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Research Paper

Mixture of Honey and Ginger Extract for Antibacterial Assessment on Some Clinical Isolates

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Abstract

The antibacterial activity of honey, methanol and ethanol extracts of ginger (*Zingiber officinale*) were investigated against some selected bacteria using the agar diffusion technique. Two Gram positive and four Gram negative bacteria were assessed for possible inhibition by the extract samples. The inhibitory potency of the extracts on the test organisms varied in the halos as inhibition effects. Though all the test organisms were susceptible to the antibacterial samples with inhibition measure between 6-3 mm, *E. coli* was the most inhibited where an inhibitory measure of 20 mm was recorded with honey, 18 mm with ginger ethanol extract and 32 mm with the mixture of honey and ginger ethanol extract. The pasture honey, the ethanol and methanol extracts of ginger were both positive for saponin and cardiac glycosides among the phytochemicals identified. While some of the commercial antibiotics (positive control) were not effective on the test organisms, gentamycin and streptomycin were effective with inhibitory halos ranging between 8-25 mm. However, the antibacterial test samples were higher in inhibition values than the reference drugs (positive control).

Keywords: *Extract, Pasture Honey, Mixture, Clinical, Antibacterial*

1. Introduction

Antimicrobial agents are the substances known to have therapeutic effect on microorganisms either as a control, prevention or cure of microbial and non-microbial disease origin. These antimicrobial agents are synthesized chemotherapeutic substances obtained majorly from microorganisms, plants and some animal products. The failure of these antibiotics has resulted for man to search for more effective sources of natural products from plants and some insects. Though some of these products perform less or higher than synthesised antibiotics, in some cases, they have been found safe and good source of pharmacological effect for man. Medicinal values are derived not only from the already available drugs in man health care system but by invention most especially into plants for the derivation properties which are medicinal. Products derived from plants have been used for medicinal purposes for centuries; at present about 80% of the world population relies on botanical preparation as medicines to meet their health needs. Many scientists have reported antimicrobial properties of several plants. The antimicrobial, anti-tumour (Khalil et al, 2005 and Akroum et al, 2009), anti-inflammatory and anti-necrotic (Lin & Huang, 2002) activities have been reported from the use of plants extracts.

The most well-known member of *Zingiber* (ginger) is *Zingiber officinale*. In many parts of the world, *Z. officinale* has medicinal and culinary values. The volatile oil gingerol and other pungent principles not only give ginger its pungent aroma, but are the most medically powerful because they inhibit prostaglandin and leukotriene formation, which are products that influence blood flow and inflammation (Longe et al, 2005). Honey produced by *Appis mellifera* is a sweet food made from the synthesis of nectar from flowers, plant saps and man waste products. Honey is a mixture of sugars, mainly fructose and glucose, having the highest percentage among other carbohydrates present. Antimicrobial agents with selective

toxicity are especially useful as a chemotherapeutic agent in treating infectious diseases and may be a function of specific receptor requirement for drug attachment or it may depend on the inhibition of biochemical events essential to the pathogen but not to the host (Omoya & Akharaiyi, 2010).

In the study of Omoya & Akharaiyi (2010) reported that a pasture honey produced by *Appis mellifera* was found to be effective against some clinical isolates and disease causing in man. Growth retardation were recorded at 1-4% V/V concentration, while a higher valued growth retardation and total inhibition and *S. dysentariae* were recorded at 4-5% V/V. Other pasture honey produced by *Apis mellipodae* were found in contrast to this observation where less inhibition at 10-20% V/V concentration was reported (Mogessie, 1994). This of course is the differences in bee species producing different types of honey (National Honey Board, 1994) and the differences in the test methods and test organisms (Mulu et al, 2004). Betts & Molan (2001) have reported a complete prevention of *S. aureus* growth at 1.8% *E. coli* at 3.7% and *P. aeruginosa* at 7.3%.

The importance of ginger (*Zingiber offficinale*) and honey cannot be over emphasized as regards their rule in health remedy. Therefore this study detailed the antibacterial and phytochemical activities of honey and ginger on selected pathogenic bacteria.

