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# Phytochemial Screening of *Acanthus montanus* (Nees) T. Anderson and *Crinium jagus* (Thomps) Dandy Extracts and their Potential for Controlling rot fungi of Stored Cocoyam (*Colocasia esculenta) (L.*) (Schott)

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## Abstract

Phytochemical screening of the crude extracts of *Acanthus montanus* (Nees) T. Anderson and *Crinium jagus (*Thomps) Dandy was carried out to determine the bioactive components of the two plants. Results revealed the presence of important compounds such as alkaloids, saponins, tannins, phenols and phytate. Only trace amounts of flavonoids was found in *A. montanus* while it was entirely absent in *C. jagus.* The effect of crude ethanolic extracts of *A. montanus* and *C. jagus* on the mycelial growth of four cocoyam (*Colocasia esculenta*) storage fungi was investigated. The rot fungi were *Sclerotium rolfsii* Sacc., *Botryodiplodia theobromae Pat, Fusarium solani*, and *Rhizopus stolonofer*. Results showed that all the extracts at different concentrations of 100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml inhibited the mycelial growth of the test fungi. Growth inhibition of the rot fungi increased with increasing concentrations of each extract. Extracts of *A. montanus* leaves had more inhibitory effect on all the four test fungi than *C. jagus* bulb extract. The significance of these results is discussed.

**Keywords**: Phytochemical, *A. montanus, C. jagus*, extract, fungi, cocoyam.

## Introduction

*Acanthus montanus* (Nees) T. Anderson also known as "mountain thistle" or "alligator plant" is a striking small shrub with sparse branches and soft stems belonging to the family Acanthaceae. *A. montanus* morphology has been described (Huxley, 1992). It grows wild in grassland woods and rocky hills. It is popular in Southern Nigeria where it is variously called "agamsoso, elele nyiju, and agamefu". It is employed in traditional medicine (Igoli *et al.,* 2004; Okoli *et al*., 2008) and documented evidence of pharmacological activities shows that the leaves of the plant possess spasmolytic, analgestic, anti-inflammatory and anti-pyretic properties (Adeyemi *et al.,* 1999, 2004; Asongalem *et al*., 2004; Okoli *et al*., 2008). In Southern Nigeria, the root poultice is popularly used by the Igede people of Benue State and Nsukka Zone of Enugu State to treat furuncles. *Crinium jagus* (Thomps) Dandy commonly called 'harmattan lily' belongs to the family Amaryllidaceae and is distributed throughout the tropics and subtropics. *C. jagus* is locally called "okonkilo inyi" which literally means elephant's potato by the Igede people of Benue State of Nigeria. The Nsukka people of Enugu State, Nigeria call the plant 'yorabas ndi muo' literally meaning 'wild onions'. The Hausa and Fulani tribes of Northern Nigeria call the plant "Ugandali" (Nwaehujor *et al.,* 2012). All the plant species have ornamental value.

In Sierra Leone, cold infusion of the fresh leaves is used to bathe young children suffering from general body debility, rickets etc., (Nwaehujor *et al*., 2012). In Ghana, a decoction of *C. jagus* is given as a vermifuge while in East Africa the decoction of the bulb is used for treatment of sores. Ode and Asuzu (2006) reported that methanol extract of *C. jagus* bulb exhibited anti-venom effects when it completely inhibited the hoemorhagic activities of *Echis ocellatus* venom at various concentrations. Most studies carried out on *A. montanus* and *C jagus* focused on the treatment of various human ailments. There is little or no report on the use of extracts of these plants for antimicrobial purposes. Chemical fungicides have commonly been employed for the control of several plant diseases. However, in view of environmental concerns, the high cost of chemical pesticides, their toxic effects on non-

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target organisms and the development of resistance against chemical fungicides, the use of chemicals is no longer popular. Plant extracts are increasingly being used in place of these chemicals (Arcury and Quandt, 2003, Deising *et al.*, 2008, Amadi, 2011).
The objective of this study is to determine the phytochemical constituents of *A. montanus and C. jagus* and evaluate the importance of their extracts as antifungal materials for the control of cocoyam (*Colocasia esculenta*) storage fungi.

## Materials and methods

### Collection of Plants and Test Fungi

Leaves of *A. montanus* and bulbs of *C. jagus* used for this study were collected from a farm in Ihakpu-Awka in Igbo-Eze South Local Government Area of Enugu State, Nigeria. The identity of the plants was confirmed by a renowned taxonomist, Prof. J.C. Okafor, a visiting Prof. at the Department of Applied Biology and Biotechnology, Enugu State University of Technology (ESUT), Enugu, Nigeria. Cultures of the fungal organisms used were obtained from the Plant Pathology and Mycology Laboratory of the Department of Botany, University of Nigeria, Nsukka, Nigeria. Test fungi are established cocoyam rot fungi namely *Sclerotium rolfsii* Sacc., *Botryodiplodia theobromae* Pat., *Fusarium solani* (Mart.) Sacc. and *Rhizopus stolonifer* (Ehren ex Fr.) Lind (Jackson and Gollifer, 1975; Arene,1980; Okeke 1981).

