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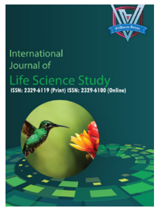

ARTICLE

MOLECULAR CHARACTERISTIC AND FUNCTION-SPECIFIC VOLATILES OF *CARYA CATHAYENSIS SHELL*

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ABSTRACT

Carya cathayensis, the pecan fruit, is a nutritionally dense fruit with a unique taste that has become popular for consumption. The Carya cathayensis shell (SCC) is a by-product of C and there is a lack of the systematic analysis on the chemical composition. The volatile organic constituent characteristics of SCC and the change law of SCC groups were investigated. The results show that the 1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-, (E) exists in SCC extracts could be used to prevent myopia and to produce fragrance. SCC could be used to extract the Vitamin E that is used in medicine and beauty products. The. gamma. -Sitosterol could be developed into an effective antidiabetic drug. The biologically active VOCs, such as Phytol, acetate, Heptacosane, and Squalene were found in the SCC extractives. The petroleum ether extractive is the highest extract content, followed by ethanol extractive and benzene/ethanol extractive, indicating that the petroleum ether extractive has a large development prospect. The results of FTIR analysis confirmed that the compound group of SCC will not be altered by organic solvent extraction. There were three obvious stages during the thermogravimetry analysis of SCC: the first stage is (30°C-90°C), the second stage is (225°C-336°C), and the third stage is (336°C-386°C). The order of quality loss is highest in the second, followed by the third and finally the first. The three temperature points (90°C, 225°C, and 386°C) could use as the theoretical basis for the analysis of pyrolysis of the SCC. A great amount of new components are produced during the pyrolysis process of SCC extracts and residues, which provides a new method for multiple uses of SCC. The GC/MS results showed that the SCC contained aromatic compounds, alkanes, ethers, organic carboxylates Acids, anhydrides and salts, esters and alcohols, and amides. The echocardiography before and after the SSC extraction showed that the surface particles were reduced after the extraction, but the overall pore structure did not. This indicated that the overall structure of the SCC was hard. These results could be used to determine if the SCC could be used as a grinding material. This initial exploration of the effective components of SCC should allow other to make full use of SCC and provide a developmental scientific basis for further development.

KEYWORDS

Carya cathayensis, volatile organic compound (VOC), GC/MS, Organic solvent extract, Molecular characteristics, thermogravimetry analysis.

1. INTRODUCTION

The Carya cathayensis (SCC) is a walnut plant, also known as the alias wild walnut, the hemp walnut, or the Thai skin. The wild walnut grows in the Qinling Mountains (China) on the northern and southern slopes that are 800 meters to 2000 meters above sea level. Walnuts are primarily distributed in the northeast elevation between 300 meters to 800 meters on both sides of the valley and foothills. This land is fertile and rich in resources. The SCC kernel, fruit, and skin could be used as medicine. The SCC bark bitter, or the Xin, could be used to treat a cold, can be heat, dampness, bowel, and an insecticide[1]. The SCC has been used for the treatment of digestive systems and tumors (specifically esophageal cancer and gastric cancer). The skin contains phenolic compounds, tannins, organic acids, alkaloids, amino acids, skin, protein, sugar, polysaccharides, glycosides (glycosides), saponins, steroids, flavonoids, coumarin, terpene Compounds, and anthraquinones[2]. The total flavonoids (TF) extracted from the SCC via immunohistochemical analysis showed that the mice treated with TFs had significantly less accumulation of macrophages in the adventitia. Studies have shown that early models of atherosclerotic lesions can be inhibited by TFs, as well as a strong effect on the inflammatory responses in vivo, which demonstrates is application in medical care[3,4]. The acetic acid extraction of CFF leafs obtained the strongest anti-tumor effect, which induced SGC-7901 cell apoptosis. This suggests that pecan leaves could have anti-carcinogenic substances[5]. CFF is a high oil content of nuts, mainly unsaturated fatty acids. The difference in gene expression during fruit ripening was examined by DDRT-PCR, in which a cDNA library related to fat metabolism was constructed by SMART. The unamplified library was titrated to show a titer of 5.0×105 pfu·mL^{-1}, the insert was greater than 0.7 kb, less than 2.5 kb, and the insert greater than 1.0 kb was

