ACTA MICROBIOLOGICA BULGARICA Volume 39 / 2 (2023)

Antifungal Activities of *Sida acuta Burm. f.* Leaf Extracts on the Fruit Rot Pathogen of *Annona muricata L.*

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Abstract

This study investigated the antifungal activities of *Sida acuta* Burm. f. leaf extracts against fruit rot fungi of *Annona muricata* (sour sop) and the phytochemical constituent of the plant. Fruits of sour sop (*A. muricata*) and leaves of *Sida acuta* used for the study were collected from different locations within Abraka community, Delta State. Qualitative and quantitative phytochemical screening and Gas Chromatography – Mass Spectrometry analysis were carried out on the extract. Fungi causing rot of *A. muricata* was isolated and antifungal activities of *S. acuta* on isolated fungi was carried out using the poisoned food method. The result showed that both methanol and chloroform extracts of the plant contained flavonoid, tannin, saponin, steroid, glycoside, alkaloid, anthraquinone, terpenoid, and reducing sugar. The GC-MS result revealed the presence of 20 compounds with 3-Trifluoromethylbenzylamine, N,N-diundecyl and L-Proline, N-(2-trifluoromethylbenzoyl)-, isohexyl ester as the most abundant in both methanolic and chloroform extracts. The study identified *Rhizopus stolonifer* as the fungi associated with the rot of *A. muricata* and showed that as the concentration of the extract increased, its antimicrobial activity was significantly higher in the organisms. The phytochemical composition and antifungal effects make *S. acuta* leaf extract a potential agent for use against fungal infections in the future.

Keywords: leaf extract, fungi rot pathogen, *Sida acuta, Annona muricata*

Резюме

Това проучване е фокусирано върху антифунгалната активност на екстракти от листа на *Sida acuta* Burm. f. срещу гъби, предизвикващи гниене на плодове от вида *Annona muricata* (кисел сок), както и техния фитохимичен състав. Плодовете на *A. muricata* и листата на *S. acuta*, използвани за изследването, са събрани от различни места в общността на Абрака, щат Делта. Извършен е качествен и количествен фитохимичен скрининг и газово-хроматографски-масспектрометричен анализ. Антифунгалната активност на екстрактите от на *S. acuta* е изследвана върху гъби, причиняващи гниене на *A. muricata* с помощта на метода на отровената храна.

Резултатът показва, че както метаноловият, така и хлороформният екстракт от растението съдържа флавоноиди, танини, сапонини, стероиди, гликозиди, алкалоиди, антрахинони, терпеноиди и редуциращи захари. Данните от GC-MS анализите на метаноловите и хлороформните екстракти доказват най-значимо наличие на 20 съединения - 3-трифлуорометилбензиламин, N,N-диундецил, L-пролин и N-(2-трифлуорометилбензоил)-изохексил естер. Проучването идентифицира *Rhizopus stolonifer* като причинител на гниенето на *A. muricata* и показа, че с увеличаването на концентрацията на екстракта неговата инхибиторна активност е значително по-висока. Фитохимичният състав и противогъбните ефекти правят екстракта от листа на *S. acuta* потенциален агент за употреба срещу гъбни инфекции в бъдеще.

Introduction

Fungi are among the prominent causal agents of plant diseases. These fungi species attack all plant parts including the leaves, branches, stem, flower, and fruits except the root causing rot diseases (Nweke and Ibiam, 2012). To colonize plants and cause disease, pathogenic fungi use distinct strategies. Fungal pathogens are known to be responsible for the post-harvest impairment of

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several economic fruits including soursop fruits (Nweke and Ibiam, 2012). Post-harvest losses of fruits can be up to 50% and diseases caused by fungi are responsible for about 70% of the total losses (Choudhury *et al*., 2018, Rezaee *et al*., 2018).

Soursop (*Annona muricata* L.) belongs to the family *Annonaceae* and is indigenous to tropical North and South America. In Nigeria, the plant is restricted to the rainforest zones of Nigeria and cultivated mainly in-home gardens in Abia, Imo, Enugu, Rivers, Ebonyi, Anambra, and Delta States (Okigbo *et al.,* 2018). Sour sop tree is a small unusually shaped fruit tree growing up to 8 m high. The flesh of its fruit is pulpy white, stringy, and sour containing shiny black seeds. Furthermore, it may serve as a source of food, sour sop has many curative properties; the juice is diuretic while the other parts have antibacterial, anti-cancerous, astringent, sedative, and other properties (Lans, 2006). The leaves had been traditionally used to treat headaches, hypertension, cough, and asthma and used as antispasmodic, sedative, and nervine for heart conditions.

