AQUEOUS *Anacardium occidentales* (CASHEW) APPLE FRUIT EXTRACT: AN EXCELLENT CARBON SOURCE FOR FUNGAL GROWTH MEDIA

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Abstract: In a bid to investigate the antifungal properties of cashew fruit (apple fruit), a fungal sensitivity test on both ethanolic and aqueous cashew apple fruit extract was conducted. The results revealed rather an experimental serendipity. The aqueous extract of the fruit gave little or no inhibition at all on the isolates, instead, it served as a better growth medium for the fungi. The ethanolic extract gave an opposite result to what was obtained from the aqueous extract. The results revealed that aqueous cashew apple fruit extracts allowed better growth of the isolates than potato dextrose agar (PDA) which served as standard growth media. The mean fungal culture growths recorded on fifth (5) day using this aqueous cashew apple fruit extract (ACAFE) was: 91.18 ± 4.4, 100.00 ± 0.00,100.00 ± 0.00 and 89.41 ± 6.65% for *Aspergillus niger*, *Rhizopus stolonifera*, *Fusarium oxysporium* and *Penicillium chrysogenum*, respectively; while minimal growths (25.50 ± 3.48,30.00 ± 4.43, 17.68 ± 2.78 and 55.88 ± 05.76%) were shown on fungal cultures from the ethanolic cashew apple fruit extract (ECAFE).Hence, this research shows discovery of a potential growth medium for fungi should further studies be considered on the aqueous extract of cashew apple fruit. It equally shows that ethanolic extract of the cashew apple fruit is a potent fungicide possible due to exposure of fungal bioactive components such as volatile oils, triterpenoids and phenolics.

Keywords: Apple, aqueous, ethanolic, cashew, fungi, fruit, growth

Introduction

The cashew plant known botanically as *Anacardium occidentale* is a common tropical green plant whose tree has high economic significance as source of food and medicine. The tree produces the oval or pear-shaped pseudocarp or false fruit which develop into the succulent drupe known as cashew apple to which is attached the popular source of snack know as cashew nut. There are myriads of claims on the efficacy its leaves and stem are applied in the treatment of many ailments. All part of the cashew plant is of great benefit. The fleshy peduncle called cashew apple when ripe is used in beverages.

The fruit is eaten fresh or made into jam or fruits juice. The kernel is consumed as roasted, fried, salted or raw nuts and may be integrated into cakes and deserts; leaves are used as vegetables and herbs while woods are used for fuel (Brijyog *et al.*, 2017). Despite abundance of cashew plantations and processing factories in Nigeria, there is still great shortfall on its finished products due to fungal attack (Esuruosu, 1974; Suleiman 2010). The cashew apple is known to possess phytochemicals such as triterpenoids and phenolics, as well as volatile oils (Ifesan *et al*., 2013) which have antifungal properties and are secondary metabolites of the apple.

The purpose of this research was to examine the effects of aqueous and ethanolic extract of cashew apple fruit on fungal isolates responsible for yam and cassava post-harvest rot.

Materials and Methods

Sample collection

Both yam and cassava samples with pronounced infestation by fungi were randomly obtained and transported sealed in separate polyethylene packages from the popular yam market, Wukari town to the Federal University Wukari, Biology Laboratory in Taraba State, Nigeria in June, 2018.

The cashew apple fruits were obtained from different location; some were purchase from new market and others from Kwararafa University Permanent site, Wukari Local Government Area of Taraba State, Nigeria. The nuts were detached from the apple fruits and the apple fruits of interest sorted and washed to remove surface dirt in clean running water. They were slit into smaller pieces before air drying indoors on clean plain sheets place on laboratory work bench tile blocks for a period of four weeks. They were dried to constant weight at room temperature of 38^{0}C.

Aqueous and ethanolic extracts

The dried cashew apple fruits were grounded in a motor grinding machine (Model-5.5hp Petrol Engine) to fine consistency. The complete extraction of both aqueous and ethanolic liquid content of *Anacardium occidentale* apple fruits was carried out using the rotary evaporator (Model-STONE, STAFFORDSHIRE ST150SA, UK). An electronic digital weighing balance (Model-Biocote SB160 UK) was used to weigh 250 g of separate grounded samplesin a transparent plastic bowl with a tight lid and to one was added 1000 ml of distilled water while the other was soaked with 1000 ml 70% ethanol. They were vigorously agitated for 90 min in their covered state and allowed to soak for 24 h, after which the samples were filtered using Whatman No. 1 filter paper. Each of the filtrate were introduce into a rotary evaporator under reduced pressure, below 78.4^{0}C set to run at the speed of 133.3224 Pa (1/760 torr) for 8 h for concentration of the ethanolic extract and below 100^{0}C set to run at the speed of 101325 Pa (1/760 torr) for 13 h for complete extraction of aqueous extract respectively.The different product were then place on analog electronic hot plate set at 100^{0}C for ethanolic extraction and 150^{0}C for aqueous extraction respectively to completely evaporate any leftover liquids in order to obtained a total concentrate of the extracts.

