

RESEARCH PAPER

ANTIFUNGAL ACTIVITIES OF A PASTURE HONEY AND GINGER (*ZIGINBER OFFICINALE*) EXTRACTS ON SOME PATHOGENIC FUNGI

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ABSTRACT

*Methanol, ethanol, ginger extracts and a pasture honey were tested on *Aspergillus flavus*, *Aspergillus fumigatus*, *Trichoderma viride* and *Candida albicans* using the well-in-agar method. The antifungal sensitivity assay indicated that the chemical solvent extracts of ginger, pasture honey and mixtures of honey and ginger extracts exerted inhibitory zones on the test fungi species except *A. Fumigatus*. However, the pasture honey displayed higher inhibitory values of 45 mm than the mixtures of honey and ethanol extract of ginger and honey and methanol extract of ginger with 40 mm and 30 mm inhibitory zones respectively. The phytochemicals present in honey were saponin and cardiac glycoside, while in the ginger sample, saponin, phlobatannin, alkaloids, flavonoids and cardiac glycoside were present. Summarily, honey and ginger extracts displayed the highest inhibitory activity on all the tested fungal isolates compared to the employed positive control antifungal (*Griseofulvin* and *Ketoconazole*).*

Keywords: *Antifungal, pathogenic, honey, ginger, solvent extraction*

INTRODUCTION

The use of spices and honey is acknowledged all over the world for their considered value in health remedy. They are natural products which have generally been tested to be safe and proven to be effective against certain ailments among other functions useful for human.

Fresh ginger rhizome contains 2.3% protein, 12.3% carbohydrate, 2.4% fibre and 1.2% minerals which includes iron, calcium and phosphorous. It also contains vitamin C, thiamine,

riboflavin and niacin (Barasch *et al.*, 2004). All these are important in health improvement. The pungent taste of ginger is due to the non-volatile phenylpropanoid-derived compounds, particularly gingerols and shogaols. The latter are formed from the former when ginger is dried or cooked. Zingerone is also produced from gingerols during this process and it is less pungent and has a spicy-sweet aroma, The volatile oil gingerol and other pungent substances not only give ginger its pungent aroma but are also medicinally powerful because they inhibit pro-

taglandin and leukotriene formations, which are products that influence blood flow and inflammation (Crawford *et al.*, 2005). The components in ginger rhizome have antifungal and antibacterial effects (Barasch *et al.*, 2004). These components have experimentally and clinically been found to possess antiemetic effect by effectively combating post-operative nausea and vomiting (Ernst and Pittler, 2000) which are symptoms of illnesses. Ginger possesses a free radical inhibiting index greater than commercial antioxidants. Ginger inhibits platelet aggregation induced by adonisin diphosphate and epinephrine, possesses anti-inflammatory effect in rodents and gastro-protective function against various chemical hazards and stressors (Mascolo *et al.*, 1998).

Honey is a substance made from the gathering of nectar, sugary deposits from plants and animals as well as human and animal wastes by honeybees (*Apis mellifera*) which in their natural scientific model synthesized, purified and stored in honey combs in a viscous or liquid or semi-solid form (Omoya and Akharaiyi, 2010). Honey is mainly fructose (about 38.5%) and glucose (31%) among others making it similar to synthetically produced inverted sugar syrup which is appropriately 48% fructose, 47% glucose and 5% sucrose. Honey contains compounds like vitamin C ascorbic acid that function as antioxidant. Cutaneous or super facial mycoses, caused through host infection by dermatophytes is one of the most common diseases of humans.

Only a small number of species of these, from the genera Epidermophyton, Microsporium and Trichophyton, regularly infect humans (Rademaker 1993). A common predisposition to some fungal infections is poor host immunity, thus bacterial infections may also be present quite often. Therefore, this work is aimed at studying the antifungal activities of honey; ethanol and methanol extracts of ginger and mixtures of honey and ginger on some pathogenic fungi.

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 four weeks at temperature of $25\pm 2^{\circ}\text{C}$ and blended with a grinder to obtain smooth powder.

