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Research Article

GC-MS analysis of fatty acids and sterols in cabbage oil and its antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

This study aimed to evaluate the antimicrobial activity of cabbage oil against *Staphylococcus aureus* and *Escherichia coli*. Cabbage oil was extracted from dried cabbage heads, appearing as a dark fluid with a refractive index of 1.456 and a specific gravity of 0.914 at 20°C. Gas chromatography-mass spectrometric (GC-MS) analysis revealed the fatty acid content of the oil, including palmitic acid, oleic acid, linoleic acid, and linolenic acid. The presence of ergosterol was also detected. The extracted oil showed significant antimicrobial activity against the tested bacteria, with mean zones of inhibition of 14.00 mm against *Staphylococcus aureus* and 11.07 mm against *Escherichia coli*. The findings suggest the potential use of cabbage oil as an antimicrobial agent, with further research needed to explore its applications in personal care products.

Keywords: Cabbage oil, antimicrobial activity, fatty acids, ergosterol, GC-MS

1. Introduction

Cabbages belong to the Cruciferae family and are related to turnips, cauliflowers, and brussels sprouts [1-3]. The exact origin of cabbage remains unclear, as it is one of the oldest cultivated vegetables, and is widely known and consumed in Nigeria. Cabbages are easily grown under a wide range of conditions and can adapt to most areas [2]. In Nigeria, although cool, moist weather results in the best quality of cabbage heads, some varieties produce acceptable heads during the warmer period of the year. Therefore, cabbages can be grown on a continuous basis in some districts in cooler areas such as the tablelands of Jos and the Mambilia Plateau in Taraba State, Nigeria.

The Brassicaceae family encompasses a wide range of plants, including various genera of cultivated crops, collectively referred to as Brassica vegetables [4]. Within the *Brassica oleracea* species, diverse types of cabbages are included, which are white [4], red [5], Savoy [2], cauliflower [6, 7], broccoli [8], Brussels sprouts, and kale [9]. These vegetables have been shown to exhibit anti carcinogenic properties [10]. Cabbage is one of the most extensively cultivated and consumed vegetables globally. Different cultivated varieties of cabbage display considerable variation in terms of leaf size, shape, color, head and texture [11, 12].

This study aimed to analyze the fatty acid and sterol composition of green cabbage oil using gas chromatography mass spectroscopy (GC-MS) and its antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria, correlating it to other vegetable oils from previous research.

2. Materials and Methods

2.1. Extraction of cabbage oil

Green cabbage heads were sourced from a local market in Udu, Delta State, Nigeria. The heads were cleaned, diced, and oven-dried at 50°C for 8 hours. The dried cabbage samples were then ground into flakes. A total of 200 g of cabbage flakes was introduced into 500 mL of 80% n-hexane. The mixture was stirred and sonicated for 30 minutes to enhance extraction. The resulting solution was centrifuged at room temperature at 9700 rpm for 40 minutes. The procedure was repeated twice, and the supernatant were pooled and stored at 5 °C in a refrigerator for further analysis.

2.2. Percentage yield of cabbage

The percentage yield of the extracted cabbage oil was calculated using the following formula:

$$\text{percentage yield} = \frac{weight\ of\ cabbage\ oil\ extract}{weight\ of\ dry\ cabbage} \times 100$$

2.3 Determination of refractive index of extracted cabbage oil

The refractive index of the cabbage oil was determined at 30 °C using an Abbe refractometer. The sample chamber was rinsed and dried before filled with the cabbage oil extract. The oil-filled cell was then inserted into the refractometer, and the refractive index reading was recorded.

2.4 Determination of specific gravity of extracted cabbage oil

The specific gravity of the cabbage oil was measured using a bulb hydrometer at 20 °C. The oil sample was measured using a bulb hydrometer at 20 °C. The oil sample was transferred into a 250 mL measuring cylinder, and the hydrometer was carefully immersed in the oil. The specific gravity was recorded at the point where the surface of the oil intersected the calibrated scale on the hydrometer, after stabilization.

2.5 GC-MS analysis of cabbage oil

The extract was analyzed using gas chromatography-mass spectrometry (GC-MS). The equipment used was a gas chromatograph equipped with a Thermo DSQ II mass spectrometer, with a DB-35MS capillary standard non-polar column (30 m x 0.25 mm ID x 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injector temperature was set at 250°. The oven temperature was initially maintained at 70 °C for 10 minutes, and then increased gradually to 280°C at a rate of 3 °C/min. Identification of components was performed by comparing the mass spectra and retention indices with those in National Institute of Standards and Technology (NIST) library integrated into the GC-MS software. The identified components and their respective retention times and concentration were tabulated.

2.6 Antimicrobial assay and agar preparation

The antimicrobial activity of the oil extract was evaluated following previously established methods [13-15]. Isolates of *Escherichia coli* and *Staphylococcus aureus* were obtained from the Microbiology Research Laboratory Niger Delta University.

To prepare the LB agar medium, 8 g of LB broth (Lennox) and 20 g of agar powder were dissolved in 1 L of deionized water. The solution was sterilized by autoclaving at 121 °C for 20 minutes. After sterilization, the medium was cooled to 55 °C and stored at 4 °C in a dark environment until use.

