Annals of Phytomedicine 13(1): 1141-1148, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.1.122

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Original Article : Open Access

Biochemical studies in Woody Pepper (*Piper pendulispicum* C.DC.): A spice with edible stem from Andaman Islands, India

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Article Info

Article history
Received 1 May 2024
Revised 20 June 2024
Accepted 21 June 2024
Published Online 30 June 2024

Keywords
Ferulic acid
Novel spice
Oleoresin
Phenolic compounds
Piperine

Abstract

A number of region-specific underutilized horticultural species have been found to contribute in the economy, ecology and well-being of the society. Woody Pepper (*Piper pendulispicum* C.DC.) is a unique genetic resource reported recently from Andaman group of Islands. The unique characteristic of this species is the presence of thick edible stems. In order to understand the biochemical parameters in this species, the present studies were carried out. Results revealed that total phenolic content (85.9-304.6 mg GAE/100 g) and oleoresin content (3.5-3.7%) in dehydrated powder varied with the thickness of the stems, *i.e.*, thin (3-5 cm), medium (5-7 cm) and thick (7-9 cm). Phenolic acid profiling was carried out in the stems of two thicknesses (1.0-2.5 cm and 2.5-4.0 cm) using LC-MS/MS, which resulted into identification of 17 phenolic acid compounds, ferulic acid being the dominant one. Proximate analysis and sensory evaluation suggested the potential of this novel spice in the humid tropical regions.

1. Introduction

Piper pendulispicum C.DC. is a species native to the Vietnam (powo.science.kew.org). The species is popularly called as *Choi Jhaal* or Woody Pepper in the Indian territory of Andaman Islands and was locally known to a few inhabitants of these islands (Waman *et al.*, 2023). However, botanical identity of it has remained debatable for quite some time (Joseph John *et al.*, 2020; Waman *et al.*, 2024). Recently, through DNA barcoding, the taxonomic confusion was resolved and authors have reported distribution of this species as a new record for India (Waman *et al.*, 2024). In order to improve the understanding about this lesser-known species, systematic studies are required. The harvested stems have limited shelf-life of only a few days under ambient conditions, and hence effort was made to prepare dehydrated powder from the same. This could not only help in increasing the shelf-life but would also improve the chances of its long-distance marketing in mainland India.

Spices are known to have antioxidant activities and the phenolic compounds are known to contribute to it (Dimitrios, 2006; Rayess *et al.*, 2023). Different solvents, when used for extraction, could influence the phenolic content (Singh *et al.*, 2014), and thereby the antioxidant capacity as well (Muzolf-Panek and Stuper-Szablewska, 2021). Further, oleoresins are known to be important ingredients for food industries worldwide (Procopio *et al.*, 2022) and are regarded as quality determinant in most spices like black pepper, nutmeg, cinnamon, ginger, *etc*. Piperine is the nitrogen containing pungency imparting alkaloid present in various plant parts of *Piper* species, which exhibits significant biological and bioavailability enhancing activities (Haq *et al.*, 2021; Tiwari *et al.*, 2020). It has been clinically well tested for its role in the prevention, management and treatment of several diseases and disorders (Tripathi *et al.*, 2022). Hence, determining these compounds in this species was envisaged in the present study.

The unique characteristic of this species is extensive thickening of the stem along with profuse rooting on each node (Waman *et al.*, 2024). These stem pieces are the economic part of the plant (Figure 1) and are mainly used in the preparation of vegetarian and non-vegetarian curries, after scrapping of the outer layer of bark, as taste imparter. The grading method adopted in the markets of Andaman Islands is based on the thickness of the stem and it is known to be a determining factor in the pricing of the produce. Suitable agro-climatic conditions and good market demand make Woody Pepper a potential crop for diversification of agriculture in the humid tropical Andaman Islands. However, due to the limitations of low shelf-life of fresh edible stems, processing into dehydrated powder forms could be beneficial. The present study deals with evaluation of the biochemical parameters (phenolic acids, oleoresin, piperine), proximate composition and sensory evaluation of dehydrated powder made from Woody Pepper stems.

