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Evaluation of the Antimicrobial Activities of Selected Plant Extracts and Honey against Clinical Isolates

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ABSTRACT: This study is aimed at assessing the in-vitro effect of *Aframomum daniellii* seed extract, Aloe vera gel, *Gongronema Latifolium* leaf extract, *Monodora myristica* seed extract and honey on *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans*. Agar well diffusion method was used for the susceptibility test. The plates were incubated at 37 ºC for 24 h. Extracts and diameter zones of inhibition were measured. All test organisms were susceptible to honey and Aloe vera gel, and a combination of both honey and Aloe vera gel. The zones of inhibition produced by Aloe vera gel against *S. aureus*, *E. coli*, *K. pneumonia* and *C. albica* were 10±0.10 mm, 11±2.50 mm, 15±1.70 mm and 35±3.26 mm respectively. For honey, they were 31±0.48 mm, 38±1.10 mm, 10±2.00 and 35±0.50 mm respectively, while for Aloe vera and honey mixture, they were 33±2.55 mm, 42±2.50 mm, 30±4.50 mm and 40±5.50 respectively. Ethanolic extract of *A. daniellii* showed inhibitory activity against *S. aureus* (15±0.24 mm), *K. pneumonia* (20±0.75 mm), *C. albicans* (13±2.70 mm) while the aqueous extract showed inhibitory activity against *S. aureus* (10±0.22 mm) and *C. albicans* (9±0.60 mm). Ethanolic extract of *G. Latifolium* showed inhibitory activity against *S. aureus* (9±0.23 mm), *E. coli* (7±0.55 mm) and *K. pneumonia* (6±0.09 mm) while the aqueous extract showed inhibitory activity against *S. aureus* (3±0.15 mm). Ethanolic extract of *M. myristica* showed activity against *S. aureus* (12±1.5 mm), *K. pneumoniae* (10±0.88 mm) and *C. albicans* (21±3.21 mm). Synergistic action was observed with honey and Aloe vera gel and was significantly different ($p<0.05$) for all test organism except *C. albicans*. The zones of inhibition produced by the test substances against all test microorganisms, were significantly different ($p<0.05$). This study has demonstrated the antimicrobial activity of natural products and synergistic action between Aloe vera gel and honey, which could prove useful for alternative natural medicine application, in the management of infectious diseases.

Keywords: Antimicrobial, Plant extracts, Honey, Clinical isolates, Pathogens, Synergy

Introduction

Decades after the breakthrough in treatment of infectious diseases using antibiotics, bacterial infections are again becoming a threat, owing to the rapid emergence of resistant bacteria (Spellberg and Gilbert, 2014). Antibiotic resistance stems from overuse and misuse of these medications, along with lack of investment into new drug development (Viswanathan, 2014). Antibiotic resistance and toxicity issues have now emerged to query the use of synthetic antimicrobials (Eggleston *et al*., 2010; Talib and Mahasneh, 2010) and is encouraging a rebirth in interest in natural antimicrobials from plants (Alviano and Alviano, 2009).

Medicinal plants have been proposed to be the unsurpassed repository for a variety of drugs according to the World Health Organization (WHO, 2002). Some of the recognized medicinal plants have proven valuable as source of bioactive compounds with the potential to be utilized as alternatives in the treatment of several infectious diseases caused by bacteria, fungi, virus and parasites (Iwu *et al*., 1999). Some of these plants include common spices such as *Aframomum daniellii*, *Gongronema latifolium* and *Monodora myristica*, and well

known medicinal plant *Aloe vera,* which are the focus of this study (Tatsadjieu *et al*., 2003; Agarry *et al*., 2005; Fasoyiro and Adegoke, 2007; Nwinyi *et al*., 2008; Lawrence *et al*., 2009; Samie *et al*., 2010; Oghenemaro *et al*., 2018).

Aframomum daniellii (African cardamom), is a specie in the ginger family (Zingiberaceae) native to Africa. Traditionally, the plant's seed is used as a spice but the plant is also valued in ethnomedicine as it has laxative, anti-parasitic, and microbial properties (Ngahang *et al*., 2017).

