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STUDY ON ANATOMICAL FEATURES OF ROOTS, STEMS, LEAVES, CHEMICAL COMPOSITION AND ANTIFUNGAL, ANTIBACTERIAL ACTIVITIES OF CINNAMON OIL (*Cinnamomum cassia* (L.) J. PRESL) PLANTED IN THANH HOA

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Abstract. The paper presents anatomical features of roots, stems and leaves of cinnamon planted in Thanh Hoa. Anatomical features of the root and stem include epidermis, cortex, phloem, xylem and pith. Petiole and midrib have the epidermis, phloem, xylem, pith and thick tissue, blade composed of the upper epidermis, lower epidermis, palisade mesophyll and spongy mesophyll. The research identified 20 chemical components of cinnamon oil, in which the major constituents includes cinnamaldehyde <E-> (76.96 %), cinnamyl acetate <E-> (11.07 %), coumarin (5.06 %). In the sample concentration of cinnamon oil samples exhibiting activity against 03 strains of *E coli*, *P. aeruginosa*, *B. subtilis* (with MIC value from 100- 200 µl/mL) with a sterile ring diameter of 10.22 mm; 16.27 mm; 14.61 mm and 01 strain of *C. albicans* (MIC = 50 µl/mL) with a sterile ring diameter of 24.45 mm.

Keywords: Anatomy, leaves, roots, stems, cinnamon oil.

1. Introduction

Cinnamon (*Cinnamomum cassia* (L.) J. Presl) belonging to family Lauraceae [1], has been utilized as a potential therapeutic agent in various cultures for centuries. In Vietnam, cinnamon planted in tropical natural forests, from the North to the South. The main products of cinnamon are cinnamon bark and cinnamon essential oil which have been widely used in pharmaceutical industry, processing industry and animal husbandry. The parts of the cinnamon such as bark, leaves, flowers, roots all contain essential oils, especially in the bark with the highest content of essential oils, sometimes reaches 4 - 5 %. Cinnamon bark is used as a spice to improve flavor of local food preparations. Cinnamon bark and leaf oils are used as flavor ingredients and also in cosmetics and pharmaceutical preparations.

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There have been many studies in the world and in Vietnam on cinnamon oil. Research by P.A.Paranagama *et al*. (2001) [2] showed that the major constituents of cinnamon fruit oil were δ- and γ- cadinene (36.0 %), T-cadinol (7.7 %) and β-caryophyllene (5.6 %). About 84 % of cinnamon fruit oil comprised sesquiterpenes, while other parts of cinnamon contained less than 9% of this group of compounds. Phenyl propanoids were the major constituents of cinnamon bark and leaf oils while root oil had monoterpenes as the major constituents (95 %).

Kamaliroosta L. *et al.* (2012) [3] indicated that Cinnamaldehyde was the predominant constituent in the isolated oil. Hoang Thi Bich *et al.* (2017) [4] has surveyed the biological activity of cinnamon leaf oil obtained from the enzyme method combined with steam distillation, the results have shown that cassia oils were obtained from the plant leaves and branches by steam-distillation (StD) and enzyme-assisted distillation (EAD) and studied on some bioactivities. Both EAD and StD oil exhibited antibacterial, antifungal, anti-inflammatory, cytotoxic activities and no antioxidant activity. Enzyme treatment of these oils with Laccase-Htec2 did not alter their biological activities.

Cinnamon is considered as one of the precious medicinal plants that have been widely used in the daily life of Vietnamese. However, the use of this medicinal plant in Vietnam is still folklore, studies on cinnamon are limited, especially those on anatomical features although the research on anatomical features contribute useful information in identifying and orienting appropriate planting sites for a variety of human purposes. Therefore, we have collected samples to analyze the anatomical features of roots, stems, leaves and essential chemical composition of cinnamon as the scientific basis for the next new research.

2. Content

2.1. Materials and methodology

**** Materials***

Stem, bark, leaves and roots of Cinnamon were collected in Trieu Son district, Thanh Hoa province in may 2019. Cinnamon is 2 years old and grows well. Cinnamon was classified according to comparative morphological method of Nguyen Nghia Thin [5].

