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Research Article

RHIZOME OF *ZINGIBER OFFICINALE*: POSSIBLE SOURCE OF ALTERNATIVE REMEDY FOR STREP THROAT

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ABSTRACT
Ginger (*Zingiber officinale*) has for long been used as an anti-sore throat remedy in African societies. This study evaluated the *in-vitro* activity of ethanol and hexane rhizome extracts of ginger against *Streptococcus pyogenes*, the most common bacterial cause of the condition and determined the levels of selected phytochemicals in the extracts. Ginger was obtained from the markets in Kampala, dried for 2 weeks, and then ground into a fine powder using mortar and pestle. After cold maceration of the powder with ethanol or hexane, the filtrates were evaporated to slurry with a rotary evaporator. Fresh crude extracts were prepared immediately prior to use. The anti-*Streptococcus pyogenes* activity was evaluated using the Agar well diffusion and the peptone water serial dilution methods and inhibition zone diameters and minimum inhibitory concentrations of the extracts were determined. Phytochemical profiles of extracts were qualitatively assessed. Both extracts inhibited *S. pyogenes* growth but activity was higher for the ethanol extract than for the hexane extract. While this difference was not statistically significant ($p > 0.05$) for the 400 mg/ml concentration of the extracts it was significant at the 800 mg/ml concentrations. The MIC for both extracts was 0.2 g/ml. Both extracts showed similar phytochemical profiles with rich tannin content and rather low levels of saponins, steroid glycosides and triterpenes. The study demonstrates the anti-*Streptococcus pyogenes* activity of the rhizome of *Z. officinale* and its potential as a possible source of a Strep throat remedy.
Keywords: *Streptococcus pyogenes*, *Zingiber officinale*, phytochemicals, rhizome, sore throat

INTRODUCTION

Pharyngitis (sore throat) accounts for 80 % of cases in children and young adults in developing countries[1]. The causes of pharyngitis may be viral, bacterial or environmental causes but the most common cause is *Streptococcus pyogenes*[2,3]. This condition is also known as Strep throat and antibiotics such as penicillin and erythromycin are used to prevent complications by *S. pyogenes* and to ensure speedy recovery[4]. NSAIDS, acetaminophen and corticosteroids like dexamethasone may also be used to relieve pain and inflammation of the pharynx and swelling of lymph nodes[5]. While antibiotics are useful in pharyngitis relief, resistance to erythromycin has been reported[6]. On the other hand penicillins possess side effects such as allergy and hypersensitivity, necessitating a search for alternative plant-based remedies. Fragrances of essential oils of lavender (*Lavandula officinalis*) have been used to relieve the condition[7]. Ayurvedic practitioners suggest gargling with a mixture of water, salt and Tumeric (*Curcuma longa*) powder[8]. Ginger (*Z. officinale*) has also been traditionally used for treatment of sore throat and studies indicate that it possesses numerous biological activities including antibacterial, analgesic and anti-inflammatory properties[9,10]. Ginger has also been used in treatment of gastric ulcers and acts by chelating *Helicobactor pylori*[11]. This study sought to determine the anti-*S. pyogenes* activity and phytochemical composition of ginger locally grown in Uganda.

MATERIALS AND METHODS

Plant material collection and extraction procedure

Fresh ginger rhizomes were purchased from Kalerwe market in Kampala and kept at room temperature. The plant specimens were identified and authenticated at the Herbarium of the Department of Botany and the voucher specimen deposited in the Pharmacology and Toxicology Research Lab of the College of Veterinary Medicine, Makerere University as PTRL 003. The rhizomes were cleaned thoroughly with running tap water, chopped into pieces using sharp knife and then spread on clean lab benches to dry for one week before they were ground into fine powder using mortar and pestle. The dry powdered spice (55 g) was macerated in 600 ml of ethanol (Zayo-Sigma Ltd, Germany) or hexane (Unilab Ltd, Kenya) in 1 L amber coloured bottles for 72 hours at room temperature with occasional shaking, and then filtered using filter paper (Whatman No. 1). The filtrate was evaporated to slurry using a rotary evaporator and further dried into a semi-solid extract which was re-dissolved using dimethyl sulfoxide (DMSO) and distilled water to make a stock solution (800 mg/ml) just prior to use.

