

# Antimicrobial Activity of Terpenoids Extracted from *Annona muricata* Seeds and its Endophytic *Aspergillus niger* Strain SH3 Either Singly or in Combination

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## Abstract

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**Keywords:** *Annona muricata*; Antimicrobial Activity; Combined extract; Endophytic Fungi and Terpenoids

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**BACKGROUND:** *Annona muricata* (Soursop) has an antimicrobial activity toward various pathogenic microorganisms which support its ethnomedicinal for the treatment of many infectious diseases.

**AIM:** Aim of the present study to evaluate the relation between antimicrobial activities of terpenoids extracted from different soursop parts with the isolated endophytic fungi.

**METHODS:** Endophytic fungal species of pulp and peel of *Annona* fruit along with those of seeds were isolated. Salkowski test was used for qualitative screening of terpenoids in plant and the isolated endophytic *Aspergillus niger* strain SH3.

**RESULTS:** Endophytic *A. niger* strain SH3 and *Annona* seed extract showed high terpenoid content indicated by the high intensity of reddish-brown colour. GC/Mass analysis revealed six compounds of terpenoids from endophytic *A. niger* strain SH3 extract and four compounds from seed extract with different retention times. The antimicrobial assay was performed using *A. niger* strain SH3 extract and *Annona* seed extract singly or in combinations against *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans*.

**CONCLUSION:** The results revealed the significant antimicrobial activity of both extracts. However, the combined extract showed some reduction in antimicrobial activity which could be attributed to the antagonistic effect exhibited by their constituents.

## Introduction

*Annona muricata* (Annonaceae) is a tropical plant species known for its edible fruits. It is called soursop, which has some medicinal merits and some toxic effects. Extracts of *A. muricata* have been famous for their antimicrobial, anti-inflammatory, anti-protozoan, antioxidant, insecticide, larvicide, and cytotoxic activities. Mechanisms of action of some pharmacological effects have been declared, such as cytotoxic, antioxidant, antimicrobial and anti-hypertensive activities [1]. *Annona* extracts from its leaves, roots, and seeds have shown antibacterial activity against a plethora of microorganisms. Endophytic fungi from medicinal plants can be considered as a reservoir of bioactive metabolites

which include terpenoids, alkaloids, flavonoids, phenolic acids, quinones, steroids, tetralones and xanthenes [2], [3].

Dicotyledonous plants such as soursop are proposed to have endophytic microorganisms which are a potential medicinal source. Endophytic microorganisms usually create symbiotic interactions with plant tissues. Several plant endophytic fungi have been shown to have antimicrobial activity [4]. The present study was performed to evaluate the antimicrobial activity of terpenoids in *Annona* seed and its fungal extracts. The combination of *Annona* seed extract and endophytic *A. niger* strain SH3 has studied also.

## Material and Methods

*Annona muricata* (Fruits and seeds) were collected from Abo-Rawash farms, Egypt. The seeds were surface-sterilised then air-dried before grinding into powder at room temperature and weighed.

#### **Isolation and identification of endophytic fungi from seed, pulp and peel of *Annona* fruit**

Plant parts were separated, two fresh fruits were washed thoroughly with tap water, surface sterilised with 70% ethanol for 1 min, 4% sodium hypochlorite for 3 min and again with 70% ethanol for 1 min, then rinsed twice with sterile distilled water. Samples were dried with sterile filter paper and cut into small pieces with sterile forceps and sterile gloves worn [5]. The fruits were then peeled, and small strips of each were obtained aseptically and plated on potato dextrose agar medium (PDA) containing chloramphenicol to suppress bacterial growth. Plates were then incubated at 25-27°C until the outgrowths of fungi from the explants were observed. The fungal growths were subcultured to produce pure culture on Czapek-dox's plates. All isolates were maintained in Czapek-dox's slants and kept at 4°C. The same procedure was applied to pulp and peel of rotten fruits where the fruits were placed in sterile polyethylene bags and stored for one week to allow deterioration. Isolation of endophytic fungi from *Annona* seeds was done by adding seed powder on the plate's surfaces, then was incubated. The endophytic fungal isolates were identified morphologically and microscopically according to Moubasher [6].