70%. The titer of the amplified library was 5.0×109 pfu·mL^{-1}, the insert was larger than 500 bp, the probability of recombination was 94.5%, and the insert longer than 1000 bp was about 66%. This study laid the foundation for the study of fat metabolism and regulation systems and the discovery of related genes[6]. Systematic analysis of fatty acid content and composition by GC/MS. The results indicate the content of unsaturated acid was 92.11%, 92.64%, and 92% of the SCC fatty acid oil. The SCC oil was primarily composed of oleic acid, suboleic acid, and falx acid. The saturated acid content was 7.8%, 7.2%, and 7.56%, which consisted mostly of palmitic acid[7]. The final study found that the SCC shell contains compounds that controls fungi and bacteria[8]. By the phosphate method, we can extract the components of activated carbon from the shell of SCC. Yu measured the adsorption capacity of activated carbon by orthogonal experiment. In the reaction experiment, in addition to the phosphate concentration, carbonization temperature and reaction time were added as influencing factors. Among them, methylene blue is used as an adsorbent, and the value of iodine and the yield are indicators. The data showed that the adsorption value of activated carbon for methylene blue was 101 mL/g, and the value of iodine was 804.37 mg/g, the yield was 53.22%. These results were produced at an activation temperature of 300, an activation time of 45 min, and a phosphate concentration of 50%. The adsorption increased alongside the temperature. Acidity makes the adsorption stronger. The data is consistent with the isotherm. The parameters indicate that the adsorption reaction is a spontaneous exothermic reaction[9-11]. If abandoned SCC shell was fully utilized, this would lessen the waste and protect the surrounding ecological environment. In this paper, an organic solvent method for the walnut shell was proposed. The aim was to explore the active ingredients of the SCC, in order to provide a scientific basis for clinical research and development. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

2. MATERIALS AND METHODS

2.1 Materials and reagents

SCC was acquired by the Biotechnology Laboratory of Central South University of Forestry and Technology. The SCC was then dried under natural conditions (about 40°C) and pulverized into a powder using a pulverizer. Processed with an AS200 sifter. Most of the reagents in the experiments were purchased from Sigma Chemical Company (USA).

2.2 Methods

2.2.1 SCC extraction

The pulverized SCC sample was taken in petroleum ether benzene/ethanol (2:1). The ratio of solid to liquid was 1:20. The mixed sample was allowed to stand at room temperature for several hours. Extract using a Soxhlet extraction device (Agilent, USA) at 76°C for 4h, then filtered with filter paper, and the residue was recycled. The extract was filtered and concentrated to 20 mL using a rotary evaporator. The extraction residue is dried. The concentrated extract and residue were all refrigerated at 4°C.

2.2.2 VOCs analysis by GC/MS

One milliliter of the extracting solution was aspirated into a 2 ml centrifuge tube, mixed with anhydrous sodium sulfate for 1 hours at 20°C, and centrifuged for 3min, speed is 1200 rpm. 700 ml of the supernatant was removed into a reagent bottle for GC/MS detection. The GC had capillary columns that were 30 m × 250 μm × 0.25 μm. The initial temperature was 50°C, and was heated at 5°C /min until 260°C, kept for 4 min, heated at 5°C /min until 260°C, and kept for 2 min. The carrier gas was He, the purity of He was 99.99%, the injection amount was 1 μL, and the column pressure was 57.4 KPa. There is no split mode. Set 280°C to the vaporization chamber temperature.

Enter the MS program, ionization: 70 eV, ionization current: 150 μA (EI). Carrier gas flow rate: 1.2 ml / min. Ion source temperature: 230°C. Quadrupole temperature: 200°C [12-14].

2.2.3 VOC analysis by TG

The Thermal Gravimetric Analyzer that was used had a balance sensitivity of 0.1 μg. The temperature control range is from 20°C to 1000°C. The SCC sample was placed in TG crucible. The temperature program starts at 20°C and increases at a rate of 10°C/min up to 600°C. The carrier gas is nitrogen, the purity is 99.99%, and the rate is 40 mL/min[15-20].

2.2.4 Analysis of SCC residues and solids by FTIR

All samples were dried at 80°C for 4 h and then stored in a dry environment to ensure accurate detection[21]. The potassium bromide was ground and passed through a 200-mesh sieve and placed in a muffle furnace. The temperature was kept at 160°C for 5 h. And then removed under a heating lamp cover. 200 mg of potassium bromide were placed in an agate mortar with a smooth surface, 0.5-2 mg of the sample is mixed with potassium bromide, and the pressed sample is tested in an infrared spectrum scanner after tableting. (SHIMADZU, IRAffinity-1)[10,11].

2.2.5 SCC solid and extracted residue analysis by SEM

The SCC sample and its extracted residue were baked at 70°C for 12h. A small amount of sample was spread onto double-backed cellophane tape that was attached to a stub, before coating the sample with gold-palladium at room temperature 32°C for 1 min. A scanning electron micrograph of the sample was taken with a scanning electron microscope (JSM-6380LV) at an accelerating voltage of 15 kV. Distance: 100 μm. The particles were imaged between 200 x and 2000 x nominal magnification.

