

Phytochemical screening and biological activities of *Hypotrigena squamuligera* raw honey

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ABSTRACT

Resistance of pathogenic microorganisms to current pharmaceuticals is on the rise, thus discoveries of alternative therapeutic agent is urgently required. Honey offers attractive options which include antimicrobial activity, wound healing and anti-inflammatory activity. Even though antimicrobial activity of honey has been extensively studied it differs according to type and source of honey. The present investigation was carried out to evaluate phytochemicals and biological activities of *Hypotrigena squamuligera* honey. Standard chemical tests, DPPH antiradical activity and agar disk diffusion methods were adopted to determine phytochemicals, antioxidant and antibacterial activity. Phytochemical screening revealed presence of tannins, alkaloids, flavonoids, alkaloids, terpenoids, anthraquinones and cardiac glycosides. *H. Squamuligera* exhibited marked antioxidant and antibacterial activity. Its antiradical activity was lower than that of ascorbic acid and quercetin at low concentrations however after a concentration of 0,05mg/ml methanolic extract exhibited higher antioxidant activity. *H. squamuligera* honey (100%) produced a greater inhibition than amoxicillin for *S. aureus*. For *E. coli* amoxicillin produced greater inhibition than *H. squamuligera* honey. Compared to *Apis mellifera* honey at a concentration of 100%, *H. squamuligera* honey revealed better inhibitory activity. These results suggest that *H. squamuligera* honey could potentially be used as an alternative pharmaceutical agent against certain microorganisms and source of natural antioxidants for promotion of health.

Keywords: *Hypotrigena squamuligera*, phytochemicals, antioxidant activity, antibacterial activity

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1. INTRODUCTION

Resistance of pathogenic micro-organism to current medicines is on the rise prompting a need to search for alternative medicines (Al-Jasser 2006; Sundqvist and Kahlmeter, 2005; Gulluce et al., 2006; Halama and van Haluwin, 2004; Rankovic et al., 2008). Honey offers attractive options which include wound healing and antibacterial activity (Mavric et al 2008; Blair and Carter 2005; Church et al 2006; Ainul Hafiza et al 2005). Although composition and antimicrobial activity of honey has been effectively established it differs according to the type and source (Tumin et al 2005; Lusby et al 2005; Basson et al 2008). Currently no extensive research on phytochemical composition and antibacterial activity of *Hypotrigona squamuligera* honey has been conducted. The objectives of this study were to conduct such studies so as to authenticate its use in folk medicine. Information obtained from social gathering indicates that infection on wounds is rapidly cleared, swelling and inflammation quickly disappears when *Hypotrigona squamuligera* honey is applied. Serious burns, stubborn wounds and cervical ulceration are said to disappear completely. Some traditionalist confessed cancer healing, analgesic and soothing ability of *Hypotrigona squamuligera* honey. It is also believed to shorten the duration of diarrhoea in patients. *Hypotrigona squamuligera* honey is widely used as medicine and food in many African countries such as Zimbabwe, Zambia, Mozambique, Botswana, Cameroon, Niger and Senegal (Eardley (2004). The insect *Hypotrigona squamuligera* and its honey are shown in Fig 1 below



Fig 1. *Hypotrigona squamuligera*, its honey and entrance

2. MATERIAL AND METHODS

2.1 Collection of Honey

The honey used in this study was obtained from Chinyani village in Murewa, Mashonaland East, Zimbabwe in November 2011. Samples were stored in a freezer before use.

2.2 Extraction

For extraction, 50g of honey was taken and added to 125mL of methanol in a conical flask. Later the solution was mixed well by vortexing and centrifuged at 3000 rpm for 10 minutes at 25°C. The extracts were filtered over Whatman No.1 filter paper into stoppard test tubes.

2.3 Phytochemical screening

Phytochemical tests were carried out on pure honey using standard chemical test reported by Sofowora, (1994) with slight modifications. Two millimeters of honey was mixed with aqueous sulphuric acid and benzene to test for presence of anthraquinone glycosides. Also,

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Mayer and Dragendorff's reagents were employed to test for presence of alkaloids. With the addition of gelatin and ferric chloride solution, test for tannins was conducted. Keller-keliani and Legal tests were carried out for cardiac glycosides, froth and haemolysis tests for saponins and 1% aluminum chloride solution in methanol was used to investigate flavonoids. Lastly, Benedict's and Fehling's solutions were added to determine presence of reducing sugars.