**** Methodology***

- *Anatomical method*

Using a sharp knife to create thin slices of the stem, roots, leaves, cut perpendicularly [6]. Select the most beautiful slices to be used as a template by double dyeing method and microscopic observation [7, 8]. All measurements were conducted on a light microscope.

- *Methods of extraction and analysis of chemical composition of cinnamon oil*

Cinnamon bark oil is collected by attractive steam distillation method for 2 hours at normal pressure according to Vietnamese Pharmacopoeia [9].

The chemical composition of cinnamon oil was analyzed at the Department of Chemical Analysis - Institute of Natural Products Chemistry - Vietnam Academy of Science and Technology. The analysis was performed by means of gas chromatography-

flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS) [10].

The HP-5MS column had dimensions of 0.25 x 30 m x 0.25 mm and HP1 had dimensions of 0.25 x 30 m x 0.32 mm. Process temperature were 60 ^{o}C/2 minutes; increased the temperature 4 ^{o}C/1 minute until 220 ^{o}C, then increased the temperature 20 ^{o}C/1 minute until 260 ^{o}C, with Helium carrier gas [11].

- Test method of antifungal, antibacterial activity of cinnamon oil

+ Microorganism strains

Bacteria Gr(-): *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027

Bacteria Gr(+): *Bacillus subtillis* ATCC 6633, *Staphylococcus aureus* ATCC 6538

Mushroom (mold): *Aspergillus niger* ATCC 9763, *Fusarium oxysporum* ATCC 48112

Mushroom (yeast): *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 16404

Standard strains (ATCC sources, Manassas, USA) are provided by the Central Institute of Drug Quality Control and kept in the Laboratory of Experimental Biology (Institute of Natural Products Chemistry).

+ Standard antibiotics

Gentamycin for bacteria Gr (-), doxycycline for bacteria Gr (+) and nystatin for mushroom (mold) and mushroom (yeast). Antibiotics are provided by Ho Chi Minh City Testing Institute.

From the original solution mixed with distilled water and then diluted to the required concentration: gentamycin (16 - 8 - 4 IU/mg), doxycyclin (0.4 - 0.2 - 0.1 IU/mg) and nystatin (12 - 6 - 3 IU/mg).

+ Test and evaluate results

Sample preparation: Dissolve the sample in DMSO (Dimethyl sulfoxit) 100 % using a vortex machine at a concentration of 1 mg/ml with pure samples.

Mix a sample with DMSO 10 % on 96 template plate in a decreasing concentration (log_2 for 5 levels of concentration). Apply a sample on 96 plate (test plate) and add microorganisms to obtain a sample concentration range of 200-100-50-25.5-12.5 µg/ml (repeat 3 times at each concentration) with raw sample (extract) and 50-25-12.5-6.25 µg/ml with sample of purified samples. Keep in the incubator at 37 °C for 24h for bacteria and 30 °C/48h for mushrooms.

Control sample: NaCl 0.9 % corresponding to the sample volume of the negative control (-) and positive control (+) as standard antibiotics.

Samples were determined to be active in the absence of microbial growth at least one test sample concentration compared to control (-) (when re-cultured at this concentration check on agar plates CFU (Colony forming unit) value < 5). The active expression sample was tested at different sample concentration ranges to determine the minimum inhibitory concentration of MIC (µg/ml) which is the lowest test concentration for which the microorganism is inhibited.

Samples were determined to be active when the MIC≤ 200 µg/ml values for raw samples and ≤ 50 µg/ml for purified samples.

2.2. Results and discussion

2.2.1. Anatomy of the roots

The analysis anatomical features of the cinnamon roots in Figure 1 indicate that: The epidermis consists of a long layer of thin-filmed cells that are often tightly packed.