Infection of fleshy fruits occurs in the field through wounds or bruises made by farm implements where the tissue is in a pre-necrotic process. Symptoms of post-harvest disease of fruits develop mainly during storage, but infection of fruit by decay-causing pathogens could occur preceding harvest or during the post-harvest handling process and storage (Gonzalez-Estrada *et al.,* 2019). Fungal pathogens responsible for the fruit rot and decline of sour sop fruits might have probably resided in dead stems and then dispersed by either rain splash or insect vectors into the growing fruits to initiate infection (Nweke and Ibiam, 2012). Several fungi have been identified that cause spoilage of sour sop fruits such as *Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae, Colletotrichum sp, Fusarium solani, Mucor sp, Penicillium chrysogenium, Penicillium* sp and *Rhizopus stolonifer* among others (Berumen-varela *et al.*, 2019).

Fungal diseases are usually controlled by chemical fungicides such as maneb, captan, and vitigran among others. However, misuse of these fungicides has resulted in environmental pollution and the development of resistance by target organisms which have led to the search for alternative products such as the use of plant extract (Chen *et al.*, 2019). Medicinal plants are known to owe their healing potential to certain biologically active substances, which exist in various parts of the plants (Ekpo and Etim, 2009). The chemicals which are considered phytochemicals include terpenoids, flavonoids, tannins, saponins, steroids, phenolic compounds, and other secondary metabolites which are present in different parts of plants: leaves, stems, roots, inflorescence, flowers, fruits, and seeds (Sathya *et al.*, 2018)

Among plants with the potential to restrain the dangers of fungal-associated deterioration is *Sida acuta* (Burm. f). *S. acuta* (common wire weed) is a shrub widely distributed in the subtropical regions belonging to the Malvacaea family of plants. Indigenous names include Iyeye (Yoruba), Tsadarlamarudu (Hausa), Nsukerra (Efik), and Udo (Igbo) and it is mainly found in bushes, farms, and habitations (Nwankpa *et al*., 2015). With increasing health challenges, surveys conducted in various places illustrated that the plant had many traditional usages (Mbajiuka and Kasim, 2019). *S. acuta* has wide application in Nigerian traditional medicine. Some herbalists/healers have claimed the traditional use of this plant to heal/cure infections such as malaria, ulcer, fever, gonorrhea, abortion, breast cancer following inflammation, and wound infections (Edeoga *et al*., 2005). *S. acuta* leaves extracts have been used *in vitro* and *in vivo* against clinical isolates therefore, it may have agricultural application as a fungicide for the control of post-harvest spoilage of sour sop fruits which has not been evaluated. The aim of this study is to investigate the antifungal activities of *S. acuta* leaf extracts against fruit rot fungi of *A. muricata*.

Materials and Methods

Source of samples

Healthy fruits of sour sop (*A. muricata*) used for the study were purchased from the main market at Abraka, Ethiope East Local Government Area, Delta State, Nigeria. The diseased one was collected from a fruit vendor opposite site 3 main gates Abraka. *A. muricata* fruits were selected on the basis of color and absence of any external injuries. Fresh *S. acuta* leaves used for the study were collected from Awhana avenue in Abraka community. Abraka lies between Latitude $5^0$47'21.96" N of the equator and longitude $6^0$6'8.45" E of the Greenwich meridian.

Preparation of plant extract

The fresh plant sample (leaves) was collected and washed under the running tap water to remove soil particles and other dust particles. The leaves were air-dried under laboratory conditions at room temperature for 15 days. The dried leaves samples were finely grounded to powder with the help of

a mixer grinder. A 10g grounded plant leaf was soaked into 50 ml organic solvents for 24 hrs in an orbital shaker at normal temperature. The extracts were filtered through the Whatmann No: 1 filter paper. The extract was allowed to dry using a rotary evaporator. The condensed extracts were stored in an airtight container at 4^0C till further investigations were carried out.