2. Materials and Methods

Ginger and honey samples were purchased from peasant farmers at Igara in Edo state, Nigeria. The ginger rhizomes were washed with clean water and rinsed several times in sterile distilled water. They were sliced to pieces, air dried for three weeks at temperature of 25$\pm$2 ^{o}C and blended with a grinder to obtain smooth powder. Three hundred grams each of the powder was weighed and extracted by soaking separately under room temperature (25$\pm$2 ^{o}C) with methanol and ethanol for 24 hours. The honey samples were filtered with a sterile seitz filter attached to a vacuum pump. The filtrate was aseptically streaked on nutrient agar plates and incubated at 37 ^{o}C for 24 hours for sterility check. The sterile samples were aseptically dispensed into sterile Pyrex sample bottles and kept at room temperature (25$\pm$2 ^{o}C) prior to its use.

2.1. Test Organisms

Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus cereus, Klebsiella pneumoniae and Pseudomonas aeruginosa.

2.2. Bio-Assay of Honey and Ginger Extracts

The bio-assay includes the use of honey, ginger, honey and ginger extract mixtures by employing the agar diffusion technique. The ginger was used by dissolving 1 g of various ginger extracts in 10 ml sterile distilled water to make a concentration of 100 mg/ml. The honey-ginger mixture was prepared by dissolving 1 g of the ginger extracts in 10 ml of pure honey to make a concentration of 100 mg/ml. Molten Mueller Hinton agar (Oxoid) prepared by suspending 3.8 g of the powder in 100 ml of sterile distilled water and brought to boiling to dissolve the medium before sterilizing with autoclave at 121 ^{o}C for 14 minutes. Inocula of the bacterial test organisms were prepared from 24 hour old cultures. The absorbance was read at 530 nm and adjusted with sterile distilled water to match that of a 0.5 Mac Farland standard solution. From this prepared bacterial solution, other dilutions with sterile distilled water were prepared to give a final concentration of about 10^{7} colony forming unit (Cfu) per millilitre. 1 ml each of the prepared bacterial solutions were pour plated with sterile agar cooled to about 45 ^{o}C. The plates were allowed to set for 2 hours. With a previously sterilized cork borer (4 mm size), wells of equal distance were bored. The ginger extracts (ethanol and methanol), honey, honey and ginger extracts mixtures were aseptically filled into the wells which were appropriately distinguished with codes. The plates were incubated at 37 ^{o}C for 24 hours. Inhibition indicated by clear halo around the wells were measured and taken as degree of susceptibility of the organisms to the samples.

2.3. Phytochemical Screening of Ginger Extracts and Honey

2.3.1. Alkaloid Test

Five grams each of the ginger extracts and 5 ml honey were stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath at 60 ^{o}C for 5 minutes. The sample was filtered with a 3 layered muslin cloth. One millilitre of the filtrate was treated with few drops of Draggendoff's reagent. Blue black turbidity serves as preliminary evidence of alkanoids.

2.3.2. Saponins Test

Five grams each of the extracts and 5 ml of honey were shaken separately with distilled water in a test tube. Frothing which persists on warming was taken as preliminary evidence of the presence of the saponins.

2.3.3. Tannins Test

Five grams each of the extracts and 5 ml of honey were stirred separately with 100 ml distilled water and filtered. One millilitre ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate was an

indication of the presence of tannins (Trease & Evans, 1989).

2.3.4. Phlobotannins Test

Deposition of red precipitate when an aqueous extract of the test samples was boiled with 1% hydrochloric acid indicated the presence of phlobotannins (Trease & Evans, 1989).

2.3.5. Flavonoids Test

Five millilitres of diluted ammonia solution was added to aqueous filtrate of the test samples followed by the addition of 1 ml concentrated H_2SO_4. A yellow colouration indicates the presence of flavonoids (Harborne & Williams, 2000).

2.3.6. Cardiac Glycosides (Keller-Killiani Test)

Five grams of each of the extracts and 5 ml of honey were dissolved separately in 2 ml glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1 ml concentrated H_2SO_4. A brown ring at the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a green ring may form which just gradually spreads throughout the layer (Trease & Evans, 1989).

2.3.7. Legal Test

Five grams of each extract and 5 ml of honey were dissolved in pyridine separately and few drops of 2% sodium nitroprusside together with few drops of 20% sodium hydroxide were added. A deep red colour, which fades to yellowish-brown, indicates the presence of Cardinolides (Trease & Evans, 1989).