Isolation of fungi

Oniyike and Maduewesi (1985) described the protocol employed in this study. A small section of the tuber tissues indicating advanced margin of rot and adjoining healthy tissue were cut using sterilized scalpel and cork borer. The peeled portions scooped using cork borers were placed on the solidified agar. Three peeled portions were placed per plate with equal distance between them. A duplicate plates for each of the rotten portion of site were made for each tuber variety. The plates were incubated at 27 ± 2^{o}C for 6 days. Fungi associated with both yam and cassava rot affected tissues were observed and the frequency of isolation determined using method of Okigbo and Ikediugwu (2000).

Preparation of pure culture

Protocol by Green (1994) was adopted in which 2.5 ml of 10% chloramphenicol was added to every 250 ml of sterile

cooled PDA prior to pour-plating so as to eliminated bacterial contamination. After solidification of medium, sub-cultures of fungal isolates were carried out and incubated at 25 ± 2ºC.

Anti-fungal activity of the extracts

The method of Sangoyomi *et al*. (2009) for studying inhibitory effect of the plant extract on fungal growth (Mycelia) was used with some modification. The extracts (10 g) were measured using an electronic digital weighing balance into a set of 250 ml beaker containing 100 ml of ethanol and sterile distilled water, respectively. The contents were mixed separately using a magnetic stirrer to obtain ethanolic and aqueous homogenous mixtures. Individual ethanolic and/or aqueous cashew apple fruits extracts (1 ml) were collected using a 5 ml syringe and dispensed into the different labeled Petri dishes and 9ml of the Potatoes Dextrose Agar media (Molten PDA) was then poured on each of the dishes containing the different extracts and gently with even dispersal within the plates, resulting to extract –culture media (PDA) complex with 10% concentration of the extract. The mixture was then allowed to solidify.

The various isolates were each cultured on the different plant extract incorporated media plates and incubated for 5 days. Each of the treatment was replicated twice. The standard (control) for the isolates was carried out by seeding different sizes of the fungi on media plates (PDA) poured on a 1 ml sterile distilled water (without the extract). All the plates were incubated at the temperature of 28^0C for 5 days and the effects of the extract on fungal growth and their daily survival were observed and the colony diameter taken as the maximum growth.

Fungi toxicity was recorded in terms of percentage colony inhibition and calculated according to the formula of Pandey *et al.* (1982) as stated below;

Growth inhibition (%) = [(DC-DT)/DC] X 100

Where DC = average diameter of control, and

DT = average diameter of fungal colony with treatment.

Fungal Growth (%) = 100 - % Growth Inhibition.

Results and Discussion

The study aimed at ascertaining the level of inhibition of Aqueous Cashew Apple Fruit Extract (ACAFE) on various fungi (*Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporium* and *Penicillium chrysogenum*) studied. Pure isolates obtained from rotted yams include; *Aspergillus niger*, *Rhizopus stolonifer* and *Fusarium oxysporium* while *Penicillium chrysogenum* was isolated from decomposing cassava tuber (Table 1). Moreover, it was discovered that instead of inhibiting the growth of fungi, the media integrated with the Aqueous Cashew Apple Fruit Extract (ACAFE) actually encouraged growth of the fungi as the bloom of fungal growth was noticed to increase with passage of time.

