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(RESEARCH ARTICLE)

Quantification of flavonoids by UPLC-MS and its antibacterial activity from *Brassica oleracea* var. *Capitata* L.

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Abstract

Foodborne diseases remain considerable topic of concern and food safety is an important health, social and economical issue. Food borne illnesses caused by the contamination of microbes increases the concerns to find alternate sources which are safe to human health and environment. This study is the first attempt designed to determine the flavonoid content by UPLC-MS in the Indian variety of *Brassica oleracea* var. *capitata* L.(organic white cabbage) cultivated in Mysuru region and their potent antibacterial property against food borne pathogens. In the results of the present study, different flavonoids such as genistein > kaempherol > naringenin and catechin was observed which possess antibacterial activity. The antibacterial results showed that the flavonoids and there derivatives have potent antibacterial activity against the gram positive *Staphylococcus aureus* and the gram negative *E. coli*. Hence it can be concluded from the study that potential for developing antibacterial from organic white cabbage appears rewarding for the development of phytomedicine to act against microbes.

Keywords: Phytochemicals; Pathogenic bacteria; Cabbage; UPLC-MS

1. Introduction

Consumption of contaminated food with pathogenic bacteria and their metabolites results in food borne diseases. Controlling the food borne pathogens by identifying and evaluating effective antibacterial agents from natural products and assure safe food supply is the major global concern [1]. Cabbage has widespread use in traditional medicine due to their antioxidant, anti-inflammatory and antibacterial properties [2]. Studies have reported that use of synthetic food preservatives use may lead to negative health consequences. Hence there is an increased interest in the possible health-promoting effects of phenolic phytochemicals including flavonoids in vegetables [3]. Researchers have shown that naturally occurring bioactive compounds from plant origin have greater antimicrobial activity than synthetic compound due to their adverse effects [4].

Flavonoids denotes the secondary metabolite class in cruciferous vegetables. Their potential as natural antioxidants has raised significant interest. The impacts of flavonoids on the human body after consumption as well as their effect as pharmaceutical supplements are therefore under exploration [5]. Their numerous physiological functions make them a promising tool for breeding purposes. General methods for the analysis of flavonoid are well established, though new compounds are still being identified. However, differences in environmental circumstances of the studies and analytical methods impede comparability of quantification results. Hence, the present study is the first attempt designed to determine the flavonoid content by UPLC-MS in the Indian variety of *Brassica oleracea* var. *capitata* L. (organic white cabbage) cultivated in Mysuru region and their potent antimicrobial property against food borne bacteria in-order to explore their application as pharmaceutical supplements.

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2. Material and methods

2.1. Sample collection

Organic white cabbages were harvested and collected from Hasiru organic farm, Mysuru district in the month of April 2017. They were cleaned immediately and freeze dried. The dried samples were kept in deep freezer (−20 °C) and used for further analysis.

2.2. Chemicals

Standards viz, genistein, kaempherol, naringenin and catechin, were purchased from Sigma-Aldrich Chemicals Co. (India). All other chemicals and solvents used were of analytical grade.

2.3. Preparation of extract

The freeze dried sample was extracted as per the procedure described by Renuka Devi and Thangam [6], with slight modification. Lyophilized sample was macerated with 80% methanol (4 mL/g of weight) and extracted for 18 hrs before filtration. The plant material was re-extracted twice with cold 80% methanol for 4 hrs each time with frequent swirling. The filtrates were combined, concentrated by drying in oven and it was used further for the analysis.

2.4. Test organisms

Bacillus subtilis (MTCC 121), *Staphylococcus aureus* (MTCC 7443), *Escherichia coli* (MTCC 7410), *Salmonella typhimurium* (MTCC 733) were used in this study.