2. Materials and Methods

2.1 Collection of samples and primary processing

Mature stems were harvested from an identified collection (WP/LM) from a farmer's field in Hawa Mahal, Middle Andaman, North and Middle Andaman district during 2019 and 2021. During 2019, the material was transported to the authors' laboratory and was

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graded as per the thickness of the stem into three classes, *viz*., thin (3-5 cm), medium (5-7 cm) and thick (7-9 cm), while during 2021, the material was graded into two classes, *viz*., small (1.0-2.5 cm) and big (2.5-4.0 cm). In both the seasons, the outer layer of bark of the graded stems was then scraped using a stainless-steel knife to remove the outer layer of tissues and adventitious roots present on the nodes. The stem pieces were then made into small bits and dehydrated at 50°C in hot air oven till the pieces became brittle. Using a grinder (500 W), the pieces were powdered, sieved through 212 µm mesh and stored at 4°C until further analysis.

Figure 1: Stem pieces of Woody Pepper harvested from home garden.

2.2 Estimation of oleoresins and total phenolic content

For determination of oleoresin content, during 2019, the known quantity of powdered sample was extracted with acetone using Soxhlet extraction apparatus and the oleoresin content was calculated on dry weight basis. The samples were cold percolated with acetone or methanol for 24 h and extracts were dried to remove the solvents. These extracts were used for determining total phenolic content following the Folin-Ciocalteau method.

2.3 Profiling of phenolic acids

The samples collected in 2021 were used for phenolic acid profiling using LC-MS/MS analysis (modified from Weidner *et al*., 2000). The solvents used were of MS grade. Accurately weighed 500 mg of sample was homogenized in methanol (80%), followed by centrifugation and volume was made up to 20 ml. The extract was vacuum evaporated at 45°C followed by dilution to 5 ml with water and further extraction with ethyl acetate thrice. The ethyl acetate extract was vacuum evaporated at room temperature. The extract was then hydrolyzed overnight after adding 4 ml of 2 N NaOH to it followed by acidification to pH 2 using 5 ml HCl (2 N) and re-extraction with 10 ml ethyl acetate. This ethyl acetate layer was evaporated to complete dryness under vacuum and the residue was finally dissolved in MS grade methanol (2 ml) and filtered through 0.2 µm nylon filter prior to injecting in the LC-MS/MS. Phenolic acids were resolved on the BEH-C18 analytical column (2.1× 50 mm, 1.7 µm, Waters India Ltd.), which was protected by Vanguard BEH C-18 (Waters, USA), The system was operated in gradient mode (Solvent -A: 0.1% formic acid in water and Solvent-B: 0.2% formic acid in methanol) with the flow rate of 0.3 ml/min. Sample (2 µl) was injected in the system and column temperature was maintained at 25°C during the analysis. The eluted phenolic acids through monitor by UPLC column effluent were pumped directly without any split into the TQD-MS/MS system (Waters, USA) optimized for the phenolic acids analysis.

2.4 Analysis of piperine by LC-MS/MS

Powdered sample (100 mg) was extracted with 80% ethanol using sonicator (Kotte *et al.*, 2012) with some modifications. It was then centrifuged (10,000 rpm, 10 min) before re-extracting with 80% ethanol. The final volume was made up to 20 ml. From this, 0.1 ml was taken and made up to 2 ml with mobile phase and filtered through 0.2 µm nylon filter prior to injection (2 µl) in the LC-MS/MS system for piperine estimation. The column details were as mentioned above. The analysis was done in isocratic mode (solvent A: acetonitrile: water: acetic acid:: 65:34.5:0.5 and solvent B: methanol: acetonitrile: water:: 10:50:40) with the flow rate of 0.15 ml/min. The sample injection volume was 2 µl and run time was 15 min. The eluted piperine through monitor by UPLC column effluent was pumped directly without any split into the TQD-MS/MS (Waters, USA) system optimized for piperine analysis.

2.5 Proximate composition and sensory evaluation

The proximate analysis was carried out using standard procedures. Moisture (%), total fat (%), total ash (%), protein (%) and crude fibre (%) were determined using AOAC (2019), while carbohydrate (%) was determined using difference method. Energy (kcal) was calculated as per Vetter and Melran (2007). Sensory evaluation of the powder was done using 7-points scale to know its acceptability.

3. Results

Woody Pepper, a novel spice, has potential for cultivation in the humid tropics. As the stems of the species are consumed as a taste imparter, biochemical analysis of stem segments is required. Moreover, the fresh produce has low shelf-life and hence, effort was made to dehydrate and process the same in the form of powder. Such powdered samples were subjected to further analysis in the laboratory. Stem pieces of thin (3-5 cm), medium (5-7 cm) and thick (7-9 cm) sizes were used for estimation during 2019, whereas the grading was done as small (1.0-2.5 cm) and big (2.5-4.0 cm) during 2021 depending on the availability of the produce.