### Preparation of Plant Materials and Extraction

Fresh leaves of *A. montanus* were sun-dried for 14 days to a constant weight. The dried leaves were ground to fine powder using Thomas Wiley Laboratory Mill. *C. jagus* bulbs were washed thoroughly under tap and rinsed with sterile distilled water. The bulbs were sliced into small pieces and sun-dried for four weeks before grinding into coarse powder using hammer mill. The powder was sieved through 1mm test sieve to obtain a fine powder used for the extraction. The extraction methods of Ogundiya *et al.* (2006) and Kolapo *et al.* (2009) were used. The solvent used for extraction was absolute ethanol. Sixty (60) grammes each of the powdered plant material were soaked separately in 600ml of absolute ethanol and allowed to stand for 48hrs on the laboratory bench. Filtration was done using Whatman No.1 filter paper. The filtrate was dispensed into sterile Petri-dishes and placed in front of a standing fan which evaporated the solvent leaving behind the solid extracts. The solid extracts were put inside sterile test tubes and stored in the refrigerator while the experiment lasted.

### Phytochemical Screening of Extracts.

The test extracts of *A. montanus* leaves and *C. jagus* bulbs were screened for the phytochemical constituents such alkaloids, tannins, phenols, saponins, phytate and flavonoids. The screening was carried out following standard procedures (Sofowora , 1984; Harbone, 1994; Omotayo and Borokini, 2012).

### Effect of Test Extracts on Mycelial Growth of Test Fungi

The plant extracts were separately dissolved in 50% concentration of dimethyl Sulphoxide (DMSO) $(CH_3)_2SO$) in the ratio of 1:10 (1g of crude extract dissolved in 10ml of DMSO) to give a concentration of 100mg/ml. Further concentrations of 75mg/ml, 50mg/ml and 25mg/ml were made from the stock concentration (100mg/ml). Two milliliters (2ml) of each extract concentration were aseptically dispensed into separate sterile Petri-dishes containing 18ml of molten potato dextrose agar (PDA) amended with streptomycin sulphate. The Petri-dishes were swirled on the laboratory bench to mix the extracts and the agar medium. Each of the Petri-dishes was inoculated at the middle with a 2mm diameter mycelial disc of any of the test fungi. Agar medium in a Petri-dish amended with 2ml of sterile distilled water served as control. Benlate, a standard fungicide with a concentration of 8.5mg/ml was used to monitor the efficacy of the plant extract (Onyeke and Maduewesi, 2005; Onyeke and Ugwoke, 2011). The experiments were laid out in a Completely Randomized Design (CRD) and replicated three times. The diameter of the growth of each fungus in a Petri-dish was measured 7 days after inoculation. The toxicity of the test extracts against the test fungi was determined as a percentage inhibition of mycelial growth with the formula:

$$F_p = \frac{F_1 - F_2}{F_1} \times 100$$

Where: $F_p$ = Percentage inhibition of mycelial growth;
$F_1$ = Mycelial growth in control Plate
and $F_2$ = Mycelial growth in treatment Plate (Onuh *et al.* 2005).

## Results

Results of the phytochemical screening of *A. montanus* leaf and *C. jagus* bulb extracts revealed the presence of bioactive secondary metabolites. These metabolites included alkaloids, saponins, tannins, phenols, flavonoids and phytates (Table 1).

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Quantitative estimation of the percentage composition of each of the phytochemicals detected showed that the metabolites occurred at different concentrations in the two plant materials. Phenol had the highest percentage composition in both *A. montanus* and *C. jagus.* Phenol percentage composition was also more in *A. montanus* than in *C. jagus*. Flavonoid had the least percentage composition in *A. montanus* but was entirely absent in *C. jagus* (Table 2).