2.5. Detection and quantification of bioactive components by UPLC/MS

LC-MS analysis was performed using a Waters (Acuity UPLC, USA) system coupled to a Q-TOF (Quadrupole time of flight) mass spectrometer (Synapt G2, USA) equipped with an electrospray ionization (negative mode) source that was used at an ion source temperature of 100 ^{o}C. The detection and quantification by UPLC/MS method was followed by our previously published method [7].The test sample was dissolved in 80 % methanol and 0.2 µL of sample was injected on acquity UPLC BEH C_{18} column (1.7 µm 1.0×50 mm) at a flow rate of 0.3 ml/min. Solvent A consisted of 0.1% Formic acid in water and solvent B consisted of acetonitrile solvent. Gradient program was used for elution: Initially, Solvent B concentration was 2% and increased to 98% at 4 min and maintained up to 6 min and finally at 8 min B concentration was 2%. The mass spectrometer was operated in $[M^{+}H]$ Positive ion mode. Capillary voltage was set at 2.5 kv and the cone voltage was optimized for each of the compounds. Molecular species were identified within the mass to charge ratio (m/z) range 50 to 1500. Data acquisition was carried out by Mass Lynx Software (Version: 4.1).

2.6. Antibacterial assay

The antibacterial activity of organic white cabbage was determined by disc diffusion method [8] against both Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli* and *Salmonella typhimurium*) bacteria. Each sterile disc (6 mm) was loaded with 25 µl of organic white cabbage (10, 25 and 50 µg $disc^{-1}$) and placed equidistantly on nutrient agar plates seeded with test bacteria (1.5 × 10^{8} CFU mL^{-1}). Streptomycin (25 µg $disc^{-1}$) and 80% methanol served as positive and negative controls, respectively. The plates were sealed using parafilm and incubated at 37±2 °C for 24 h and zone of inhibition was measured.

3. Results

The present study UPLC-MS profile highlighted the presence of flavonoids where each compound was identified mainly based on the mass spectrometric data and by using corresponding standards the comparison was made. Each compound was analyzed on mass spectrometry under positive modes. The chromatograms and mass spectra of organic white cabbage are illustrated in Figure 1.