Table 1: Morphology/cultural characteristics of some fungi isolated in this study

S/N	Isolates	Macroscopic view	Microscopic view
1.	*Aspergillus niger*	The growth of *A. niger* on PDA is rapid and fast. It is powdery and black coloured almost covering the plates after 5 days.	It is non-septate with conidiophores arising from thick-walled foot cells. Each conidiophores ends in a terminal enlarged spherical swellings. Conidia borne by phialides arising from a terminal swelling on the conidiophores.
2.	*Rhizopus stolonifera*	The colony grew rapidly with grey colour which field the whole perimeter of the culture plates within three days on aqueous treament. The grey colour darkened with age.	It has broad hyphae, non or scarcely septate ;rhizoids and stolons are present with brown sporangiophores which is solitary or in tuftson the stolons diverging from the point at which the rhizoids form
3.	*Fusarium oxyporium*	*F. oxysporium* also grow rapidly on PDA agar with White aerial mycelium tinged with pink purple colour.	There is a presence of micro and macro conidia the macro conidia is slightly sickle-celled with apical cell and foot shaped basal cell, chlamydospores are also present and singled with some in pairs.
4.	*Penicilliumchrysogenum*	Moderately rapid to rapid, texture velvety to powdery; Green, blue-green, gray-green, white, yellow, and pinkish on the surface. Some appear white to yellowish, sometimes red and brown when the plate is reverse upside down.	Hyphae septet, hyaline. Conidiophores are simple branch. Conidia unicellular, round to ovoid, hyaline and pigmented, rough and smooth walled, in chains phial ides grouped in branch-like clusters (penicillin) at the end of the conidiophores.

Inhibition on the other hand by the integrated media, showed great decline from the first day to the fifth day of the experiment. Thus inhibition of media on *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporium* and *Penicillium chrysogenum* had similar pattern of decline between first day and fifth day as follows: 91.17 ± 0.84 - 8.82 ± 4.47%, 88.24 ± 9.64-0%, 97.00 ± 4.16 – 0% and 80.00±4.99- 10.59±06.65%. The values in Table 3 showed there was no significant inhibition on fungal growth by the media even though the greatest inhibition (10.59±06.65%) was against *Penicillium chrysogenum.* There was absolutely no inhibition of this integrated media against *Rhizopus stolonifer* and *Fusarium oxysporium* as shown in Table 2.

Growth of fungi was achieved from twenty four hours of incubation to the fifth day. Within twenty four hours of inoculation of media, the growth recorded for *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporium* and *Penicillium chrysogenum* were: 8.83 ± 0.84, 11.76 ± 9.64, 3.00 ± 4.16 and 20.00±4.99% which rose to 91.18 ± 4.47, 100.00 ± 0.00, 100.00 ± 0.00 and 89.41±6.65%, respectively five days later. The result of fungal growth in aqueous cashew apple fruit extract (ACAFE), was comparable with that recorded in the standard (Potato Dextrose Agar (PDA) media only) Table 6. In the standard, after five days growth of *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporium* and *Penicillium chrysogenum*, their values were; 100.00 ± 0.00, 85.83 ± 1.81, 100.00 ±0.00, 100.00 ±0.00 and 95.88 ± 03.83. These values are comparable with those of the ACAFE integrated PDA media. As a matter of fact *Rhizopus stolonifera* recorded greater growth in *ACAFE* integrated

media (100.00 ± 0.00 %) than that recorded in PDA (85.83 ± 1.81%) after five days of growth. Similarly, *Fusarium oxysporium* recorded equal growth (100.00 ± 0.00 %) in both media. The discovery made as herein above illustrated showed that aqueous cashew apple fruit extract (ACAFE) could be incorporated as a carbon source for media development for industrial production of useful fungal media. Thus, aqueous apple fruit extracts are indisputably excellent sources of carbon as shown by the work carried out by Dos Santos Lima*et al*. (2012). Cashew apple bagasse samples analysis carried out by Dos Santos Lima *et al*. (2012) on HPLC gave results showing saccharification of biomass with glucose (1537.49 mg/L), xylose (3823.22 mg/L) and arabinose (7131.11 mg/L) constituents.

Unlike the Aqueous Cashew Apple Fruit Extract (ACAFE) which encouraged fungal growth, Ethanolic Cashew Apple Fruit Extract (ECAFE) is a real growth inhibitor as was discovered in this research. There was 100.00±0.00% inhibition by ECAFE integrated media against *Aspergillus niger*, *Rhizopus stolonifer*, and *Fusarium oxysporium* consistent for two days, while *Penicillium chrysogenum* showed 89.43±3.50 and 88.24±2.76% within twenty four and forty eight hours respectively. There was little non statistical decline in *Aspergillus niger*, *Rhizopus stolonifer* and *Fusarium oxysporium* from the second day to the fifth day (Table 5). The inhibition maintained in the fifth day against the prior illustrated fungi were 74.50±3.48, 70.00±4.43 and 82.32±2.78%. However, statistically significant reduction in inhibition of ECAFE against *Penicilliumchrysogenum* was noted, with about 44.12±05.76 % inhibition.