2.4 Determination of antioxidant activity

The radical scavenging activities of *Hypotrigona squamuligera* honey against 2,2-Diphenyl-1-picryl hydrazyl radical (DPPH) (Sigma-Aldrich) were determined by UV spectrophotometry at 517 nm. The following concentrations of the honey extracts were prepared, 0.0125, 0.025, 0.05 and 0.1 mg/ml in methanol. Quercetin and ascorbic acid dissolved in methanol were used as standard antioxidants. One milliliter of the extract was placed in a test tube, and 3 ml of methanol was added followed by 0.5 ml of 1 mM DPPH in methanol. The radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging effect} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c is the absorption of the blank sample (methanol + DPPH) and A_s is the absorption of the sample, methanol + DPPH.

2.5 Antibacterial Activity of Methanolic Extract

The extracts were diluted with absolute methanol to produce the following concentrations: 10, 30, 50 and 100% (v/v). Nutrient agar was melted in a steam bath set at 30°C to prevent solidification. Six Petri dishes were pre-inoculated with *E.coli* and another six with *S. aureus* in the following manner. One milliliter of the bacterial suspension was pipetted into the appropriately labeled Petri dish to which 25ml of molten agar was then added followed by thorough mixing of the bacteria and molten agar. The agar was allowed to set for 1hr. Holes of 3 X 4mm wide were then made in the agar using a borer. Extracts (25µl) of a specific concentration was introduced into each of the holes in an appropriately labeled Petri dish using a sterile micropipette. Amoxicillin (10µg/ml) was used as a positive control and absolute alcohol as a negative control. The dishes were then incubated at 25°C for 24hours after which zones of inhibition were measured and recorded. The zone of inhibition was taken to be the diameter of the zone visibly showing the absence of bacterial growth including the 4mm hole. Where there was no inhibition, the value of 0 mm was assigned to the test sample.

3. RESULTS AND DISCUSSION

Since ancient times *Apis mellifera* honey has been used for its medicinal properties in many societies. Information on the use of honey for the treatment of many human ailments can be found in magazines, beekeeping journals and natural products leaflets however little is known about *Hypotrigona squamuligera* honey. The phytochemical analysis carried out on

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methanolic extract showed the presence of tannins, alkaloids, flavonoids, alkaloids, terpenoids, anthraquinones and cardiac glycosides as shown in Table 1.

Phytochemical component	Remarks
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Terpenoids	+
Cardiac glycosides	+
Anthraquinones	+

+ = present, - = absent

Table 1: Phytochemical components of the extracts in honey

The result of antioxidant activity of different concentrations of methanolic extract and standard antioxidant (Quercetin and ascorbic acid) is shown in Table 2. The extract exhibited marked antioxidant activity by scavenging DPPH (free radical) and converting it into DPPHH. The extract has shown concentration dependent radical scavenging activity. The scavenging activity of standards was higher than that of methanol extract at low concentrations however after a concentration of 0,05mg/ml methanolic extract exhibited higher antioxidant activity. Phytochemicals such as flavonoids, tannins and saponins Aliyu et al (2008) constitute a major group of compounds that act as antioxidants. They consist of high redox potentials which enable them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Kahkonen et al., 1999). Delocalization of electrons over the phenolic ring and stabilization by resonance effect of the aromatic nucleus makes the antioxidant radical unreactive (Tsao and Akhtar, 2005). The antioxidant effect of *Hypotrigona squamuligera* honey may be rationalized in terms of the large number of phytochemicals present. (Phillips et al 2009; Gheldof et al 2002) carried studies and found out that honey consisted of various phenolic compounds which differ according to origin of the honey. The antioxidant capacity of honey was as a result of the combined activity of a wide range of compounds including phenolics, peptides, organic acids and enzymes.

Concentration (mg/ml)	% radical scavenging activity		
	Methanolic extract	Ascorbic acid	Quercetin
0.0125	7.35	85.80	89.70
0.0250	76.40	83.80	82.60
0.0500	100	80.20	82.10
0.1000	100	99.00	84.40

Table 2: Antioxidant activity of methanolic extract of *Hypotrigena squamuligera* honey, ascorbic acid and quercetin by DPPH free radical scavenging assay

The result of antibacterial activity of methanolic extract of *Hypotrigena squamuligera* honey is shown in Fig 2 and Table 3. The results were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth.