#### **Extraction of terpenoids from *Annona* seeds**

Terpenoids extraction from seeds were performed by agitation with ethanol (250 mL / 20 g of seed powder) three times for 48 hours on an orbital shaker. The extract was concentrated under vacuum till dryness. The concentrated extract was then stored in a vacuum desiccator at room temperature for further use.

#### **Extraction of terpenoids from *Annona* endophytic fungi**

The isolated fungi were grown in 2-litre standard flasks containing 500 ml of Potato Dextrose Broth. After 3 weeks of culturing at 25°C, the culture fluids were passed through four layers of cheesecloth to remove solids. To the culture filtrate, 0.25 g sodium carbonate was added with frequent shaking to reduce the number of fatty acids that may contaminate the culture; then terpenoids were extracted with two equal volumes of ethyl acetate solvent. Ethyl acetate layers were collected, concentrated and evaporated to dryness. Residues were stored for subsequent

analysis [7].

#### **Qualitative assay of terpenoids**

Test for terpenoids (Salkowski test): Five ml of each extract was mixed with 2 ml of chloroform, then concentrated H<sub>2</sub>SO<sub>4</sub> (3 ml) was carefully added to form a layer. A reddish-brown colouration at the interface was formed, indicating positive results for the presence of terpenoids [8].

#### **The GC-MS analysis of fungal and seed terpenoids**

The analysis was carried out using a GC/MS (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD Agilent 7000) equipped with an apolar Agilent HP- 5 ms (5%-phenyl methyl polysiloxane) capillary column (30 m × 0.25 mm i.d. and 0.25 µm film thickness). The carrier gas was helium with a linear velocity of 1 ml/min. The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature [9].

#### **Assay of antimicrobial activity**

Antimicrobial activities of the terpenoids extracted from fungal *Aspergillus niger* SH3 and seed extract singly and in combination were tested against one Gram +ve bacterial species (*Staphylococcus aureus*), two Gram -ve bacterial species (*Escherichia coli* and *Pseudomonas aeruginosa*) and one fungal species (*Candida albicans*). The assay was performed using the Kirby-Bauer disc diffusion method [10], [11]. Nutrient agar (NA) medium was used for testing of bacteria, while candida agar (CA) medium was used for fungi.

The pathogens were inoculated by streaking over the surface of the sterilised media. Fungal and *Annona* extracts were applied at the surfaces of plates at 5 mg/disc dissolved in DMSO. Petri dishes were incubated at 37°C for 48 h for bacterial species and 25°C for 72 h for *Candida*. Sensitivity was then determined by measuring the mean diameter of the inhibition zones in mm. Ampicillin (5 mg/disc) was used as a positive control for bacteria. Amphotericin B (5 mg/disc) was used as a positive control for *C. albicans*. A control test for the solvent only was also performed.

#### **Determination of relative activity**

The relative activity of the tested extract concerning positive control was calculated by using



in Table 4 revealed that the two extracts had antimicrobial activity against gram +ve bacterial species and gram -ve bacterial species, but *C. albicans* was resistant towards any of them singly and in combination. *P. aeruginosa* was strongly susceptible to the inhibitory action of both extracts. Moreover, *E. coli* and *S. aureus* were very sensitive, respectively. The combined extract showed a reduction in antimicrobial activity which could be attributed to the antagonistic effect between both seed and endophytic *A. niger* strain SH3 extracts. It is worth noting that the inhibitory action of the seed endophytic fungal species was more than that of the seed extract itself which clarifies that the endophytic microorganisms may be the source of the biological activity of the higher plant by its 2ry metabolites or at least intensify these activities.