Three hundred grammes each of the powder was weighed and extracted by soaking separately under room temperature ($25\pm 2^{\circ}\text{C}$) with method and ethanol for 24hr. 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°C for 24 hr for sterility check. The sterile samples were aseptically dispensed into sterile pyrex sample bottles and kept at room temperature ($25\pm 2^{\circ}\text{C}$) prior to its use.

Test organisms

The isolates included: *Candida albicans*, (from human urine), *Aspergillus flavus*, *Aspergillus fumigatus* and *Trichoderma viride* (from food samples). They were tested against a wistar rat and fruits to ascertain their pathogenicity.

Antifungal determination with crude extracts

The bio assay of the ethanol, methanol ginger extracts and honey was carried out by the agar diffusion method. One ml each of the fungal test organisms was taken from a concentration of 10^7 Spore/ml and pour plated using Potato dextrose agar. The plates were allowed to set for 2 hr to allow the organisms to be well established in the medium. With a previously sterilized cork borer (4mm), wells of equal distance were bored. The ginger extracts (ethanol and methanol); honey, honey (5ml) and the ginger extract (1g) mixtures were aseptically filled into the well respectively and were appropriately labelled and incubated at $28\pm 2^{\circ}\text{C}$ for 84hr. At every 12 hr, the antifungal test samples were replenished in the wells where evaporation is noticed. Inhibitory zones were meas-

ured after incubation as degree of sensitivity of the test organisms to the test samples.

Antifungal sensitivity test with commercial antibiotics

Ketoconazole and griseofulvin, as known standard antifungal drugs were prepared by dissolving a capsule of 500mg in 5ml of sterile distilled water to make 100mg/ml. The bored well on the fungal seeded plates were filled respectively with the standard antifungal samples and incubated at $28 \pm 2^{\circ}\text{C}$ for 84hr. At every 12hr, the standard antifungal samples were replenished in their appropriate well. Inhibitions observed at the end of incubation were then measured.

Phytochemical Screening of Ginger Extracts and Honey

Alkaloid test

Five grammes each of the ginger extracts and 5ml honey were stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath at 60°C for 5min. The sample was filtered with a 3 layered muslin cloth. One milliliter of the filtrate was treated with few drops of Dragendoff's reagent. Blue black turbidity serves as preliminary evidence of alkanoids.

Saponins test

Five grammes each of the extracts and 5ml 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.

Tannins test

Five grammes each of the extracts and 5ml of honey were stirred separately with 100ml 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 and Evans, 1989).

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 and Evans, 1989).

Flavonoids test

Five milliliters of diluted ammonia solution was added to aqueous filtrate of the test samples followed by the addition of 1ml concentrated H_2SO_4 . A yellow colouration indicates the presence of flavonoids (Harborne, 1973).

Cardiac glycosides (keller-killiani test)

Five grammes of each of the extracts and 5ml of honey were dissolved separately in 2ml glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1ml 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 and Evans, 1989).

Legal test

Five gramme of each extract and 5ml 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 Cardenolides (Trease and Evans, 1989).

Salkoski test

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

Lieberman's test

Five grammes of the extracts and 5ml of honey were mixed with 2ml of acetic anhydride and cooled. After which 0.5ml 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 and Evans, 1989).

RESULTS AND DISCUSSION

Antifungal activity of the extracts on tested fungal isolates

Aspergillus flavus and *Trichoderma viride* were the most inhibited in the antifungal assay. Zones between 10 and 45mm were observed as the inhibitory affinity on *A. Flavus*. The honey sample displayed the highest zone of 45mm followed by the mixtures of honey and ethanol-ginger extract (40mm); honey and methanol-ginger extract (30mm); ethanol-ginger extract (10mm); and was least in methanol-ginger extract (0.9mm). *Trichoderma viride* highest inhibition was observed in the mixtures of honey and ethanol-ginger extract (45mm), followed by ethanol-ginger extract (25mm), mixtures of honey and methanol-ginger extract (20mm), methanol-ginger extract (18mm) whereas the least activity was recorded in honey treated samples (0.9mm). *Candida albicans* was inhibited by ethanol-ginger extract (28mm), methanol-ginger extract (20mm) while mixture of honey and ethanol-ginger extract showed no zone of inhibition, while *Aspergillus fumigatus* could not be inhibited by any of the antifungal agents. It was observed