Aloe barbadensis Mill (Aloe vera), is a cactus-like plant of the family Asphodelaceae, which grows in hot and dry climates (Suleyman and Sema, 2009). Aloe vera is widely cultivated and used locally as part of skin care regime and for the treatment of skin ailments, inflammation, gastro-intestinal problem, radiation injury, ulcer, diabetes, wound and burns (Johnson *et al*., 2012).

Gongronema latifolium is a climbing plant of the family Apocynaceae and the subfamily Asclepiadaceae, native to tropical Africa (Nwinyi *et al*., 2008; Osuagwu *et al*., 2013). *Gongronema latifolium* is most valued in African cuisines as a vegetable spice but its medicinal properties have been well reported (Enyi-Idoh *et al*., 2017).

Monodora myristica belongs to the order Magnoliales, family Annonaceae. *Monodora myristica* seeds are used for preparing soup for the control of uterine hemorrhage in nursing mothers (Fattouch *et al*., 2007) and for treating arthritis (Ogunmoyole *et al.*, 2013)

Honey, a natural product of honeybees produced from the nectar of flowers, is a viscose, complex liquid mixture whose composition and properties differ by botanical plant and nectar foraged by the bees producing it (Machado de-Melo, 2018). Honey has been valued as a food and medicine from time immemorial and among its known medicinal properties is its action against pathogenic microorganisms (Mavric *et al*., 2008; Ocampo *et al*., 2014).

The efficacy of synthetic antibiotics in treating infectious diseases is being threatened by resistance, and several plant species exist from which new molecules can be obtained for the management of these infectious diseases, with probably lesser side effects. This study therefore is aimed at assessing the antimicrobial properties of natural products (honey, Aloe vera, *A. daniellii*, *G. latifolium* and *M. myristica*) against three bacterial pathogens (*Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*) and the yeast *Candida albicans*, a common fungal pathogen.

Materials and methods

Sample collection: The plants used in this study, Aloe vera, *Aframomum daniellii*, *Gongronema latifolium* and *Monodora myristica* were purchased from Opolo market in Yenagoa, Bayelsa State, Nigeria. Taxonomically identification of the plants was carried out at the Department of Biological Sciences, University of Africa, Toru-Orua, Bayelsa State. Honey was purchased from Mandhiz honey, Obudu, Cross River State, Nigeria.

Preparation of plant samples: Freshly collected leaves of Aloe vera were washed properly with distilled water, then dissected longitudinally to expose the colourless parenchmatous tissue (Aloe gel), which was scoop out with care using a sterile knife. The gel was then transferred into a sterile container and stored till it was used.

Fresh leaves of *G. latifolium* were carefully separated from the stalk, washed properly with distilled water and exposed to dry in a hot air oven for 15 minutes at 40 °C. All dried leaf samples were ground using electric blender and sieved to powder using a 1mm sieve, preparatory for extraction.

Fresh seeds of *M. myristica* and *A. daniellii* were separated from their pod, washed properly with sterile distilled water, air dried, weighed and ground into powder using electric blender, preparatory for extraction.

Preparation of plant extract: Extraction followed method described by Stanley *et al*. (2016) with modification. One hundred grams (100g) of dried material was steeped in 200ml of 95% (w/v) ethanol at room temperature or in 200ml of sterile distilled water, for ethanolic or aqueous extraction respectively, and then allowed to stand for 48 hours. The mixture was filtered with Whatman's No 1 filter paper, before the filtrate was evaporated to dryness using a rotary evaporator, to get the crude extracts used for the susceptibility test. Extracts were stored in a refrigerator till they were needed for analysis.

Preparation of test organisms: Pure cultures of *S. aureus*, *E. coli, K. pneumoniae* and *C. albicans* were obtained from the Medical Microbiology Laboratory of Glory Land Hospital, Opolo, Yenagoa, Bayelsa State, Nigeria. Test organisms were tested for purity and confirmed by their cultural, microscopic and biochemical features following methods described by Cheesbrough (2007).