***Table 1: Qualitative Phytochemical Screening of the Leaf and Bulb Extracts of Acanthus montanus and Crinium jagus***

| **Constituents** | ***A. montanus*** | ***C. jagus*** |
|---|---|---|
| Alkaloids | + + | + + |
| Saponins | + + | + + |
| Tannins | + + | + + |
| Phenols | + + | + + |
| Flavonoids | + | - |
| Phytate | + | + + |

**+ + = Highly Present; + = Present - = Absent**

***Table 2: Quantitative Phytochemical Screening of the Leaf and Bulb Extracts of Acanthus montanus and Crinium jagus***

| **Constituents** | ***A. montanus*** | ***C. jagus*** |
|---|---|---|
| Alkaloids | 5.58 | 3.77 |
| Saponins | 3.07 | 4.04 |
| Tannins | 5.84 | 3.63 |
| Phenols | 11.38 | 6.84 |
| Flavonoids | 0.73 | - |
| Phytate | 2.75 | 2.25 |

It was observed in this study that the two test extracts had inhibitory effects on the mycelial growth of all the cocoyam rot fungi tested. The effects were uniform on all the fungi though the percentage inhibition varied from one fungus to another. Statistical analysis showed that the effects on mycelial growth were significant at 5% ($P \leq 0.05$). It was also observed that the inhibitory effects on mycelial growth by the plant extracts increased with increase in concentrations. The extracts of *A. montanus* showed higher potential for inhibiting mycelial growth than the *C. jagus* extracts at all concentrations. On the whole, the percentage inhibition of mycelial growth by the two test extracts in this study at the highest concentration of 100mg/ml compared favourably with the effect of Benlate at a lower concentration. The individual effects of the extracts on the different fungi are presented in Tables 3 – 6. The two test extracts recorded the highest inhibition of mycelial growth of 100% and 89.49% in *F. solani* for *A. montanus* and *C. jagus*, respectively, at the concentration of 100mg/ml.

***Table 3: Effects of Ethanolic Extracts of Acanthus montanus and Crinium jagus on Mycelial Growth (mm) of S. rolfsii***

| Concentration (mg/ml) | *Acanthus montanus* Mycelial Growth (mm) | % Inhibition | *Crinium jagus* Mycelial Growth (mm) | % Inhibition |
|---|---|---|---|---|
| 100 | 0.00 | 100 | 0.72 | 89.46 |
| 75 | 1.16 | 83.02 | 2.48 | 63.70 |
| 50 | 2.88 | 57.83 | 4.27 | 37.48 |
| 25 | 3.75 | 45.39 | 5.33 | 21.96 |
| Benlate | 0.00 | 100 | 0.00 | 100 |
| Control | 6.83 | - | 7.11 | - |
| **LSD ($P \leq 0.05$)** | 0.67 | | 0.54 | |

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**Table 4: Effects of Ethanolic Extracts of *Acanthus montanus* and *Crinium jagus* on Mycelial Growth (mm) of *Botryodiplodia theobromae***

| | *Acanthus montanus* | | *Crinium jagus* | |
|---|---|---|---|---|
| Concentration (mg/ml) | Mycelial Growth (mm) | % Inhibition | Mycelial Growth (mm) | % Inhibition |
| 100 | 0.76 | 88.53 | 1.49 | 81.76 |
| 75 | 1.87 | 75.17 | 2.18 | 73.32 |
| 50 | 3.03 | 5976 | 3.62 | 55.69 |
| 25 | 4.14 | 45.02 | 4.75 | 41.86 |
| Benlate | 0.00 | 100 | 0.00 | 100 |
| Control | 7.53 | - | 8.17 | - |
| **LSD (P ≤ 0.05)** | 0.72 | | 0.56 | |

***Table 5: Effects of Ethanolic Extracts of Acanthus montanus and Crinium jagus on Mycelial Growth (mm) of Fusarium solani***

| | *Acanthus montanus* | | *Crinium jagus* | |
|---|---|---|---|---|
| Concentration (mg/ml) | Mycelial Growth (mm) | % Inhibition | Mycelial Growth (mm) | % Inhibition |
| 100 | 0.00 | 100 | 0.86 | 89.49 |
| 75 | 1.13 | 86.29 | 1.93 | 76.41 |
| 50 | 2.94 | 61.04 | 3.02 | 63.08 |
| 25 | 4.16 | 49.51 | 4.89 | 40.22 |
| Benlate | 0.00 | 100 | 0.00 | 100 |
| Control | 8.24 | - | 8.18 | - |
| **LSD (P ≤ 0.05)** | 0.68 | | 0.55 | |

***Table 6: Effects of Ethanolic Extracts of Acanthus montanus and Crinium jagus on Mycelial Growth (mm) of Rhizopus stolonifer***

| | *Acanthus montanus* | | *Crinium jagus* | |
|---|---|---|---|---|
| Concentration (mg/ml) | Mycelial Growth (mm) | % Inhibition | Mycelial Growth (mm) | % Inhibition |
| 100 | 0.00 | 100 | 0.00 | 100 |
| 75 | 0.00 | 100 | 1.49 | 80.03 |
| 50 | 1.31 | 82.71 | 2.85 | 61.80 |
| 25 | 3.47 | 54.22 | 4.14 | 44.50 |
| Benlate | 0.00 | 100 | 0.00 | 100 |
| Control | 7.58 | - | 7.46 | - |
| **LSD (P ≤ 0.05)** | 0.53 | | 0.47 | |

