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Research Article

ADVANCING CHEMICAL RESEARCH AT THE DEPARTMENT OF CHEMISTRY, FEDERAL UNIVERSITY OTUOKE, BAYELSA

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Abstract

The preliminary qualitative phytochemical screening, quantitative alkaloids, flavonoids and saponins and antioxidant activity have been carried out on both the n-hexane, HCP and the ethyl acetate, ECP extracts obtained from honeybee residue collected from Chikun in Kaduna State, Nigeria. The crude honeybee was strained and its residue subjected to both phytochemical and antioxidant profiling using established procedures. The qualitative Phytochemical screening revealed the presence of both steroids and terpeniods in HCP while alkaloids, flavonoids and saponins were present in addition in ECP. Quantitatively, alkaloids (0.89±0.00 mg/g AE), flavonoids (9.69±0.40 mg/g RE) and saponins (1.08±0.08 mg/g DE) were evaluated in ECP. Both extracts exhibited antioxidant activity in a concentration dependent gradient. Crude honeybee residue is an alternative source of phytochemicals with relevance therapeutical potentials.

Keywords: Honeybee residue, phytochemicals, antioxidant

1.0 Introduction

The clinical world of humans is being threatened by the evolutionary forces of multidrug resistance microbes. This has prompted several research and development at ensuring that the evolutionary nature of pathogens is being curtained. The search for alternative therapeutical substances is verged at exploring available sources of bioactive substances.

Crude honeybee residue is one of the abundant sources of phytochemicals that could serve as alternative sources of medicine. Phytochemically, it has been reported to posssess steroids, terpeniods, quinines, saponins and alkaloids (Odokwo and Salawu, 2021; Hamilton-Amachree and Odokwo). Phytocompounds such as ferulic acid, rutin, apigenin, quercetin and kaempferol have been isolated from crude honeybee residue. However, with these sets of phytochemicals there is need to further probe crude honeybee residue across different locations with the view of establishing their neutraceuticals benefits.

Material and Methods Collection of Material

The crude honeybee was collected from the wild in Chikun Local Government Area of Kaduna state, Nigeria. The crude honeybee in its raw form was strained and the residue obtained.

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Extraction of Phytochemicals

50 g strained crude honeybee residue was grinded**,** subjected to gradient extraction starting with 100 ml of n-hexane for 72 hrs, decanted and concentrated under presure. The marc was further extracted using ethyl acetate for another 72 hrs, decanted and concentrated under pressure, Bothe n-hexane extract, HCP and the ethyl acetate extract, ECP were subjected to preliminary qualitative and quantitative phytochemical and antioxidant profiling.

Qualitative Phytochemical Profiling of HCP and ECP

Samples of both HCP and ECP were profiled separately using established methods as obtained in literatures. Classes of phytochemicals such as: alkaloids, flavonoids, phenols, steroids, diterpenes, quinones, coumarins, anthocyannins, cardiac glycosides, tannins and saponins were evaluated qualitatively.