3.1 Oleoresin content

Oleoresins are admixtures of essential oils and resinous matter present in most of the spices (Sowbhagya, 2019). The oleoresins represent the complete flavour and quality of whole spices in the concentrated form and hence, are desirable components in food preparations (Procopio *et al*., 2022). Thus, the oleoresin content is considered as a quality index in the spices industry. Analysis of data presented in Figure 2 revealed that oleoresin content was 3.5% in thin samples (3-5 cm wide), while it showed non-significant increment (3.7%) in medium samples (5-7 cm wide) and remained the same in thick samples (7-9 cm wide).

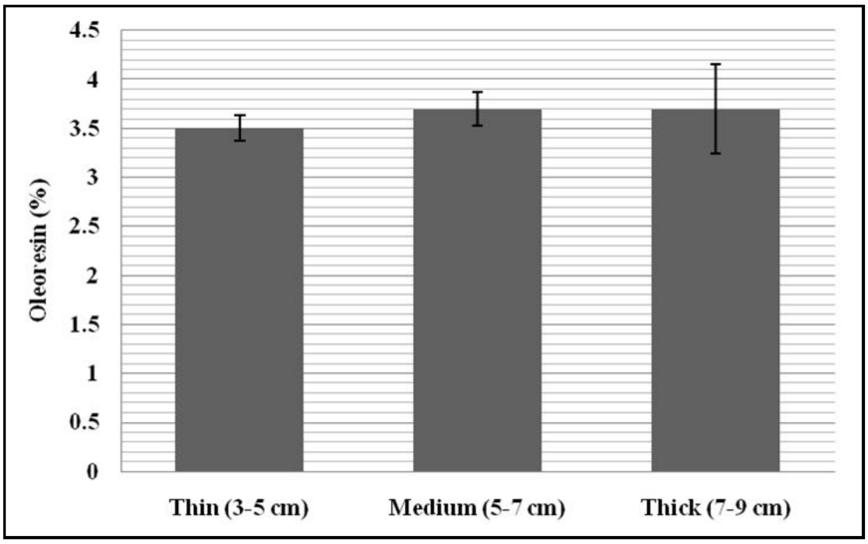

Figure 2: Oleoresin (%) in Woody Pepper stem of different thicknesses.

3.2 Total phenolic content

In the present study, total phenolic content (TPC) was influenced by both the thickness of the stem as well as the solvent used for extraction (Figure 3). In case of acetone extracts, no trend was observed as the lowest total phenolic content of 85.9 mg GAE/100 g extract was observed in medium sized stem samples, while it was the highest (183.0 mg GAE/100 g extract) in thin stems. In case of methanol extract, the total phenolic contents decreased from 304.6 mg GAE/ 100 g extract to 77.9 mg GAE/ 100 g extract with increase in stem thickness. Nevertheless, all the extracts showed presence of phenolic contents, indicating that the spice has antioxidant activity in all the grades of stem thickness. Thus, the produce of different stem thicknesses arriving in the Andaman markets would offer these benefits to the consumers.

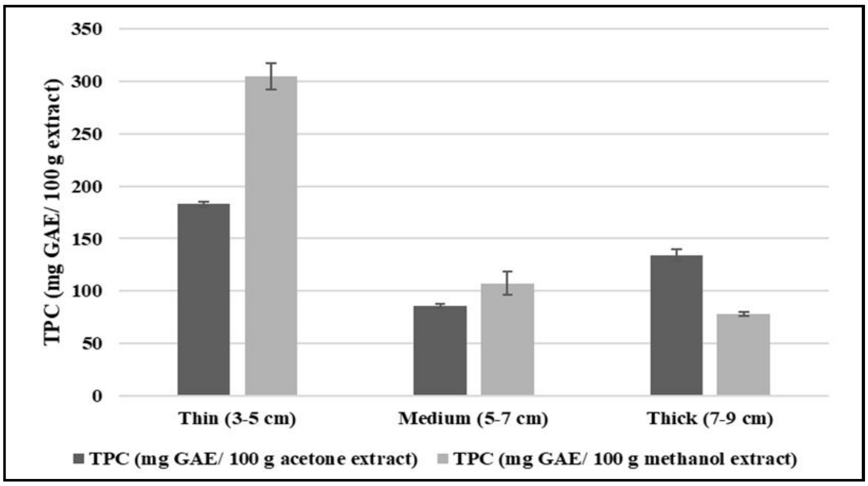

Figure 3: Total phenolic content in acetone and methanolic extracts of Woody Pepper stem of different thicknesses.

