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CAFFEIC ACID: A PHARMACOLOGICALLY ACTIVE PHENOLIC ACID FOUND IN PROPOLIS OBTAINED FROM NIGERIA

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ABSTRACT

Studies reveal that chemical composition of propolis is influenced by vegetation surrounding the beehive. This study set out to isolate and characterize chemical compounds from propolis using hexane and ethyl acetate solvents, and to assess its antimicrobial activity in varying polarities; hexane, acetone, ethyl acetate and methanol. Isolation of chemical compounds was carried out by subjecting extract to column chromatography by gradient elution using two solvent mixtures - hexane: ethyl acetate and ethyl acetate: methanol. Isolated compounds were identified using ^{1}H and ^{13}C-NMR spectroscopy and Mass spectrometry with further verification of data on the compound by comparison with literature reports. Microbial assay was carried out using standard procedures. Isolated compound was identified as caffeic acid, a known phenolic acid useful for anti-oxidation, anti-inflammation and anti-carcinogenesis. Percentage (%) yield of propolis in the four solvents was high in hexane (85.60%) and acetone (64.95%). Methanol extract of propolis was active against *Escherichia coli* and *Salmonella typhi* at concentrations of 100 mg/mL, 50 mg/mL and 25 mg/mL. Ethyl acetate extract showed activity on *Escherichia coli* at 100 mg/mL, 50 mg/mL and 25 mg/mL concentrations while *Salmonella typhi* was inhibited at 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL concentrations of propolis in ethyl acetate solvent. This suggests that, the more polar the solvent used for extraction of propolis obtained from Gboko, the stronger the antimicrobial activity.

Keywords: propolis, anti-microbial activity, column chromatography, ^{1}H-NMR, ^{13}C-NMR, caffeic acid

INTRODUCTION

Man will always resort to help from the environment. This has marked the importance of natural products over the years, particularly plants. Plant products come in various forms including propolis. Propolis, an exudate resin from plants has found usefulness in various climes. Extensive research has been carried out on it in the temperate regions including South America. Research on propolis is gradually becoming popular in the tropics also. Recently, studies on Nigerian propolis have yielded pharmacologically active compounds such as amyrins [1] and lupeol [2]. [3] isolated ten phenolic compounds including calyocosin, liquiritigenin, medicarpin, vestitol from propolis obtained from Rivers state in Nigeria. Phenolic acids such as caffeic acid are known to exhibit antioxidant, anti-inflammatory and anti-carcinogenic activities [4]. Hydroxycinnamic acid in human diet is largely obtained from caffeic acid [4][5][6] as a result of secondary metabolism of vegetables including fruits, olives, potatoes and carrots [4][5][6][7].

Organic acid and sugar esters, glycosides and amides are caffeic acid in simple forms, whereas dimers, trimers and flavonoids and its derivatives are its complex forms [5]. Cell wall of vegetables contain proteins and other polymers and may also contain caffeic acid [8]. Caffeic acid has been reported to inhibit growth of insects, fungi and bacteria, and is also reported to be involved in the plant defense mechanism against predators, infections and pests [8]. Plant leaves are protected from ultraviolet radiation B by caffeic acid [8].

We had earlier reported other compounds isolated from this propolis sample [1][2]. Here, we report the isolation and characterization of this pharmacologically useful compound, caffeic acid, from propolis obtained from Benue State, Nigeria and the antimicrobial property of propolis using different solvents.

MATERIALS AND METHODS

Experimental Procedures

Silica gel 60 (0.040-0.063 mm) (200-425 mesh ASTM) was used to carry out column chromatography while Thin –layer chromatography was done using Aluminum sheets pre-coated with silica gel F250 (Merck, Germany). Nuclear Magnetic Resonance (NMR) analyses were carried out on a Bruker AVIII (500 MHz) spectrophotometer with the use of $CDCl_3$ as solvent and TMS as internal standard. Mass spectral data was acquired using JEOL MStation JMS-700 mass spectrometer.

Preparation of Extract of Propolis

Propolis sample was sourced from an apiary in Gboko, Benue state which lies between longitude (7^o 47’ 0” and 10^o 0’ 0” E) and latitude (6^o 25’ 0” and 8^o 8’ 0” N). Sample was confirmed by Professor John O. Igoli of Joseph Sarwuan Tarka University, Makurdi, Nigeria. *Maranthes polyandra, Detarium microcarpum* and *Burkea africana* plants mostly formed the surrounding of the beehive and were suspected to be major sources of this propolis. Propolis sample was homogenized and placed in the thimble of the Soxhlet extractor. Successive reflux with hexane and ethyl acetate at a maximum temperature of 40 °C was carried out for 24 hours. The liquid extract was evaporated to dryness using rotary evaporator at 40^{o}C. Sample was then dried and dissolved in silica gel for column chromatography.

