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PHYTOCHEMICAL AND ANTIFUNGAL PROPERTIES OF *Gongronema latifolium* and *Heinsia crinita*. LEAF EXTRACTS AGAINST FUNGAL PATHOGENS ASSOCIATED WITH POST-HARVEST ROT OF SELECTED FRUITS.

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ABSTRACT

Antifungal potentials of *Gongronema latifolium* and *Heinsia crinita* leaf extracts were evaluated against some fruit rot pathogens. Preliminary phytochemical screening revealed among others the presence of alkaloids, flavonoids, saponins, tannins and cardiac glycoside at varying amounts in both ethanolic and aqueous extracts of the plants. Inhibitory activities of the extracts against the mycelial growth and spore germination of an array of fungi that cause fruit rots showed that their antifungal potentials were concentration dependent. Thus, at the highest extract concentrations of 750 mg/mL, highest inhibitions were observed and the least activities were obtained for concentration of 250 mg/mL extract. This is attributed to the bioactive phytochemical constituents at different extract concentrations particularly the alkaloids and cardiac glycosides. The extracts of *G. latifolium* leaves at highest concentrations of 750 mg/L proved highly effective, with zones of inhibition that ranged from 12.0 to 21.0 mm, for inhibiting spore germination in all the test organisms except *Talaromyces verruculosus* and *Candida utilis* while that of *H. crinita* ranged from 12.0 to 20.0 mm and was able to inhibit spore germination of all test organisms except *Rhizopus oligosporus* and *Absidia corymbifera*. Evidently, these plant extracts have great potential as natural antifungals which can be safely used against post-harvest rot of fruits**.**

Key words: Antifugal, pathogens**,** inhibition, phytochemical, spore germination

1. Introduction

Fruits have high nutritional value as they are rich in sugars, minerals, vitamins and other biotic molecules. They help in repair and control of body processes. They also serve as a good source of income for the people. However, the contamination of fresh fruits with plant and human pathogens can cause considerable economic losses for the industry, apart from being the origin of food borne diseases (Ebong *et al*., 2014). Over the last two decades, biological control of plant pathogens has emerged as a viable disease control strategy (Elad and Stewart, 2004; Lahlali et al., 2022) Numerous factors are responsible for increasing interest in biological control including the negative effects of fungicides on human health (White, 1998), increased regulatory restrictions (Janisiewicz and Korsten, 2002), traceability protocols for crop protection practices, residue tolerance in some export markets, continued interest in organics, pathogen resistance to commonly used fungicides (Rosslenbroich and Stuebler, 2000) and a lack of replacement products. Inhibition of spoilage and/or human pathogenic fungi on fruits by extracts of medicinal plants and their subsequent application as bio preservatives will be a good alternative to chemical compounds and fungicides used in fruit preservation (Elmer *et al*., 2005).

This study was aimed at assessing the antifungal potential of ethanol and aqueous leaf extracts of two indigenous medicinal plants; (*Gongronema latifolium* and *Heinsia crinita*) in Akwa Ibom State, Nigeria against fungal pathogens associated with post-harvest rots of selected fruits.

2. Materials and Methods

Isolation and identification of fungal pathogens from the fruits

Fungal isolates were obtained from diseased fruits of *Persia americana, Citrus sinensis, Carica papaya, Annona muricata* and *Lycopersicon esculentum.* The fruits were obtained from local markets in the three senatorial districts of Akwa Ibom State, Nigeria. Prior to use, the fruits were washed with clean water and surface sterilized in 10 % hypochlorite solution. Sterile scalpel was used to cut 3 mm × 3 mm sections of the fruits, moving from healthy portions to the diseased portions where the fungi were likely to be more active, and aseptically plated on Potato dextrose agar (PDA) medium and incubated at ambient temperature of 28^{o}C ± 2^{o}C.

Pure cultures of each isolate were obtained after series of inoculations unto PDA and incubation at 28^{o}C ± 2^{o}C for five days as the fungi became fully grown and covered the Petri dishes. Preliminary identification was carried out by wet-mounting the fungal mycelium on lacto phenol-cotton blue and observing under × 40 objective lens of the phase contrast microscope. Colony colour, growth pattern on plates, details of philiades and spores were used as identification parameters (Cheesebrough, 2005).

