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# EFFECT OF ETHANOL AND PETROLEUM ETHER EXTRACTS OF SOME MEDICINAL PLANTS ON *SCLEROTIUM ROLFSSII*, CAUSAL AGENT OF POSTHARVEST ROT OF YAM (*DIOSCOREA* SPP.)

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## ABSTRACT

Ethanol and Petroleum ether of leaves of *Azadirachta indica* A. Juss, *Citrus aurantifolia* (Christm.) Swingle, *Jatropha curcas* L., *Anacardium occidentale* L., seeds of *Garcinia kola* Heckel and peels of *Citrus sinensis* Osbeck were studied for *in vitro* activity against mycelial growth and sclerotium germination of *Sclerotium rolfsii*, Sacc., the causal agent of postharvest yam rot. The phytochemical screening of the extracts of the medicinal plants revealed the presence of alkaloids, flavonoids, glycosides, steroids, saponins, tannins and terpenoids. The ethanol and petroleum ether extracts showed varying levels of fungitoxicity activity with the ethanol extracts being more effective. Mycelial growth of *S. rolfsii* was significantly ( $P < 0.05$ ) reduced by ethanol extracts of *C. sinensis* (89.63 – 100%), *C. aurantifolia* (67.04 – 83.28%) and *A. indica* (53.70 – 69.38%) during the period of incubation. Sclerotium germination was also significantly ( $P < 0.05$ ) reduced by ethanol extracts of *C. sinensis* (87.50%), *C. aurantifolia* (62.50%) and *A. indica* (50%). This study showed the potentials of extracts of some plant products to control *S. rolfsii* in yam.

**Keywords:** Ethanol extracts, Petroleum ether extracts, *Sclerotium rolfsii*, Postharvest rot of yam, Medicinal plants, Fungitoxicity.

## 1. INTRODUCTION

Yams (*Dioscorea* spp) are among the oldest food crops grown and consumed in Nigeria. The most cultivated species in Nigeria are *D. rotundata* (white yam), *D. cayenensis* (yellow yam) and *D. alata* (water yam) [1]. *Dioscorea rotundata* and *D. cayenensis* are indigenous to West Africa while *D. alata* is native to Asia. Yam tubers are harvested in Nigeria mostly between June and September and most of which are stored in different storage facilities depending on the cultural and traditional advancement of the people of such area [2]. During storage, substantial losses in weight, quantity and quality occur. Apart from the metabolic processes, such as sprouting and

respiratory activities and loss of moisture content by evaporation, storage rot is the major factor responsible for the deterioration of stored yams [3].

Microbial rotting of yam tubers accounted for a substantial proportion of the annual loss in yam production in Nigeria [4]. Olurinola, et al. [5] estimated microbial post harvest losses in yam at 40% while Okigbo and Ikediugwu [6] indicated that between 20 and 39.5% of stored tubers may be lost to decay. Studies have shown that fungal rot is the greatest cause of tuber loss in storage [7]. The principal species of microorganisms associated with yam rot in Nigeria are *Sclerotiumrolfsii*, *Botryodiplodiatheobromae*, *Fusariumoxysporum*, *Pencilliumoxalicum* and *Aspergillusniger* [6, 8, 9]. Synthetic chemicals have potentials to control these pathogens. However, the cost and harmful side effects of chemical use have necessitated the need for alternative methods such as the use of plant extracts [10]. Studies carried out by some workers through in vitro investigation have confirmed the fungicidal potential of extracts of some plant species [11-15]. Plant extracts are eco-friendly, accessible to rural peasant yam producers, less costly and little technical knowledge is needed in handling the extracts [16]. This investigation was therefore aimed at determining, *in vitro*, whether Petroleum ether and ethanol extracts of six medicinal tropical plants, *Citrusaurantifolia* (Christm.) Swingle, *Azadirachtaindica* (A Juss), *Jatropha curcas* (L.), *Anacardiumoccidentale* (L.), *Garcinia kola* (Heckle) and *Citrus sinensis* (Osbeck) have inhibitory effect on *S. rolfsii*, which is a major pathogen of postharvest yam rot.