GC-MS analysis

GC-MS analysis was done at Mifor Consult Laboratory, Calabar, A Shimadzu GCMS-QP 2010 Plus system was used. The GC-MS has operated under the following conditions: Column oven temperature: 70°C; Injection temperature: 250°C; Injection mode: split; Pressure: 104.1 kPa; Total flow: 6.2 ml/min; Column flow: 1.59 ml/min; Linear velocity: 46.3 cm/sec; Purge flow: 3.0 mL/min; and Split ratio: 1.0. The generated chromatogram was recorded. The identification of the components was carried out using the peak enrichment technique of reference compounds and computer matching with those of NIST.05 library mass spectrum.

Phytochemical screening

Phytochemical screening was performed to identify phytochemicals in the extracts of plant leaves used in the study. The phytochemicals were detected by color tests using the method of Chukwuma *et al.* (2016).

Isolation and identification of fungi

Isolation and identification of fungi from diseased *A. muricata* fruits were carried out using the method adopted by Ilondu and Bosah (2015). Sections, 4 mm long, excised from the margins of diseased spot with a sterile razor blade were surface-sterilized for 2 mins in 2% aqueous solution of commercial bleach (sodium hypochlorite solution), rinsed in two changes of sterile distilled water. The disinfected tissue pieces were blotted between sterile Whatman No. 1 filter paper and aseptically plated on potato dextrose agar (PDA) plates (3 pieces per plate). The plates were then incubated at room temperature (32±2°C) for five days. Any observed mycelial growth was repeatedly transferred to fresh PDA plates until pure cultures of isolates were obtained. Identification was based on cultural characteristics and microscopic examination.

Antifungal activity of leaf extracts

Effect of Plant Extracts on Mycelium Growth. The poisoned food technique was adopted in the preliminary screening of plant extracts for their antifungal properties. First, mycelia growths were determined in 60 mm Petri dishes filled with PDA and SDA solid medium amended with the plant extract (0.0625, 0.125, 0.25, 0.5, and 1.0%). Subsequently, the middle of each Petri dish was inoculated with about 5 mm diameter disc of fungal pathogen mycelium, taken from a 7-day-old pure culture. All inoculated dishes were then incubated at 25°C for 6 days. After that time, the radial mycelial growth was measured. For each treatment, three replicates were done. Finally, the antifungal activity of the extract was calculated in terms of the inhibition percentage of mycelia growth using the formula:

$$\% \text{ inhibition} = (dc - dt)\ /dc \times 100$$

Where dc is the average increase in mycelia growth in control and dt is the average increase in mycelia growth in treated plates (Masoud, 2010).

Statistical analysis

Data obtained from the study were recorded and subjected to statistical analysis using Microsoft Excel and the values were expressed as means with their standard error of means.

Results

Phytochemical and GC-MS screening

The results of qualitative and quantitative phytochemical screening of *S. acuta* using methanol and chloroform as extraction solvents are presented in Tables 1 and 2. The methanolic leaf extract of *S. acuta* revealed the presence of high concentrations of flavonoid, tannin, saponin, and terpenoid with moderate concentrations of steroid and alkaloid while glycoside, anthraquinone, and reducing sugar were present in low concentrations.

Table 1. Qualitative phytochemical screening of *Sida actua* leaf extract

	Phytochemicals	Methanol extract	Chloroform extract
1	Flavonoid	+++	+
2	Tannin	+++	+
3	Saponin	+++	+
4	Steroid	++	+
5	Glycosides	+	+
6	Alkaloids	++	+
7	Anthraquinone	+	+
8	Terpenoids	+++	+
9	Reducing Sugar	+	+

Key: + = Low; ++ = Moderate; +++ = High

Table 2. Quantitative phytochemical screening of *Sida acuta* leaf extracts

	Phytochemicals	Methanol (mg/ml)	Chloroform (mg/ml)
1	Tannin	2.2	2.3
2	Flavonoid	3.1	3.0
3	Saponin	3.3	1.6
4	Alkaloids	2.3	2.0

Chloroform extract revealed high concentration of flavonoid and low concentration of saponin, tannin, steroid, glycoside, alkaloid, anthraquinone, terpenoids, and reducing sugar (Table 1). The quantification showed that saponin was higher in methanol and lowest in chloroform extract (Table 2).