Antimicrobial susceptibility testing: Sterile Mueller-Hinton agar plate was streaked with pure culture of the standardized microbial cell suspension. Holes, 8 mm in diameter, created using sterile cork borer, were filled with the plant extract and allowed to stand for one hour to ensure pre-diffusion of the extract prior to incubation at 37±2°C for 24 hours. Susceptibility was indicated by the zone of clearance (inhibition) measured in millimeter (Cheesbrough, 2000). All plates were in triplicate.

Determination of the minimum inhibitory concentration (MIC): The MIC was determined using tube dilution method with stock solutions of the extracts and honey diluted to 50 mg/ml 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml. Each concentration was inoculated with 0.1 ml of microbial cell suspension and incubated at 37±2°C for 24 hours. The lowest concentration of the plant extracts and honey that did not give any growth was recorded as the Minimum Inhibitory Concentration (MIC) (Cheesbrough, 2007).
Statistical analysis: Zones of inhibitions were measured as mean ± SD. One-way analysis of variance (ANOVA) was used to compare the mean differences between the zones of inhibition of the extracts and honey. A p-value less than 0.05 was taken as significantly different.

Results

Antimicrobial susceptibility testing: Table 1 shows the susceptibility pattern of the Aloe vera gel and honey against the test isolates (*S. aureus, K. pneumonia, E. coli* and *C. albicans*). *Candida albicans* was more susceptible to Aloe vera, honey and a blend of both with diameter zones of inhibition of 35±3.26 mm, 35±0.50 mm and 40±5.50 mm respectively. Aloe vera and honey blend showed more activities against all the microorganisms.

Table 1: Susceptibility pattern of Aloe vera gel and honey at 100 mg/ml

Isolate	Diameter of zone of inhibition (mm)			
	Aloe vera gel	Honey	Aloe vera gel + Honey	
S. aureus	10±0.10	31±0.48	33±2.55	p< 0.05
E. coli	11±2.50	38±1.10	42±2.50	p< 0.05
K. pneumoniae	15±1.70	10±2.00	30±4.50	p< 0.05
C. albicans	35±3.26	35±0.50	40±5.50	p>0.05
	p< 0.05	p< 0.05	p< 0.05	

The results from the antimicrobial susceptibility testing of *M. myristica, G. latifolium and A. daniellii* extracts against the test isolates are shown in Tables 2. *S. aureus, K. pneumonia* and *C. albicans* were susceptible to ethanolic extract of *M. myristica* while aqueous extract showed no activity against the test microorganisms. Ethanolic extract of *G. latifolium* produced activities against *S. aureus, E. coli* and *K. pneumonia* while aqueous extract produced activity against *S. aureus*. Ethanolic extract of *G. latifolium* produced activities against *S. aureus, C. albicans* and *K. pneumonia* while aqueous extract showed activity against *S. aureus* and *C. albicans*. Of all test organisms, *S. aureus* was the most susceptible to the test substances and *E. coli* was the least.

Table 2: Susceptibility pattern of ethanolic and aqueous plant extracts at 100 mg/ml

Isolate	Diameter of zone of inhibition (mm)						
	M. myristica		*G. latifolium*		*A. daniellii*		
	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	
S. aureus	12±1.50	0	9±0.23	3±0.15	15±0.24	10±0.22	p< 0.05
E. coli	0	0	7±0.55	0	0	0	p< 0.05
K. pneumoniae	10±0.88	0	6±0.09	0	20±0.75	0	p< 0.05
C. albicans	21±3.21	0	0	0	13±2.70	9±0.60	p< 0.05
	p< 0.05	p< 0.05	p< 0.05	p< 0.05	p< 0.05	p< 0.05	

Minimum inhibitory concentration: Table 3 shows that the MIC of plant extracts. The MIC of *A. daniellii* ethanolic extract against *S. aureus, K. pneumonaie, C. albicans* was 50 mg/ml, 100 mg/ml and 50 mg/ml correspondingly while MIC against *S. aureus* was 50 mg/ml for the aqueous extract. The MIC of *G. latifolium* ethanolic extract was 100 mg/ml while MIC against *S. aureus* was 50 mg/ml for the aqueous extract. The MIC of *M. myristica* ethanolic extract against *S. aureus, K. pneumonaie, C. albicans* was 50 mg/ml, 50 mg/ml and 25 mg/ml. The MIC of Aloe vera against *S. aureus, E. coli, K. pneumonaie, C. albicans* was 50 mg/ml, 50 mg/ml 25 mg/ml and 100 mg/ml correspondingly. The MIC of honey *S. aureus, E. coli, K. pneumonaie, C. albicans* was 6.25 mg/ml, 3.125 mg/ml, 50 mg/ml and 3.125 mg/ml respectively. The MIC of honey and Aloe vera blend was 6.25mg/ml for all isolates.