2.3.8. Salkoski Test

Five grams of the extracts and 5 ml of honey were dissolved in 20 ml of chloroform. Few drops of sulphuric acid were carefully added to form a layer at the lower part. A reddish-brown colour at the interface indicates the presence of steroids nucleus (Trease & Evans 1989).

2.3.9. Lieberman's Test

Five grams of the extracts and 5 ml of honey were mixed with 2 ml of acetic anhydride and cooled. Later one, 0.5 ml of sulphuric acid was carefully added. A colour change from violet to blue to green indicates the presence of a steroids nucleus (i.e. a glycone portion of the cardiac glycoside) (Trease & Evans, 1989).

2.4. Standard Antibiotic Bioassay

1 ml each of 24 hours broth culture of the test organisms at a concentration of 10^6 Cfu/ml, was pure plated with Muller Hinton agar. On establishment of the seeded organisms after 2 hours, standard antibiotic disc were aseptically placed with a sterile forceps at the centre of the seed of agar plates. The arm of the antibiotics was firmly pressed down to rest on the agar plates for even diffusion of their contents. The plates were incubated at 37 °C for 24 hours. Zones of inhibition were measured and reported as value of inhibition.

3. Results and Discussion

The cultural characteristics (such as colony colour, edge, surface, elevation) physiological (indole, motility, catalase, methyl red, voges proskauer tests) and biochemical tests (involving the organisms ability to utilize carbohydrates) carried out identified the test organisms as *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus cereus* and *Klebsiella pneumoniae*. The phytochemical tests carried out on the used pasture honey identified positive test for saponin and cardiac glycosides (Table1).

Table 1. Phytochemical Screening of Honey

Phytochemical	Inference
Saponin	+
Tannin	-
Phlobertannin	-
Alkaloid	-
Anthaquinone	-
Flavonoid	+
Cardiac Glycosides	
Keller-kiliiani's Test	-
Salkwoski's Test	+
Leiberman's Test	+
Legal Test	+

+ = positive, - = negative

The methanol extract of ginger was positive for saponin, phlobatinnin, flavonoids and cardiac glycosides (Table 2) while the ethanol extract of ginger was positive for saponin, alkanoides, flavonoids and cardiac glycoses (Table 3). Among the bacterial test isolates, *E. coli* was the most inhibited with the pasture honey (20 mm), ginger ethanol extract (18 mm) and the mixture of honey and ginger ethanol extract (32 mm). *S. aureus* was most inhibited with the mixture of honey and ginger methanol

extract with 3.0 mm followed by the mixtures of honey and ethanol ginger extract with 26 mm inhibitory potency. *B. cereus*, *S. typhi* and *K. pneumoniae* was also inhibited under that trend as 24 mm 22 mm, 26 mm 22 mm, and 18 mm 20 mm respectively (Table 4).

Table 2. Phytochemical Screening of Methanol Extract of Ginger

Phytochemical	Inference
Saponin	-
Tannin	-
Phlobertannin	+
Alkaloid	-
Anthraquinone	-
Flavonoid	+
Cardiac glycosides	
Keller-kiliani's Test	+
Salkwoski's Test	+
Lieberman's Test	+
Legal Test	+

+ = *positive*, - = *negative*

Table 3. Phytochemical Screening of Ethanol Extract of Ginger

Phytochemicals	Inference
Saponin	+
Tannin	-
Phlobertannin	-
Alkaloid	+
Anthraquinone	+
Flavonoid	+
Cardiac Glycosides	
Keller-kiliani's Test	+
Salkwoski's Test	+
Lieberman's Test	+
Legal Test	+

+ = *positive*, - = *negative*

Some of the standard antibiotics as tetracyclin, ampicillin, cotrimoxazole, cloxacillin and penicillin were not effective on the test bacterial isolates. All the test organisms were susceptible to gentamycin with inhibitory zones of between 10-25 mm and streptomycin between 8-20 mm (Table 5). Though the test organisms were susceptible to other antibiotics, their susceptibility to the mixture of honey and the ginger extracts had higher values.

This study emphasized honey and ginger extract as having antibacterial activity on some pathogenic bacteria isolated from human samples. The inhibitory potency of honey and ginger extract at 100 mg/ml on the test bacterial species were similar. Though majority of the test isolates were Gram negative bacteria, the Gram positive bacteria were both inclusive in valuable inhibitions with the pasture honey and ginger extracts.