Isolation of Compounds

Hexane and ethyl acetate were used to extract the sample to dryness. The extract was subjected to column chromatography using silica gel in a glass column after being combined based on TLC similarity. Column was packed wet in a hexane: ethyl acetate (90:10) mixture and eluted gradiently starting with 10 % ethyl acetate in hexane while increasing the amount of ethyl acetate by 10% until 100% ethyl acetate producing 10-ml vials. Further elution with ethanol in ethyl acetate starting with ethyl acetate: ethanol (90:10) mixture was carried out. TLC was used to examine fractions and similar ones were combined and allowed to dry in a fume cupboard. ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry were used to analyze the compounds.

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Fractions labeled SGP-1 to SGP-117, totaling one hundred and seventeen 10 mL vials were collected. Similar fractions were pooled together using Thin-layer chromatography (TLC). Fifteen fractions in total were collected based on TLC similarity. These fractions were stored at room temperature and then characterized using ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry.

Antimicrobial Assay

Antimicrobial assay was carried out using an agar well diffusion and serial dilution method on some local clinical isolates including *Salmonella typhi* and *Escherichia coli*, at different concentrations: 6.25, 12.5, 25, 50 and 100 mg/mL of propolis using methanol, ethyl acetate, acetone and hexane as solvents of extraction.

RESULTS AND DISCUSSION

The compound, SGPH 34 (SGP 2130D) was obtained as white solid powder/crystals from a pool of vials labelled SGPH 34-36. It showed one spot on TLC (solvent system, 80:20 of Hexane : ethyl acetate) with an R_f value of 0.55. The melting point value of this fraction was 224-226 ^{o}C.

EI-MS m/z (relative intensity): 179 (0.62) [M]+, 161 (5.74), 135 (2.37), 102 (5.45), 79.112 (100). The ^{1}H-NMR (400MHz, DMSO-d_6) showed two doublets at δ_H 6.17 (1H, d, *J*= 16.0, H-7) and 7.42 ppm (1H, d, *J* = 16.0, H-8). Three aromatic proton signals at 6.96 (1H, dd, *J* = 8.2, 2.1, H-2), 6.76 (1H, d, *J* = 8.2, H-3) and 7.02 (1H, d, *J* = 2.1, H-6). A deshielded signal at δ_C168.3 ppm typical of a carboxylic acid carbonyl group (C-9), two olefinic CH at 115.7 and 145.0, three aromatic CH at 121.7, 116.3 and 115.2, two phenolic carbons at 148.6 and 146.1 and a quaternary carbon at 126.2 ppm appeared on the ^{13}C-NMR spectrum. The compound was identified as caffeic acid and its NMR spectral data were in agreement with literature report [9].

