylopia aethiopica look like small, twisted bean-pods which are dark brown, cylindrical, 2.5 to 5cm long and 4 to 6mm thick. Each pod houses about 5 to 8 kidney-shaped seeds of approximately 5mm length. *Xylopia aethiopica* are dark brown, cylindrical, 2.5 to 5 cm long and 4 to 6 mm thick. Each pod houses about 5 to 8kidney-shaped seed grains of approximately 5mm length.

Mycology studies

Preparation of mycological medium

Sterilization of conical flask,slides,petri dishes and all the equipment needed for the experiment was carried out in the laboratory. The glass wares were sterilized in the oven at 120℃ for an hour after washing with soap, while other equipment were surface sterilized with 70% ethanol to reduce microbial contamination (Okogbule *et al*., 2024). Inoculating loops and scalpels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The mycological medium used was Sabouraud Dextrose Agar prepared in a conical flask using the standard method. The mouth of the flask was plugged with non-absorbent cotton wool and wrapped with aluminum foil. The conical flask containing the mycological medium was autoclaved at 121℃ and pressure of 1.1kg cm-3 for 15 minutes. The molten agar was allowed to cool to about 40℃ and dispensed into Petri dishes at 15mls per plate and allowed to further cool and solidify.

Isolation of fungi

A three fold serial dilution was used in accordance to the method of Mehrotra & Aggarwal, (2003) where 1g of the spoilt Negro pepper samples were transferred into the first test tube containing 9mls of normal saline. 1ml of the solution was transferred to the second test tube and finally from the second to the third. 0.1ml aliquot from the second and third dilutions were plated onto Sabouraud Dextrose Agar in Petri dishes containing ampicillin to hinder the growth of bacteria and this was done in triplicate. The inoculated plates were incubated for 5 days at ambient temperature of 25℃ ± 3℃ (Okogbule *et al*, 2021). The entire set up was observed for 7 days to ensure full grown organisms. Pure culture of isolates were obtained after a series of isolations.

Identification of fungal organisms

Microscopic examination of fungal isolates was carried out by the needle mount method (Cheesebrough, 2000).The fungal spores were properly teased apart to ensure proper visibility. The well spread spores were stained with cotton blue in lacto phenol and examined microscopically using both the low and high power objective. The fungi were identified based on their spore and colonial morphology, mycelia structure and other associated structures using the keys of (Barnett & Hunter, 1998).

Determination of percentage Incidence

The percentage incidence of fungal occurrence was determined by the formulae stated below (Okogbule *et al.*, 2023)

= % incidence

Where;

X=total number of each organism in a variety

Y=total number of all identified organism in a variety

RESULTS

Qualitative Phytochemical Screening

The ethyl acetate and methanol extracts of the plant were subjected to examination for the detection of phytochemical compounds by employing the procedures of Harborne (A.J. Harborne, Berlin, Germany, 1998) and Evans. The qualitative detection of the various phytochemicals was carried out by using Mayer's and Wagner's reagents (alkaloids). Other tests carried out include the modified Keller–Killiani test for glycosides, foam test (saponins), Salkowski and Liebermann

Burchard's tests (steroids and triterpenoids), stain test (fat and oil), and ferric chloride (phenols and tannins) and lead acetate (flavonoids) tests.

Quantification of Total Tannin Content

Tannin determination was evaluated according to the method depicted by Van Buren and Robinson with slight alteration as illustrated by Kaur and Arora ("BMC complementary and Alternative Medicine, vol.9, no.1, P. 30, 2009 using tannic acid as standard. Two hundred and fifty milligrams (250 mg) of the extracts was added to 50 mL of distilled water in a conical flask. The mixture was agitated for 1 h by using a mechanical shaker and subsequently filtered into a 50 mL volumetric flask and made up to the final volume by addition of distilled water. An aliquot (1 mL) of the filtrate was mixed with 4 mL of distilled water and treated with 2 mL (10-fold dilution) of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M potassium ferrocyanide. The resultant solution was mixed thoroughly and allowed to stay for 10 minutes; the absorbance was measured at 605 nm against the blank. The quantification was carried out based on the 7-point standard calibration curve of tannic acid (20, 40, 60, 80, 100, 140, 200 mg/L) in distilled water. The tannin content was articulated as tannic acid equivalents (TAEs) in milligram per 100 grams of the dry material.

Phosphomolybdate Assay

The total antioxidant ability of C. citrinus extracts was examined by phosphomolybdate technique with ascorbic acid as standard. A portion (0.1 mL) of the sample extracts was added with 1 mL of reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. Different tubes containing the mixture were covered with an aluminium foil and incubated in a water bath at 95°C for 90 min. The test tubes were brought out of the water bath and allowed to cool to ambient temperature; the absorbance of the mixture was then measured at 765 nm against the blank, using ascorbic acid as the standard.