For the antimicrobial assay, the LB broth medium was used to culture the bacteria isolates at 37°C for 24 hours. Bacterial suspensions were standardized to 5.6 × 106 colony-forming units (CFU)/mL for *S. aureus* and 1.33 × 108 CFU/mL for *E. coli*.

Each LB agar was inoculated with 0.1 mL of the respective bacterial suspension. The cabbage oil extract was then tested at a concentration of 0.10 mg/mL for antimicrobial activity by measuring zones of inhibition.

3. Results and Discussion

3.1 Physicochemical properties of cabbage oil and compounds identified by GC-MS

The physicochemical properties of extracted cabbage oil were evaluated, and the results are presented in Table 1. The GC-MS analysis revealed a broad range of fatty acids in cabbage oil, including palmitic acid, oleic acid, linoleic acid, and linolenic acid (Table 2). These fatty acids have been reported to exhibit antimicrobial activity through various mechanisms, including disrupting cell membranes and inhibiting fatty acid synthesis [16, 17].

The presence of unsaturated fatty acids, such as linoleic acid and linolenic acid, may contribute to the antimicrobial activity of cabbage oil, as these compounds have been reported to exhibit greater antimicrobial activity compared to saturated fatty acids [17, 18].

Ergosterol, a plant sterol present in edible fungi [19], was identified in the GC-MS results of cabbage oil. This sterol has been found to exhibit substantial health benefits and therapeutic potential [19]. As a provitamin, ergosterol is converted to vitamin D2 upon exposure to UV radiation [19]. Studies have proven that ergosterol possesses a broad spectrum of medicinal properties, including antimicrobial, anti-oxidative, antitumor, antidiabetic, and neuroprotective effects [19].

Table 1. Physio-chemical properties of extracted cabbage oil

Parameters	Value
Percentage yield (%)	45
Colour	Dark brown
Refractive index	1.456 (unitless)
Specific gravity	0.914 (unitless)

Table 2. Retention time of some compounds in cabbage oil identified via GC-MS

S/N	Retention time (min)	Peak Area (%)	Common name	IUPAC name	Molecular formula (MF)
1	6.896	11.506	Palmitic acid	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
2	7.548	7.355	Oleic acid	Octadec-9-enoic acid	$C_{18}H_{34}O_2$
3	7.605	2.971	Linoleic acid	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$
4	7.691	7.590	Linolenic acid	9,12,15 Octadecatrienoic acid	$C_{18H}H_{30}O_2$
5	14.466	4.278	Ergosterol	Ergost-25-ene-3,5,6- triol	$C_{16}H_{11}NO_2$

3.2 Antimicrobial studies

The antimicrobial activity of cabbage oil against *Staphylococcus aureus* and *Escherichia coli* was evaluated. Cabbage oil exhibited significant antimicrobial effect against both bacteria, with mean zones of inhibition measuring 14.00 mm against *S. aureus* and 11.07 mm against *E. coli* (Table 3).

In Table 4, a comparison with a literature reference [20], cabbage oil demonstrated comparable or superior antimicrobial activity to various oils, including sunflower oil (8.667 mm and 8.333 mm), safflower oil (14.000 mm and 9.667 mm), and canola oil (12.667 mm and 9.667 mm) against *S. aureus* and *E. coli*, respectively (Table 4). Notably, rice bran oil exhibited the highest antimicrobial activity against S. aureus (20.333 mm), while cottonseed oil showed significant activity against both bacteria (17.667 mm and 12.000 mm).

Table 3. Cabbage oil zone of inhibitions against bacterial strains

Sample	Plate 1 (mm)	Plate 2 (mm)	Plate 3 (mm)	Mean (mm)
Escherichia coli	11.10	10.80	11.31	11.07
Staphylococcus aureus	13.99	14.01	14.00	14.00
Control(blank disc)	0	0	0	0

Table 4: Comparison of zones of inhibition of cabbage oils against *Escherichia coli* and *Staphylococcus aureus* with literature reference [20]

Sample	Zones of inhibition (mm)		Reference
	Staphylococcus aureus	*Escherichia Coli*	
Cabbage oil	14.00	11.07	Current Study
Sunflower oil	8.667	8.333	[20]
Safflower oil	14.000	9.667	[20]
Canola oil	12.667	9.667	[20]
Soybean oil	13.000	11.667	[20]
Cotton seed oil	17.667	12.000	[20]
Grape seed oil	16.667	10.000	[18]
Flax seed oil	15.333	11.000	[20]
Perillartine oil	11.000	9.667	[20]
Avocado oil	14.667	10.667	[20]
Chia seed oil	16.000	8.667	[20]
Inca inchi oil	10.333	10.667	[20]
Sesame oil	16.000	11.000	[20]
Rice bran oil	20.333	9.667	[20]

4. Conclusion

Cabbage oil exhibited significant antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, potentially due to its diverse fatty acid and sterol composition. Further research is needed to explore its potential applications in areas such as personal care products.

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Author Contributions

Chinaemerem Ebenezer Iheme: Conceptualization, Methodology, Investigation, Data Analysis, Writing - Original Draft; **Enizibe Zedie Williams:** Investigation, Methodology, Data analysis, Review; **Ifeoma Blessing Amadi:** Investigation, Methodology, Data analysis, Review

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