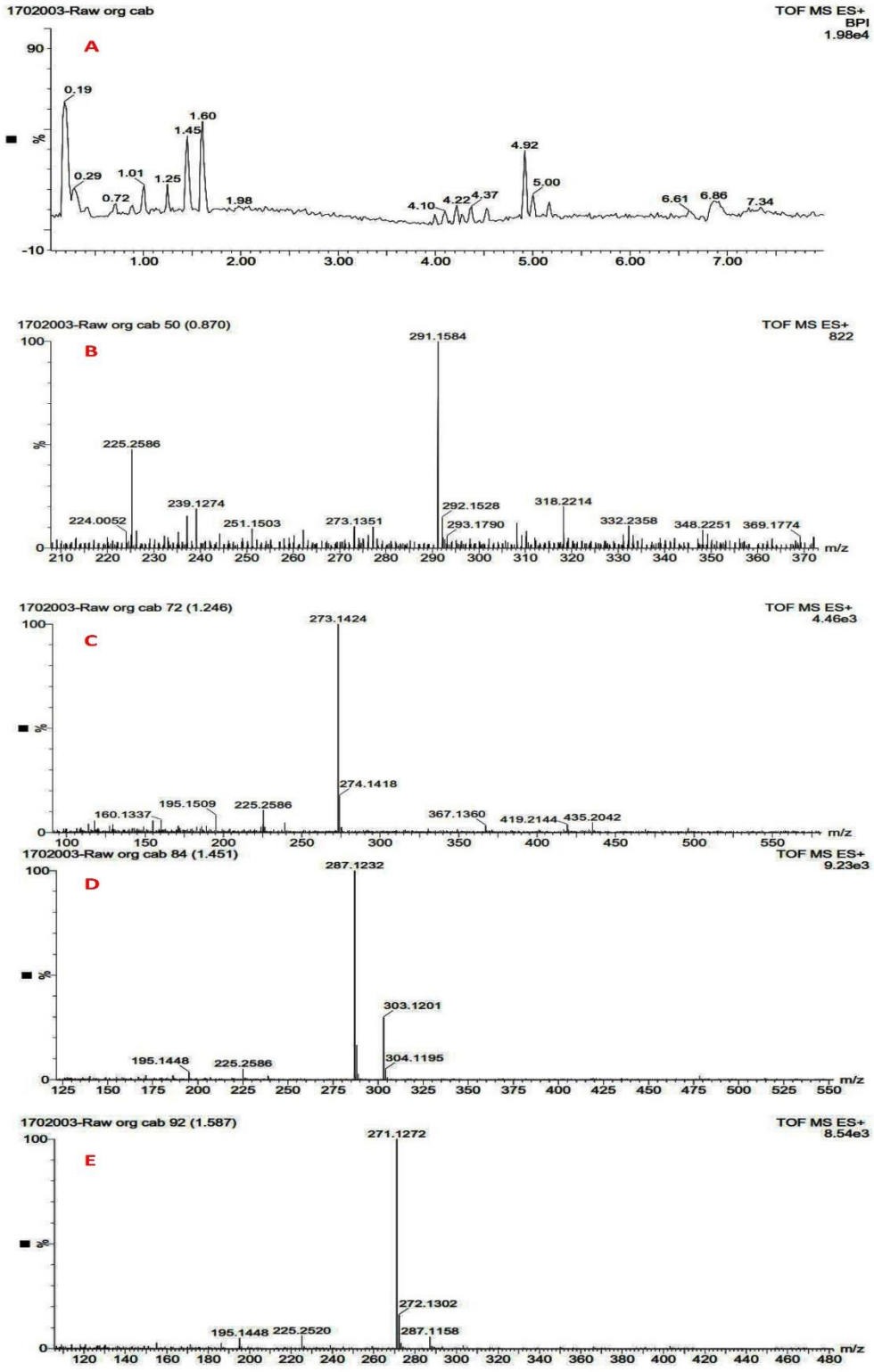

Figure 1 The chromatogram and mass spectra of organic white cabbage by UPLC/MS. (A) Chromatogram of Organic white cabbage (B) Mass spectra of Catechin (C) Naringenin (D) Kaempherol and (E) Genistein.

The retention time, molecular mas and quantity are summarized in Table 1. Among these, genistein (1.632 mg g^{-1}) content was the highest followed by kaempherol (1.229 mg g^{-1}), naringenin (0.585 mg g^{-1}), catechin (0.168 mg g^{-1}) on wet basis.

Table 1 Identification and quantification of flavonoid content in organic white cabbage estimated by UPLC-MS

Cruciferous vegetable extract (80% methanol)		Catechin	Naringenin	Kaempherol	Genistein
Organic white cabbage	t_R (min)	0.87	1.24	1.45	1.58
	$[M^+H]^+$	291.18	273.17	287.15	271.15
	mg/gm	0.168	0.585	1.229	1.632

t_R (min): Retention time; $[M^+H]^+$: Molecular weight; mg/gm: expressed on wet basis

The antibacterial activities expressed as inhibition zone diameters of the organic white cabbage at different concentrations (10, 25 and 50 µg $disc^{-1}$) against the *E. coli, Salmonella typhi, Bacillus subtilis* and *Staphylococcus aureus* are in (Figure 2 and Table 2). It was observed that no antibacterial activity was found at 10 µg $disc^{-1}$. It was noted that the extract of 50 µg $disc^{-1}$ from organic white cabbage offered maximum inhibition zone of 19 ± 0.35 mm and 17 ± 0.3 mm against the gram positive *Staphylococcus aureus* and the gram negative *E. coli* respectively. The extract concentration at 50 µg $disc^{-1}$and streptomycin at 25 µg $disc^{-1}$(positive control) had comparable antibacterial activity.