In all the tests carried out, there was significant (P≤0.05) difference in inhibition of the mycelial growth between the leaf extracts of *A. montanus* and the bulb extracts of *C. jagus* with *A. montanus* being slightly more potent in inhibiting fungal mycelial growth than *C. jagus.*

## Discussion

Investigations into the phytochemical constituents of the leaf and bulb extracts of *Acanthus montanus* and *Crinium jagus*, respectively, revealed the presence of alkaloids, saponins, tannins, phenols, flavonoids and phytates. Similar bioactive constituents

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have been reported in the leaf extracts of *Landolphia owariensis* (Nwaogu *et al*., 2007), *Ageratum conyzoides* (Sazada *et al*., 2009), bulb extracts of *Crinium jagus* (Ode *et al*., 2010), and *Crinium asiaticum* (Win, 2011). Flavonoid was however, absent in the bulb extract of *C. jagus* in the present study and only a trace amount (0.73%) in *A. montanus*. Phenols had the highest percentage content of 11.38 and 6.84 for *A. montanus* and *C. jagus*, respectively.

Many studies have shown that natural antioxidants from plant sources can effectively inhibit oxidation of food and reduce the risk of age-dependent diseases (Burda & Oleszek 2001; Zou *et al*., 2004). Flavonoids, abundant in fruits, vegetables, teas, medicinal plants, have attracted the greatest attention and have been studied extensively, because they are a kind of highly effective antioxidants with a lower toxicity than synthetic antioxidants such as butylated hydroxyanisole (BHA) and the related compound butylated hydroxytoluene (BHT) (Pekkarinen *et al*., 1999). BHA and BHT are phenolic compounds that are often added to foods to preserve fats. Cai *et al.* (2010) have reported flavonoids and phenols in *Opuntia milpa alta*, a subtropical plant of the Cactaceae family. A. *montanus* and *C. jagus* exhibited high degrees of antifungal activities against four cocoyam rot fungi namely *S. rolfsii*, *B. theobromae*, *F. solani* and *R. stolonifer*. *A. montanus* also showed more potency against the rot fungi than *C. jagus*. This demonstration of higher potency could be as a result of higher concentration of the active principles in the leaves of *A. montanus* than in the bulbs of *C. jagus.* Generally, efficacy of plant extracts in antimicrobial experiments is concentration-dependent. Leaf extracts of *Azadirachta indica* (neem tree) and C*hromolaena odorata* (Siam weed) have been reported to be fungitoxic against some yam rot fungi (Okigbo *et al*., 2010). Amadi *et al.* (2010) have reported that extracts of African Basil (*Ocimum gratissimum* L.) impaired radial growth in *Aspergillus repens*, *Curvularia lunata* and *Fusarium moniliforme* and that the test organisms differed in their reactions to the different extracts. Various plant extracts have been shown to have *in vitro* and *in vivo* biological activities and can be used as bio-fungicidal compounds (El-Mougy and Alhabeb, 2009; Fawzi *et al.*, 2009; Amadi, 2011; Onyeke and Ugwoke, 2011). Yanar *et al.*, (2011) reported total inhibition of *Phytophthora infestans* mycelial growth by the extracts of four plants. Chiejina and Onaebi (2013) have reported inhibitory effects of ethanolic extracts of *Chromolaena odorata* L. and *Moringa oleifera* Lam. on the mycelial growth of *Fusarium oxysporum*, *Aspergillus niger*, *Rhizopus stolonifer*, *Geotrichum candidum and Mucor micheli*.

Of major interest in the findings of this study is the fact that *A. montanus* and *C. jagus* plant extracts compared favourably with Benlate, a standard chemical fungicide in inhibiting mycelial growth of cocoyam rot fungi. Benlate was shown to be highly effective in reducing rot in stored yam and cocoyam (Ogundana, 1972, Ogundana and Dennis 1981, Eze and Maduewesi, 1990). It is this ability of plant extracts to compare favourably with known standard fungicides coupled with their environmentally friendly qualities that makes their use as antimicrobials very attractive and convenient.

## Conclusion

Having established the efficacy of *A. montanus* and *C. jagus* extracts in inhibiting activities of cocoyam rot fungi, their use as protectant fungicides should be encouraged by way of agricultural extension. Leaf litters of these plants could be applied as manure to control soil-borne organisms as earlier suggested by Okigbo *et al.*, (2010). With the results of this study a positive indication has been made as to the fungicidal potentials of these plants. Future research efforts should therefore be directed towards producing organic fungicides from these plants as they are safer and readily available in good quantity in our localities and more so, conventional chemical fungicides are very expensive and environmentally unsafe.

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