## 2. MATERIALS AND METHODS

### 2.1. Isolation of Inoculum

*Sclerotiumrolfsii* was isolated from rotted yam tubers collected from Ogbegonogo and Cable Point markets in Asaba, Delta State, Nigeria. Tissue segments (1-2 mm diameter) were cut from advancing edge of infected portions of the tuber using sterilized scalpel. The cut tissues were surface-sterilized in 10% sodium hypochlorite (NaOCl) solution to remove surface contaminations and rinsed twice in sterile distilled water [17]. Four sections of the sterilized tissue pieces were plated out on Potato Dextrose Agar (PDA). The plates were incubated at 28°C for 7 days. Three subcultures were made on PDA to obtain pure culture of the fungus. Pathogenicity test was carried out and the pathogen was identified as *Sclerotiumrolfsii* with the aid of compound microscope and identification manual [18].

### 2.2. Preparation of Plant Extracts

The following plant products were used for the preparation of the extracts: leaves of *Citrus aurantifolia* (Christm.) Swingle (lime), *Azadirachtaindica* A Juss (neem), *Jatropha curcas* L. (Physic nut), *Anacardiumoccidentale* L. (cashew), seeds of *Garcinia kola* Heckle (bitter cola) and peels of *Citrus sinensis* Osbeck (sweet orange). These plant products were surface-sterilized (10% NaOCl for 2 min), air dried for 10 days and ground using a Tower blender (Model BL-NC-6802D) to obtain 500g powder of each plant species. Each of the powdered plant materials (100g) was

packed into a soxhlet apparatus (2L) and extracted exhaustively and successively with 500ml of petroleum ether (60-80°C) and ethanol for 6 hours. The extracts were evaporated to dryness using a water bath. The test solution of each extract was prepared by dissolving 5g of extract separately in 50 ml of sterile distilled in a 250 ml Erlenmeyer flask in a water bath at 80°C. Extracts were subsequently filtered through four folds of cheese cloth.

### 2.3. Phytochemical Screening

Phytochemical screening was carried out on part of the pulverized plant materials to reveal the presence of secondary metabolites in them using the method of Poongothai, et al. [19].

### 2.4. In Vitro Tests

The effect of the plant extracts on radial mycelia growth and sclerotium germination of the pathogen was determined using the poisoned food technique described by Okigbo, et al. [17]. One milliliter of each plant extract was pipette separately and aseptically into 9 ml of cool molten PDA medium in each of the Petri dishes. Each plate was swirled on the laboratory bench to ensure even dispersion of extracts. The PDA-extract medium was allowed to solidify and 2 mm diameter mycelia disc obtained from 4 day-old culture of *S. rolfsii* was aseptically transferred to the centre of the solidified PDA-extract medium contained in a Petri dish that was previously marked at the bottom with perpendicular lines passing through at 28°C and growth inhibition was determined after 2,4 and 6 days by measuring the growth of the fungal colonies along two preset diametral lines. To determine the toxicity of extracts against sclerotium germination, each plate containing PDA-extract medium was inoculated with 10 sclerotia obtained from 7 day-old culture of *S. rolfsii* previously grown on plain PDA medium. Germination was assessed after 3 days incubation at 28°C. Plant extract-free PDA plates similarly inoculated with mycelia discs or sclerotia served as controls. Three replicates were maintained for each experiment. Percentage inhibition of mycelial growth or sclerotium germination was calculated according to the formula:

$$\% \text{ inhibition} = \frac{dc - dt}{dc} \times 100$$

Where:

dc = average diameter of fungal colony/average number of sclerotium germination in control plates

dt = average diameter of fungal colony/average number of sclerotium germination in treated plates

### 2.5. Experimental Design and Analysis

The experiment was laid out in a Completely Randomized Design (CRD) with three replicates. Data collected were subjected to analysis of variance (ANOVA) using [20] and significant means

were separated with the Duncan's Multiple Range Tests (DMRT) ( $P < 0.05$ ).