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Preparation of aqueous and ethanolic leaf extracts

The leaves of *Gongronema. latifolium and Heinsia crinita.* were separately cut into pieces and dried in shades for 48hrs and then ground into a coarse powder. The powdered dried leaves were placed in maceration tanks and water or ethanol added for aqueous and ethanolic extractions respectively. The macerated leaves were allowed to stand for 24 h (aqueous) and 72 h (ethanol) before filtration. The liquid crude extract was placed in a rotary evaporator to concentrate. The rotary evaporator was kept cooled by passing chilled water through the chilling chambers. When most of the solvent had evaporated, the extract was transferred into large beakers and placed in a water-bath at 45^{o}C until it was fully dried. The dried crude extract was then transferred into 100mls beakers, covered with aluminum foil and stored in a refrigerator at 4 ^{o}C till required. The percentage yield of extract was calculated using the following formula:

$$\frac{weight\ of\ extract}{weight\ of\ dried\ leaves}\ x\frac{100}{1}$$

Phytochemical constituents and functional group characterization of the extracts

The phytochemical constituents of the crude extracts of the plant leaves were analysed using the methods described by Sofowora (1993), Trease and Evans (2009) and Harborne (1998). The following phytochemicals were screened for: alkaloids, tannins, saponins, flavonoids, cardiac glycosides, anthraquinones, resins, deoxy-sugar, protein and phlobatanins. The functional groups in the extracts were determined using Fourier Transform Infra-red (FT-IR) spectrometer Varian 660 MidIR Dual MCT/DTGS Bundle with ATR.

Determination of inhibitory activity of extracts on fungal spore germination

The fungal isolates were inoculated into sterile conical flasks containing 100 mL Potato. Dextrose Broth (PDB), covered with sterile cotton wool and aluminum foil and placed in a water bath with a shaker and gently shaken for 48 hours at 30^{o}C. Fungal spores of each isolate were obtained by filtering with sterile filter paper (0.45 µm – pore-size filter, Millipore).

Inhibitory activity of the extracts on fungal spore germination was carried out using the agar-well diffusion method. To determine the inhibitory activity of the leaf extracts against fungal spore germination, 10^{6} conidia/ml of the fungal spores were dispensed into different sterile Petri dishes and freshly prepared PDA poured unto it and rocked gently for even distribution of the spores. These were allowed to solidify. Then, four wells of 8mm per plate were made using a cork borer. One millilitre aliquots of the leaf extracts at various increasing concentrations (250 mg/mL, 500 mg/mL and 750 mg/mL) were added to three wells while the fourth well received 1mL of the solvent dimethylsulfoxide (DMSO), and served as control. The experiment was carried out in triplicates and the plates incubated at 28^{o}C ± 2^{o}C for 72 h. The zones of inhibition were measured using a transparent meter rule.

Use of the leaf extracts as bio control

Fresh healthy fruits were purchased from the market. They were surface sterilized by washing in running tap water and soaking in 10% hypochlorite for 30 min. The fruits were divided into two groups. One group was soaked inside antifungal leaf extracts at different concentrations (250 mg/mL, 500 mg/mL and 750 mg/mL) for 20 min, allowed to air-dry under the laminar flow sheet while the other group was left without the leaf extract. Fungal spores (10^{6}conidia/ml) obtained via the preparation of fungal inoculums of the fungal isolates were applied unto the surface of all the fruits using a sterile plastic spreader. The fruits were wrapped loosely with sterile aluminum foils and left at ambient temperature for 14 days. The appearance of fungal growth on the surface of the fruits was monitored.

3. Results

Percentage yields of plant extracts

The extract yields ranged from 14.9 to 27.7 % for the aqueous and ethanolic extracts. Highest extract yield was detected in the *G latifolium* irrespective of the extraction solvent used (Table 1).

Table 1: Percentage yields of the plant extracts

	Extract yield (%)	
	Aqueous extract	**Ethanol extract**
Gongronema latifolium	26.7	27.7
Heinsia Crinita	14.9	24.0

Phytochemical constituents of the extracts

The phytochemical constituents identified in the ethanol leaf extracts of *G. latifolia* were cardiac glycosides, saponins, tannins, deoxy-sugar alkaloids, flavonoids, resin and proteins which were detected at various concentrations while there was no detection of either free or combined anthraquinones, and phlobatanins. In the case of the ethanol leaf extracts of *H. crinita*, alkaloids, saponins, tannins, flavonoids, cardiac glycosides, deoxy-sugar and proteins were detected (Table 2).