Culture of *S. pyogenes*

The *Streptococcus pyogenes* bacteria were cultured, Gram-stained and tested to confirm their identity. The bacterial stock culture was maintained on Mueller-Hinton agar plates[12]. The test organism was inoculated overnight into the plates and incubated for 24 h at 35°C. Gram staining was done on the stock culture after inoculation and colonial morphology was examined to identify the culture characteristics after incubation. Gram staining was re-done on the colonies to determine presence of bacterial growth. Sub-culturing was then done to obtain pure colonies of *S. pyogenes* followed by further Gram staining. Biochemical tests i.e. Catalase test in which *S. pyogenes* was negative and Bacitracin test for which it was sensitive confirmed the identity of the bacteria.

Evaluation of the anti-*Streptococcus pyogenes* activity of the extracts

The anti-*Streptococcus pyogenes* activity of the extracts was evaluated using Agar well diffusion and the peptone water serial dilution methods. The Agar well diffusion method involved inoculation of Mueller-Hinton agar plates with *S.*

pyogenes by spreading the bacterial inoculums on the media. Wells (6 mm diameter) were punched in the agar and filled with 800 mg/ml ethanol or hexane ginger extracts. Each plate also had wells filled with 800 mg/ml penicillin solution (positive control) and 0.9 % normal saline (negative control). The plates were incubated at 37°C for 24 h and the inhibition zone diameter (mm) were measured with a divider and ruler. The assays were done in duplicates and mean inhibition zone diameters (± SEM) were calculated. For MIC determination, the method described by Thongson *et al*. was used[13]. Two-fold serial dilutions of ethanol and hexane extracts were prepared by dilution in peptone water to achieve decreasing concentrations of extracts i.e. 800 mg/ml, 400 mg/ml, 200 mg/ml and 100 mg/ml. A 90 µl volume of each dilution was added aseptically into Mueller Hinton agar plates inoculated with *Streptococcus pyogenes* by spread plate method. The plates were incubated overnight and then checked for inhibition of bacterial growth. The lowest concentration of extract showing a clear zone of inhibition was taken as the MIC (g/ml). All experiments were done in duplicates. Means ± SEM values of inhibition zone diameters and MICs for the different extract concentrations were calculated with Microsoft Excel®. The Tukey multiple comparison test was used to compare these values and they were considered statistically significant at $p < 0.05$.

Phytochemical screening

Phytochemical screening was done on both ethanol and hexane ginger extracts using methods described by Harborne[14]. The abundance of the phytochemicals in the extracts was determined qualitatively i.e. depending on the intensity of the colour change.

Alkaloids

The extracts (20 ml) were separately transferred to a capsule and evaporated on a boiling water bath and 5 ml of 10 % HCl were added to the residue. The alkaloids are salts of mineral acid from the aqueous solution, precipitated as bases by adding 5 ml of 10 % ammonia and then extracted with chloroform. The chloroform solution was evaporated to dryness in a capsule and the residue dissolved in 1.5 ml of 20 % HCl. The acidic solution (in which the alkaloids are in salt form), was divided in three test tubes i.e. one was the reference and in the other two tubes 2-3 drops of Mayer's reagent were added and observed for colour change, which would indicate presence of alkaloids.

Saponins and tannins

Diluted solutions (1:1) of extract (2 ml) were separately shaken in a 1.6 cm diameter test tube for 15 minutes. The test tube was observed for the presence of foam that would indicate presence of saponins. For tannins the extracts (1 ml) were separately diluted with 2 ml of water in a test tube and 3 drops of diluted solution of Ferric Chloride were added. The solution was observed for a colour change to blackish blue that would indicate presence of tannins.

Flavonoids, steroid glycosides and triterpenes

The extracts were hydrolyzed by adding 15 ml of 10 % HCl to 25 ml of the extracts by refluxing and then heated up for 30 minutes. During the hydrolysis, the solution became opalescent due to the precipitating aglycones obtained by the degradation of the glycosides. After cooling, the solution was extracted 3 times in a separating funnel with 10 ml of ethyl ether. The ether extracts were collected together (30 ml) and dehydrated with anhydrous Na_2SO_4. This resulted in formation of the aqueous fraction which was discarded and the ether fraction which was used to test for presence of flavonoids, steroid glycosides and triterpenes. The presence of flavonoids was confirmed by evaporating 5 ml of the ether fraction of the extracts to dryness and the residue was dissolved in 2 ml of 50 % methanol by heating. Magnesium metal and 6 drops of concentrated hydrochloric acid were added and an observation was made for a colour change, which would indicate presence of flavonoids. For steroid glycosides and triterpenes, 10 ml of the ether fraction were evaporated to dryness and the residue dissolved in 0.5 ml acetic anhydride and 0.5 ml of chloroform. The solutions were transferred to a dry test tube using a pipette and 2 ml of concentrated H_2SO_4 was added at the bottom. The test tube was observed for presence of a reddish-brown ring at the separating level of the two liquids and a bluish-green superior layer which would indicate presence of steroid glycosides and triterpenes.