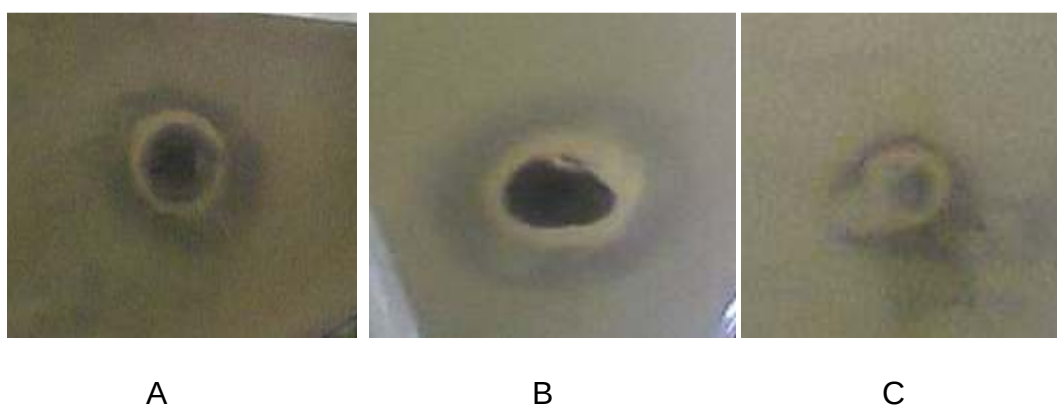


Fig 2 Antibacterial activity of methanolic extract of *Hypotrigena squamuligera* honey against *E. coli* (A), *S. aureus* (B) and *E. coli* for the positive control with Amoxicillin (10µg/ml) (C).

Test organism	Diameter of zone of inhibition (mm) n = 6						
	0% (-control)	10%	30%	50%	100%	Amoxicillin (10µg/ml)	<i>Apis mellifera</i> (100%v/v methanol)
<i>E. coli</i>	0	8	13	15	19	21	17
<i>S. aureus</i>	0	11	12	15	24	19	15

Table 3: Antibacterial activity of methanolic extract of *Hypotrigena squamuligera* honey for extract concentration 0, 10, 30, 50, 100% and *Apis mellifera* 100% (v/v)

The data obtained showed a dose dependent inhibitory action of *H. squamuligera* honey. The results suggest that *S. aureus* is more susceptible to *H. squamuligera* honey than *E. coli* with diameter zone of inhibition of 24 and 19mm respectively. *H. squamuligera* honey (100%) produced a greater inhibition than amoxicillin for *S. aureus*. For *E. coli* amoxicillin produced greater inhibition than *H. squamuligera* honey. Compared to *Apis mellifera* honey at a concentration of 100%, *H. squamuligera* honey revealed better inhibitory activity. Differences in antibacterial activity of *H. squamuligera* can be as a result of differences in differential susceptibility of bacterial cell walls, which is a result of slight differences inherent in the cell wall structure (Zhao et al. 2001). Gram-positive and gram-negative microorganisms differ in many features other than the structure of their cell walls, for example the presence of lipoproteins and lipopolysaccharides in gram-negative bacteria form a barrier to hydrophobic compounds Mazutti et al. (2008). Antimicrobial activity of honey has been attributed to hydrogen peroxide, which is produced by naturally occurring glucose oxidase, and phenolic compounds (Taormina et al 2001). When honey is used in wound dressing hydrogen peroxide is produced by dilution of the honey with body fluids resulting in it being released slowly and acts as an antiseptic. Honey chelates and deactivates free iron, which would otherwise catalyze the formation of oxygen free radicals from hydrogen peroxide, leading to inflammation. Also, the antioxidant constituents in honey help clean up oxygen free radicals present.

