Communication in Physical Sciences, 2022, 8(4):647-651

Phytochemical Profiling of 30% Acetone Honeybee Crude Extract Collected From Ondo West Forest, Ondo State, Nigeria

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Received: 02 June 2022/Accepted 20 December 2022/Published online: 30 December 2022

Abstract: *Chemical data on the phytochemistry of crude honeybee residue may be useful in the development of therapeutic substances for alternative medicines. This has necessitated the present study, which involves the probing of 30% acetone crude extract of crude honeybee residue (collected from the Ondo North forest of Ondo state, Nigeria). The crude honeybee residue was assayed for its phytoconstitutions via qualitative phytochemical screening and quantitatively through high-pressure liquid chromatography(HPLC). The results obtained from the phytochemical investigation indicated the presence of alkaloids, terpenoids, flavonoids, phenols and glycosides. However, HPLC spectrum reveals the presence of quercetin (27.84%) and kaempferol (23.31%) showing retention times of 36.64 and 38.70 min respectively. The identified phytochemicals were the major constituents and are known for their medicinal significance.*

Keywords*: Crude honeybee residue, phytoconstitutions, quercetin, kaempferol*

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1.0 Introduction

The search for combinatory, functional or alternative medicines is a driving force for research and development in combing the global challenge and menace posed by multidrug-resistant related issues in the clinical world. Crude honeybee residue remains highly untapped with vast phytoconstitutions whose distributions are ecological functions of the surrounding vegetation (Abel and Banjo, 2012; Bankova *et al*., 2000).

Phytochemicals such as flavonoids, phenolics, coumaric acids, steroids, terpenes and terpenoids, flavonoids, phenolics, coumaric acids and essential oils have been reportedly identified in crude honey (Alvarez-Suarez *et al*., 2010; Jose and Vassya, 2010; Feng *et al*., 2009). For example, Odokwo and Salawu, (2021) reported the presence of steroids, quinones, saponins and alkaloids in crude honeybee residue collected from the Takum Local Government area of Taraba state. The investigated honeybee residue was observed to display antibacterial activity against strains of *Staphylococcus aureues*, *Eschericia coli*, *Bacillus subtillis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumonae*, in a dose concentration gradient. Literature is scanty on the exact or active constituents of the product, especially concerning its suspected medicinal applications. Such information can only be gotten from chemical analysis and quantification. Consequently, the present study seeks to complement literature claims on the phytochemical constituents of this product and to carry out HPLC analysis for the identification of the active phytochemicals that can exert antimicrobial activities.

2.0 Materials and Methods

Crude honeybee was harvested from the forest in Ondo West Local Government Area of Ondo State, South-West geopolitical zone of Nigeria. The reagents and chemicals used in the course of this work were of analytical grade and products of Sigma-Aldrich. The reagents and chemicals include acetone, ethyl acetate,

distilled water, methanol, acetic acid, caustic soda, sulphuric acid, chloroform, aluminium chloride, ferric chloride, hydrochloric acid, ethanol and Wagner's reagent.

2.1 Separation of crude honeybee residue

The crude honeybee was strained with a sieve, and dried at room temperature and the solid residue was preserved in glassware.

Plate 1 Crude honeybee residue

2.2 Preparation of 30% acetone in ethyl acetate

30% acetone in ethyl acetate was prepared by transferring 300 ml of acetone into a volumetric flask (1000 ml) containing 700 ml of ethyl acetate and the solution was thoroughly mixed and labelled.

2.3 Extraction of crude honeybee residue

100 g of the solid crude honeybee residue was extracted using 30% acetone in ethyl acetate and freeze-dried below room temperature. The percentage yield, PY was calculated using the relationship:

$$PY = \frac{Ye}{Ys}100\% \quad (1)$$

where: *Ye* represent the weight in grams of the dried extract and *Ys* is the weight in grams of the crude honeybee residue

2.3 Phytoconstitution profiling

The 30% acetone extract of the crude honeybee residue was investigated for its phytoconstitution via HPLC and qualitative Phytochemical screening techniques.

2.3.1 *HPLC analysis*

The identification of the candidates present in the extract was done using reverse phase, RP HPLC separation with gradient elution with water-methanol-acetic acid and detection at 280 nm.

2.4 Phytochemical screening

The qualitative profiling of secondary metabolites was done by standard methods as reported by Odokwo and Salawu, 2021. The crude extract was screened for the following classes of secondary metabolites: alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides and phenols.