Gas chromatography-Mass spectrometry

GC-MS of methanolic and chloroform extracts of *S. acuta* extract revealed the presence of twenty (20) different chemical constituents (Tables 3 and 4). The most abundant compound in the methanolic extract of *S. acuta* was 3-Trifluoromethylbenzylamine, N,N-diundecyl with 23.652% relative abundance followed by 1,3- Benzene, p-bis(4-phenylbutyl)- which recorded 9.577% relative abundance (Table 3). In the chloroform extract, L-Proline, N-(2-trifluoromethylbenzoyl)-, isohexyl ester was the most abundant with 48.546% relative abundance, followed by Emodin, 3TMS derivative with 7.872% relative abundance (Table 4). The chromatogram for both extraction solvents is presented in Fig. 1.

Isolation of fungi and antifungal activities

The study isolated and identified *R. stolonifer* as the fungi associated with fruit rot of *A. muricata* (Table 5). The study showed that as the concentration of both the methanol and chloroform extract increased from 0.625 to 1.0 mg/ml, the effect of the extract was significantly higher on the organisms (Table 6). There was no significant difference in the antifungal activity of the chloroform and methanol extract at the same concentration on the test fungi.

Discussion

Isolation of *R. stolonifer* as the fungi associated with fruit rot of *A. muricata* in this study is in line with the work of Berumen-Varela *et al.* (2019) who isolated the fungi among others. *R. stolonifer* has been known to cause decay in plant products during transit or storage and can cause human diseases such as mucormycosis (Zheng *et al*., 2007). The phytochemical screening of *S. acuta* leaf extract has shown that the leaves of the plants possess different secondary metabolites in the form of phytochemicals. Alkaloids, tannins, saponins, flavonoids, steroids, terpenoids, anthraquinone, reducing sugar, and cardiac glycosides were found in amounts of medicinal value. These secondary bioactive metabolites are known to possess antioxidant, anti-bacterial, anti-inflammatory, anti-sickling, hypoglycaemic, and immunomodulatory properties (Egba *et al.,* 2012).

Studies on the presence of phytochemicals in plant species such as *S. acuta* include that of Ekpo and Etim (2009) who stated that the analysis of the leaves extract of *S. acuta* revealed that it contains the following phytochemical active agents in different proportions. They include alkaloids, saponins, authraquinones, cardiac glycosides, tannins, and flavonoids.

It was observed from the study that the methanolic leaf extract of *S. acuta* revealed the presence of high concentrations of flavonoid, tannin, saponin and terpenoid with moderate concentration of steroid and alkaloid while glycoside, anthraquinone and reducing sugar were present in low concentrations. This is in agreement with the work of Karou *et al.* (2007), who attributed the great potential of *S. acuta* to the presence of these different components. Akinnibosun and Pela (2015) also reported the presence of phytochemicals such as alkaloids, saponins, tannins, steroids, glycosides, polyphenols, oxalate, and flavonoids from ethanolic extracts of *S. acuta*. Raimi *et al.* (2014) also observed the presence of these phytochemicals in *S. acuta*.

The potential of *S. acuta* leaf extract as a potent antifungal species could be attributed to its natural ability to provide a large number of lead compounds used for developing new drugs. Natural product drugs include aromatic polyketides, polyethers, coumarins, flavonoids, terpenoids, alkaloids, and aminoglycosides. Flavonoids are known to have medicinal properties and play a major role in successful medical treatments from ancient times and their use has been persevered to date. Tannins possess antibacterial, antiviral, and potent against degenerative diseases. Steroids are important in pharmaceuticals and play an important role in the functions of sex hormones. Saponin has been shown to have hypocholesterolemic, hypotensive, and cough-depressant activities. Saponins are active agents against fungal infections. Glycosides are potent signal transducers acting on several intracellular targets thus modulating the activities of enzymes and hormones. Terpenes have been reported to have both antimalarial and hypoglycaemic effects (Tijjani *et al.,* 2012).