### 3. RESULTS

Phytochemical screening of the plant extracts revealed that all the plant materials had tannins. In addition, *C. sinensis* peel contained alkaloids, glycosides and steroids. *Citrus aurantifolia* leaf contained flavonoids and glycosides. *Azadirachta indica* leaf contained alkaloids, flavonoids, glycosides, saponins and terpenoids. *Garcinia kola* seed contained flavonoids and saponins. *Jatropha curcas* leaf contained flavonoids, saponins and glycosides. *Anacardium occidentale* leaf contained alkaloids and terpenoids. The component, anthraquinones was not detected in the plant materials tested (Table 1). The petroleum ether and ethanol extracts of the six plant materials screened, *in vitro*, showed varying levels of toxicity to *S. rolfssii*, expressed as percentage inhibitions to mycelia growth and sclerotium germination. Results in Table 2 showed that ethanol extract of *C. sinensis* peel recorded the highest growth inhibition of *S. rolfssii* (89.63 – 100%) during the period of incubation. This was followed by ethanol extract of *C. aurantifolia* leaf with inhibition range of 67.04 – 83.28% water extract of *C. sinensis* peel, 66.67 – 74.53 and there ethanol extract of *A. indica* leaf, 53.70 – 69.38%. The least was water extract of *A. occidentale* leaf with inhibition range of 14.33 – 20.31%. Ethanol extracts were more effective in inhibiting the growth of the pathogen than water extracts. The inhibitory effects of the extracts decreased as the period of incubation increased for the test plant materials.

**Table-1.** Phytochemical analysis of the plant extracts

Phytochemical	<i>Citrus sinensis</i>		<i>Citrus aurantifolia</i>		<i>Azadirachta indica</i>		<i>Garcinia kola</i>		<i>Anacardium occidentale</i>		<i>Jatropha curcas</i>	
	E	PE	E	PE	E	PE	E	PE	E	PE	E	PE
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	+	+	+	+	+	-	-	-	+	-	-	-
Flavonoids	-	-	+	+	+	-	+	-	-	-	+	+
Glycosides	+	+	+	+	+	+	-	-	-	-	+	+
Steroids	+	+	-	-	-	-	+	+	-	-	-	-
Saponins	-	-	-	-	+	-	-	-	-	-	+	-
Tannins	+	+	+	-	+	-	+	+	+	+	+	+
Terpenoids	-	-	-	-	+	+	-	-	+	+	-	-

Keys: + = Present E = Ethanol  
- = Absent PE = Petroleum ether

**Table-2.** Percentage inhibition of radial growth of *Sclerotium rolfssii* by plant extracts on PDA at different dates

Plant materials	Inhibition (%)					
	Petroleum ether extract			Ethanol extract		
	2	4	6	2	4	6
<i>Citrus sinensis</i> peel	74.53 <sup>f</sup>	70.38 <sup>e</sup>	66.67 <sup>f</sup>	100 <sup>f</sup>	100 <sup>f</sup>	89.63 <sup>f</sup>
<i>Citrus aurantifolia</i> leaf	62.50 <sup>e</sup>	53.75 <sup>d</sup>	46.67 <sup>e</sup>	83.28 <sup>e</sup>	75.38 <sup>e</sup>	67.04 <sup>e</sup>
<i>Azadirachta indica</i> leaf	47.73 <sup>d</sup>	42.50 <sup>c</sup>	38.82 <sup>d</sup>	69.38 <sup>d</sup>	66.75 <sup>d</sup>	53.70 <sup>d</sup>
<i>Garcinia kola</i> leaf	37.50 <sup>c</sup>	41.25 <sup>c</sup>	20.37 <sup>c</sup>	57.81 <sup>c</sup>	55.00 <sup>c</sup>	44.44 <sup>c</sup>
<i>Jatropha curcas</i> leaf	39.06 <sup>c</sup>	14.13 <sup>b</sup>	14.82 <sup>b</sup>	56.72 <sup>c</sup>	46.63 <sup>b</sup>	41.41 <sup>c</sup>
<i>Anacardium occidentale</i> leaf	20.31 <sup>b</sup>	18.75 <sup>b</sup>	14.33 <sup>b</sup>	46.86 <sup>b</sup>	42.50 <sup>b</sup>	38.15 <sup>b</sup>
Control	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>