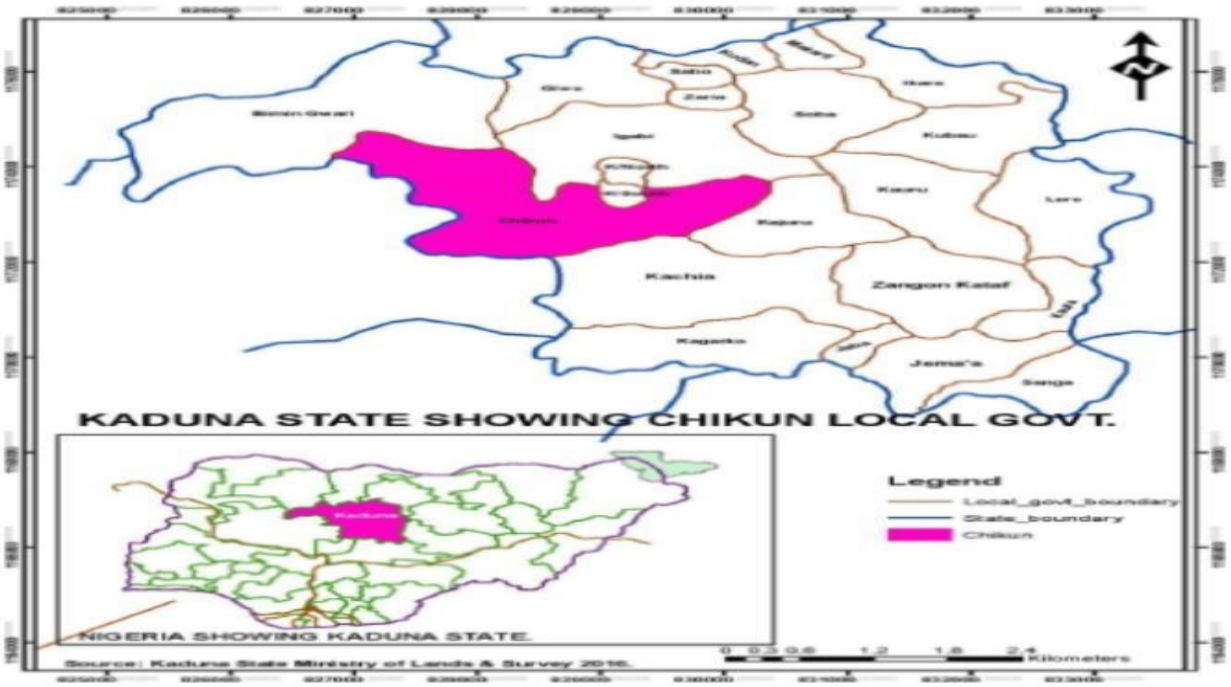

Fig. 1: Map of Kaduna State, showing Chikun Local Government Area (Source: Ugya, and Imam, 2018)

Test for Alkaloids

Few drops of Hager's reagent (saturated solution of picric acid) to 2 ml extract. The presence of a yellow colouration was an indication of alkaloids.

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Test for Flavonoids

1 ml extract added to 10% lead acetate (Pb (OAc)$_4$. The presence of a yellow colouration was an indication of the presence of flavonoids.

Test for Phenols

5 drops of iron (iii) chloride to ethanolic fraction of the extract. A yellow-green precipitate indicates the presence of phenols.

Test for Steroids (Salkowski test)

To 2 ml extract add chloroform (2ml) and concentrated H_2SO_4. An appearance of a reddish-brown ring was an indication of the presence of steroids.

Test for Terpenoids

2 ml of acetic acid anhydride and 2-3 drops of concentrated H_2SO_4 was added 2 ml of extract. The appearance of a deep red colouration was an indication of the presence of terpeniods.

Test for Quinones

1 ml extract was added 1 ml concentrated H_2SO_4. The formation of a red colouration was an indication of the presence of quinines (Firdouse and Alam, 2011).

Test for Coumarins

To 2ml extract was added 3 ml 10% sodium hydroxide. A yellow precipitation was indication of the presence of coumarins.

Test for Anthocyannins

To 2ml test extract was added 2 ml of 2M HCl. A pinkish-red to a bluish-violet coloration was an indicator of the presence of anthocyannins.

Test for Cardiac Glycosides

Dried chloroform fraction of the filterate of the aqueous alcoholic lead actetate and 2 ml acetic acid was dissolved in alkaline solution of 2 ml pyridine solvent and 2 ml of sodium nitropruside.

The formation of a pink colouration was an indication of the presence of cardiac glycosides.

Test for Tannins

10 ml of Br_2/H_2O was added to 500 mg aqueous extract. The decolourization of Br_2/H_2O indicates the presence of tannins (Gul *et al*., 2017).

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Test for Saponins

5.0 ml of distilled water was mixed with 200 mg aqueous extract in a test tube. The mixture was mixed thoroughly and then was added few drops of olive oil, shaken further. The appearance of foam showed the presence of saponins (Gul *et al*., 2017).

Quantitative Analysis of MCP

Quantitative Estimation of Alkaloids: To 1ml of MCP mixture, was added 5 ml phosphate Buffer (pH 4.7), 5 ml BCG solution and 4 ml of chloroform. The blank was prepared with the same procedure but without adding MCP. The absorbance of the complex in chloroform was read at 470 nm. Atropine is used as a standard material and compared the assay with Atropine equivalents. All quantifications were carried out in triplicates (Mythili *et al*., 2014).

Determination of Total Flavonoids: 100 µl MCP in methanol was mixed with 100 µl of 20 % alcoholic $AlCl_3$. A drop of acetic acid was added to the solution, made up to 5 ml using methanol and left for 40 min. The absorbance of the complex was read at 415 nm against the blank. The absorbance of the standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All quantifications were carried out in triplicates (Mythili *et al*., 2014).

Determination of Total Saponins: To 80% methanolic solution of MCP was added 2ml ethanolic Vanilin, 2ml of 72% sulphuric acid solution, mixed well and heated on a water bath at 60 ^{0}C for 10min. The absorbance was measured at 544 nm against reagent blank. Diosgenin was used as the standard material. All quantifications were carried out in triplicates (Akbari *et al*., 2019).

Antioxidant Profiling

The antioxidant profiling of MCP was carried out using the 2,2-diphenyl-1-picrylhydrazyl, DPPH method (Oloyede *et al*., 2014) with absorbance read at 517 nm and ascorbic acid used as standard, in a triplicate concentration dose range. The percentage inhibition, %I was calculated using the relationship:

$$\%I = \frac{A_{BLANK} - A_{MCP}}{A_{BLANK}}$$

Where: %I – percentage inhibition,

A_{BLANK} – Blank absorbance,

A_{MCP} – MCP absorbance

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3.1 Statistical Analysis

Data obtained were expressed as mean of triplicates determinations ± standard deviation (SD). The Statistical Package for Social Scientists (SPSS version 20.0) was used for all data analysis.