Table 3. Major identified constituents of *S. acuta* methanol extract

Peak No.	Retention time (min)	Relative abundance (%)	Compound name	Compound formula	Mol mass
1	12.550	3.147	Methyl 4-O-acetyl-2-O-methyl-3,6-dideoxy-d-gluco-pyranoside	$C_{10}H_{18}O_5$	218
2	13.196	2.963	1-Pyrenecarboxaldehyde	$C_{17}H_{10}O$	230
3	14.139	3.470	3,4-Dihydroxyphenylpropionic acid, ethyl ester, di-PFP	$C_{17}H_{12}F_{10}O_6$	503
4	14.517	3.638	Mordant yellow 12, O,O'-di(tert.-butyldimethylsilyl)-	$C_{25}H_{39}N_3O_3Si_2$	485
5	15.041	2.911	Methyl triphenylstannanedithiocarboxylate	$C_{20}H_{18}S_2Sn$	442
6	15.797	2.995	Furan, tetracyano-	C_8N_4O	168
7	19.470	23.652	3-Trifluoromethylbenzylamine, N,N-diundecyl	$C_30H_{52}F_3N$	483
8	19.662	9.577	Benzene, p-bis(4-phenylbutyl)-	$C_{26}H_{30}$	342
9	19.778	4.291	Phenylpyruvic acid, ethyl ester,O- trimethylsilyl-	$C_{14}H_{20}O_3Si$	264
10	19.866	4.673	2-Pyrimidinamine, 5-bromo-N,N-dimethyl-4-(methylthio)-	$C_7H_{10}BrN_3S$	247
11	20.034	4.845	Chromium, bis(1,4-di(trifluoromethyl)benzene-	$C_{16}H_8CrF_{12}$	480
12	20.227	5.500	Silane, methylvinyldi(hexadecyloxy)-	$C_{35}H_{72}O_2Si$	552
13	20.622	3.598	Tritriacontane, 3-methyl-	$C_{34}H_{70}$	478
14	21.751	3.048	{Methanediylbis[(3,4,6-trichlorobenzene-2,1-diyl) oxy]}bis(trimethylsilane)	$C_{19}H_{22}C_{l6}O_2Si_2$	548
15	22.706	2.975	2-([4-Chloro-3-(trifluoromethyl)phenyl]imino)-3-methyl-4-oxo-N-(4-propoxyphenyl)-1,3-thiazinane-6-carboxamide	$C_{22}H_{21}ClF_3N_3O_3S$	499
16	22.869	4.747	1-Butoxypervinylhomohexasilsesquioxane	$C_{18}H_{30}O_{11}Si_7$	618
17	24.650	4.700	Benzenehexacarboxylic acid, hexamethyl ester	$C_{18}H_{18}O_{12}$	426
18	24.685	3.226	Silane, diethylbutoxy(pentachlorophenoxy)-	C14H19Cl5O2Si	422
19	24.743	2.942	Cobalt, (η5-2,4-cyclopentadien-1-yl) [[(2,3,4,5-η)-2,4-cyclopentadien-1-yl]benzene]-	$C_{10}H_{18}O$	266
20	24.859	3.102	Ursane-3,16-dione, (18α,19α)-	$C_{30}H_{48}O_2$	440