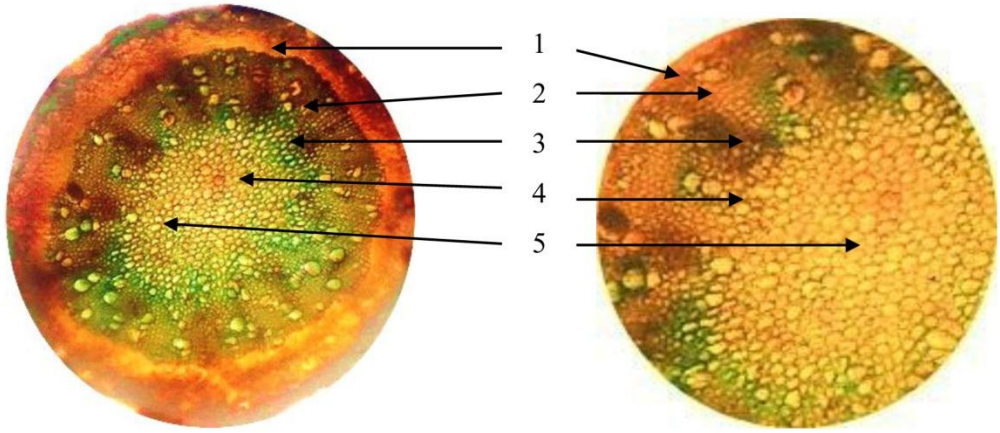


Figure 1 . Anatomy of the roots

1. Epidermis; 2. Cortex; 3. Phloem; 4. Xylem; 5. Pith

The cortex is massive and consists of thin-walled cells with rounded or polygonal parenchyma cells having sufficiently developed intercellular spaces among them. The parenchyma cells of the cortex contain abundant starch grains in them.

The transport system consists of separate bundles of phloem and xylem arranged alternately. The xylem forms discrete strands, alternating with the phloem strands. Sometimes the xylem occupies the centre, with the strand-like parts projecting from the central core like ridges. The phloem is also centripetally differentiated, the protophloem occurring closer to the periphery than the metaphloem.

Ground tissue of the pith accounts for a small proportion of the innermost part, soft tissue consists of cells with thin membranes and relatively large size, close together so there is no space between the cells.

2.2.2. Anatomy of the stem

The result in Figure 2 shows anatomical features of cinnamon stem, the structure consists of: The epidermis consists of a single layer of cells and is the outermost layer of the stem. It contains stomata and the cells are compactly arranged and do not possess intercellular spaces. In transverse section the cells appear almost rectangular. It serves mainly for restricting the rate of transpiration and for protecting.

The cortex lies below the epidermis, it consists of large parenchyma cell and cells are generally regular in shape, have comparatively thin walls, and are not greatly elongated in any direction. They are living cells and contain a moderate amount of protoplasm. The innermost layer of the cortex is the endodermis consisting of barrel-shaped, elongated, compact cells, having no intercellular spaces among them.

The phloem strands are found on extreme ends of the vascular bundle. The phloem occurs in two patches, towards the periphery, the outer phloem, and towards pith, the inner phloem. Each strand of phloem consists of sieve tubes, companion cells and phloem parenchyma. Fibres and ray cells are absent.

Xylem occupies the central position of the vascular bundle, consisting of pitted vessels towards periphery of the metaxylem, and on the inner side of narrow vessels which form the protoxylem. In the xylem, ray and xylem parenchyma are also present. The xylem vessels are not arranged in radial rows.