In aqueous extract of the *G. latifolia*, alkaloids, saponins, tannins, flavonoids, cardiac glycosides, deoxy-sugar, resin and proteins were detected in different concentrations, while anthraquinones and phlobatanins were not detected. Similarly, in the aqueous extracts of *H. crinita*, none of the phytochemicals were detected in a very high concentration. Alkaloids, saponins, tannins were detected in a moderately high concentration, while flavonoids, cardiac glycosides and deoxy sugar were detected in a low concentration (Table 3).

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Table 2: Phytochemical constituents of ethanol leaf extracts of the plants

Constituents	**Tests**	**Observation / Inference**	
		G. latifolia	***H. crinita***
Alkaloids	Picric test	+	+++
	Drangendorff's	++	+++
	Mayer's	+	++
Saponins	Frothing	++	++
	Sodium bi-carbonate	+	+
Tannins	Ferric chloride	+	+++
	Bromine water	++	++
Flavonoids	Shinoda reduction	+	+
	Alkaline reagent	+	++
	Aluminum chloride	+	+
Cardiac glycoside	Salkowski's	++	+
	Lieberman's	+++	+
	Keller-killiani's	+++	++
Anthroquinones	Borntrager's for free anthraquinones	-	-
	Borntrager's for combined anthraquinones	-	-
Resin	Acetone water	+	-
Deoxy sugar	Benedict	+	+
	Fehling	++	+
Proteins	Xanthoproteic	+	+
	Biuret	+	+
Phlobatanins	General	-	-

'+++', '++', '+' and '-'represent detected very high concentration, detected in moderately high concentration , detected in low concentration and not detected, respectively

Table 3: Phytochemical constituents of aqueous leaf extracts of the plants

Constituents	**Tests**	**Observation / Inference**	
		G. latifolia	***H. crinita***
Alkaloids	Picric test	+	+
	Drangendorff's	+	++
	Mayer's	+	++
Saponins	Frothing	++	++
	Sodium bi-carbonate	+	+
Tannins	Ferric chloride	+	++
	Bromine water	+	++
Flavonoids	Shinoda reduction	+	+
	Alkaline reagent	+	++
	Aluminum chloride	+	+
Cardiac glycoside	Salkowski's	+	+
	Lieberman's	++	+
	Keller-killiani's	++	+
Anthroquinones	Borntrager's for free anthraquinones	-	-
	Borntrager's for combined anthraquinones	-	-
Resin	Acetone water	+	-
Deoxy sugar	Benedict	+	+
	Fehling	++	+
Proteins	Xanthoproteic	+	-
	Biuret	+	-
Phlobatanins	General	-	-

'+++', '++', '+' and '-'represent detected very high concentration, detected in moderately high concentration , detected in low concentration and not detected, respectively

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Fourier Transform Infrared Spectroscopic Analysis of Extracts

The FTIR spectrum of the aqueous extract of *H. crinita* manifested prominent bands located at 3908, 2931, 1641 and 1704 cm^{-1} (Fig. 1), while that of the ethanolic extracts of *H. crinita* had intense peak bands due to its polarity at 3404, 2928, 1693, 1600 and 1074 cm^{-1}. In comparison to the bands of the aqueous extract, there were relative shifts in peak positions and intensity indicative of the dipole moments and number of the bonds present..