2.4.1 Test for alkaloids

1 ml of the crude honeybee extract was stirred with 5 ml of 1% aqueous HCl in a steam bath and filtered while hot. Distilled water was added to the residue and 1ml of the filtrate was treated with a few drops of Wagner's reagent. The formation of a reddish-brown precipitate with Wagner's gives a positive test for alkaloids (Odokwo and Salawu, 2021).

2.4.2 Test for saponins

3ml of aqueous extract from the crude honeybee was transferred into a test tube and was vigorously shaken, the formation of froth/foam indicates the presence of saponins (Odokwo and Salawu, 2021).

2.4.3 Test for tannins

10% alcoholic ferric chloride was added to the extract of crude honeybee (Brayer's Test) (Odokwo and Salawu, 2021).

2.4.4 Test for terpenoids (Salkowski Test)

5 ml of extract was mixed with 2 ml of chloroform, and 3ml concentrated H_2SO_4 was carefully added. A reddish-brown colouration

of the interface was formed to show positive results for the presence of terpenoids (Odokwo and Salawu, 2021).

2.4.5 Test for flavonoids

A few drops of dilute sodium hydroxide solution were added to 5 ml of extract, the presence of yellow colour indicates the presence of flavonoids. 3 ml of 1% Aluminium chloride solution was added to 5 ml of each extract. A yellow colouration was observed indicating the presence of flavonoids (Odokwo and Salawu, 2021).

2.4.6 Test for glycosides (Keller – Killani Test)

5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution, and then a few drops of concentrated sulphuric acid was added into the solution. A violet-green ring appearing below the brown ring, in the acetic acid layer, indicates the positive presence of glycoside (Odokwo and Salawu, 2021).

2.4.7 Test for phenols

5ml of ethanol was added to 15ml of extract of the crude honeybee and 5 drops of iron (iii) chloride were added to the solution. A yellow-green precipitate indicates the presence of phenols (Odokwo and Salawu, 2021).

3.0 Results and Discussion

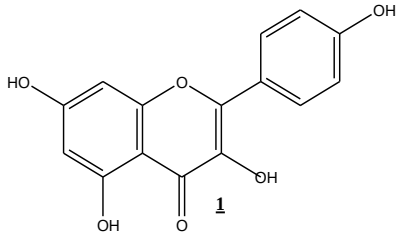
The calculated percentage yield of the dried crude using 30% acetone in ethyl acetate was 60%. The physical appearance of the crude extract was yellowish. The outcome of the qualitative phytochemical profiling is reported in Table 1. The results indicate the presence of alkaloids, terpenoids, flavonoids, phenols and glycosides in the crude honeybee residue, which suggest that the reported utilization of the residue for medicinal purposes may be associated with its phytochemical constituent. Terpenoids, flavonoids, glycosides and phenols had earlier been reported to be present in crude honeybee residue at levels closely related to the levels reveal in this study (Hamilton-Amachree and Odokwo, 2022).

Table1: Qualitative phytochemical screening

Secondary Metabolite	Observation
Alkaloids	+
Saponins	-
Tannins	-
Terpenoids	+
Flavonoids	+
Glycosides	+
Phenols	+

***+: presence, - : absence**

Quercetin (1) and kaempferol (2) showed significant concentrations of 27.84 and 23.31% under observed retention times of 36.64 and 38.72% respectively. were two notable flavonoid candidates present in the 30% acetone crude extract of the crude honeybee residue as identified using HPLC. The presence of quercetin and kaempferol had been reportedly in the crude honeybee residue alongside ferulic acid, rutin and apigenin (Hamilton-Amachree and Odokwo, 2022).



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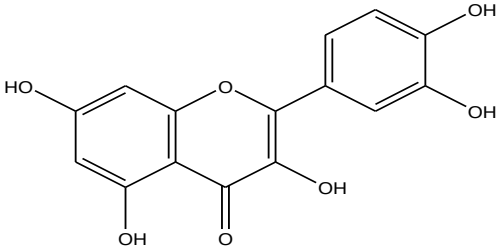

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Quercetin (**1**) is a flavonol found in fruits: berries, grapes, beverages: red wine; build: onion and olive oil (Kumar and Pondey, 2013).