**Table 4: Antimicrobial activities of extracts of *A. niger* strain SH3 isolated from *A. muricata* and seed extract (singly and in combination)**

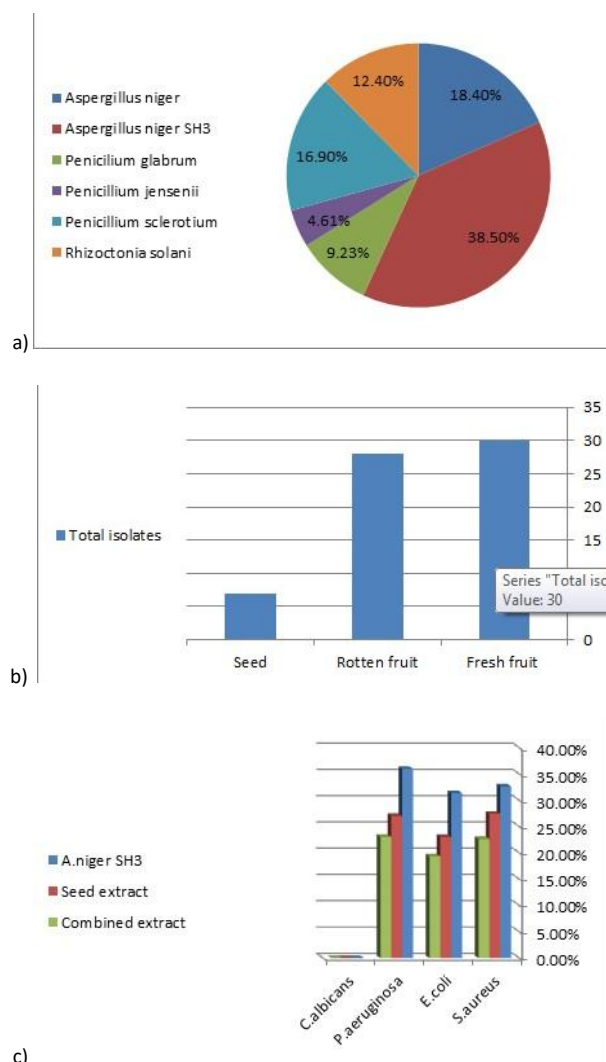
Pathogenic Microorganism	Inhibition zone diameter (mm)				
	Negative Control	Positive Control	<i>A. niger</i> strain SH3 extract	Seed extract	Combined Extract
Gram +ve bacteria					
<i>S. aureus</i>	0 <sup>a</sup> ± 0.0	21 <sup>a</sup> ± 0.1	12 <sup>b</sup> ± 0.5	11 <sup>a</sup> ± 0.0	10 <sup>a</sup> ± 0.0
Gram -ve bacteria					
<i>E. coli</i>	0 <sup>a</sup> ± 0.0	25 <sup>b</sup> ± 0.0	14 <sup>a</sup> ± 0.0	12 <sup>a</sup> ± 0.0	11 <sup>b</sup> ± 0.5
<i>P. aeruginosa</i>	0 <sup>a</sup> ± 0.0	25 <sup>a</sup> ± 0.1	15 <sup>a</sup> ± 0.0	13 <sup>b</sup> ± 1.5	12 <sup>a</sup> ± 0.0
Yeast					
<i>C. albicans</i>	0 <sup>a</sup> ± 0.0	21 <sup>b</sup> ± 0.0	0 <sup>a</sup> ± 0.0	0 <sup>a</sup> ± 0.0	0 <sup>a</sup> ± 0.0

The study supports the ethnomedicinal use of *A. muricata* for treatment of many infections. The results of antimicrobial activity of *A. niger* strain SH3 extract, seed extract and in combination were compared with positive control either Ampicillin or Amphotericin B for evaluating their relative percentage inhibition, where *A. niger* strain SH3 extract exhibits maximum relative percentage inhibition against *P. aeruginosa* (36%), (32.65%) against *S. aureus* (31.37%) against *E. coli* followed by seed extract and combined extract showed the least percentage (Table 5).

**Table 5: Relative activity compared to the standard positive control**

Pathogenic Microorganism	Relative activity (%)		
	<i>A. niger</i> strain SH3 Extract	Seed Extract	Combined Extract
<i>S. aureus</i>	32.65	27.43	22.67
<i>E. coli</i>	31.37	23.04	19.36
<i>P. aeruginosa</i>	36.00	27.05	23.05
<i>C. albicans</i>	0.00	0.00	0.00

Mathew *et al.*, [20] proved the effectiveness of *A. muricata* leaf extract as an antibacterial agent against *Enterococcus faecalis*.



