

Fungicidal activity of *Solanum nigrum* and *Physalis angulata* extracts against *Macrophomina phaseolina*, a fruit rot pathogen of melon (*Citrullus colocynthis* (L.) Schrad)

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ABSTRACT

Acetone and methanol leaf extracts fractions of *Solanum nigrum* and *Physalis angulata* at various concentrations (1000, 500, 250, 100, 50 mg/ml) in PDA were tested against *Macrophomina phaseolina*, a pathogen of fruit rot disease of melon. All the extract concentrations showed significant ($P<0.05$) activity with dose and species-dependent effect against the phytopathogen. Acetone extracts had superior activity over methanol extracts with 100% inhibition of fungal growth at 500mg/ml for *S. nigrum* and 1000mg/ml for *P. angulata*. Phytochemical tests of the extracts revealed the presence of secondary metabolites in varying degrees with high concentrations of flavonoids and tannins which may be responsible for observed fungitoxic activity. The results of this study have uncovered the potentials of the Solanaceous weeds as antifungal agents. We therefore suggest field formulations and trials of these extracts as soil drench in agricultural farms against soil-borne pathogens.

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INTRODUCTION

Melon *Citrullus colocynthis* L. Schrad is a tropical crop with a creeping stem. It is always grown as a sole crop or along with other crops like yam, cassava and maize. It is an important crop because of its ability to provide ground cover for effective weed control and ability to fix nitrogen (Chuku *et al.*, 2010). Melon seed is a good source of protein and oil, carbohydrate, ash and crude fibre. Melon seed consumed in various forms in Nigeria is an important source of protein and oil. The forms are as condiment in Nigeria local soups, melon ball snacks and as “ogiri” (fermented melon) for seasoning variety of dishes (chiejina, 2006). *Physalis angulata* L. (Solanaceae) includes about 120 species with herbal characteristics and perennial habits distributed throughout tropical and subtropical regions of the world. It has a broad spectrum

of biological activity like antibacterial (Donkor *et al.*, 2012), anti-parasitic and anti-viral (Silva *et al.*, 2005) activities. *Solanum nigrum* L. commonly known as black nightshade is a dicot weed in the Solanaceae family. It is an annual herbaceous plant 10-60 cm high with a green, smooth and semi-climbing stem. It is a rather common species in wet woods, near river, waste land, old field, roadside and cultivated land (Prakash and Jain, 2011; Gogoi and Islam, 2012). Plants are usually exposed and threatened by a variety of pathogenic microorganisms present in their environment. Phytopathogenic fungi are the most problematic pest of agricultural crops worldwide (Savary *et al.*, 2006). Plant diseases caused by soil-borne pathogens play an important role in the destruction of natural resources in agriculture. *Macrophomina phaseolina* (Tassi) Goid ia a soil-borne fungal

pathogen that cause charcoal rot, seedling blight, root rot, stem rot, pod rot on more than 500 species of plant (Ahmed *et al.*, 2009) including *Cucumis melo* (Salari *et al.*, 2012), cowpea (Javaid *et al.*, 2012). Control of such diseases depends mainly on fungicidal treatments. Hence, chemical control of *M. phaseolina* has been achieved (Choudhary *et al.*, 2004). However, inappropriate and nondiscriminatory use of chemicals has put human and animal health at risk apart from contaminating the environment (Kumar *et al.*, 2007, Yang *et al.*, 2010). The biological inhibitions of different natural substances such as plant extracts have been investigated on fungal activities by various researchers (Ilondu, 2013; Ahila Devi *et al.*, 2013; Al-Rahmah *et al.*, 2013; Rastegar and Gozari, 2017). The objective of this work was to evaluate the fungitoxic effect of extracts from *S. nigrum* and *P. angulata* against *M. phaseolina* isolated from charcoal rot of melon fruits.

MATERIALS AND METHODS

Test Organisms

Microphomina phaseolina was previously isolated and identified from fruit rot of melon in a farm at Eku, Delta State (Ogba, 2014). The pure culture was maintained in PDA slants at 40C in Department of Botany Laboratory. Upon collection, the fungus was revived twice on PDA before use for antifungal activity assay.