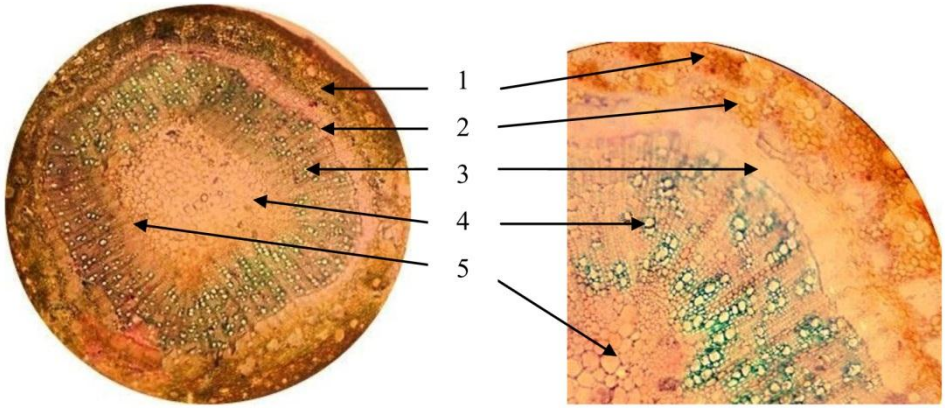


Figure 2. Anatomy of the stem

1. Epidermis; 2. Cortex; 3. Phloem; 4. Xylem; 5. Pith

Pith occupies the whole centre of the stem. It extends outwards in between the bundles up to the pericycle. Pith consists of thin-walled rounded or oval parenchymatous cells having well defined intercellular spaces among them.

2.2.3. Anatomy of leaves and petioles

**** Petiole***

Anatomy of the cross-section of petiole: The epidermis consists of rectangular cells arranged according to the length of the stem, the outer layer is thin, epidermis cells are single layered and are arranged regularly in both sides. Epidermis is covered with a smooth cuticle.

Thick tissue is located close to the epidermis including small cells layers and is responsible for supporting the petiole. Soft tissue consists of irregular cells, thin walls, containing many chloroplasts, between cells with small intercellular space. Petiole of cinnamon consists of vascular bundles in the middle and a small single bundle in each corner. Middle and small vascular bundles are surrounded by parenchyma cells.

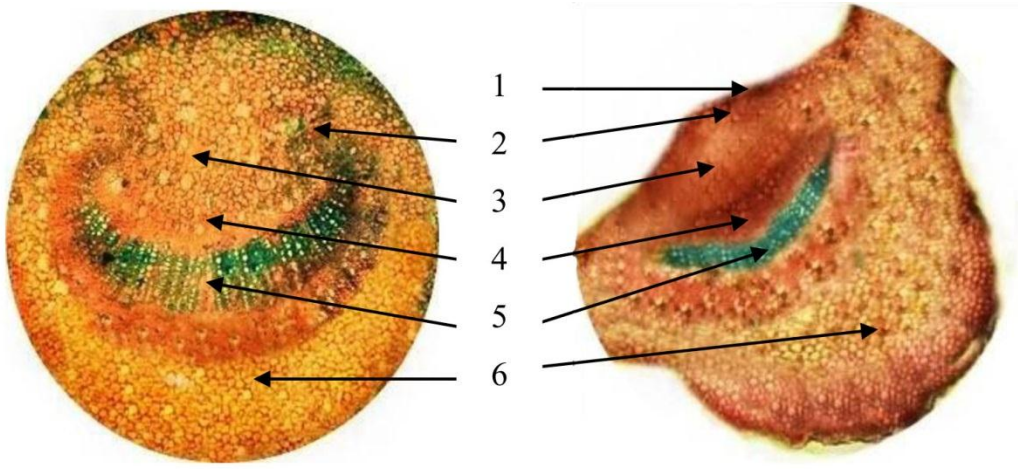
**** Midrib***

Midrib is concave on the upper surface and convex on the lower one. The lower epidermis is relatively regular cells; thick tissue is a layer of cells with irregular wall thickness, with a transition to soft tissue; soft tissue with many layers of thin-walled cells, irregular size. The phloem consists of cells that are many times smaller than ground tissue cells, arranged in discrete clusters around the xylem. Xylem consists of large bundles at the bottom and small bundles at the top, alternating with clumps of ground tissue cells.

**** Blade***

From the upper surface of the leaf, it consists of the upper epidermis, soft tissue and lower epidermis. the epidermis consists of cells which have no chloroplast, the outer membrane is usually thicker and is covered with a thin layer of cuticle, The lower epidermis has more stomata than the upper epidermis.