**Figure 1: a) Frequency percentage of endophytic fungal species isolated from different parts of *A. muricata*; b) Total isolates of endophytic fungi isolated from different parts of *A. muricata*; c) Relative activity % of *A. niger* strain SH3 and seed extracts either singly or in combination**

## Discussion

In relations to our study, the fungal genera *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus* were isolated from *A. muricata* as endophytes [13], [14]. We can conclude that among the endophytic flora, *Aspergillus* was found to be the core group fungus from *Annona* with a colonisation frequency of 56.9%.

Salkowski test was more precise for checking terpenoids [15]. The appreciable amount of terpenoids may be due to the precise extraction process which influences the number of secondary metabolites [16].

Abdelhamid *et al.*, [17] reported that GC-MS chromatogram of the ethanolic extract of *Nelumbo nucifera* seed showed thirty-eight peaks which indicates the presence of thirty-eight phytochemical

constituents including [[trimethylsilyl]oxy] methyl]ethyl ester, Anodendroside E2 monoacetate, Betulin and Cholestan-3-one, cyclic 1,2-ethanediylacetal, (5 $\alpha$ ) exhibited various biological activities including Antiinflammatory, Antitumor, Antiviral, Cytotoxic and Hypolipemic [18]. Venkatachalam and Jyothiprabha, 2016 also reported antimicrobial activity of cinnamon (*Cinnamomum Verum*) extracts against Vancomycin-Resistant Enterococcus due to the presence of thirty major antimicrobial compounds identified by GC-MS analysis including Anodendroside F.

In this field, *Annona muricata* leaf extracts at a potency 20 mg/ml showed antimicrobial activity when tested against *P. aeruginosa*, *E. coli*, *S. aureus* and *C. albicans* with inhibition zones ranging from 20 to 42 mm [19].

The synergism of flavonoids, terpenoids, and alkaloids found in the extracts of *A. muricata* explains its antibacterial activity [1], [21]. It is reported that endophytic fungi from the same host plant could contain identical bioactive compounds but showed different activity [22]. Also, the combination of ethanolic extract of sour soup with antibiotic treatment increased the effectiveness of the antibiotic against multidrug-resistant strains of *E. coli* and *S. aureus* [2], [16].

In conclusion, *Annona muricata* with its endophytic fungi have an important role as antimicrobial agents against certain microorganisms. So, *Annona muricata* can be used for treatment of many infections which could be attributed to presence of terpenoids.