Table 3: Minimum Inhibitory Concentration (MIC)

Antimicrobial Agent	Organism	Concentration (mg/ml) 3.125	6.25	12.5	25	50	100
A. danielli ethanol extract	*S. aureus*	+	+	+	+	-	-
	K. pneumonaie	+	+	+	+	+	-
	C. albicans	+	+	+	+	-	-
A. danielli aqueous extract	*S. aureus*	+	+	+	+	-	-
G. latifolium ethanol extract	*S. aureus*	+	+	+	+	+	-
	E .coli	+	+	+	+	+	-
	K. pneumonaie	+	+	+	+	+	-
G. latifolium aqueous extract	*S. aureus*	+	+	+	+	-	-
M. myristica ethanol extract	*S. aureus*	+	+	+	+	-	-
	K. pneumonaie	+	+	+	+	-	-
	C. albicans	+	+	+	-	-	-
Aloe vera	*S. aureus*	+	+	+	+	-	-
	E. coli	+	+	+	+	-	-
	K. pneumonaie	+	+	+	-	-	-
	C. albicans	+	+	+	+	+	-
Honey	*S. aureus*	+	-	-	-	-	-
	E. coli	-	-	-	-	-	-
	K. pneumonaie	+	+	+	+	-	-
	C. albicans	-	-	-	-	-	-
Honey + *Aloe vera*	*S. aureus*	+	-	-	-	-	-
	E. coli	+	-	-	-	-	-
	K. pneumonaie	+	-	-	-	-	-
	C. albicans	+	-	-	-	-	-

Key (+) = Growth; (-) = No growth

Discussion

The increase in microbial resistance to and toxicity of conventional antibiotics have compelled the prospecting for novel and inexpensive bioactive substances as better alternatives for the management of infectious diseases. In this study, different natural products (*Monodora myristica, Gongronema latifolium*, *Aframomum daniellii*, Aloe vera gel extract and honey) with presumed antimicrobial agents from reports in ethnomedicine were evaluated against four clinical isolates namely *S. aureus, E. coli, K. pneumoniae and C. albicans*.

The results from the antimicrobial sensitivity evaluation of *M. myristica, G. latifolium*, *A. daniellii*, Aloe vera gel and honey used in this study as shown by the zones of inhibition of the growth of test organisms, is a further reassertion of their relative antimicrobial potential as previously reported in literature (Tatsadjieu *et al*., 2003; Agarry *et al*., 2005; Fasoyiro and Adegoke, 2007; Nwinyi *et al*., 2008; Lawrence *et al*., 2009; Samie *et al*., 2010; Oghenemaro *et al*., 2018). The ethanolic extracts had the highest inhibitory effect compared to the aqueous extracts. The ethanolic extracts of *M. myristica, G. latifolium and A. daniellii* inhibited the growth of *S. aureus* at 12±1.50 mm, 9±0.23 mm and 15±0.24 mm respectively; *K. pneumoniae* at 10±0.88 mm, 6±0.09 mm and 20±0.75 mm; *E. coli* at 7±0.55 mm for only *G. latifolium*, and *C. albicans* at 21±3.21 mm and 13±2.70 mm for *M. myristica* and *A. daniellii*. The ethanolic extracts of *A. daniellii* and *M. Myristica* had no inhibitory effect on *E. coli*. The ethanolic extract of *G. latifolium* had no inhibitory effect on *C. albicans.* The aqueous extract of *A. daniellii* inhibited the growth of *S. aureus and C. albicans* at 10±0.22 mm and 9±0.60 mm respectively and had no inhibitory effect of *K. pneumoniae* and *E. coli.* The aqueous Extract of *G. latifolium* showed inhibitory effect on only *Staphylococcus aureus* at 3±0.15 mm. *Monodora myristica* aqueous extract had no inhibitory activity on all test microorganisms. The zones of inhibition produced by the test substances against all test organisms, were significantly different ($p<0.05$).