that the honey exhibited 45mm on *A. flavus* and 0.9mm on *T. viride*. The mixtures of honey and ethanol-ginger extract exhibited inhibitory zone of 40mm on *A. flavus* and 45mm on *T. viride*. This observation emphasised that honey antifungal effect on the test organisms was higher than ginger extract. Also, the honey and the extracts mixture with honey displayed no inhibitory effect on *C. Albicans* (Table 1).

Phytochemical screening of the honey and ginger extracts

The phytochemical screening of honey had only Saponin and Cardiac glycosides (Table 2) compared to ginger which had the presence of Saponin, Phlobatannin, Alkanoid, Flavonoid and Cardiac glycosides (Table 2). These phytochemical properties give plants their medicinal value which is appreciated by human beings because of their great importance in health care of individuals and communities (Akharaiyi and Boboye, 2010). From the phytochemical screening, it is evident that the antifungal activities of the ginger extracts were effective due to its phytochemical components such as saponnin, tannins and alkaloids known to show medicinal activities as well as exhibiting physiology activity for therapeutic value. The antifu-

Table 1: Zones of inhibition from the antifungal activity of the extracts and antibiotic sensitivity of tested fungal isolates

Tested Isolates	Extracts					Antibiotics	
	Honey+ methanol Extract	Honey + ethanol Extract	Methanol ginger Extract	Ethanol ginger Extract	Honey Extract	<i>Griseofulum</i>	<i>Ketoconazole</i>
<i>Aspergillus flavus</i>	30mm	40mm	0.9mm	10mm	45mm	No zone	26mm
<i>Aspergillus Fumigates</i>	No zone	No zone	No zone	No zone	No zone	No zone	19mm
<i>Candida albicans</i>	No zone	No zone	20mm	28mm	No zone	17mm	20mm
<i>Trichoderma Viride</i>	20mm	45mm	18mm	25mm	0.9mm	15mm	15mm

Table 2: Qualitative phytochemical screening of honey and Ginger extracts

Phytochemicals	Honey	Methanol ginger extract	Ethanol ginger extract
Saponin	+	+	+
Tannin	-	-	-
Phlobertannin	-	+	+
Alkaloid	-	+	-
Anthraquinine	-	-	-
Flavonoids	-	+	+
Gardiac glycosides			
Keller-kilani's test	-	+	+
Salwoski's test	+	+	+
Lieberman's test	+	+	+
Legal test	+	+	+

+ = positive - = negative

ngal activity of the ginger extract could also depend on the high molecular weight of the phytochemicals where diffusion is faster and at a quicker rate (Ekwenye and Elegam, 2005), and also the presence of gingerol and shogaols (Okunbobwa and Edema 2007).

Antibiotic sensitivity of the fungal isolates

The antibiotic sensitivity assay revealed that *A. flavus* and *A. fumigatus* were resistant to griseofulvin. However, ketoconazole inhibited the two fungi species with zones of 26 mm and 19 mm respectively. However, the two commercially employed antifungal drugs (Griseofulvin and Ketoconazole) inhibited *T. viride* and *C. albican* with zones >10mm (Table 1). Despite the fact that the ginger extract and honey could not effect inhibitory potency on *A. Fumigatus*, ketoconazole displayed inhibitory zone of 19 mm. The antifungal activities of honey and ginger mixtures are comparable to the activities of the employed commercial antifungal agent, in the sense that inhibitory zones of between 0.9 and 45mm were exhibited.

CONCLUSION

In view of the obtained results, it is evident that ginger and honey are potential antifungal agents, and mixtures of honey and ethanol-ginger extracts showed a wider spectrum as anti

fungal agent than honey.

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