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 6-3mm, *E. coli* was the most inhibited where an inhibitory measure of 20mm was recorded with honey, 18mm with ginger ethanol extract and 32mm 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 8-25mm. However, the antibacterial test samples were higher in inhibition values than the reference drugs (positive control).

Keywords: Extract, pasture honey, mixture, clinical, antibacterial.

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 (Akroum *et al*., 2009; Amjad *et al*., 2005), anti-inflammatory and anti-necrotic (Lin and Huang, 2002) activities have been reported from the use of plants extracts.

The most well-known member of Zingiber (ginger) is *Zingiber officinale* (Crawford *et al*; 2005). 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 (Crawford, *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 and Akharaiyi 2010).

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.

Materials and methods

The 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 severally in sterile distilled water. They were sliced to pieces, dried for three weeks at room temperature and blended with a grinder to attain smooth powder. 300g each was weighed and extracted with methanol and ethanol. 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 37^0 C for 24h for sterility check. The sterile samples were aseptically dispensed into sterile Pyrex sample bottles and kept at room temperature prior use.

Test organisms

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

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 1g of the various ginger extracts in 10ml sterile distilled water to make a concentration of 100mg/ml. The honey-ginger mixture was prepared by dissolving 1g of the ginger extracts in 10ml of pure honey to make a concentration of 100mg/ml. Molten Mueller Hinton agar (Oxoid) prepared by suspending 3.8gram of the powder in 100ml of sterile distilled water and brought to boiling to dissolve the medium before sterilizing with autoclave at 121^0 C for 14min. The molten agar cooled to about 45^0 C was used to pure plate 1ml each of the test organisms at a concentration of 10^7 Cfu/ml. The plates were allowed to set for 2h. With a previously sterilized cork borer (4mm) 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 37^0 C for 24h. Inhibition indicated by clear halo around the wells were measured and taken as degree of susceptibility of the organisms to the samples. .

Phytochemical screening of ginger extracts and honey.

Alkaloids test

5g each of the ginger extracts and 5ml of honey was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath. 1ml of the filtrate was treated with few drops of Draggendoff's reagent. Blue black turbidity serves as preliminary evidence of alkaloids.

Saponins test

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

Tannins test

5g each of the extracts and 5ml of honey was stirred with 100ml distilled water and filtered. Ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate determines the presence of Tannins (Trease and Evans, 1989).

Phlobotannins test

Disposition of red precipitate when an aqueous extract of the test samples was boiled with 1% hydrochloric acid determines the presence of phlobotannins (Trease and Evans 1989).

Flavonoids test

5ml of diluted ammonia solution was added to aqueous filtrate of the test samples followed by the addition of concentrated H_2SO_4. A yellow coloration observation determines the presence of flavonoids.

Cardiac glycosides (keller-killiani test)

5g of each of the extracts and 5ml of honey was dissolved in 2ml glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1ml concentrated H_2SO_4. A brown ring of 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 just gradually spread throughout this layer (Trease and Evans, 1989).

Standard antibiotic bioassay.

1ml each of 24h 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 2h, 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 37^0 C for 24h. Zones of inhibition were measured and reported as value of inhibition.

RESULTS AND DISCUSSION

The cultural characteristics physiological and biochemical tests 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). 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. (Table3). Among the bacterial test isolates, *E. coli* was the most inhibited with the pasture honey (20mm), ginger ethanol extract (18mm) and the mixture of honey and ginger ethanol extract (32mm). *S. aureus* was most inhibited with the mixture of honey and ginger methanol extract with 3.0mm followed by the mixtures of honey and ethanol ginger extract with 26mm inhibitory potency. *B. cereus*, *S. typhi* and *K. Pneumoniae* was also inhibited under that trend as 24mm 22mm, 26mm 22mm, and 18mm 20mm respectively (Table 4).

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 10-25mm and streptomycin between 8-20mm (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 100mg/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 100mg/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 100mg/ml displayed respectively 27.0%

and 27.8% inhibitory potency on the test isolates. Though there was no growth retardation of the bacterial species at inhibition < 6mm, *Escherichia coli*, *Staphylococcus aureus*, were the most inhibited with zones of inhibition >12mm to 32mm. The pasture honey and ginger extract at 100mg/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.

In the study of Omoya and 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 (Andargarchew *et al* 2004). Molan and Betts (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 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*., 2003) and so effective in combating post-operative nausea and vomiting (Ernst and 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 10mm with honey and 8mm with ginger extract but a higher value of 26mm 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. The Kani tribe in India are said to usually prepare medicines from a combination of severally substances as they believed that combinations of several plants and substances cure diseases rapidly. In substances where remedies for ailment require boiling certainly, there will be decrease in inhibition potency. In such instances, the antimicrobial potency of single substances could not be dependent on its bioactive components hence some could be heat labile. The heat stable components though they could be active agents their concentrations might be too low to solely respond for a valued therapeutic effect. Therefore, combinations of substances with medicinal values will guide against lost, low concentration and enhancement of bioactive components for rapid cure and prevention. 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 1: Phytochemical screening of methanol extract of ginger

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

+ = positive, - = negative

Table 2: Phytochemical screening of methanol extract of ginger

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

Saponin	+
Tannin	–
Phlobertannin	–
Alkaloid	+
Anthraquinone	–
Flavonoid	+
Cardiac glycosides	
keller-kiliani's Test	+
Salkwoski's Test	+
Lieberman's Test	+
Legal Test	+

+ = positive, - = negative

Table 4: Antibacterial Activity of honey and ginger extracts.

Test organisms	Honey	Methanol extracts of ginger 100mg/ml	Ethanol extracts of ginger 100mg/ml	Honey + methanol extracts of ginger 100mg/ml	Honey + ethanol extracts of ginger 100mg/ml
Staphylococcus aureus	14mm	21mm	16mm	30mm	26mm
Bacillus cereus	10mm	11mm	11mm	24mm	22mm
Pseudomonas aeruginosa	6mm	12mm	14mm	14mm	14mm
Salmonella typhi	10mm	8mm	9mm	26mm	22mm
Escherichia coli	20mm	17mm	18mm	28mm	32mm
Klebsiella pneumoniae	11mm	8mm	11mm	18mm	20mm

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**
Escherchia 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

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