Aloe vera gel extract showed inhibitory effect on *S. aureus, K. pneumoniae* and *E. coli* at 10±0.10 mm, 11±2.50 mm and 15±1.70 mm respectively and had no inhibitory effect on *C. albicans*. Lawrence *et al.* (2009) similarly reported that Aloe vera has inhibitory effect against *S. aureus, K. pneumoniae* and *E. coli* but with zones of inhibition ranging from 9.2± 1.5 mm - 10.25± 0.25 mm, 7.1± 0.08 mm - 8.8 ± 0.08 mm and 8± 0.25 - 10.1± 0.08 mm respectively. Lawrence *et al.* (2009) in their study, reported that Aloe vera contains several chemicals that act synergistically, as several fractions obtained after chromatographic separation inhibited microbial growth.

Honey produced inhibitory effect on *S. aureus, E. coli, K. pneumoniae* and *Candid albicans* at 31±0.48 mm, 38±1.10 mm, 10±2.00 mm and 35±0.50 mm respectively. The inhibitory effect of honey on bacteria (both Gram positive and Gram negative) and yeast, indicates its broad spectrum of activity. The differences in zones of inhibition is statistically significant ($p<0.05$) for all isolates except *C. albicans*. The blend of Aloe vera and honey showed additive inhibitory effects on *S. aureus, E. coli, K. pneumoniae* and *Candid albicans* at 33±2.55 mm, 42±2.50 mm, 30±4.50 mm and 40±5.50 mm. Oghenemaro *et al*. (2018) likewise reported a synergy between honey and Aloe vera in inhibiting the growth of *S. aureus* and *E. coli* isolated from wound, with honey showing a better inhibition efficiency compared to Aloe vera gel.

The Minimum Inhibitory Concentration (MIC) of all extracts that showed inhibitory effect was determined. The MIC of *A. daniellii* ethanolic extract against susceptible organisms varied between 50 mg/ml and 100 mg/ml and 50 mg/ml for aqueous extract; MIC for *G. latifolium* ethanolic extract was 100 mg/ml and 50 mg/ml for the aqueous extract; MIC for *M. myristica* ethanolic extract varied between 25 mg/ml and 50 mg/ml; for Aloe vera it varied between 25 mg/ml and 100 mg/ml; for honey it varied between 3.125 mg/ml and 50 mg/ml whereas for honey and Aloe vera blend it was 6.25 mg/ml for all isolates. The antimicrobial property exhibited by plants is presumed to be due to the bioactive phytochemicals existing in them (Yebpella *et al.,* 2011). The above results supports the use of natural products at low dosage for the control of human pathogens.

The comparison of the results of the antimicrobial sensitivity testing and MIC shows that honey possesses excellent antimicrobial property which agrees with the report of Mavric *et al.* (2008) and Morroni *et al*. (2018). Honey has antibacterial activity and inhibits a broad spectrum of bacterial species, owing to its osmotic properties (Ocampo *et al.,* 2014). Antimicrobial properties of honey might be conferred by the low pH, hydrogen peroxide concentration, flavonoid and other phytochemicals (Ghori and Ahmad, 2009; French *et al.,* 2005). Specifically, the compound 1,2-dicarbonyl methylglyoxal present in Manuka honey has been reported to be a potent antimicrobial compound (Mavric *et al*., 2008).

Conclusion

The present study has reaffirmed that natural products contain antimicrobial compounds that can be beneficial in the control of infections caused by *S. aureus, E. coli, K. pneumoniae and C. albicans*. The results from this study showing that honey inhibited the growth of all the test organisms at low concentrations and giving a better inhibitory results when mixed with Aloe vera gel extract could encourage the use honey and Aloe vera gel as nutraceuticals.

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