3.3 Phenolic acid profiling

In order to know the phenolic acids present in the stem of Woody Pepper, the analysis was carried out in stems of two thicknesses (Table 1, Figures 4 and 5). Analysis suggested that TPC identified by LC-MS/MS were higher 1142.82 µg/g in the small samples (1.0-2.5 cm wide) than that observed in the big samples (2.5-4.0 cm wide), *i.e.,* 1113.10 µg/g. Seventeen compounds were detected in Woody Pepper, relative quantities of which varied with the stem thickness studied. Ferulic acid was the most dominant one with higher content in big samples (867.95 µg/g) than the small ones (699.04 µg/g). Apart from this, *p*- Coumaric acid was the other compound, which was found at 99.62 and 188.51 µg/g concentrations in big and small samples, respectively. The other compounds present in comparatively lower quantities were *o*-coumaric acid, *t*-cinnamic acid, gallic acid, caffeic acid, vanillic acid and sinapic acid, while remaining compounds were present in minute quantities.

Table 1: Phenolic acid profiling (µg/g) of dehydrated stem pieces of Woody Pepper (µg/g)

Phenolic acids	Small (1.0-2.5 cm)	Big (2.5-4.0 cm)
Benzoic acid	5.42 ± 0.027	2.93 ± 0.016
p-hydroxy benzoic acid	0.69 ± 0.003	0.35 ± 0.002
Salicylic acid	1.92 ± 0.106	2.64 ± 0.006
3-Hydroxy benzoic acid	2.07 ± 0.022	0.88 ± 0.017
t-Cinnamic acid	41.31 ± 0.601	35.92 ± 0.160
2,4-dihydroxybenzoic acid	7.28 ± 0.165	1.30 ± 0.014
Gentisic acid	2.89 ± 0.172	2.99 ± 0.064
Protocatechuic acid	3.38 ± 0.073	2.02 ± 0.119
p-Coumaric acid	188.51 ± 0.665	99.62 ± 0.880
o-Coumaric acid	68.40 ± 0.756	25.10 ± 0.094
Vanillic acid	19.94 ± 0.118	11.39 ± 0.037
Gallic acid	35.57 ± 0.198	23.03 ± 0.082
Caffeic acid	39.57 ± 0.222	29.23 ± 0.524
Ferulic acid	699.04 ± 1.147	867.95 ± 1.760
Syringic acid	1.66 ± 0.007	0.97 ± 0.006
Sinapic acid	25.15 ± 0.082	6.74 ± 0.132
Ellagic acid	0.06 ± 0.001	0.05 ± 0.004
Total	**1142.86 ± 1.398**	**1113.10 ± 0.177**

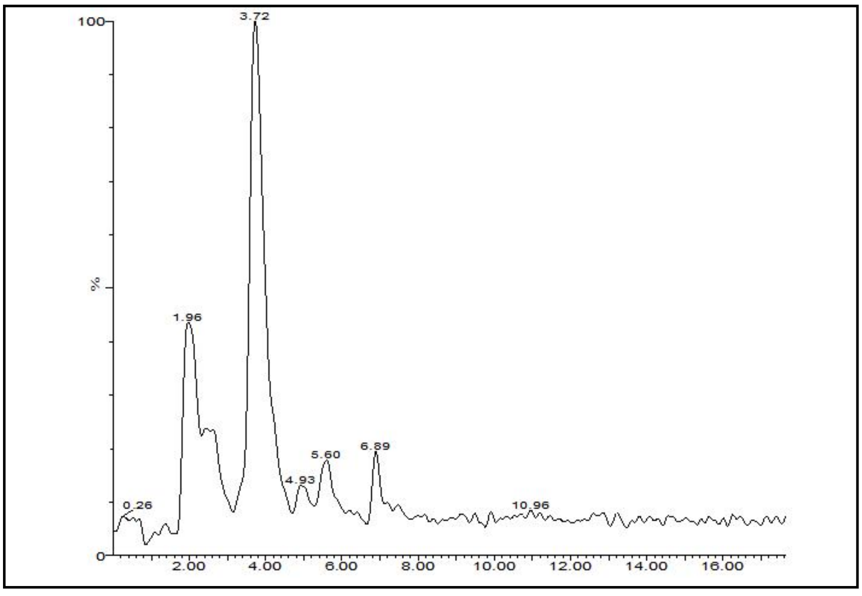

Figure 4: Chromatograph of big size stem of Woody Pepper showing phenolic acids.