Ethical considerations

This study was approved by the Ethical Review Committee of the College of Veterinary Medicine, Animal Resources and Bio security of Makerere University and ethical clearance number VAB/REC/12/006 was issued.

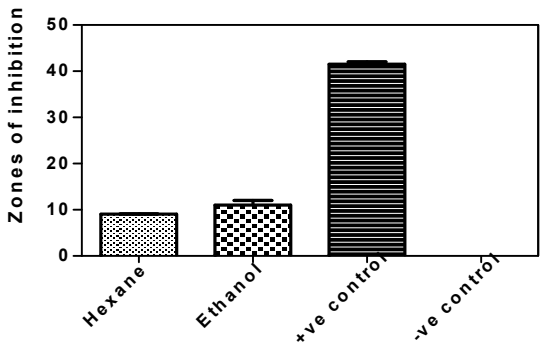

Figure 1: Inhibition zones for 400 mg/ml concentration of hexane and ethanol ginger extracts

The *in-vitro* anti-*S. pyogenes* activity of the ethanol and hexane ginger extracts was evaluated using the Agar well diffusion method. Penicillin was used as positive control and normal saline was negative control.

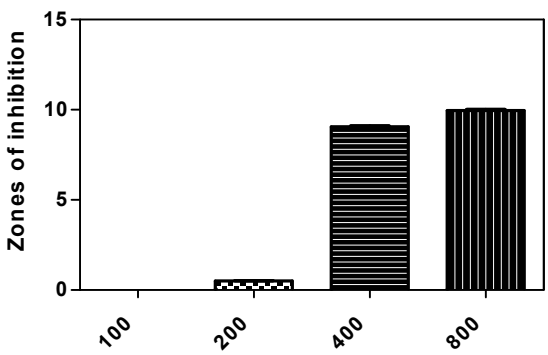

Figure 2: Comparison of inhibition zones for different concentrations of the hexane extract

The inhibition zone diameter increased significantly with increasing extract concentrations.

Table 1: Anti-*Streptococcus pyogenes* activity of the *Zingiber officinale* extracts

Extracts	Mean inhibition zone diameter ± SEM				
Concentrations	100	200	400	800	Penicillin
Hexane	0^{aA}	0.51 ± 0.014^{aB}	9.05 ± 0.0707^{aC}	9.96 ± 0.0846^{aD}	42 ± 0.707107
Ethanol	0^{aA}	0.805 ± 0.00707^{aA}	11 ± 1.4142^{aB}	13.04 ± 0.07071^{aB}	43 ± 1.414214

Small letters (a) indicate statistically significant difference between the two extracts ($p < 0.05$) while capital letters (A, B, C and D) indicate statistically significant difference within extract concentrations

Table 2: Abundance of selected phytochemicals in ginger extracts

Phytochemical	Ethanol extract	Hexane extract
Alkaloids	-	-
Flavonoids	-	-
Saponins	+	+
Tannins	+++	+++
Steroid glycosides and tritepenes	+	+