The pasture honey exhibited 14.1% inhibitory potency on the test organisms while the ginger methanol and ethanol extracts at 100 mg/ml exhibited 14.3% and 14.7% respectively. However, mixtures of honey and ginger methanol extract and mixtures of honey and ginger methanol extract at 100 mg/ml displayed 27.0% and 27.8% inhibitory potency respectively on the test isolates. Though there was no growth retardation of the bacterial species at inhibition <6 mm, *Escherichia coli*, *Staphylococcus aureus*, were the most inhibited with zones of inhibition >12 mm to 32 mm. The pasture honey and ginger extract at 100 mg/ml exerted antibacterial activity on all the test organisms which were resistant to some common standard antibiotics such as ampicillin, cloxacillin, tetracyclin, penicillin, contrimoxazole and crythromycin.

The ginger extracts having chemical compounds such as saponin, alkanoids and flavonoids have been reported to have antifungal and antibacterial activities in-vitro (Barasch et al, 2004) and so effective in combating post-operative nausea and vomiting (Ernst & Pittler, 2000). Its combination with honey displayed valued, potency on the test organisms than when used in single form. This emphasised that combination of two or more substances with medicinal values could be better if their components will not cause a reaction that could cause health disaster than healing. The synergisms of more than one medicinal plant have been in practice. It will be remedying of multiple actions, hence some illnesses by certain pathogens in man. It is noted in this study that *S. typhi* was inhibited with a zone of 10 mm with honey and 8 mm with ginger extract but a higher value of 26 mm resulted with the mixture of the two substances. Nevertheless, higher inhibitory values were recorded with mixtures of the two substances on all the test organisms than when used in single form.

This experiment also showed that honey and ginger extracts possess differences in antibacterial activities. Honey in its saturated solution of sugar will cause osmotic effect on the bacteria and ginger in its spicy nature with free radical inhibitions index performs other toxic factors which of course responded to the antibacterial effect observed in the study.

Table 4. Antibacterial Activity of Honey and Ginger Extracts (100 mg/ml)

Test Organisms	Honey (mm)	Methanol Extracts of Ginger (mm)	Ethanol Extracts of Ginger (mm)	Honey + Methanol Extracts of Ginger (mm)	Honey + Ethanol Extracts of Ginger (mm)
Staphylococcus aureus	14	21	16	30	26
Bacillus cereus	10	11	11	24	22
Pseudomonas aeruginosa	6	12	14	14	14
Salmonella typhi	10	8	9	26	22
Escherichia coli	20	17	18	28	32
Klebsiella pneumoniae	11	8	11	18	20

Table 5. Antibacterial Activity of Standard Antibiotic Disc

Test Bacteria	Zones of Inhibition (mm)							
Gram Negative Bacteria	**GEN**	**NAL**	**NIT**	**COL**	**STR**	**TET**	**AMP**	**COT**
Escherichia coli	17	22	15	11	10	R	R	R
Salmonella typhi	14	19	14	10	13	R	R	R
Pseudomonas aeruginosa	10	R	R	10	9	R	R	R
Gram Positive Bacteria	**GEN**	**PEN**	**CHL**	**CXC**	**STR**	**TET**	**AMP**	**ERY**
Bacillus cereus	15	R	10	R	13	R	R	R
Staphylococcus aureus	25	R	13	R	20	13	R	26
Klebsiella pneumoniae	14	R	R	R	8	R	R	R

R=Resistant (no Zone of Inhibition)
Key: ***GEN**-Gentamycin, **NAL**-Nalicillin, **NIT**-Nitrofuratin, **STR**-Streptomycin, **TET**-Tetracyclin, AMP-Ampicillin, **COT**-Cotrimoxazole, **ERY**-Erythromycin, **CHL**-Chloramphenicol, **CXC**-Cloxacillin*

4. Conclusion

The obtained results in this study approved honey and *Zingiber officinale* having antibacterial potency able to establish valuable inhibition zones in vitro. While some phytochemical constituents known for inhibition of microorganisms were observed in *Zingiber officinale*, honey also possessed traces of sapinin and cardiac glycoside. Effective antimicrobial was possible for this reason with *Zingiber officinale* while it was also possible in conjunction of the sugar contents in honey. Majority of the bacterial test organisms were not susceptible to many of the employed standard antibiotics but the honey, *Zingiber officinale* and their combinations demonstrated varied inhibitory zones on all the test bacterial organisms.

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