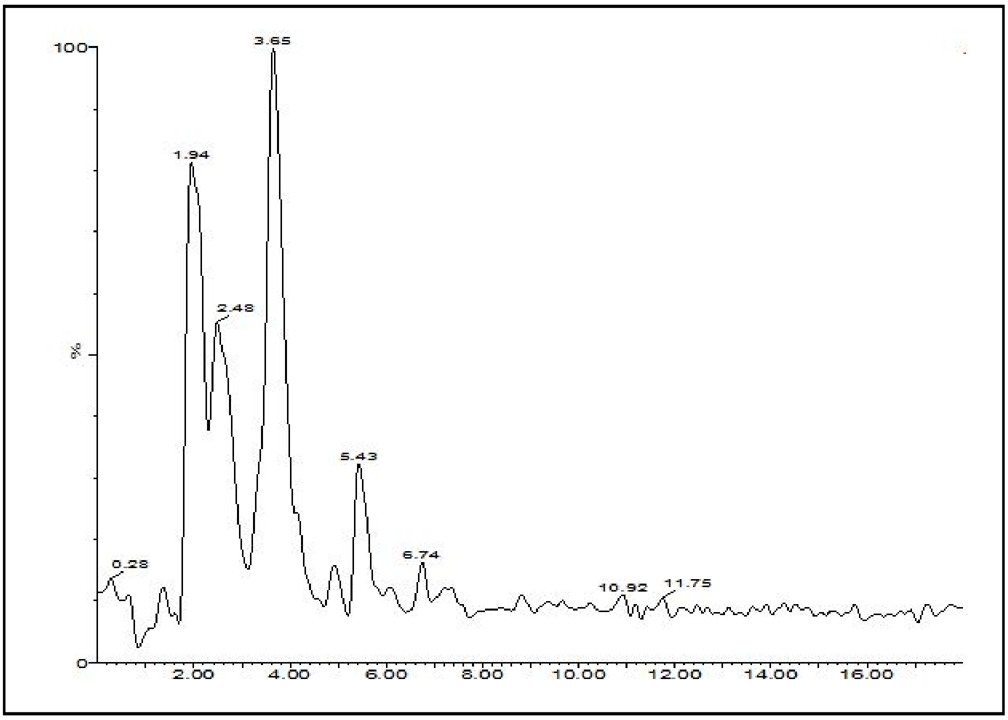

Figure 5: Chromatograph of small size stem of Woody Pepper showing phenolic acids.

3.4 Piperine content

Piperine is the pungency imparting compound reported to occur in various plant parts such as fruits, leaves, roots and stem of several *Piper* species (Haq *et al.*, 2021; Tripathi *et al.*, 2022). In the present study, piperine content (Figures 6-8) of the small (1.0-2.5 cm wide) Woody Pepper stem was 6.23 mg/g and it increased significantly to 6.654 mg/g in big samples (2.5-4.0 cm wide).

3.5 Proximate composition and organoleptic evaluation

Proximate analysis of the dehydrated Woody Pepper was carried out using standard procedures (Table 2). Results revealed that the powdered samples had carbohydrates (48.69%) as the dominant constituent. Further, the species was also found to be rich in crude fibre (31.89%). Total ash (9.30%), protein (7.34%) and fat (2.78%) were the other constituents in the tested sample. The product had 249 kcal energy per 100 g of produce. Further, the sample was subjected for sensory evaluation using consumer panel to know the acceptability of the product. It had woody taste in the beginning with a strong pungent aftertaste. Organoleptic evaluation on a 7 points hedonic scale revealed the mean scores of 5.5 ± 0.17 and 6.0 ± 0.22 for colour and taste, respectively. The overall acceptability score of the product was 6.1 ± 0.15.