CONCLUSION

The results of the present research show that *H. squamuligera* honey consist of many bioactive compounds, significant antioxidant and antibacterial activity. It showed better antioxidant activity than standard antioxidants, ascorbic acid and quercetin. Compared with *Apis mellifera* honey, *H. squamuligera* honey showed superior antibacterial activity. Significant potency of *H. squamuligera* honey against certain microorganisms suggests its potential to be used as an alternative therapeutic agent for a number of medical conditions, particularly bacterial infections and wound healing.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ainul Hafiza, A.H., Yusof, N., Maimon, A. (2005). Potential of Malaysian local honey as an antibacterial agent. *Sains Malaysiana*, 34(1),17-20.
2. Aliyu, A.B., Musa, A.M., Abdullahi, M.S., Oyewale, A.O. (2008). Phytochemical and antibacterial properties of *Ludwigia suffruticosa* (Willd.) Oliv. ex. O. Ktze (Onagraceae). *Int. J. Appl. Sci*, 2(4), 1-5. (IJPAS Online).
3. Al-Jasser, A.M. (2006). *Stenotrophomonas maltophilia* resistant to trimethoprim-sulfamethoxazole: An increasing problem. *Ann Clin Microbiol Antimicrob*, 5, 23.
4. Basson, N.J., Grobler, S.R. (2008). Antimicrobial activity of two South African honeys produced from indigenous *Leucospermum cordifolium* and *Erica* species on selected micro-organisms. *BMC Complement Altern Med*, 8, 41.
5. Blair, S.E., Carter, D.A. (2005) The potential for honey in the management of wounds and infections. *J Australian Infect Control*, 10(1), 24-31.
6. Church, D., Elsayed, S., Reid, O., Winston, B., Lindsay R. (2006) Burn wound infections. *Clin Microbiol. Rev*, 19(2), 403-434.
7. Eardly, C.D. (2004). Taxonomic revision of the African stingless bees. *African plant protection*. 10, 63-96.
8. Gheldof, N., Engeseth, N.J. (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *J Agric Food Chemistry*, 50, 3050–3055
9. Gulluce, M., Asian, A., Sokmen, M., Sahin, F., Adiguzel, A., Agar, G., Sokmen, A. (2006). Screening the Antioxidant and Antimicrobial Properties of the Lichens *Parmelia saxatilis*, *Parmelia glauca*, *Ramalina pollinaria*, *Ramalina polymorpha* and *Umbilicaria nylanderina*. *Phytomedicine*, 13(7), 515-521.
10. Halama, P., van Haluwin, C. (2004). Antifungal Activity of Lichen Extracts and Lichenic Acids. *BioControl*, 49(1), 95-107.

11. Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* 47, 3954-3962.
12. Lusby, P.E., Coombes, A.L., Wilkinson, J.M. (2005). Bactericidal activity of different honeys against pathogenic bacteria. *Arch Med Res*, 36(5), 464-467.
13. Mavric, E., Wittmann, S., Barth, G., Henle, T. (2008). Identification and quantification of methylglyoxal as the dominant antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zealand. *Mol Nutr Foods Res*, 52(4), 483-489.
14. Mazutti, M., Mossi, A.J., Cansian, R.L., Corazza, M.L., Dariva, C.J., Oliveira, V. (2008). Chemical profile and antimicrobial activity of Boldo (*Peumus boldus* Molina) extracts obtained by compressed carbon dioxide extraction. *Braz. J. Chem. Eng.* 25 (02) , 427- 434, April – June.
15. Phillips, K.M, Carlsen, M.H, Blomhoff, R. (2009). Total antioxidant content of alternatives to refined sugar. *J. Am Diet Assoc.* 109(1), 64-71
16. Rankovic, B., Mišić, M., Sukdolak, S. (2008). The Antimicrobial Activity of Substances Derived from the Lichen *Physcia aipolia*, *Umbilicaria polyphylla*, *Parmelia caperata* and *Hypogymnia physodes*. *World J. Microbiol. Biotechnol.* 24(7), 1239-1242.
17. Sofowora, E.A. (1994). *Medical Plant and Traditional Medicine in Africa*. University of Ife Press. Nigeria.1, 1-23.
18. Sundqvist, M., Kahlmeter, G. (2005). *E. coli* and *S. aureus*– antimicrobial resistance surveillance and the effect of excluding duplicate isolates in a consecutive database 1990–2003. 15th European Congress of Clinical Microbiology and Infectious Diseases. Copenhagen / Denmark, April 2-5.
19. Taormina, P.J., Niemira, B.A., Beuchat, L.R. (2001). Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power, *Int. J Food Microbiol.* 69(3), 217-25.
20. Tsao, R. and Akhtar. M.H. (2005). Nutraceuticals and functional foods I: Current trend in phytochemical antioxidant research. *Agric. Food Chem. Environ.*, 3,10-17.
21. Tumin, N., Halim, N.A., Shahjahan, M., Noor Izani, N.J., Sattar, M.A., Khan, A.H., Mohsin, S.S.J. (2005). Antibacterial activity of local Malaysian honey. *Malaysian Journal of Pharmaceutical Sciences* , 3(2),1-10.
22. Zhao, W.H., Hu, Z.O., Okubo, S., Hara, Y., Shimamura, T. (2001). Mechanism of synergy between epigallocatechin gallate and β lactams against methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 45, 1737- 1742.