Antifungal Activities of Ethyl Acetate and Methanol Extracts of *Annona Muricata* Aerial Part

Tojola O.B.
Ph.D. Student, Department of Chemistry, Federal University of Technology Akure Ondo State, Nigeria

Lajide L.
Lecturer, Department of Chemistry Federal, University of Technology Akure, Ondo State, Nigeria

Owolabi B.J
Lecturer, Department of Chemistry, Federal University of Technology, Akure, Ondo State, Nigeria

Olaleye M.T
Lecturer, Department of Biochemistry, Federal, University of Technology, Akure, Ondo State, Nigeria

Okoh S.O
Chief Research Officer, Department of CFE, Federal Institute of Industrial Research Oshodi, Nigeria

**Abstract:**
The medicinal plants are the plants people use with the intention of maintaining good health and as they are known to contain bioactive compounds that have therapeutic properties. The antifungal activities of *Annona muricata* aerial part extracts against *Candida albicans*, *Penicillium notatum*, *Aspergillus niger*, *Rhizopus stolonifer* were investigated. The medicinal plants were dried and extracted sequentially using hexane, ethyl acetate and methanol respectively. The ethyl acetate extract of *Annona muricata* aerial part at 100mg/ml concentration exhibited the highest value of zone of inhibition of 19.0±1.00mm against *Candida albicans*, *Aspergillus niger* and *Rhizopus stolonifer* respectively, while the methanol extract had the highest zone of inhibition at 100mg/ml concentration was 15.0±1.00mm against *Candida albicans*. Ethyl acetate extract of *Annona muricata* aerial part exhibited zone of inhibition in all the concentrations while its methanol extract did not exhibit zone of inhibition at 12.5mg/ml, 6.25mg/ml and 3.125mg/ml concentrations. Ethyl acetate had the minimum inhibitory concentration (MIC) of 1.25mg/ml against all the test organisms. In this research work the ethyl acetate extract of *Annona muricata* aerial part proved to have a better antifungal property against *Aspergillus niger*, *Penicillium notatum* and *Rhizopus stolonifer*.

**Keywords:** Medicinal plant, zone of inhibition, maceration, antifungal drug

1. Introduction
A medicinal plant is a plant that is used with the intention of maintaining health, to be administered for a specific condition, or both, whether in modern medicine or in traditional medicine[1][2]. Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value[3]. The diseases that have been managed traditionally using medicinal plant include epilepsy, malaria, convulsion, dysentery, fungal and bacterial infections[4]. It was reported that the minimum inhibitory concentration of ethanolic extract of *A. muricata* was found to be 1 mg/ml, and shows that the *A. muricata* could be used as the potential antitherapeutic drugs[5]. The extracts of *A. muricata* possess potent in vitro antioxidant activity as compared to other *Annona* species, suggesting a role as an effective free radical scavenger[6]. The seeds of *A. muricata* has been found to be potent against internal or external parasites, head lice, and worms[7]. Traditionally, *Annona muricata* leaves had been used to treat headaches, hypertension, cough, asthma and used as antispasmodic, sedative, and nerve for heart condition[8,9]. *A. muricata* leaf extract exhibits a broad spectrum of activity against a panel of bacteria (*B. subtilis*, *Staph aureus*, *K. pneumonia*, *P. vulgaris*, etc.) responsible for common bacterial diseases like pneumonia, diarrhea, UTIs and skin infections[10]. The leaves of *A. muricata* has been found to exhibit a significant inhibition against some selected groups of fungi as *Alternaria solani*, *Alternaria albicans*, *Aspergillus fumigatus* and *Penicillium chrysogenum*[11]. Due to the problem of antimicrobial resistant by the organisms, there is need for research and development on medicinal plants as to solve the problem of fungi infections. The main research objective was to investigate and compare the antifungal properties of the ethyl acetate and methanol extracts of *A. muricata*. 
2. Materials and Methods

2.1. Collection and Authentication of Plant Materials

The plant was obtained locally from a farmland in Lagos, Lagos State, Nigeria and the plant specimens were identified by a taxonomist in the Department of Microbiology, University of Lagos, Lagos, Herbarium. The voucher specimen was deposited at the Herbarium of Department of Microbiology, University of Lagos, Lagos State.