Collection of Plant Materials

Solanum nigrum and *Physalis angulata* were collected randomly from the Delta State University, Abraka. Plants were identified using voucher specimens deposited at the Dept of Botany Laboratory (Akobundu and Agyakwa, 1998). The plant materials were washed thoroughly for 3-4 times with running tap water and twice with sterile distilled water, shade dried at room temperature. After complete drying the plant materials were powdered using the blender and stored in separate airtight bottles and used for preparation of acetone and methanol extracts.

Phytochemical analyses

Various classes of secondary metabolites were identified using standard prescribed methods

of Trease and Evans (1999) and Edeoga *et al.* (2005).

Antifungal activity of plant extracts

Antifungal activity was evaluated using the poisoned food technique as described by Ahila Devi *et al.* (2013). Different concentrations (1000, 500, 250 100 and 50 mg/mL) were prepared from acetone and methanol extracts of *S. nigrum* and *P. angulata*. One millilitre of each concentration was aseptically incorporated into 20ml of cool molten PDA in sterile test tube. Each medium was homogenized by gentle agitation before dispensing into sterile 9cm Petri dishes. The control was set up using extract free PDA plates. The plates were allowed to set for 3 h. The effect of the extracts on fungal growth was determined by inoculating at the centre of 90cm Petri plates with a mycelia disc (4mm) obtained from the colony edge of 7-day old culture of the test fungus. Three replicates of both the control and PDA-extract plates were incubated at room temperature ($30\pm2^{\circ}\text{C}$) and radial growth was measured with a metric ruler daily for seven days. Colony diameter was taken as the mean along two directions on two perpendicular lines drawn on the reverse of the plates. The percentage inhibition was calculated by the method of Javaid *et al.* (2012).

Data Analysis

Data obtained was subjected to Analysis of Varian (ANOVA) using Statistical Package for Social Sciences (SPSS) version 17.0 and differences among the means were determined for significance at $P<0.05$ using Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The phytochemical composition of the plant extracts are presented in Table 1. The presence of these secondary metabolites may have been responsible for the antifungal activities of the extracts. Some plant extracts act as contact fungicides; some disrupt cell membrane integrity at different stages of fungal development while the others inactivate important enzymes and interfere with metabolic processes (Aye and Matsumoto, 2011). Tannins and flavonoids were found to

be in high concentration of acetone and methanol extracts of *S. nigrum*. Tannins prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them. Similarly, the growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Gogoi and Islam, 2012). Kanwal *et al.* (2010) observed a significant reduction in the growth of five phytopathogenic fungi including *M. phaseolina* due to the action of flavonoids isolated from mango leaves.

Table 1. Preliminary phytochemical constituents of *Physalis angulata* and *Solanum nigrum*

Phytochemicals	<i>Physalis angulata</i>		<i>Solanum nigrum</i>	
	Acetone	Methanol	Acetone	Methanol
Tannins	+	-	+++	+
Alkaloids	+	+	++	++
Saponins	+	-	++	++
Flavonoids	++	+	++	+++
Anthraquinone	-	-	+	-
Reducing sugar	-	-	-	-
Terpenes	-	-	-	-
Steroids	+	++	+	-
Glycosides	-	-	-	-

Key: - = absent; + = Low concentration; ++ = Moderate concentration; +++ = High concentration

The antifungal activities of the plant extracts are presented in Table 2. Acetone and methanol extracts fractions of *S. solanum* and *P. angulata* were screened *in-vitro* to evaluate them for the control of *M. phaseolina*. The assay showed that the extracts significantly inhibited the mycelia growth of the test fungus over the control. Antimicrobial activity of *Physalis angulata* extracts have been reported (Osho *et al.*, 2010; Yanor *et al.*, 2011; Donkor *et al.*, 2012). Similarly, extract of *S. nigrum* has been found to possess antibacterial and Figure 1. Percentage growth inhibition of *M. phaseolina* at various concentrations of leaf extract from *S. nigrum* and *P. angulata* 7 days after incubation on agar plate antifungal property (Prakash and Jain, 2011; Parameswari *et al.*, 2012). The extracts exhibited antifungal activity with concentration dependent effect. Concentration dependent observation agreed with the findings of Aslam *et al.*, (2010) who reported that higher concentrations of extracts exhibited greater antifungal activity than lower concentrations.