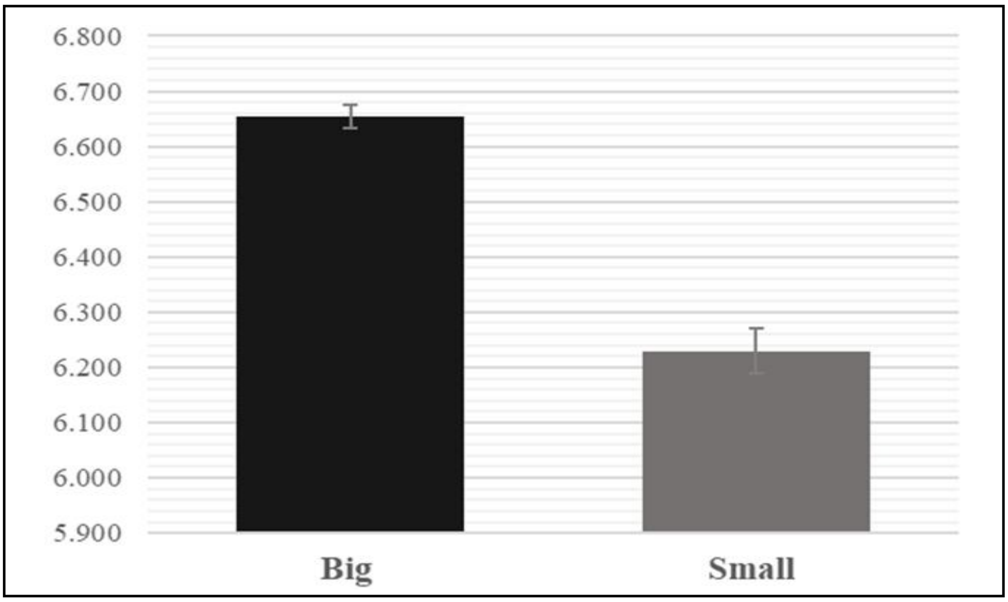

Figure 6: Piperine content (mg/g) in the stem of Woody Pepper as influenced by stem thickness.

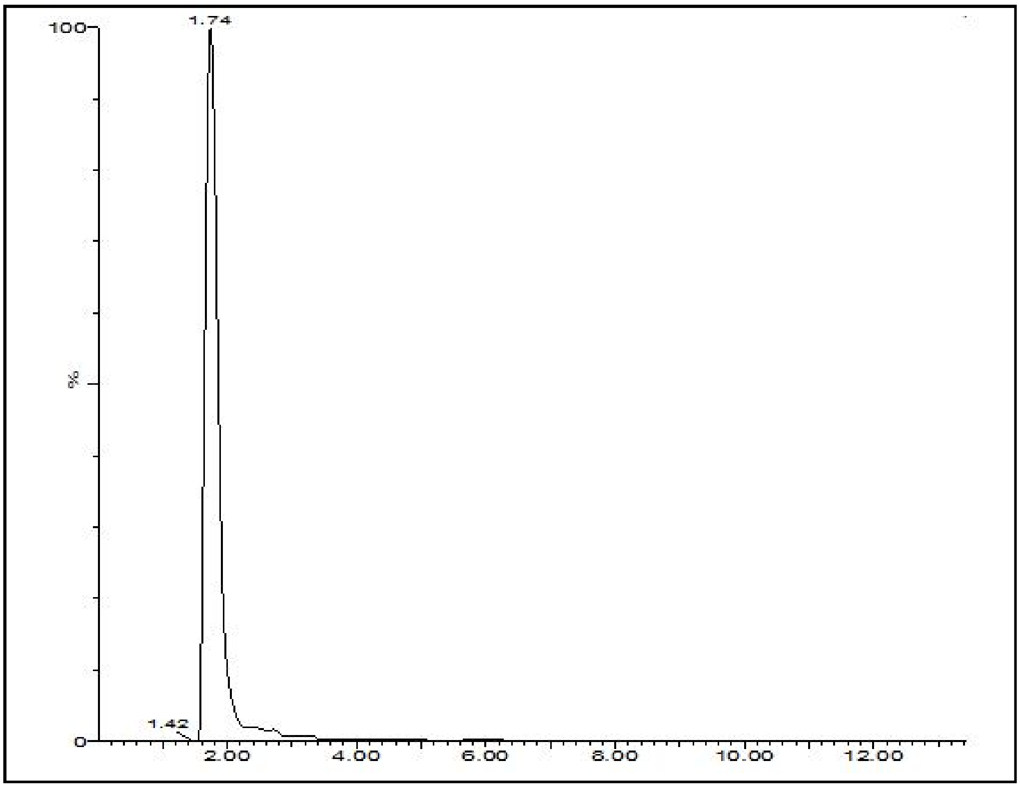

Figure 7: Chromatograph of big size stem of Woody Pepper showing piperine content.