Key: +++ Present in high concentration; + Present in low concentration; - Absent

RESULTS

Both hexane and ethanol extracts of *Z. officinale* (ginger) showed statistically significant inhibition of *S. pyogenes* bacterial growth on comparison with the negative control ($p < 0.05$). At the 400 mg/ml and 800 mg/ml concentrations of the extracts, the inhibition of *S. pyogenes* growth was higher for the ethanol extract than for the hexane extract (Table 1, Figure 1). While this difference was not statistically significant ($p > 0.05$) for the 400 mg/ml extracts, at the 800 mg/ml concentration, the ethanol extract showed a significantly higher inhibition of bacterial growth than the hexane extract ($p < 0.05$). When the inhibition zone diameters obtained for both extracts were compared with the positive control (penicillin, 800 mg/ml) they were found to be significantly lower for the extracts ($P < 0.05$) as shown in Table 1. The anti-*S. pyogenes* effect of the hexane extract was concentration-dependent i.e. inhibition zone diameters significantly increased ($p < 0.05$) with increasing extract concentrations for the hexane extract (Table 1, Figure 2). However for the ethanol extract there was no significant difference ($p > 0.05$) between the inhibition zones diameters for the 400 mg and 800 mg concentrations and between the 100 mg and 200 mg concentrations (Table 1). However, the minimum inhibitory concentration (MIC) for both extracts was 0.2 g/ml. Both the crude ethanol and hexane rhizome extracts of *Zingiber officinale* showed similar phytochemical profiles. For both extracts, tannins were highly present while alkaloids and flavonoids were absent (Table 2). Saponins, steroid glycosides and triterpenes were present in rather low quantities.

DISCUSSION

Streptococcal pharyngitis, caused by *S. pyogenes*, is one of the commonest illnesses requiring medical attention in children and young adults[1]. The results of this study show that both ethanol and hexane ginger extracts are active against *S. pyogenes*. Studies on the comparative effect of leaf and root ginger ethanol and water extracts on *S. pyogenes* and *S. aureus* in Nigeria demonstrate that the root ethanol extract showed greater effect on *S. aureus* and *S. pyogenes* compared to the leaf ethanol extract, leaf and root water extracts[15]. The ginger ethanol extract has also demonstrated antibacterial activity against a range of other bacteria inhibiting growth of Gram positive (*S. aureus*, *S. epidermidis*, *B. cereus*) and Gram negative bacteria (*K. pneumonia*, *P. aeruginosa*, *E. coli*)[16]. In this study, the ginger ethanol extracts showed better activity than isopropyl-hexane (7:3) extracts. Another study showed that methanol and ethanol ginger extracts exhibit a broad antibacterial spectrum activity while control experiments with only the solvents showed no inhibition of any bacteria, indicating that ginger itself and not the solvent inhibited the growth of the bacteria[17]. The antibacterial activity is most likely due to the phytoconstituents present in ginger. Phytochemicals with antibacterial activity are more concentrated in the ginger root (which is more widely used in traditional remedies) than in the leaf[17]. However, the levels of phytoconstituents in ginger vary depending on the climatic conditions and soil properties and whether the rhizomes are fresh or dry[18]. Our study shows that Ugandan ginger has tannins as the most abundant phytoconstituent, with moderate levels of saponins, steroid glycosides and terpenoids. In other studies, methanol extracts of the ginger rhizome in India yielded the presence of terpenoids, flavonoids, alkaloids and tannins[17]. The antibacterial properties of ginger phytoconstituents have been reported[19]. Tannins have antibacterial activity and thus, could probably explain most of the anti-*S. pyogenes* activity of the extracts[20]. Recent studies also indicate that steroidal glycosides from plants possess potent activity against a number of Gram-positive and Gram-negative bacteria[21]. Saponins not have stimulatory effects on the components of specific immunity, but also on non-specific immune reactions such as inflammation which may be useful in conditions like pharyngitis[22]. While this effect is demonstrated *in-vivo*, it probably contributes to the reported potent anti-Streptococcal pharyngitis activity of ginger. *In-vitro* studies in Nigeria indicate that crude ginger extracts possess significant antioxidant properties[23]. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules (proteins, lipids, DNA etc) and cause protein/DNA damage along with lipid peroxidation in cells[24]. This contributes to the pathogenesis of inflammatory conditions such as pharyngitis. Tannins due to their antioxidant properties are capable of preventing oxidative damage by scavenging free radicals[25]. Terpenoids on the other hand have antimicrobial, anti-inflammatory and immunomodulatory properties and are useful in bronchitis[26]. The anti-inflammatory potential of some terpenoids is superior to that of standard drugs such as indomethacin[27]. Steroid glycosides have anti-inflammatory,

antioxidant, anti-asthmatic and antibacterial effects which may be useful in pharyngitis[28].

CONCLUSION

The results of this study indicate that ginger is a potential source of a sore throat remedy, which validates its continued traditional use for this purpose in the community. Further fractionation of the extracts could lead to isolation of active constituents that may serve as leads for the development of new pharmaceuticals against Streptococcal pharyngitis.

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