Ground tissue of leaves includes palisade mesophyll and spongy mesophyll. The palisade mesophyll is located just below the upper epidermis, which is composed of a rectangular layer of cells, arranged close to the end of the leaf blade almost perpendicular to the epidermal cells, the palisade mesophyll cells often contain many forces. The spongy mesophyll cells is located below the palisade mesophyll cells and adjacent to the lower epidermis of leaves, which are round, smaller than palisade mesophyll cells, arranged in clusters separated to reveal large spaces.



a. Petiole *b. Midrib*

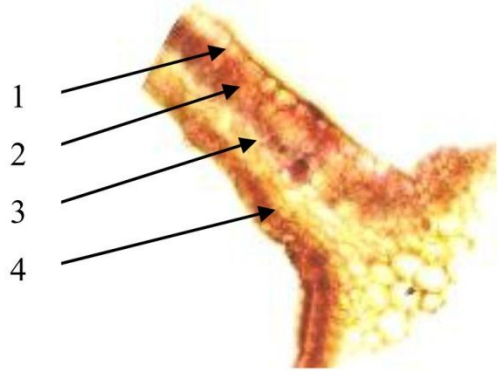


Figure 3. Anatomy of leaves

Petiole and midrib: *1. Upper epidermis; 2. Thick tissue; 3. Cortex; 4. Phloem;5. Xylem; 6. Pith*
Blade: *1. Upper epidermis; 2. Palisade mesophyll; 3. Spongy mesophyll; 4. Lower epidermis*

The transport system is submerged in soft tissue, and the bundle also distinguishes between the xylem and phloem, which conduct water, minerals and photosynthesis products in the leaves.

2.2.4. The chemical composition of cinnamon oil

The results of spectral analysis of Table 1 and chromatography spectrum of Figure 4 shows that the major constituents of cinnamon bark oil includes cinnamaldehyde <E-> (76.96 %) (Table 1), cinnamyl acetate <E-> (11.07 %), coumarin (5.06 %). The remaining compounds account for 0.11 % to 0.92 %, of which the lowest is styrene 0.11 %. Cinnamaldehyde (3-phenyl-2-propanal) represents the main constituent of the cinnamon bark oil that contributes to about 49.9 - 62.8 % of the total amount. It provides protection against metabolic syndromes such as cardiovascular complications and diabetes.

Table 1. The chemical composition of cinnamon oil

No.	Time	RI	Hit %	Chemical name	%
1	8.92	899	72	Styrene	0.11
2	10.98	967	74	Benzaldehyde	0.77
3	13.76	1052	82	Salicyaldehyde	0.40
4	17.88	1171	72	Benzenpropanal	0.92
5	18.50	1188	83	Benzofuran <2-Methyl->	0.50
6	19.95	1230	94	Cinnamaldehyde <Z->	0.62
7	21.91	1287	54	Cinnamaldehyde <E->	76.96
8	22.94	1317	96	Cinnamyl alcohol <E->	0.84
9	24.61	1368	79	Eugenol	0.24
10	25.34	1390	80	Copaene <a->	0.19
11	25.69	1400	55	Bourbonene <b->	0.13
12	26.85	1437	34	Caryophyllene <E-> (=Caryophyllene <b->)	0.13
13	27.38	1454	82	Cinnamyl acetate <E->	11.07
14	27.47	1457	90	Coumarin	5.06
15	28.18	1479	12	Caryophyllene <9-epi-(E)->	0.13
16	29.37	1518	73	Bisabolene <b->	0.14
17	29.94	1538	75	Selinene <7-epi-a->	0.16
18	30.13	1544	77	Methoxycinnamaldehyde <(E)-o->	0.64
19	30.95	1571	39	Nerolidol <E->	0.81
20	31.97	1606	0	Caryophyllene oxide	0.17
Total					**99.99**