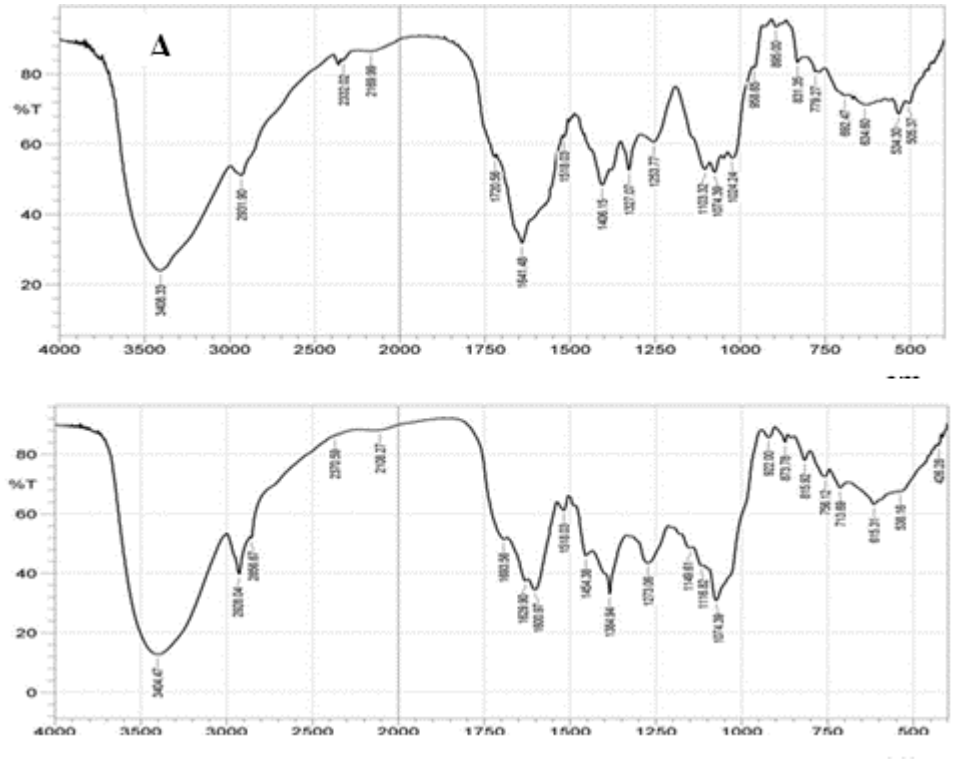

Fig. 1: Absorption bands of the *H. crinita* extracts: A and B represent aqueous and ethanol extracts, respectively

The aqueous and ethanolic extracts of *G. latifolium* had similar features or presence of biomolecules as the previously discussed plant extract but with fewer prominent absorption bands (Fig. 2).

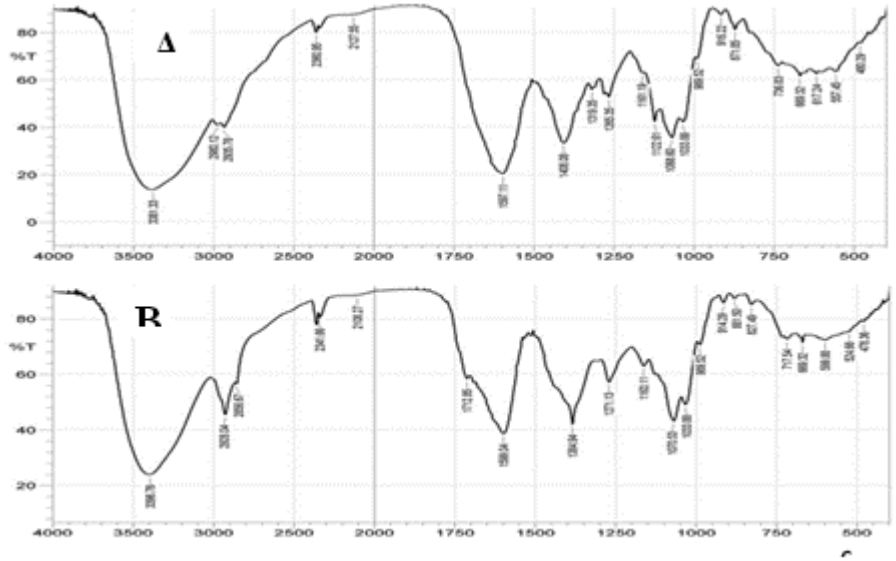

Fig. 2: Absorption bands of the *G. latifulium*: A and B represent aqueous and ethanol extracts, respectively

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Antifungal activities of leaf extracts on spore germination of fungal isolates

Inhibition of the fungal spore germination in presence of the *G. latifolium* extracts was observed to be greater with the ethanol extract than the aqueous. This observation was irrespective of the extract concentration that was used. None of the concentrations used was observed to inhibit the spore germination of *Talaromyces verrucosus* and *Candida utilis.* Across both extracts, degree of inhibition was directly proportional to the concentration of extract used. Generally, highest zone of inhibition of 21 mm was observed for *Mucor racemosus* at ethanol extract concentration of 750 mg/mL (Table 4).