It is known for its pharmacological activities such as anti-oxidant (Boots *et al*., 2008; McAnulty *et al*., 2008; Beatty *et al*., 2000), anticancer (Murakami *et al*., 2008), reduction of high blood pressure (Edwards *et al*., 2007) and alleviates chronic prostatitis (Loke *et al*., 2008; Dhar and Shoskes, 2007). Kampferol (**2**), a flavonol with an additional –OH group has similar pharmacological activities and sources as those of quercetin (Kumar and Pondey, 2013).

4.0 Conclusions

The phytochemical spectrum of interest has established that crude honeybee residue collected from Ondo North forest is riched in various classes of natural products. The presence of essential flavonoid candidates such as: quercetin and kampferol as identified via HPLC are strong justification for the ethnomedicinal claims associated with crude honeybee residue.

5.0 References

Hamilton-Amachree, A. & Odokwo, O.E. (2022). Phytoconstitution, anti-oxidant and iron chelating profiling of 30% acetone extract of crude honey residue in Obudu Local Government Area of Cross Rivers State, Nigeria. *World Scientific News*, 172, pp.165-178.

Abel, A.A. & Banjo, A.D. (2012). Apitheraphy in Southern Nigeria: An assessment of therapeutic potentials of some honeybee products. *Journal of Pharmaceutical and Biomedical Sciences*, 2,2, pp. 9-15.

Bankova, V., Castro, S.L. & Marcucci, M.C. (2000). Propolis: Recent advances in Chemistry and plant origin. *Apidologie*, 31, pp. 3-15.

Alvarez-Suarez, J.M., Tulipani, S., Romandini, S., Bertoli, E. & Battino, M. (2010). Contribution of honey in nutrition and human health: a review. *Mediterr J Nutr Metab*., 3, pp.15-23.

Jose, M.S. & Vassya, B. (2010). Propolis: Is there a potential for development of new drugs. *Journal of Ethnopharmacology*, 133, pp. 253-260.

Feng, L., Suresh, A., Hongyan, Z., Yasuhiro, T., Hiroyasu, E. & Shigetoshi, K. (2009). Chemical constituents of propolis from Myannar and their preferential cytotoxic against a human pancreatic cancer cell line. *Journal of Natural Products*, 72, pp. 1283-1287.

Odokwo, E. O. & Salawu, R. A. (2021).
Chemical and biological profiling of n-hexane extract of crude honeybee residue. *World Scientific News*, 152, pp. 69-81.

Dhar, N. B. & Shoskes, D. A. (2007). New therapies in chronic prostatis. *Curr Urol Rep*., 8, 4, pp. 313-318.

Edwards, R. L. Lyon, T. & Litwin, S. E. (2007). Quercetin reduces blood pressure in hypertensive subjects. *J Nutr* 137, pp. 2405-2411.

Kumar, S. & Pondey, A.K. (2013). Chemistry and activities of flavonoids: An overview. *Scientific World Journal*, 2013, pp. 1-16.

Loke, W.M., Hodgson, J. M., Proudfoot, J. M., McKinley, A. J., Puddey, I. B. & Croft, K. D. (2008). Pure dietary flavanoids quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *Am J Clin Nutr* 88, 4, pp. 1018-1025.

McAnulty, S. R., McAnulty, L. S., Nieman, D. C., Quindry, J. C., Hosick, P. A., Hudson, M. H., Still, L., Henson, D. A., Milne, G. L., Morrow, J. D., Dumke, C. L., Utter, A. C.,Triplett, N. T. & Dibarnardi, A. (2008). Chronic quercetin ingestion and exercise induced oxidative damage and inflammation. *Appl Physiol Nutr Metals* 33, 2, pp. 254-262.

Beatty, E. R. O'Reilly, J. D., England, T. G., McAnlis, G. T., Young, I. S., Geissler, C. A., Sanders, T.A. & Wiseman, H. (2000). Effect of dietary quercetin on oxidative DNA damage in healthy human subjects. *British Journal of Nutrition*, 84, 6, pp. 919-925.

Boots, A. W., Haenen, G. R. & Bast, A. (2008). Health effect of quercetin: from antioxidant to nutraceutical. *Eur J Pharmacol.* 585, 2,3,pp. 325-337.

Murakami, A. Ashida, H. & Terao, J. (2008). Multitargeted cancer prevention by quercetin. *Cancer Lett.* 269, 2, pp. 315-325.

Consent for publication

Not Applicable

Availability of data and materials

The publisher has the right to make the data Public

Competing interests

The authors declared no conflict of interest.

.Funding

There is no source of external funding

Authors' contributions

The entire work was designed, conducted and reported by the author.