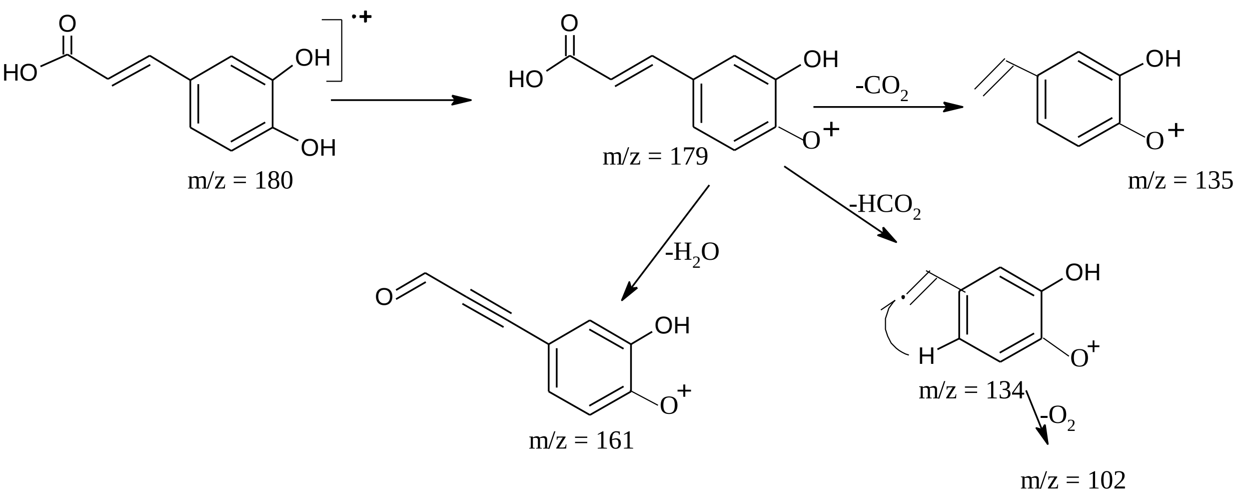

Figure 1: Possible Fragmentation pattern for Caffeic acid

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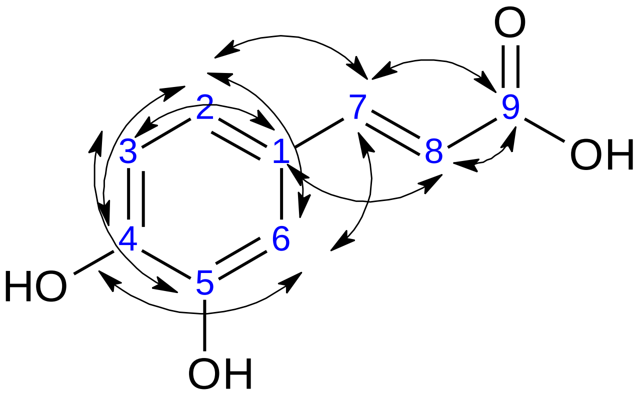

Figure 2: HMBC of Caffeic acid

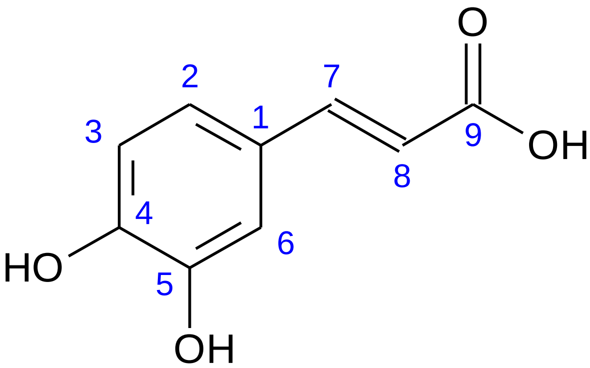

Figure 3: Caffeic acid

Table 1: ^{1}H and ^{13}C NMR Spectrum of SGPH 34 (SGP 2130D) Experimental in Comparison with Data from Literature.

Position	Experimental		Literature [9]		HMBC
	^{1}H (δ ppm)	^{13}C (δ ppm)	^{1}H (δ ppm)	^{13}C (δ ppm)	
1	-	126.2	-	124.71	-
2	6.96 (d, *J* = 8.2, 2.1)	121.7	6.69	123.18	C-4, C-6, C-7
3	6.76 (d, *J* = 8.2)	116.3	6.69	119.25	C-1, C-5

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4	-	148.6	-	145.00	-
5	-	146.1	-	143.83	-
6	7.02 (d, *J* = 2.1)	115.2	6.45	106.05	C-2, C-4, C-7
7	6.17 (d, *J* = 16.0)	115.7	6.08	106.05	C-2, C-6, C-9
8	7.42 (d, *J* = 16.0)	145.0	6.96	143.35	C-1, C-9
9	-	168.3	-	168.77	-
9-COOH	8.12 (s)	-	10.39		-
4-OH	9.51 (s)	-	9.55		-
5-OH	9.11 (s)	-	9.24		-

Antimicrobial Activity

Table 2: Percentage Yield of Propolis in Different Solvents

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Table 3: Antimicrobial Properties of Extracts of Propolis Against Test Bacteria

Solvent	Amount (g)	Yield (%)
Hexane	41.8	85.60
Methanol	29.4	60.25
Ethyl acetate	29.4	60.25
Acetone	31.7	64.95