When the *H. crinita* was used, none of the extracts was observed to show inhibition against *Trichoderma koningiopsis, Rhizopus oligosporus and Absidia corymbifera.* This observation was irrespective of the extract concentration used for investigation. When tested against *A. paraciticus,* growth was not inhibited in presence of the aqueous extracts. Growth of *A. paraciticus* was however inhibited in presence of ethanol extract concentrations of 500 and 750 mg/L. Across the different concentrations investigated, highest inhibition was observed against *Fusarium solani* (20 mm) and *A. niger* (18 mm) with concentration of 750 mg/mL of the ethanol and aqueous extracts, respectively (Table 5).

Use of leaf extracts as bio-control

Fruits that were preserved with the respective extracts were observed to remain relatively healthy up to fourteen days of post-treatment storage while the unprotected fruits showed signs of spoilage within five days of storage.

4. Discussion

The selected medicinal plants in these studies were *Gongronema latifolia* and *Heinsia crinita* and their choice was informed by earlier reports on their antimicrobial activities (Eleyinmi, 2007; Adegoke and Adebayo-Tayo, 2009; Oboh *et al*., 2016). Antimicrobial potentials observed in both selected medicinal plants were concentration dependent in line with earlier reports (Adegoke and Adebayo-Tayo, 2009; Adegoke and Inyang, 2009). At higher extract concentration of 750mg/mL, more active ingredients were expected to be present which translated to higher activity. The activity observed at 750 mg/mL and all the other concentration can be attributed to the effect of the extract and not the solvent, as there was no zone of activity when only the solvent was used. Antimicrobial activity was less in aqueous extract than in ethanolic extract of *Gongronema latifolia* and *Heinsia crinita* especially at low concentrations of 250 mg/mL and 500 mg/mL. This disparity in activity based on solvent was in tandem with the report of Nwinyi *et al*. (2009). Their profile also agreed with reports by previous researchers (Eleyinmi, 2007; Adegoke *et al*., 2010; Oboh *et al*., 2016). Generally, low activity of ≤ 15 mm observed in both plants extract used against fungal spores in this study was due to higher resistance a fungal spore usually shows compared to that of vegetative cells. This level of inhibition observed makes the extract to be possible sources of potent preservative (Njimoh *et al*., 2015).

Table 4: Antifungal activities of ethanol and aqueous leaf extracts of the *G. latifolium* on fungal spore germination