2.2. Preparation of Extracts

The plant material was air dried under shade, grounded to coarse powder. The dried plant material was sequentially extracted using hexane, ethyl acetate and methanol respectively using the method of maceration at normal room temperature for three days according to Handa et al., 2008[12]. The extract was filtered and then distilled off the extracting solvent by drying it on an evaporating dish under a mild temperature.

2.3. Microorganisms Used

Four strains of fungus were used for this study and they were Candida albicans, Penicillium notatum, Aspergillus niger, Rhizophus stolonifera. The fungus was maintained on the prepared sterile Sabouraud dextrose agar medium.

2.4. Antifungal Activity Assay

Antifungal activities of the ethyl acetate extract and methanol extract of Annona muricata were determined by using surface plate method (agar diffusion) on a sterile Sabour and Dextrose Agar. A sterile Sabourand Dextrose Agar was prepared accordingly and aseptically poured into the sterile petri dishes in duplicates and allowed to set properly. 0.2ml of the diluted organism (10^-2) was spread on the agar using a sterile cork borer of the 6mm diameter. Tioconajole (70%) was used as the positive control while the solvent of extraction was used as the negative control. In each of the wells the graded concentrations of the sample were introduced into the wells including the controls. The plates were then left on the bench for 2 hrs. so as to allow the sample to diffuse properly into the agar. The plates were incubated uprightly in the incubator for 48 hrs. at 26-28°C. The fungi plates were observed after 48hrs of incubation and the clear zones of inhibition were measured in millimeter.

2.5. Minimum Inhibition Concentration (MIC)

The Minimum inhibitory concentration of the extracts was determined using agar dilution method, 2mls each of the prepared concentrations was added to 18mls of Muller Hinton Agar, shook gently and poured into the sterile petri dishes, which means both the agar and sample were poured together and allowed to set inside the sterile plates. The diluted organisms (10^-2) to be used were then streaked on the agar at different concentrations of the same sample. The fungi plates were incubated for 48hrs at 26-28°C. The fungi plates were observed after 48hrs for the presence of growth in the plates.

3. Results

3.1. Antifungal Activity

| S/N | Concentration (mg/ml) | Zone of Inhibition (mm) |
|-----|-----------------------|-------------------------|
|     |                       | Candida albicans | Penicillium notatum | Aspergillus niger | Rhizophus stolonifer |
| 1   | 100.0                 | 19.0 ±1.00 | 18.0±0.00 | 19.0±1.00 | 19.0±1.00 |
| 2   | 50.0                  | 17.0±1.00 | 16.0±0.00 | 17.0±1.00 | 17.0±1.00 |
| 3   | 25.0                  | 15.0±1.00 | 14.0±0.00 | 15.0±1.00 | 15.0±1.00 |
| 4   | 12.5                  | 14.0±0.00 | 12.0±0.00 | 14.0±0.00 | 14.0±0.00 |
| 5   | 6.25                  | 12.0±0.00 | 10.0±0.00 | 12.0±0.00 | 12.0±0.00 |
| 6   | 3.125                 | 10.0±0.00 | 10.0±0.00 | 10.0±0.00 | 10.0±0.00 |
| 7   | +Ve                   | 27.0±1.00 | 26.0±0.00 | 26.0±0.00 | 27.0±1.00 |