Quantification of Total Phenolic Content

The phenolic contents of both extracts were examined via a spectrophotometric method. About 1 mg/mL of each extract in 1 mL of solvent was added to 1 mL of Folin–Ciocalteu reagent in different test tubes, and the mixture was left for about 4 minutes, and then, 10 mL of 7% Na_2CO_3 solution and 13 mL of deionized distilled water were added to the above mixture. The tubes were vortexed for about 25 seconds and kept in the dark at 25°C for colour development; absorbance was read at 750 nm. The analyses were done in triplicate, and the results were expressed as mg GAE/100 g of gallic acid using a prepared calibration curve with linear equation presented as follows: $y = 0.009x + 0.012$ ($R^2 = 0.999$), where x is the concentration and y is the gallic acid equivalent.

Quantification of Total Flavonoid Content

The method of Ordonez was employed to determine the total flavonoid content. About 0.5 mL of 2% $AlCl_3$ in ethanol solution was mixed with 0.5 mL of the extracts and kept at 25°C for 1 h; absorbance was measured at 420 nm, and the flavonoid content was expressed as mg RE/100 g of rutin using the equation as follows: $y = 0.023x + 0.022$ ($R^2 = 0.982$), where x is the concentration and y is the rutin equivalent.

Quantification of Total Flavonol Content

The evaluation of the total flavonol content in the leaf extracts was done in accordance with the method of Kumaran [40], in which 2.0 mL of the sample, 3.0 mL of sodium acetate (50 g/L), and 2.0 mL of aluminium trichloride prepared in ethanol were mixed together. The absorbance of the mixture was measured at 440 nm after 2.5 h at 20°C. Total flavonol content was then estimated as

mg QE/100 g of quercetin equivalent (QE) from the calibration curve using the equation: y = 0.003x − 0.003, R2 = 0.998, where x is the concentration and y is the quercetin equivalent.

RESULTS

TABLE 1: FUNGAL CHARACTERIZATION

Fungal Isolates
Macroscopic examination
Microscopic examination
Probable organism
% Incidence

Isolate A
Colonies with a small circular, convex, undulate, smooth opaque and creamy.
Budding, spherical to enlongate cells, forming pseudomycelium Candida sp60

Isolate B
Growth rate is rapid and texture of colonies are powdery and produced a radial fissures in the agar.
Surface colony colour was initially white becoming black.
Hyphae are septate and hyaline and conidial heads are radiate and subglobose with metulae that supports the phialides. Aspergillus niger20

Isolate C
Distinctive green colony colour with granular surface, radial rugae and white apron at the periphery.
Filamentous and septate with erect conidiophores terminating in whorls of phailides. Penicillium sp.20

Table 2: Qualitative Screening of the plant species studied.

Tanin	Phylate	Phenol	Flavonoids	Terpenoid	Oxalate	Alkanoids
2.87	9.8.	34.51.	14.1.	11.4	0.57.	N9.65

DISCUSSION

Phytochemicals are non-nutritive chemicals found in plants, having protective characteristics from diseases which have been considered to be useful and beneficial to the health of humans.
Based on the present study, this phytochemical content in the plant species indicates that the plant is of medicinal values as reported by other researchers,
The qualitative screening for phytochemicals revealed that the plants that had the highest quantity in Phenol (34.51 – 0.72), Flavonoids (14.1 – 2.7), Terpernoid (11.4 – 3.40), Phylate (9.8 – 1.23), Alkanoids (9.65 – 0.75) , Tanin (2.87 – 0.52) and Oxalate (0.57- 0.22) had the lowest quantity in the plant (Table 2).
Fungi Isolation
The fungi associated with the spoilage of Negro pepper sold in Mile 3 Market, port Harcourt, Rivers State, Nigeria were studied and the result revealed the presence of a teeming population of

fungi. In (Table 1) the highest colony count was Candida sp 60% followed by 20% of Aspergillus niger and 20% of Penicillium spp..

Phytochemical Screening

The phytochemical study of the Negro pepper extracts both revealed the presence of different bioactive compounds such as alkaloids, terpenoids, flavonoids, phenols, and tannins. Bioactive compounds stored in plant possess biological and antibacterial activities that can be used as an alternative medicine for the treatment of bacterial infections in man (Nnodim et al, 2013). These compounds have been reported to bestow resistance in opposition to microbial pathogens and this could be accountable for the exhibition of antibacterial activity by both extracts in this present study. Also, secondary metabolites like terpenoids have been reported to have antiinflammatory, antimalarial, antibacterial, and antiviral activities and reported to inhibit cholesterol synthesis (Mahato et al, 1997). Alkaloids are believed to have a broad range

CONCLUSION AND RECOMMENDATION

Conclusion

This study shows that the plant species contains the bioactive constituents (Phenol, alkanoid, flavonoids, saponin, tannin, terpernoid).

These plant chemicals give the plant medicinal value to humans as they are applied in the treatment and application of many health conditions such as malaria, asthma, skin irritations etc.

The phytochemical screening showed that the aqueous extracts prepared from the fruits of *Xylopia aethiopica* revealed the presence of phenols, tannins and flavonoids. The presence of a variety of secondary metabolites in *Xylopia aethiopica* fruits contributes to the biological and pharmacological effects associated with the consumption of this plant.

Recommendation

I recommend that extensive studies should be done on the plant species to help ascertain other phytochemicals and nutritive compositions present in the plant and their effectiveness in the production of the pharmaceuticals and also in food.

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