Fungal isolates	**Ethanol extract (mg/mL)**			**Aqueous extract (mg/mL)**		
	250	**500**	**750**	**250**	**500**	**750**
Talaromyces verruculosus	NZ	NZ	NZ	NZ	NZ	NZ
Lasiodiplodia theobromae	12.3 ±1.52	15.7 ±1.53	19.3 ±2.31	9.3 ±1.15	14.3 ±0.58	17.0 ±1.73
Trichoderma koningiopsis	10.3 ±1.53	14.0 ±1.73	16.3 ±1.53	8.7 ±1.15	12.0 ±0.00	14.3 ±1.53
Penicillium citrinum	10.7 ±1.15	14.0 ±1.00	17.3 ±1.53	NZ	NZ	9.7 ±1.53
Pichia kudriavzevii	9.0 ±0.00	11.7 ±2.08	12.7 ±1.15	NZ	NZ	10.7 ±1.53
Rhizopus oligosporus	11.3 ±1.53	13.0 ±1.00	17.0 ±2.00	NZ	10.3 ±1.53	13.7 ±0.58
Aspergillus niger	10.7 ±0.58	13.0 ±1.00	15.7 ±1.15	10.0 ±1.00	13.0 ±1.73	16.0 ±2.65
Fusarium culmorum	NZ	NZ	10.7 ±1.53	NZ	NZ	NZ
Fusarium solani	NZ	14.3 ±1.53	18.3 ±1.53	NZ	NZ	NZ
Aspergillus carbonarius	10.7 ±0.58	13.0 ±1.73	15.7 ±2.08	8.7 ±0.58	12.0 ±1.73	13.3 ±1.53
Aspergillus. Paraciticus	12.0 ±2.00	14.7 ±1.15	18.0 ±1.75	9.0 ±1.0	11.7 ±1.15	16.0 ±1.53
Moniliella suaveolens	8.3 ±0.58	11.0 ±1.73	14.7 ±0.58	8.0 ±0.00	9.7 ±1.15	11.7 ±1.53
Mucor racemosus	12.3 ±1.53	15.3 ±1.53	21.0 ±3.61	10.0 ±2.64	12.0 ±1.00	16.0 ±1.73
Aspergillus sclerotiorum	NZ	NZ	12.0 ±2.65	NZ	NZ	NZ
Geotrichum candidum	10.7 ±0.58	13.0 ±1.00	16.0 ±1.00	8.7 ±0.58	11.7 ±2.08	14.3 ±2.08
Candida tropicalis	9.3 ±0.58	13.0 ±1.00	17.3 ±2.31	7.7 ±0.58	11.0 ±1.73	14.7 ±0.58
Absidia corymbifera	13.0 ±1.00	14.0 ±0.00	21.0 ±1.73	9.7 ±0.58	14.0 ±2.65	15.0 ±2.00
Candida utilis	NZ	NZ	NZ	NZ	NZ	NZ
Aspergillus aculeatus	11.3 ±1.53	16.0 ±2.65	20.0 ±2.65	10.0 ±0.00	13.0 ±1.00	15.7 ±2.08
Aspergillus nomius	8.7 ±0.58	13.7 ±0.58	17.3 ±2.52	8.3 ±0.58	11.3 ±0.58	13.7 ±2.52
Purpureocillium lilacinum	9.7 ±1.15	13.7 ±1.15	16.7 ±0.58	8.7 ±0.58	10.0 ±0.00	12.7 ±1.15
Candida. pseudotropicalis	NZ	NZ	NZ	NZ	NZ	NZ

Values represent zones of inhibition and ± standard deviation of means mm. NZ = No Zone of inhibition

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Table 5: Antifungal activities of ethanol and aqueous leaf extracts of the *H. crinita* on fungal spore germination

Fungal isolates	**Ethanol extract (mg/mL)**			**Aqueous extract (mg/mL)**		
	250	**500**	**750**	**250**	**500**	**750**
Talaromyces verruculosus	10.7 ±2.08	14.7 ±2.08	18.7 ±2.08	8.3 ±0.58	11.3 ±0.58	14.3 ±1.53
Lasiodiplodia theobromae	10.3 ±0.58	14.0 ±1.00	18.0 ±0.00	12.0 ±1.00	13.0 ±2.00	17.0 ±2.65
Trichoderma koningiopsis	NZ	NZ	14.7 ±1.53	NZ	NZ	NZ
Penicillium citrinum	11.3 ±1.53	14.0 ±1.73	17.7 ±2.08	8.3 ±0.58	11.7 ±1.53	15.3 ±2.52
Pichia kudriavzevii	12.0 ±0.00	14.3 ±1.15	19.3 ±2.31	11.0 ±1.73	13.0 ±2.65	16.0 ±3.00
Rhizopus oligosporus	NZ	NZ	NZ	NZ	NZ	NZ
Aspergillus niger	10.3 ±0.58	15.3 ±0.58	17.0 ±2.0	12.0 ±2.00	14.0 ±1.00	18.0 ±1.73
Fusarium culmorum	8.0 ±0.58	13.3 ±1.53	15.7 ±1.15	8.7 ±0.58	10.0 ±1.00	13.3 ±0.58
Fusarium solani	12.3 ±0.58	15.3 ±1.53	20.0 ±1.73	9.7 ±0.58	10.7 ±0.58	15.7 ±2.08
Aspergillus carbonarius	9.7 ±1.15	13.7 ±1.15	18.3 ±0.58	8.0 ±0.00	10.3 ±0.58	12.7 ±1.15
Aspergillus. Paraciticus	NZ	10.3 ±0.58	12.0 ±1.00	NZ	NZ	NZ
Moniliella suaveolens	10.7 ±0.58	15.0 ±0.00	17.7 ±2.08	8.7 ±0.58	11.0 ±1.73	14.7 ±2.08
Mucor racemosus	11.3 ±0.58	13.7 ±0.58	17.3 ±1.53	9.7 ±1.53	12.3 ±1.53	15.0 ±1.73
Aspergillus sclerotiorum	8.0 ±0.00	12.7 ±1.15	16.0 ±0.00	10.0 ±0.00	11.7 ±0.58	13.3 ±0.58
Geotrichum candidum	NZ	9.3 ±1.15	14.7 ±1.53	NZ	NZ	8.3 ±0.58
Candida tropicalis	9.7 ±1.15	15.0 ±1.73	16.0 ±1.73	8.7 ±0.58	10.3 ±0.58	14.7 ±0.58
Absidia corymbifera	NZ	NZ	NZ	NZ	NZ	NZ
Candida utilis	11.7 ±1.53	14.0 ±1.00	15.7 ±1.15	10.0 ±0.00	11.3 ±0.58	13.0 ±1.73
Aspergillus aculeatus	11.0 ±0.00	14.7 ±0.58	17.3 ±.52	11.0 ±1.0	12.7 ±0.58	16.0 ±2.65
Aspergillus nomius	11.3 ±1.15	14.0 ±0.00	16.3 ±1.53	10.3 ±1.15	12.7 ±0.58	15.0 ±0.00
Purpureocillium lilacinum	10.7 ±0.58	13.0 ±0.00	15.0 ±1.00	8.7 ±0.58	11.3 ±1.15	13.7 ±1.15
Candida. pseudotropicalis	NZ	NZ	NZ	NZ	NZ	NZ