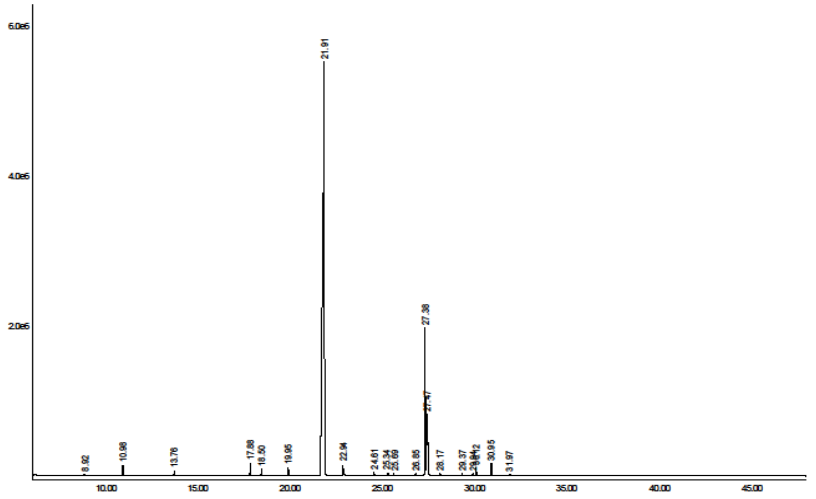


Figure 4. Chromatogram for the chemical composition of cinnamon oil

Research results of the individual chemical components of cinnamon oil grown in Thanh Hoa are consistent with the analysis of the chemical composition of cinnamon leaf oil and *C. cassia* bark in Yen Bai by Hoang Thi Bich *et al.* (2017) there are about 28 compounds found in cinnamon leaf essential oil, of which trans-cinnamaldehyde is the main compound (69.74 %), followed by cinnamyl acetate (17.2 %).

The research results on the chemical composition of cinnamon essential oil are very important, contributing to providing more data as a scientific basis for new studies on cinnamon in Thanh Hoa in particular and the whole country in general as applications in the manufacture of medicinal pharmaceuticals.

2.2.5. Test results of antifungal, antibacterial activity of cinnamon essential oil

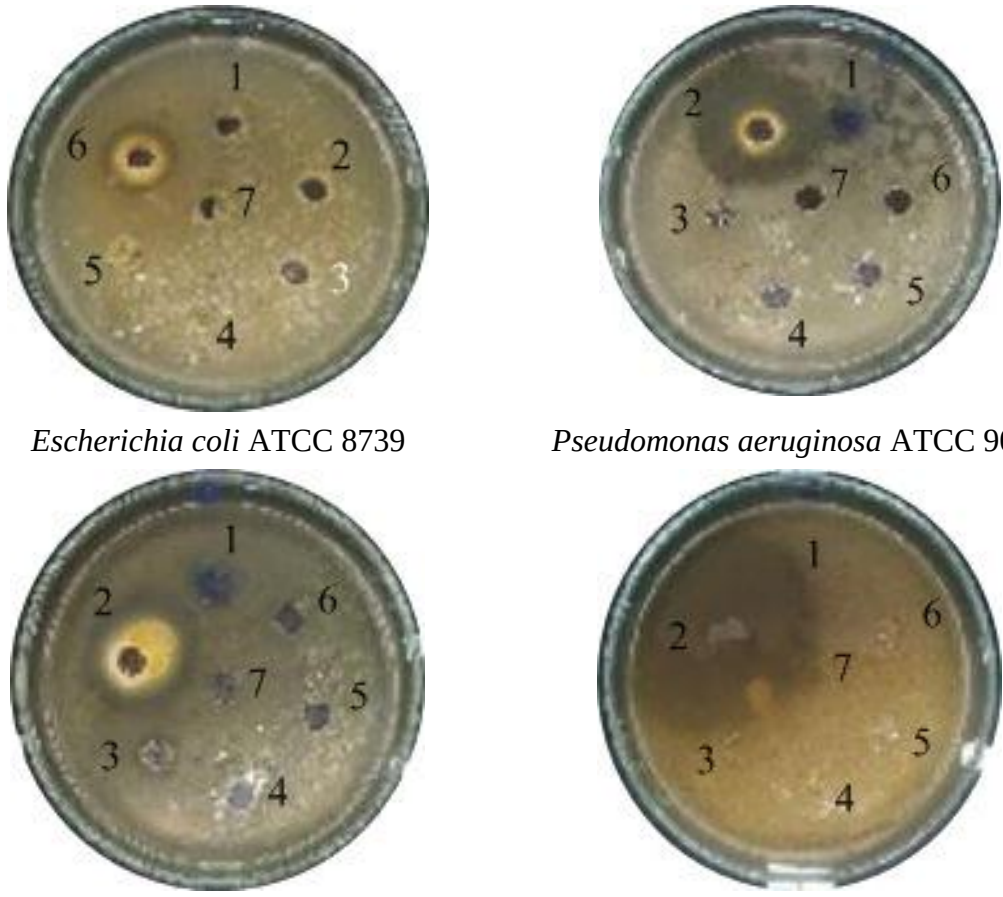
Table 2. Minimum inhibitory concentration