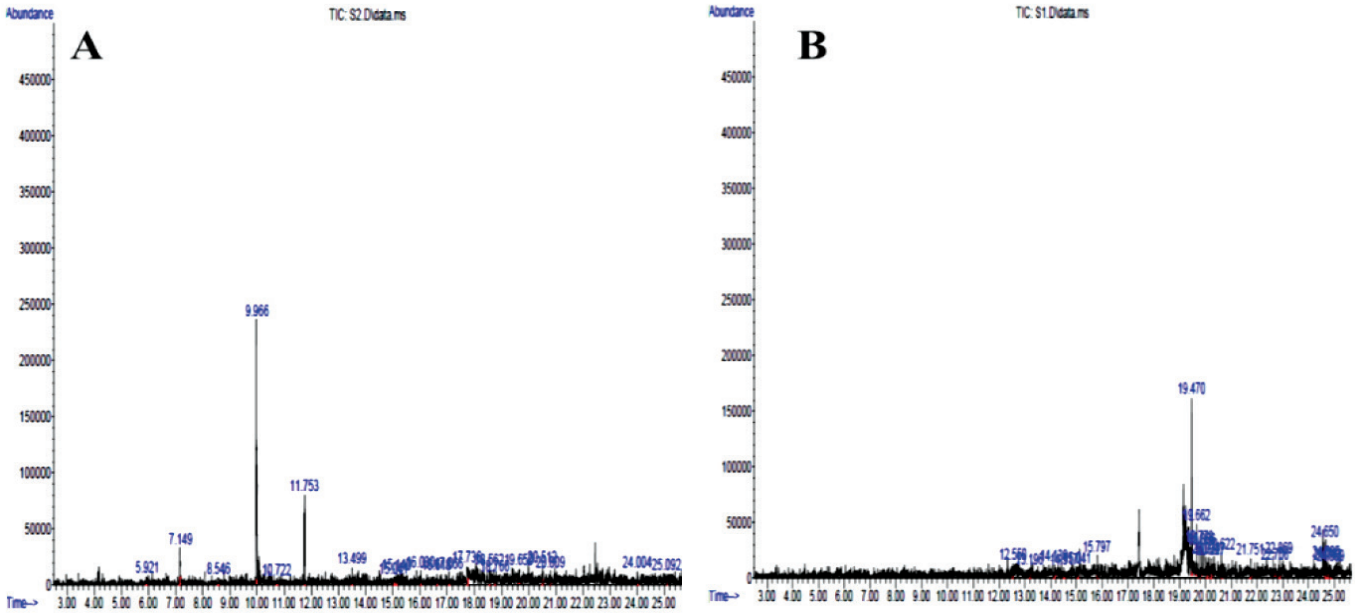

Fig. 1. GC-MS chromatogram for *S. acuta*: (A) chloroform extract (B) methanol extract

Table 4. Major identified constituents of *S. acuta* chloroform extract

Peak No.	Retention time (min)	Relative abundance (%)	Compound Name	Compound formula	Mol. mass
1	5.921	1.624	5-(p-Aminophenyl)-4-(m-iodophenyl)-2-thiazolamine	$C_{15}H_{12}IN_3S$	393
2	7.149	7.475	3-(4-Iodo-phenyl)-6-nitro-chromen-2-one	$C_{15}H_8INO_4$	393
3	8.546	1.606	Mercury, chloro(3,17-dioxoandrosta-1,4,6-trien-2-yl)-	$C_{19}H_{21}ClHgO_2$	518
4	9.966	48.546	L-Proline, N-(2-trifluoromethylbenzoyl)-, isohexyl ester	$C_{19}H_{24}F_3NO_3$	371
5	10.722	1.648	Chloroacetamide, N-ethyl-N-pentyl-	$C_9H_{18}ClNO$	191
6	11.753	7.872	Emodin, 3TMS derivative	$C_{24}H_{34}O_5Si_3$	486
7	13.499	2.097	Copper, [2,8,12,18-tetraethyl-3,7,13,17-tetramethyl-21H,23H-porphinato(2-)-N21,N22,N23,N24]-, (SP-4-1)-	$C_{32}H_{36}CuN_4$	539
8	15.041	1.749	Tungsten, dicarbonyl-(η-4-pinocarvone)[1,2-bis(dimethylphosphino)ethane]	$C_{18}H_{30}O_3P_2W$	540
9	15.140	1.988	Cholest-2-eno[2,3-b]indole, 1‘-formyl-5‘-methoxy-	$C_{35}H_{51}NO_2$	517
10	16.030	2.198	N‘-(3-Allyl-2-hydroxybenzylidene)-3-phenyl-1H-pyrazole-5-carbohydrazide ditbdms	$C_{32}H_{46}N_4O_2Si_2$	574
11	16.618	1.972	1-Hexacosanol, TMS derivative	$C_{29}H_{62}Osi$	112
12	17.066	1.825	Silane, diethyl(cis-4-methylcyclohexyloxy)octadecyloxy-	$C_{29}H_{60}O_2Si$	468
13	17.736	4.726	2,3,20,21-Dibenzo-1,4,7,10,13,16,19,22,25,28-decaoxacyclotriacontan-2,20-diene	$C_{28}H_{40}O_{10}$	536
14	18.562	2.110	N-tert-Butyl-2,3,3,3-tetrafluoro-2- (1,1,2,3,3,3-hexafluoro-2-heptafluoropropyloxypropoxy) propionamide	$C_{13}H_{10}F_{17}NO_3$	551
15	18.766	1.937	Beryllium, hexakis[μ-(2-methylpropanoato-O:O‘)]-μ4-oxo-tetra-	$C_{24}H_{42}Be_4O_{13}$	574
16	19.650	1.863	1‘H-Cholest-3-eno[3,4-b]indol-6-one, 1‘-methyl-, (5β)-	$C_{34}H_{49}NO$	487
17	20.512	2.383	1-Cyclohexyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrole-3-dicarboxyllic acid, 2-benzyloxy(trimethylsilyloxy) methylene-, trimethylsilyl ether, ethyl ester	$C_{27}H_{41}NO_6Si_2$	531
18	20.809	2.472	Pyrrol-2(5H)-one, 4-acetyl-3-hydroxy-5-(3-nitrophenyl)-1-[2-(1-piperazinyl)ethyl]-	$C_{18}H_{22}N_4O_5$	374
19	24.004	1.988	Methanesulfonylazide	$CH_3N_3O_2S$	121
20	25.092	1.919	4-Amino-2-methyl-5,6-trimethylenepyrimidine	$C_8H_{11}N_3$	149