## References

- Coria-Téllez AV, Montalvo-González E, Yahia EM, Obledo-Vázquez EN. *Annona muricata*: a comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arabian J Chem*. 2016; 1:1-4.
- Sandhu S, Gupta D, Mandal R, Puttey SJ. Screening of endophytic fungi isolated from some medicinal plants in Jabalpur region for antibacterial activity. *World J Pharm Pharm Sci*. 2014; 3:1655-1666.
- Tan RX, Zou WX. Endophytes: a rich source of functional metabolites. *Nat Prod Rep*. 2001; 8:448-459. <https://doi.org/10.1039/b100918q> PMID:11548053
- Katoch M, Singh G, Sharma S, Gupta N, Sangwan PL, Saxena AK. Cytotoxic and antimicrobial activities of endophytic fungi isolated from *Bacopa monnieri* (L.) Pennell (Scrophulariaceae). *BMC Complement Altern Med*. 2014; 14:1-8. <https://doi.org/10.1186/1472-6882-14-52> PMID:24512530 PMID:PMC3930298
- Nath A, Joshi SR. Ultrastructural effect on mastitis pathogens by extract of endophytic fungi associated with ethnoveterinary plant, *Hibiscus sabdariffa* L. *J Micro Ultra*. 2015; 3:38-43. <https://doi.org/10.1016/j.jmau.2014.10.001> PMID:30023180 PMID:PMC6014217
- Moubasher AH, Moustafa AF. A survey of Egyptian soil fungi with special reference to *Aspergillus*, *Penicillium* and *Penicillium* related genera. *Trans Br Mycol Soc*. 1993; 54:35-44. [https://doi.org/10.1016/S0007-1536\(70\)80121-8](https://doi.org/10.1016/S0007-1536(70)80121-8)
- Kalaiselvam M, Elavarasi A, Rathna GS. Taxol producing mangrove endophytic fungi *Fusarium oxysporum* from *Rhizophora anomalayana*. *Asian Pac J Trop Biomed*. 2012; S:1081-1085. [https://doi.org/10.1016/S2221-1691\(12\)60365-7](https://doi.org/10.1016/S2221-1691(12)60365-7)
- Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plant. *Afr J Biotechnol*. 2005; 4:685-688. <https://doi.org/10.5897/AJB2005.000-3127>
- Santanal PM, Mirada M, Payrol JA, Silva M, Hernández V, Peralta E. Gas Chromatography-Mass Spectrometry Study from the leaves fractions obtained of *Vernonanthura patens* (Kunth) H. Rob. *Int J Org Chem*. 2013; 3:105-109. <https://doi.org/10.4236/ijoc.2013.32011>
- NCCLS. Reference Method For Broth Dilution Antifungal Susceptibility Testing of Conidium-Forming Filamentous Fungi: Proposed Standard M 38-A. NCCLS. 2002; 22:1-30.
- National Committee for Clinical Laboratory Standards. Method for antifungal disk diffusion susceptibility testing of yeast: proposed guideline M44-P. 2003; 24:1-23.
- Gaurav K, Karthik L, Bhaskara Rao KV. In vitro anti-Candida activity of *Calotropis gigantea* against clinical isolates of *Candida*. *J Pharm Res*. 2010; 3:539-542.
- Ajiboye AE, Ajuwon AB, Adedayo MR. Physicochemical and microflora associated with the spoilage of soursop fruits (*Annona muricata*). *Adv Biotechnol Res*. 2014; 1:1-9.
- Okwulehie IC, Alfred NK. Fungi associated with deterioration of soursop (*Annona muricata*, Linn) fruits in Abia State, Nigeria. *Afr J Microbiol Res*. 2010; 4:143-146.
- Agu KC, Okolie N. Proximate composition, phytochemical analysis, and in vitro antioxidant potentials of extracts of *Annona muricata* (Soursop). *Food Sci Nutr*. 2017; 5:1029-1036. <https://doi.org/10.1002/fsn3.498> PMID:28948021 PMID:PMC5608983
- Biqiku L, Lupidi G, Petrelli D, Vitali LA. Antimicrobial Activity of Single and Combined Extracts of medicinal plants from Cameroon. *J Pharm Biol Sci*. 2016; 4:86-90. <https://doi.org/10.9790/3008-1104048690>
- Abdelhamid MS, Kondratenko EI, Lomteva NA. GC-MS analysis of phytochemicals in the ethanolic extract of *Nelumbo nucifera* seeds from Russia. *J App Pharm Sci*. 2015; 5:115-118. <https://doi.org/10.7324/JAPS.2015.50419>
- Venkatachalam P, Jyothiprabha V. Phytochemical screening and antibacterial activities of Cinnamon against Vancomycin resistant *Enterococcus*. *Int J Sci Res*. 2016; 5:309-312. <https://doi.org/10.20546/ijcmas.2016.502.013>
- Olugbuyiro JAO, Omotosho OE, Taiwo OS, Ononiwu FO, Banwo AS, Akintokun OA, Obaseki OS, Ogunleye OM. Antimicrobial activities and phytochemical properties of *Annona muricata* leaf. *Coven J phys life Sci*. 2017; 5:40-49.
- Mathew J, George R, Theruvil R, Padavil TC, Tomy L, Kurian A. Antibacterial Activity of Leaf Extract of *Annona muricata* and *Simarouba glauca* on *Enterococcus faecalis*. *J Contemp Dent Pract*. 2016; 17:650-653. <https://doi.org/10.5005/jp-journals-10024-1906> PMID:27659082
- George VC, Kumar DR, Suresh PK, Kumar RA. Antioxidant, DNA protective efficacy and HPLC analysis of *Annona muricata* (soursop) extracts. *J Food Sci Technol*. 2015; 52: 2328-2335. <https://doi.org/10.1007/s13197-014-1289-7> PMID:25829616 PMID:PMC4375238
- Kaur R, Kapoor K, Kaur H. Plants as a source of anticancer agents. *J Nat Prod Plant Resour*. 2011; 1:119-124.