Findings from experiment showed that growth was non-existent on ECAFE integrated media against *Aspergillus niger*, *Rhizopus stolonifer* and *Fusarium oxysporium* in the first two days of inoculation (Table 4). As a matter of fact there was no growth of *Fusarium oxysporium* consistently for four days; a clear indication of great inhibitory activity of ECAFE. On the fifth day, the growth recorded in the media by *Aspergillus niger*, *Rhizopus stolonifera*, *Fusarium oxysporium* and *Penicilliumchrysogenum* were 25.50 ± 3.48, 30.00 ± 4.43, 17.68 ± 2.78 and 55.88 ± 05.76%, respectively. Apart from *Penicilliumchrysogenum* with growth of 55.88 ± 05.76 %, other fungi were considered inhibited by Ethanolic Cashew Apple Fruit Extract (ECAFE) integrated media.

Table 2: Percentage growth of fungi in aqueous cashew apple fruit extract (ACAFE)

Days	*(% Growth ± SD) A. niger*	*(% Growth ± SD) R. stolonifera*	*(% Growth ± SD) F. oxysporium*	*(%Growth ± SD) P. chrysogenum*
Day1	8.83 ± 0.84^{a}	11.76 ± 9.64^{a}	3.00 ± 4.16^{a}	20.00±4.99^{a}
Day 2	60.01 ± 4.25^{b}	61.18 ± 5.25^{b}	51.18 ± 8.04^{b}	43.23±09.57^{b}
Day 3	76.47 ± 3.27^{c}	80.00 ± 6.28^{c}	68.24 ± 4.92^{c}	60.00±1.65^{c}
Day 4	88.24 ± 2.63^{d}	91.76 ± 3.65^{d}	70.59 ± 4.59^{c}	75.29±4.65^{d}
Day 5	91.18 ± 4.47^{d}	100.00 ± 0.00^{e}	100.00 ± 0.00^{d}	89.41±6.65^{e}

All similar alphabets in the same column are not significantly different

Table 3: Percentage mean inhibition of fungi in aqueous cashew apple fruit extract

Days	*(% inhibition ± SD) A. niger*	*(% inhibition ± SD) R. stolonifera*	*(% inhibition ± SD) F. oxysporium*	*(% inhibition ± SD) P. chrysogenum*
Day1	91.17 ± 0.84^{a}	88.24 ± 9.64^{a}	97.00 ± 4.16^{a}	80.00±4.99^{a}
Day 2	39.99 ± 4.25^{b}	38.82 ± 5.25^{b}	48.82 ± 8.04^{b}	56.77±09.57^{b}
Day 3	23.53 ± 3.27^{c}	20.00 ± 6.28^{c}	31.76 ± 4.92^{c}	40.00±1.65^{c}
Day 4	11.76 ± 2.63^{d}	8.24 ± 3.65^{d}	29.41 ± 4.59^{c}	24.71±04.65^{d}
Day 5	8.82 ± 4.47^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	10.59±06.65^{e}

All similar alphabets in the same column are not significantly different

Table 4: Mean percentage growth of fungi in ethanolic cashew apple fruit extract (ECAFE)

Days	*(% Growth ± SD) A. niger*	*(% Growth ± SD) R. stolonifera*	*(% Growth ± SD) F. oxysporium*	*(% Growth ± SD) P. chrysogenum*
Day 1	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	12.57 ± 3.50^{a}
Day 2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	11.76 ± 2.76^{a}
Day 3	12.50 ± 6.22^{b}	4.78 ± 4.12^{a}	0.00 ± 0.00^{a}	18.09 ± 7.28^{b}
Day 4	21.00 ± 7.22^{c}	11.76 ± 2.40^{b}	0.00 ± 0.00^{a}	48.23 ± 03.93^{c}
Day 5	25.50 ± 3.48^{c}	30.00 ± 4.43^{c}	17.68 ± 2.78^{b}	55.88 ± 05.76^{d}

All similar alphabets in the same column are not significantly different

Table 5: Mean percentage ethanolic cashew apple fruit extract (ECAFE) inhibition

Days	*(% inhibition ± SD) A. niger*	*(% inhibition ± SD) R. stolonifera*	*(% inhibition ± SD) F. oxysporium*	*(% inhibition ± SD) P. chrysogenum*
Day 1	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	89.43±3.50^{a}
Day 2	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	88.24±2.76^{a}
Day 3	87.50±6.22^{b}	95.22±4.12^{a}	100.00±0.00^{a}	81.91±07.28^{b}
Day 4	79.17±7.22^{c}	88.24±2.40^{b}	100.00±0.00^{a}	51.77±03.93^{c}
Day 5	74.50±3.48^{c}	70.00±4.43^{c}	82.32±2.78^{b}	44.12±05.76^{d}