Extract Concentration *(mg/ml)*	Solvent/Test Bacteria: Acetone		Ethyl Acetate		Hexane		Methanol	
	E. coli	***S. typhi***	***E. coli***	***S. typhi***	***E. coli***	***S. typhi***	***E. coli***	***S. typhi***
100	-	-	+	+	-	+	+	+
50	-	-	+	+	-	-	+	+
25	-	-	+	+	-	-	+	+
12.5	-	-	-	+	-	-	-	+
6.25	-	-	-	+	-	-	-	+
Control (Ciprotab 200mg)	+	+	+	+	+	+	+	+

Key: + = sensitive; - = Resistant

Percentage yield of propolis in different solvent was highest in hexane, while methanol, acetone and ethyl acetate yielded approximately similar proportions. This may be related to the polarity and solvent properties of these various compounds. Methanol, a solvent commonly used for extraction due to its high polarity gives high extraction yields. Ethyl acetate is also widely used because of its chemical and biological functions such as medium polarity and minimal cell toxicity. Also, ethyl acetate exhibits biphasic action which enables extraction of both polar and non-polar compounds [16]. Probably because of high extraction capacity for active compounds (more polar compounds), methanol and ethyl acetate extracts had high effective action on the organisms, while hexane and acetone had less effect on the organism.

Propolis exhibits antimicrobial activity, which has been harnessed by humans for pharmaceutical purposes [17]. Propolis also has antibacterial, antifungal and antiviral properties and many other useful biological activities [17].

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Antimicrobial activities of various plant extracts have been reported severally [18]. In the present study, some extracts exhibited activity against all the test bacteria and others selectively active. Some were inactive. The methanol and ethyl acetate extracts especially were found to be most active against the test bacteria. This implies that it can inhibit bacteria growth because methanol and ethyl acetate are polar in nature. The Propolis extracts in acetone and hexane were not effective against the two bacteria tested. This may be as a result of the non-polar nature of these solvents which affected their ability to extract in sufficient quantities the antimicrobial components of propolis. These two observations suggest that antimicrobial activity may be dependent on polarity of solvent. Propolis has a range of compounds such as flavonoids, alkaloids, terpenoids and coumarins [19]. [16] also reported that propolis contains flavonoids, terpenoids and phenolic acids such as cinnamic and caffeic acids. These compounds have been reported to exhibit antimicrobial, antitumor and antioxidant activities [20].

Reports have also shown that compositions and proportions of propolis depend on the place and time of collection [2]. [21] reported that propolis from different places showed varying proportions of phenolic acid esters and flavonoids. Polyphenols, aromatic acids and diterpene acids are very essential for biological activities [18]. In this study, the methanol and ethyl acetate extract of propolis showed better efficacy against *Salmonella* and *E. coli.* [22] studied effects of various types of propolis on Methicillin Resistant *Staphylococcus aureus* (MRSA) strain and found that they all had antimicrobial activity against the organism, but the best activity was found in the propolis with greater amount of flavonoids. This also highlights the efficacy of polar solvents which largely extract polar compounds [1][23].

The antimicrobial property of propolis was reported to be equal to or slightly more effective than two common antibiotics; erythromycin and amoxicillin, in killing *S. aureus* and *Streptococcus faecalis* [24][25]. The control antibiotic, Ciprofloxacin (Ciprotab), in this study, was generally more effective than the propolis extract against the Gram-negative bacteria tested. This was also observed by [24], who reported higher antibacterial activity of ethanol extract of propolis against gram-positive cocci (*S. aureus*) and weak activity against gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) and yeast (*Candida albicans*). This may mean that propolis has relatively more activity against gram-positive bacteria. *E. coli* shows resistance to many antimicrobials by a number of mechanisms [26]. However, that polar (ethyl acetate and methanol) extracts of propolis sample were active against *E. coli* shows great promise in development of future antimicrobials against this organism and hopefully others.

Numerous pharmacological activities of caffeic acid and its derivatives have been reported including antibacterial [6], antiviral [7][10], antioxidant [8][10], anti-inflammatory [8][10], anti-atherosclerotic [6][8], immunostimulatory [8][11], anti-diabetic [7][10], cardioprotective [7][12], anti-proliferative [8][13],

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hepatoprotective [8][14], anti-cancer [7][10] and anti-hepatocellular carcinoma activity [8][15].

CONCLUSION

The isolation of caffeic acid, a hydroxycinnamic acid from this propolis sample suggests promise. The effectiveness of methanol and ethyl acetate extract of propolis as alternative for treating infection is noteworthy and should be harnessed. This further underscores the ethnomedicinal use of this bee product.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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