Table 5. Macroscopic and microscopic characteristics of fungi isolated from rotten *A. muricata* fruit

Macroscopic characteristics	Microscopic characteristics	Probable fun
Fast growing, grayish, cottony	Coenocytic mycelium, hyphae have stolon, rhizoids and unbranched erect sporangiophores	*R. stolonife*

Table 6. Length of mycelial growth (cm) and percentage inhibition of *Rhizopus* sp. isolated from *A. muricata* fruit exposed to different concentrations of *S. acuta* leaf extracts

Extract concentration (mg/ml)	Average mycelia diameter± SEM (mm)/Inhibition of mycelia growth (%)	
	Methanol	Chloroform
0	4.3 ± 0.15/0.0	4.3 ± 0.15/0.0
0.0625	0.0 ± 0.00/100.0	0.0 ± 0.00/100.0
0.125	0.1 ± 0.01/97.67	0.0 ± 0.00/100.0
0.25	0.3 ± 0.10/93.0	0.0 ± 0.00/100.0
0.5	0.5 ± 0.10/88.0	0.2 ± 0.05/95.0
1.0	0.5 ± 0.10/88.0	0.3 ± 0.05/93.0

The difference in the GC-MC extracted compounds by methanol and chloroform is due to the differences in the polarity of the solvents as the type of solvent used determines the kind of biologically active compound that will be extracted from the plant (Tiwari *et al.*, 2011)

The mycelia inhibition diameter was concentration dependent for both the methanol and chloroform extracts and the activity of the extracts varied from one concentration to another. The antifungal activities of *S. acuta* leaf extract against fungal isolate showed that the extract was able to inhibit the activities and mycelial growth of *Rhizopus* species isolated from sour sop fruit rot. There was no significant difference in the antifungal activity of the chloroform and methanol extract at the same concentration on the test fungi, which implied that both solvents are good for extracting the antimicrobial constituents of *S. acuta* leaf. The antifungal potential of *S. acuta* extract has previously been reported by Jindal *et al.* (2012). The plant exhibited good antifungal activity against tested fungi. Among 8 extracts tested, 7 were found to be active at tested concentration.

Conclusion

The study isolated and identified *R. stolonifer* as the fungi associated with fruit rot of *A. muricata*. The study has documented the phytochemical constituents of *S. acuta* leaf and the antifungal potentials of *S. acuta* leaf extract against *R. stolonifer*. Phytochemicals such as alkaloids, tannins, saponins, flavonoids, steroids, terpenoids, anthraquinone, reducing sugar, and cardiac glycosides were found in amounts of medicinal value in the leaves of *S. acuta.* 3-Trifluoromethylbenzylamine, N,N-diundecyl and L-Proline, N-(2-trifluoromethylbenzoyl)-, isohexyl ester were the most abundant compounds in both methanolic and chloroform extracts of the plant leaves.

S. acuta leaf extract has shown a potential antifungal effect against the test fungal pathogen. The phytochemical composition and antifungal effects make *S. acuta* leaf extract to possess the potential for use against fungal infections in the future. Further research to test the efficacy of the plant extract against diseased sour sop fruits *in vivo* would be very ideal.

Acknowledgement

Technical input and support from members of staff of Mifor Consult Laboratory, Calabar is greatly appreciated. However, no financial support was received by the authors in the course of this research. The authors also declare that there is no conflict of interest associated with this research work.

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