All similar alphabets in the same column are not significantly different

Table 6: Percentage growth of fungi in plates with only PDA

Days	*(% Growth ± SD) A. niger*	*(% Growth ± SD) R. stolonifera*	*(% Growth ± SD) F. oxysporium*	*(% Growth ± SD) P. chrysogenum*
Day1	10.59 ± 1.66^{a}	5.30 ± 0.84^{a}	8.24 ± 1.02^{a}	12.65 ± 3.89^{a}
Day2	33.53 ± 5.80^{b}	26.47 ± 9.6^{b}	46.67± 4.17^{b}	80.59 ± 1.14^{b}
Day3	69.57 ± 9.98^{c}	50.59 ± 2.63^{c}	72.36 ± 4.16^{c}	89.71 ± 01.89^{c}
Day4	94.12 ±1.66^{d}	60.01 ±4.97^{d}	91.18 ± 0.84^{d}	92.35 ± 01±81^{d}
Day5	100.00 ± 0.00^{e}	85.83 ± 1.81^{e}	100.00 ±0.00^{e}	95.88 ± 03.83^{e}

All similar alphabets in the same column are not significantly different

Retardation of growth and antifungal activity of the plant extracts are a function of the presence of requisite chemical compounds with high potency to do so as well as use of appropriate solvents to dislodge these phyto-fungicidal constituents present in plants. These potent fungicidal properties in plant extracts are viable synthesized secondary metabolites produced in the plants.

The work by Rajesh Kannan *et al*. (2009) showed 95.45% maximum percentage inhibition against *Fusarium* sp. after three days inoculation in ethanolic extracts of *A. occidentale* L. nut extracts. Their findings gave credence to the result of this research in which after three days inoculation in ethanolic cashew apple fruit extract (ECAFE) inhibition percentage was 100· Possibly, the anti-fungal bioactive components such as volatile oils, triterpenoids and phenolics as reported by Ifesan *et al*. (2013) is high in the ethanolic cashew apple fruit extract (ECAFE). At the same time, these toxic antifungal phenolic compounds with great lethality against fungi and whose action is due to greater quantities of hydroxyl groups and site(s) of the phenol resulting to increased toxicity due to more hydroxylation ability (Geissman, 1963) may be in very negligible quantity in the aqueous cashew apple fruit extract (ACAFE) thereby permitting growth of the examined fungi.

According to Rajesh Kannan *et al*. (2009) *A. occidentale* exhibited fungi static effect which ranged from 41.37 to 95% in their finding and *Fusarium* sp. was very susceptible to the ethanol extracts toxicity and the percentage of inhibition was above 94%. This is in concordance with the research finding in this work (Table 5).

In this research, ethanol was able to expose basic fungicidal compounds (volatile oils, triterpenoids and phenolics) embedded in the cashew apple fruit which was not possible with aqueous exposure. Broad spectrum of activity towards fungal isolates (*Aspergillus niger*, *Rhizopus stolonifer*, and *Fusarium oxysporium)* and higher percentage of activity (inhibition) was also recorded in the ethanol extract.

Differences in inhibitory activities could be alluded to variations in the quantity of bioactive components in the sample or synergistic action of various phytochemicals from the extracts (Govindachari *et al.,* 1998). The broad and narrow spectrum inhibitory action of the plant extracts on the fungus are correlation to the potency and nature of the chemical compounds (Rajesh Kannan *et al.,* 2009).

Conclusion

In this research, the ethanolic cashew apple fruit extract (ECAFE) could be said to have broad spectrum of inhibitory activities against the fungi while aqueous cashew apple fruit extract (ACAFE) exhibited narrow and/or no spectrum of inhibitory activities.

The result of interest in this work is that of the cashew apple fruit extract (ACAFE) which showed a comparable ability to support growth of fungi studied. The growth recorded in this growth integrated media was as good as that of the standard PDA media. Thus, further research could lead to discovery of a specialized media with composites of aqueous cashew apple fruit extract (ACAFE) with other media nutrients which could be of commercial interest.

Conflict of Interest

Authors declare that there is no conflict of interest.

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