Results and Discussion Table 1. Qualitative phytochemical profiling of MCP

CLASSES OF PHYTOCHEMICAL	EXTRACT	
	HCP	ECP
Alkaloids	-	+
Flavonoids	-	+
Phenols	-	-
Steroids	+	+
Terpeniods	+	+
Quinones	-	-
Coumarins	-	-
Anthocyannins	-	-
Cardiac glycosides	-	-
Tannins	-	-
Saponins	-	+

+:* present, -: absent

Table 1 reveals the present of two (2) classes of phytochemicals in the n-hexane crude extract, HCP. The two classes were steroids and terpeniods. Alkaloids, flavonoids and saponins were detected in the moderately polar ethyl acetate extract, ECP in addition to the steroids and terpeniods found in the preliminary investigation of HCP. Quantitatively, the amount (table 2) of alkaloids, flavonoids and saponins were determined. The total flovonoids content in mg/g of rutin was the higher in amount than either the alkaloids or the saponins present. Crude honeybee residue investigated in the present study is rich in flavonoids. Flavonoids are known classes of phytochemicals with established pharmacological activities such as antioxidant, antiviral, antibacterial, anti-inflammation, anti-ageing, antidiabetic, analgesic, anti-cancer, hepatoprotective and cardioprotective (Pandey, 2017; Kumar and Pandey, 2013; Sekher-Pannala *et al.*, 2001; Tapas et al., 2008; Zhu *et al.*, 2012; Brusselmans *et al.*, 2003; Hans *et al.*, 2007).

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Table 2. Quantitative phytochemical profiling of ECP

S/N.	Phytochemicals	Amount
1.	Alkaloids (mg/g AE)	0.89±0.00
2.	Total flavonoids (mg/g RE)	9.69±0.40
3.	Total saponins (mg/g DE)	1.08±0.08

AE-atropine equivalent, RE-rutin equivalent, DE-diosgenin equivalent

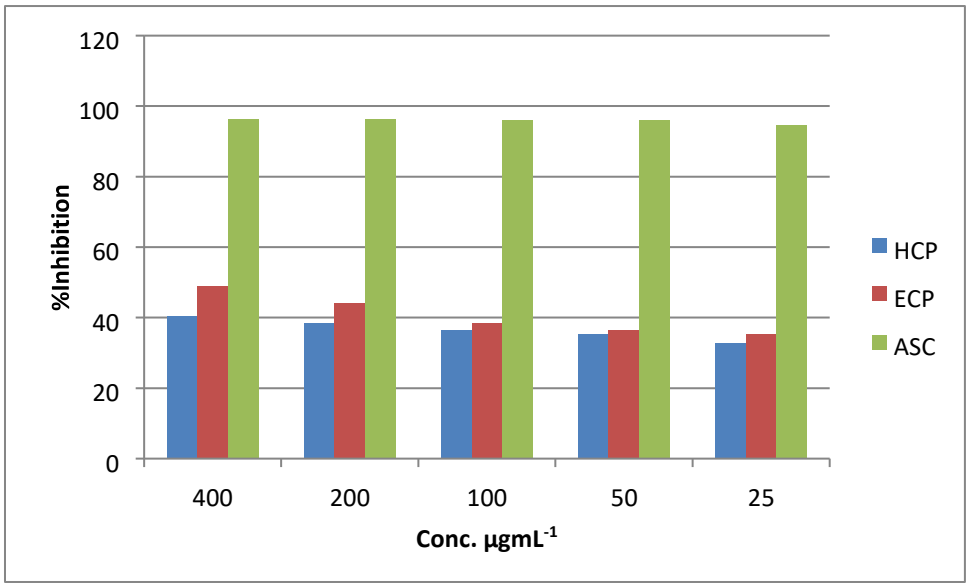

Fig. 1 Antioxidant profiling of HCP and ECP against Ascorbic acid, ASC standard

The antioxidant profiling (fig. 1) of both HCP and ECP were lowered than those of the absorbic acid standard used. The crude extracts have shown relative antioxidant activities whose mode of action could be via reduction of the concentration of ROS, lytic or chain breaking reactions, chelation and termination of initiating radicals in biochemical systems (Shiva, 2011). The percentage inhibition of ECP was rekatively higher than those of HCP due to the present of the flavonoids, alkaloids and saponins classes of secondary metabolites.

Conclusion

Crude honeybee extracts have been investigated to possess phytochemicals such as the alkaloids, flavonoids, saponins, steroids and terpenoids. The presence of these phytochemicals tends to establish the pharmacological significant of crude honeybee residue. The crude honeybee extracts were antioxidant active.

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