Means in the same column with different superscripts are significantly different ( $P < 0.05$ )

**Table-3.**Percentage inhibition of *Sclerotium rolfsii* germination of *Sclerotium rolfsii* by plant extracts

Plant material	Inhibition (%)	
	Petroleum ether extract	Ethanol extract
<i>Citrus sinensis</i> peel	58.02 <sup>e</sup>	87.50 <sup>e</sup>
<i>Citrus aurantifolia</i> leaf	41.67 <sup>d</sup>	62.50 <sup>d</sup>
<i>Azadirachta indica</i> leaf	39.17 <sup>d</sup>	50.00 <sup>c</sup>
<i>Garcinia kola</i> leaf	25.00 <sup>c</sup>	47.50 <sup>c</sup>
<i>Jatropha curcas</i> leaf	12.50 <sup>b</sup>	33.33 <sup>b</sup>
<i>Anacardium occidentale</i> leaf	8.33 <sup>b</sup>	29.17 <sup>b</sup>
Control	0 <sup>a</sup>	0 <sup>a</sup>

Means in the same column with different superscripts are significantly different (P < 0.05)

On the effect of extracts on the sclerotium germination of *S. rolfsii* (Table 3), the highest percentage inhibition in sclerotium germination was recorded in ethanol extract of *C. sinensis* peel (87.50%). This was followed by ethanol extract of *C. aurantifolia* leaf (62.50%) water extract of *C. sinensis* peel (58.02%) and ethanol extract of *A. indica* leaf (50%). The least percentage inhibition of sclerotium germination was recorded with water extract of *A. occidentale* leaf (8.33%). Ethanol extracts were relatively more effective than water extracts of the plant materials.

#### 4. DISCUSSION

The extracts of the plant products studied were found to contain one or more of the following phytochemical compounds alkaloids, flavonoids, glycosides, steroids, saponins, tannins and terpenoids. Other investigators [21-25] have reported the presence of these components in members of the families Rutaceae, Meliaceae, Guttiferae, Euphorbiaceae and Anacardiaceae to which the plants used in this study belong. The inhibitory effects of these plant products on *S. rolfsii* may be due to the presence of the above phytochemical components. Scientists have shown that phytochemicals play defensive roles in the plants producing them. For example, Haralampidis, et al. [26] reported that secondary metabolites have been implicated as chemical defence against attack by soil fungi. In the same paper they further reported that many plants synthesize secondary metabolites as part of their normal programme of growth and development often sequestering them in tissues to protect them against microbial attack.

Results from this study indicate that fungitoxic compounds are present in the different plant extracts tested. This is in agreement with the reports of some earlier workers [27-30]. The inhibitory effects of plant extracts on different pathogens of crops have been widely reported [13, 15, 31, 32]. Among the six plant materials screened, *C. sinensis* peel extracts gave a very remarkable result especially the ethanol extract which recorded maximum fungitoxic effect on the growth of the test rot causing pathogen up to the 4<sup>th</sup> day of culture. The fungitoxic effect of *C. sinensis* has been reported against *Fusarium oxysporum* [25] and postharvest pathogens Sharma and Tripathi [33]. Ethanol extracts exhibited relatively stronger fungitoxicity than petroleum ether extracts on the test organism. The differences in fungitoxicity between the extraction

medium can be attributed to the solubility of the active compound(s) in the extracting solvents [34, 35].

Extracts of the test plants were most effective in reducing the radial growth of the pathogen after 2 days of culture, which decreased as incubation period increased indicating that the efficacy of the active compounds of the plant materials were not persistent in the culture or they depreciated in toxicity after two days of culture.

## 5. CONCLUSION

This study shows the potential of organic extracts of medicinal plants in controlling *Sclerotium rolfsii* in yam. The study also found out that the best plant extracts that could suppress the growth and germination of this rot causing pathogen is *C. sinensis*, followed by *C. aurantifolia* and *A. indica*.

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