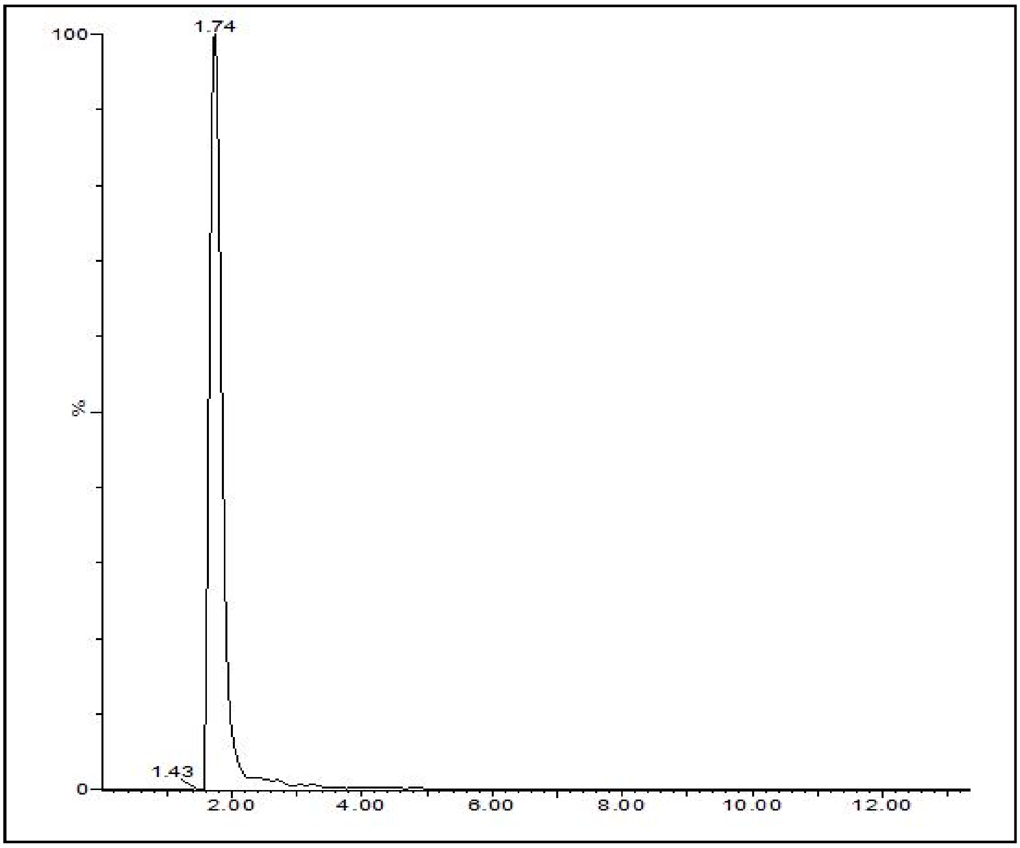

Figure 8: Chromatograph of small size stem of Woody Pepper showing piperine content.

Table 2: Proximate composition of dehydrated Woody Pepper powder

Parameter	Composition
Moisture (%)	9.64
Total fat (%)	2.78
Total ash (%)	9.30
Protein (%)	7.34
Crude fibre (%)	31.89
Carbohydrate (%)	48.69
Energy (kcal)	249.14

4. Discussion

Woody Pepper is a novel spice that has been reported to occur from the Andaman Islands in the Bay of Bengal (Waman *et al*., 2024). As limited information is available in this species, efforts were made to know more about it in the present study. Grading is an important operation that governs the quality of the produce in a number of medicinally valued plant species (Amrutanand *et al*., 2021). In Woody Pepper, the grading is mainly based on thickness of the stem. However, as the produce is not uniform, the grading and pricing is governed by the conditions at the time of marketing. That means if the demand is more, even relatively thinner stem pieces would get more prices.