Values represent zones of inhibition and ± standard deviation of means in mm. NZ = No Zoneof inhibition

In this study, the detection of varying concentrations of alkaloids, saponins, tannins, flavonoids, and cardiac glycosides from very high concentration, to moderately high concentration and low concentration depicted the inherent active antimicrobial ingredient applicable for fruit preservation (Maatalah *et al*., 2012; 2013; Mujeeb *et al*., 2014). Maatalah *et al*. (2012) reported the antimicrobial activity of the alkaloids and saponin extracts on some *Candida* species. Antifungal activities observed in this research can be adduced to the presence of high concentration of alkaloids in *H. crinita* (Maatalah *et al*., 2012).

Multitudinous application of cardiac glycoside as antifungal, anticancer and preservatives have been described confirming this phytochemical as potential preservative against fruit rot (Prassas and Diamandis, 2008). There have been reports of antibiotic and antifungal resistance by some food/water microbial isolates (Adegoke et al., 2020) but the secondary metabolites detected in our studies have been reported to have appreciable activity against some resistant strains (Adegoke et al., 2010).

The rich dynamism of these leaf extracts is clearly depicted by the high absorption bands. Prominent absorption bands by *H. crinita* in the regions of 2930 – 2928, 1693 – 1600, 1406 and 1074 cm-1 of both extracts were ascribed to –C-H stretching in alkanes, -C=O and -C=C aromatic and –C-O stretching of ester respectively as corroborated by Singh et al. 2021. The hydroxy (OH), -C-H, -C=C and –CO functional groups of *G. latifolium* corresponding to peaks at approximately 3380, 2900, 1600 and 1070 cm-1 respectivelyis equally high but lower than those of *H. crinite*. These showcase the plants as potential sources of antifungals, following more research input.

5. Conclusion

The inhibitory activity of the two plant extracts (*Gongronema latifolium and Heinsia crinita*) were evaluated against the mycelial growth and spore germination of an array of fungi that caused rots in fruits from Akwa Ibom State. All the concentrations of the plant extracts brought about significant inhibition in the mycelial growth or spore germinations of these pathogenic fungi. The highest concentrations caused maximum inhibition in the mycelial growth, and spore germination, followed by the lower concentrations. The extracts of *G. latifolium* leaves at the highest concentration of 750 mg/ml proved highly effective in inhibiting the mycelial growth and spore germination of all these pathogenic fungi, followed by the other plant extract. These two plants thus may have potentials as the new natural fungicide for management of fungal diseases of fruits.

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