Table 2. Effect of acetone and methanol leaf extracts of *physalis angulata* and *Solanum nigrum* on the radial mycelia growth (cm) of *Macrophomina phaseolina* on agar plates 7-days after inoculation

Extracts conc. (mg/ml)	<i>Physalis angulata</i>		<i>Solanum nigrum</i>	
	Acetone	Methanol	Acetone	Methanol
0	4.30 ^a	4.30 ^a	4.30 ^a	4.30 ^a
50	2.73 ^b	3.30 ^b	2.80 ^b	3.00 ^b
100	1.20 ^c	2.17 ^c	1.90 ^c	2.36 ^c
250	0.71 ^d	1.03 ^d	0.50 ^d	1.80 ^d
500	0.34 ^e	0.42 ^e	0.00 ^e	0.54 ^e
1000	0.00 ^f	0.18 ^f	0.00 ^e	0.12 ^f

Values with the same superscript(s) in the same column are not significantly different at $p>0.05$ by Duncan's Multiple Range Test (DMRT).

Plate 1. Radial mycelial growth inhibition of *Macrophomina phaseolina* by leaf extract (mg/ml) of *Physalis angulata*

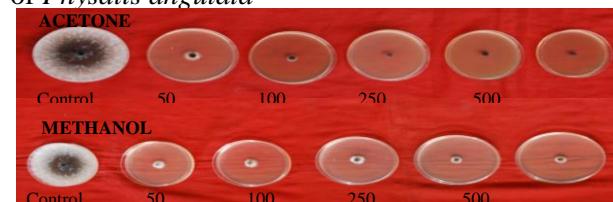
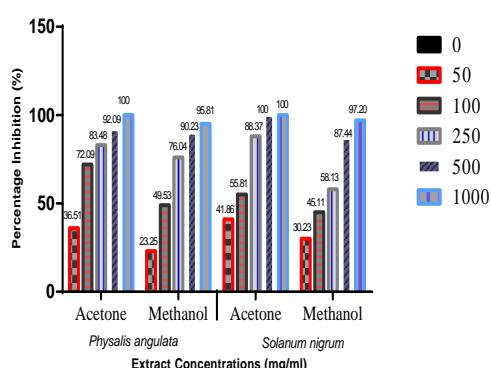
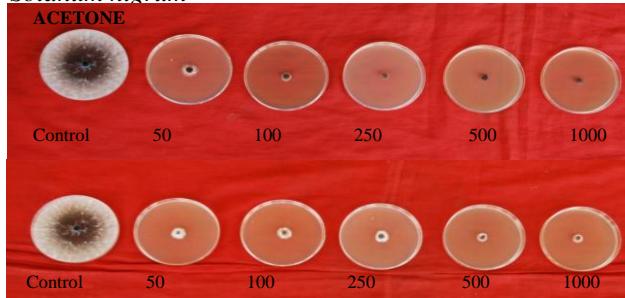


Plate 2. Radial mycelial growth inhibition of *Macrophomina phaseolina* by leaf extract (mg/ml) of *Solanum nigrum*



The acetone extracts of the two plants species have a superior effect over the methanol extracts. Complete mycelia growth inhibition was recorded at 500 and 1000 mg/ml of *S. nigrum* and *P. angulata* respectively. The sensitivity of the fungus varied greatly according to plant species and extracting solvent. This is similar to the report of Sridhar *et al.* (2011), Ilondu, (2013) and Rastegar and Gozari (2017). The variation of the fungitoxicity of the extracts may also be due to variation in their constituents, methods of extraction and time of harvest of plant materials (Okungbowa and Edema, 2007; Okigbo and Odurukwe, 2009). This work has demonstrated that acetone and methanol extracts of *S. nigrum* and *P. angulata* exhibit strong fungicidal activity against *Macrophomina phaseolina*. These extracts could be exploited in the formulation of inexpensive and environmental friendly biofungicides for the control of fruit rot of melon caused by *Macrophomina phaseolina* in agricultural farms.

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