Oleoresin content has been determined in the spice samples as a part of quality analysis and hence, it was determined in the present study. Results revealed variations among the treatments though non-significant. Yields of oleoresins have been reported to vary with various factors such as genotype, extraction conditions, form of produce (dry/ fresh), country of origin, edaphic factors, production techniques adopted, *etc*. (Ratnambal *et al*., 1987; Vernin and Parkanyi, 2005; Nagaraju *et al*., 2020; Waman *et al*., 2020; Khasanah *et al*., 2017). Oleoresin content also varies greatly with the species, *e.g*., 7.8-11.5% in cinnamon (Waman *et al*., 2020), 3.80-6.69% in ginger rhizomes (Anisha Babu *et al*., 2021), 5.82-12.73% in *Piper nigrum* berries (Sruthi *et al*., 2013) *etc*., have been reported. Further studies with produce sourced from various sources and varied thicknesses would reveal interesting findings in future. Total phenolic content is an indication of the antioxidant activity of most of the natural products. Strong positive correlation of TPC with the antioxidant activity has been well documented (El Rayess *et al*., 2023). In Woody Pepper, TPC content was determined to know its antioxidant capacity, and both stem thickness and solvents used in the study were found to influence it. Influence of solvent on phenolic content has been reported in a number of spices and medicinal plant species (Sruthi and Zachariah, 2016; Muzolf-Panek and Stuper-Szablewska, 2021; Singirikonda *et al*., 2022; El Rayess *et al*., 2023; Sachin *et al*., 2022).

Globally more than 500 polyphenols have been documented in various food commodities, major sources of those being various horticultural products including fruits, vegetables, spices, beverages (teas/ decoctions/ wines) *etc*. (Singh and Yadav, 2022). Among those, spices are the dense sources of these compounds and a number of health promoting activities have been reported from their consumption (El Rayess *et al*., 2023). Phenolic acids are the important group of phenolic compounds, which have significant presence in various spices (Muzolf-Panek and Stuper-Szablewska, 2021; El Rayess *et al*., 2023). The composition of phenolic acids and their relative concentration varies with the species. Ferulic acid and *p*- Coumaric acid were found to be the dominant phenolic acids in Woody Pepper and their content varied with the stem thickness. In earlier study, Ferulic acid was found to be present only in three (bay leaf, clove and garlic) of the tested 13 spices, while in others, the compound was not observed (Muzolf-Panek and Stuper-Szablewska, 2021). Ferulic acid has also been reported to occur in *P. nigrum*, *P. longum* and *P. chaba* (Sruthi and Zachariah, 2016). Studies have shown that the ferulic acid and coumaric acid exhibit good radical scavenging activity (Wang *et al*., 2020). Further, a number of polyphenols have been reported to show antidiabetic potential by playing important role in transporter stimulation mechanism in the body (Vetrani *et al*., 2020).

In the present study, stems of Woody Pepper were found to be a source of piperine, an important constituent known to impart pungency to several *Piper* species. However, generally the compound has mainly been reported from the fruits and rarely from the stem. Several reports have claimed the health promoting and medicinal benefits of piperine through *in vitro* and *in vivo* studies (Tiwari *et al*., 2020; Haq *et al*., 2021; Tripathi *et al*., 2022; Mehrotra, 2021). Thus, Woody Pepper could provide these health benefits to the consumers. Though spices are not used as sources of primary nutrients, proximate composition is known to provide the potential nutritive value of any food including medicinally important plant species (Hait *et al*., 2023; Devi *et al*., 2023). Further, the organoleptic evaluation gives an idea about the market potential of newly developed products. Woody Pepper powder was found to be acceptable to the consumers and hence, could be used as taste imparter in food preparations mainly to sprinkle on fruit chats, boiled eggs, beverages, *etc*.

Thus, it could be concluded that, the stem pieces of Woody Pepper have good potential to be processed into dehydrated powder form. The spice has phenolic compounds, oleoresins and piperine, which would contribute to the antioxidant activity, and has good acceptability by the consumers.

Acknowledgements

Senior author is thankful to the Department of Biotechnology, Government of India for providing financial assistance (BT/PR34255/ NDB/39/666/2019). Authors are thankful to the Director, ICAR-CIARI, Port Blair for providing necessary facilities for carrying out the work, to the farmer Mr. Lankeshwar Mandal, Middle Andaman for providing the material and to the SAIF, ICAR-IIHR, Bengaluru for their help in chromatographic studies.

Conflict of interest

The authors declare no conflict of interest relevant to this article.

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Citation Ajit Arun Waman, Pooja Bohra and R. Karthika Devi (2024). Biochemical studies in Woody Pepper (*Piper pendulispicum* C.DC.): A spice with edible stem from Andaman Islands, India. Ann. Phytomed., 13(1):1141-1148. http://dx.doi.org/10.54085/ap.2024.13.1.122.