Table 2 Inhibitory activity of organic white cabbage against different pathogenic bacteria

Test organisms	Zone of inhibition (mm)				
	10 mg/disc	25 mg/disc	50 mg/disc	Positive control	Negative control
E. coli	0.0	12 ± 0.4	17 ± 0.3	17 ± 0.32	0.0
Salmonella typhi	0.0	12 ±0.23	14 ± 0.32	15 ± 0.6	0.0
Bacillus subtilis	0.0	10 ± 0.6	12 ± 0.45	12 ± 0.62	0.0
Staphylococcus aureus	0.0	13 ± 0.42	19 ± 0.35	16 ± 0.2	0.0

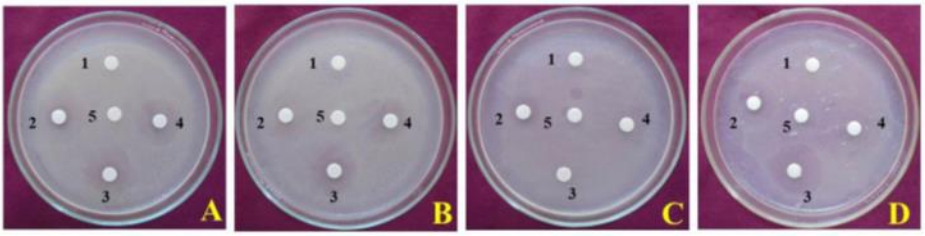

Figure 2 Inhibitory activity of organic white cabbage against test pathogens by disc diffusion method. A-*E. coli*; B- *S. typhi;* C- *B. subtilis*; D- *S. aureus*; 1-10 mg/ ml; 2- 25 mg/ ml; 3-50 mg/ ml; 4- Positive control; 5-Negative control

4. Discussion

Flavonoids are ubiquitous in photosynthesizing cells and are commonly found in fruit, vegetables, nuts, seeds, tea, stems, flowers, honey, wine and propolis. For centuries, to treat human diseases, preparations containing these compounds (the principal physiologically active constituents) have been used [9].The present experiment analyzed phytochemicals in organic white cabbage and has identified the presence of different flavonoids such as genistein, kaempherol, naringenin and catechin which have been attributed to its potent antibacterial activity. Flavonoids act as antimicrobial agents in different ways including direct antibacterial activity, combined effect with antibiotics and virulence suppression [10]. Flavonoids such as kaempherol and quercetin have antibacterial activities against *Propionibacterium*, inhibitory effects of apigenin against *S. typhi*, *Proteus mirabilis* and *P. aeruginosa* [11] and selective

toxicity of apigenin and luteolin against *S. aureus* including the methicillin-resistance *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* strains (MSSA) [12].

It has been postulated that cell membrane of gram negative bacteria contains many condensed fat layers compared with gram positive bacteria and there is difficulty in penetration of chemicals, antibiotics or antiseptics. To overcome this, flavonoid extract were used which contains hydroxyl group, reacting with the phospholipids of bacterial cell wall. This leads to the distraction of cell membrane and increase the permeability of cell membranes of antibacterial compounds by denaturing the cell protein [13].

Our previous study has analyzed the presence of glucosinolates which includes allyl isothiocyanate, iberin and indole-3-carboxyaldehyde in higher amounts which are rich in sulphur containing compounds [14]. It was reported that sulfhydryl groups easily bind with specific enzymes essential for microbial growth and survival. This binding restricts the enzymes activity causing reduction in the cellular levels of important thiol groups, leading to the formation of oxygen and other free radicals [15], which reduces the viability of bacterial cells. Studies have shown that, the quantity of kaempherol varied from 0 to 1.2 mg/100 g in Chinese cabbage, white cabbage and red cabbage [16]. In our study the content of kaempherol and quercetin varied slightly. Hence, it can be concluded that the difference in the production of secondary metabolites are affected quantitatively as well as qualitatively by a range of environmental factors, including temperature, light intensity, relative humidity, soil type, irrigation water quality and day length, as well as by genetic factors.

5. Conclusion

It could be concluded from the present study that different agro-climatic conditions influence the phytonutrient content, composition and antibacterial activity of the organic white cabbage. Hence, from the results obtained appears that potential for developing antimicrobials from cruciferous vegetables appears rewarding as it will lead to the development of phytomedicine to act against microbes. Plant-based antimicrobials can serve the purpose of synthetic antimicrobials have enormous therapeutic activity. Therefore, further in-depth studies to develop antimicrobials from cruciferous vegetables are needed.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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