Name of the template	**Minimum inhibitory concentration (MIC, µg/mL)**							
	Bacteria Gr (-)		**Bacteria Gr (+)**		**Mold**		**Yeast**	
	E. coli	*P. aeruginosa*	*B. subtillis*	*S. aureus*	*A. niger*	*F. oxysporum*	*S. cerevisiae*	*C. albicans*
TDQ	200	100	100	> 200	> 200	> 200	> 200	50

Table 3. Test results on agar plates

Concentration test	**Sterile ring diameter (D-d, mm)**							
	Bacteria Gr (-)		**Bacteria Gr (+)**		**Mold**		**Yeast**	
	E. coli	*P. aeruginosa*	*B. subtillis*	*S. aureus*	*A. niger*	*F. oxysporum*	*S. cerevisiae*	*C. albicans*
12.5 µg/mL	-	-	-	-	-	-	-	-
25.5 µg/mL	-	-	-	-	-	-	-	-
50 µg/mL	-	-	-	-	-	-	-	24.45
100 µg/mL	-	16.27	14.61	-	-	-	-	-
200 µg/mL	10.22	-	-	-	-	-	-	-

(-): Not determined (does not exhibit activity at test concentration)



Escherichia coli ATCC 8739 *Pseudomonas aeruginosa* ATCC 9027

Bacillus subtillis ATCC 6633 *Candida albicans* ATCC 10231

Figure 5. Test results on agar plates

1,2,3,4,5: Test sample ; 6: Control sample (-); 7: Control sample (+)

The activity test results showed that, in the concentration range of the sample of cinnamon oil expression of activity against 03 strains of *E coli*, *P. aeruginosa*, *B. subtilis* (with MIC value from 100- 200 µl/mL) with a sterile ring diameter of 10.22 mm; 16.27 mm; 14.61 mm and 01 strain of *C. albicans* (MIC = 50 µl/mL) with a sterile ring diameter of 24.45 mm.

3. Conclusions

The paper presents anatomical features of roots, stems and leaves of cinnamon planted in Thanh Hoa. Anatomical features of the root and stem include epidermis, cortex, phloem, xylem and pith. Petiole and midrib have the epidermis, phloem, xylem, pith and thick tissue, blade composed of the upper epidermis, lower epidermis, palisade mesophyll and spongy mesophyll.

The research identified 20 chemical components of cinnamon oil, in which the major constituents of cinnamon bark oil includes cinnamaldehyde <E-> (76.96 %), cinnamyl acetate <E-> (11.07 %), coumarin (5.06 %), the remaining compounds account for 0.11% to 0.92 %, of which the lowest is styrene 0.11 %.

In the sample concentration of cinnamon oil samples exhibiting activity against 03 strains of *E. coli, P. aeruginosa, B. subtilis* (with MIC value from 100 - 200 µl/mL) and 01 strain *C. albicans* (MIC = 50 µl/mL). These results are an important scientific database contributing to new research on cinnamon planted in Thanh Hoa.

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