Table 1: Antifungal Activity of Ethyl Acetate Extracts of Annona muricata against Four Fungal Strains at Different Concentrations
Table 2: Antifungal Activity of Methanol Extracts of Annona Muricata against Four Fungal Strains at Different Concentrations

| S/N | Concentration (mg/ml) | Candida albicans | Pencillium notatum | Aspergillus niger | Rhizophus stolonifer |
|-----|-----------------------|------------------|-------------------|------------------|---------------------|
| 1   | 100.0                 | 15.0±1.00        | 14.0±0.00         | -                | -                   |
| 2   | 50.0                  | 13.0±1.00        | 12.0±0.00         | -                | -                   |
| 3   | 25.0                  | 10.0±0.00        | 10.0±0.00         | -                | -                   |
| 4   | 12.5                  | -                | -                 | -                | -                   |
| 5   | 6.25                  | -                | -                 | -                | -                   |
| 6   | 3.125                 | -                | -                 | -                | -                   |
| 7   | -Ve                   | -                | -                 | -                | -                   |
| 8   | +Ve                   | 28.0±0.00        | 27.0±1.00         | 27.0±1.00        | 27.0±1.00           |

Figure 1: Comparison of the Minimum Inhibitory Concentrations of the Extracts

3.2 Antifungal Activity

The antifungal properties of ethyl acetate and methanol extracts of Annona muricata aerial part was carried out using four organisms at different extract concentrations, while Tioconajole (70%) was used as standard drug (positive control). At 50.0mg/ml concentration, the ethyl acetate extract as shown in Table 1 exhibited 17.0±1.00mm zone of inhibition against Candida albicans, Aspergillus niger, Rhizophus stolonifer while it exhibited 16.0±0.00mm zone of inhibition against Penicillium notatum. In Table 2, the methanol extract showed no zone of inhibition against Aspergillus niger and Rhizophus stolonifer at all concentrations, while at 25.0mg/ml concentration; it exhibited 10.0±0.00mm zone of inhibition against Candida albicans and Penicillium notatum. The results in the two tables showed that the zone of inhibition exhibited by the extracts varied directly with the extract concentrations.

3.3 Minimum Inhibitory Concentration

The results of the minimum inhibitory concentrations of the two extracts against Candida albicans, P. notatum, A. niger and R. stolonifera are shown in Figure 1. The ethyl acetate extract exhibited 1.25mg/ml concentration as its minimum inhibitory concentration for all the organisms. The results showed that the methanol extract only exhibited minimum inhibitory concentration against two of the test organisms. It exhibited 5.0mg/ml concentration as its minimum inhibitory concentration against Candida albicans and P. notatum.

4. Discussion

The plant A. muricata is known to have a wide spectrum of biological activities, the medicinal property of this plant is due to the presence of various bioactive constituents it possesses. Since creation man has been depending on plants and plant extracts as a source of medicine, food, shelter etc.[13]. In this study, the antifungal activities of the ethyl acetate and methanol extracts against Candida albicans, Penicillium notatum, Aspergillus niger and Rhizopus stolonifera were determined using surface plate method (agar diffusion) on a sterile Sabouraud Dextrose Agar. In Table 1, the ethyl acetate extract in all the concentrations was active against the fungi used while in Table 2, the methanol extract was only active against Candida albicans and Penicillium notatum at higher concentrations. It was not active against Aspergillus niger and Rhizopus stolonifera in all the concentrations. It was reported that A. muricata plant extract possess antimicrobial activities.[14]. The ethyl acetate extract was more active against the organisms than the methanol extract, at 100.0mg/ml concentration it exhibited the highest zone of inhibition of 19.0±1.00mm against Candida albicans, Aspergillus niger and Rhizopus stolonifera. The aqueous extract of Annona reticulata showed only 41.0% inhibition against Botrytis cinera, only
11.0% against Aspergillus niger and 38.0% against Rhizopus stolonifer, [15]. The A. muricata and A. squamosa have been found to have better insecticidal properties in their family [16]. The degree of activity of the extracts against the organisms varies with the extract concentrations and the two extracts were found less active than the standard fungi drug used.

5. Conclusion
This study has shown that the ethyl acetate extract of A. muricata aerial part was more active than the methanol extract of Annona muricata aerial part. In comparison, with the standard fungi drug used, the two extracts have low activity, and this has proved that the ethyl acetate and methanol extracts of Annona muricata aerial part do not have antifungal property. Therefore, for further work, I recommend the determination of antifungal property of the other parts of A. muricata plant, as they may possess bioactive compounds of good antifungal property.

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