3. RESULTS AND ANALYSIS

3.1 VOCs characteristics of SCC extractives

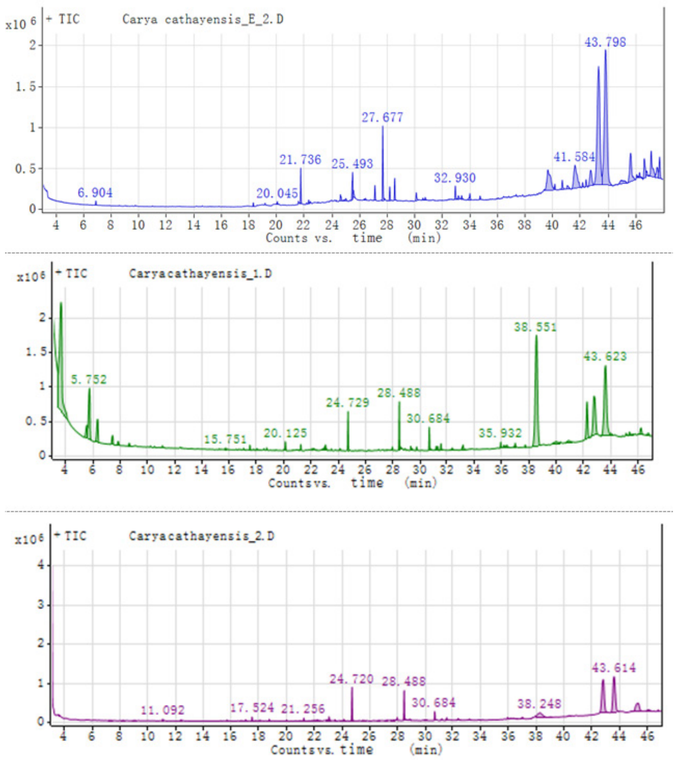


Figure 1: Carya cathayensis_E_2.D, Carya cathayensis_1.D and Carya cathayensis_2.D are GC/MS chromatogram of VOCs from SCC. They represent three extraction solvents: ethanol, petroleum ether and benzene/ethanol.

Si compounds appear in all samples. 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- is present in the VOC of the SCC extract. The extract contents of ethanol, petroleum ester and benzene/ethanol were 0.95%, 5.69% and 1.83%, respectively. The extraction efficiency of the naphtha tropisol that was extracted with petroleum ether was higher in the three extraction solvents. The results suggested that the nerolidol was more soluble in petroleum ether. The retention time was 24.729 min. Note, nerolidol is a type of aromatic organic compound (formula =$C_{15}H_{26}O$) [22].

Vitamin E was present in the VOCs of two SCC extracts. The content extracted with petroleum ester and benzene/ethanol was 26.8% and 16.19%. The retention time was 43.623 min. Vitamin E is a vitamin. The hydrolysate is a preferred antioxidant. Since oxidized low-density lipoprotein is found to be involved in the formation of atherosclerosis, vitamin E can be used as a nutrient for the treatment of coronary artery disease. Vitamin E is also used to treat mild cognitive impairment[23, 24].

The γ-sitosterol was present in the VOCs of two SCC extracts. The content extracted by ethanol and petroleum ester was 4.27% and 9.01%. The extraction efficiency of the petroleum ether was higher. The retention time was 45.36 min. Experimental studies of γ-sitosterol with four different target proteins have shown that γ-sitosterol can be well docked with various targets associated with diabetes. Gamma-sitosterol can be used as a drug for the treatment of diabetes[25].

Phytol acetate was present in the VOCs of two SCC extractives. The content extracted by petroleum ester and benzene/ethanol was 1.91% and 1.22%. The extraction efficiency of the two extraction solvents for Phytol showed that the rates were similar and the extraction rate was low. The retention time was 30.684 min.

Heptacosane was present in the VOCs of two SCC extractives. The content extracted by petroleum ester and benzene/ethanol was 26.9% and 12.84%. The petroleum ether was more efficient in extracting Heptacosane. is more efficient. The retention time was 42.787 min.

Squalene was present in the VOCs of the phenylethanol extract. The content extracted by phenylethanol was 0.19%. According to various studies, olive oil has a role in promoting cancer treatment compared to other vegetable oils. The squalene detected in olive oil may be part of the beneficial effects of olive oil[26, 27].

Table1: VOCs of three COSC extractives.

No.	Retention time/ min	Compounds	Relative content/%		
			Ethanol	Petroleum ether	Phenylethanol
1	5.578	cis-Bicyclo[4.2.0]octa-3,7-diene			1.96
2	6.349	1,3,5-Cyclooctatriene			2.9
3	24.729	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	0.95	5.69	1.83
4	30.684	Phytol, acetate		1.91	1.22
5	31.19	Squalene			0.19
6	42.263	Heptacosane			4.58
7	42.787	Heptacosane		26.9	8.26
8	43.623	Vitamin E		26.8	16.19
9	7.278	1-Octanol, 2,7-dimethyl-		0.25	
10	8.243	Bicyclo[3.1.1]heptan-3-amine,2,6,6-trimethyl-		0.22	
11	9.051	Bicyclo[3.1.1]heptan-3-amine,2,6,6-trimethyl-		0.2	
12	11.092	Octane, 2,4,6-trimethyl-		0.32	
13	12.424	2-butyl-1-octanol		0.43	
14	17.074	2-butyl-1-octanol		0.3	

15	17.524	Dodecane, 2,6,11-trimethyl-		0.65
16	17.754	2-butyl-1-octanol		0.14
17	18.057	Tridecane		0.17
18	18.765	Dodecane, 2,6,11-trimethyl-		0.32
19	23.075	Tetradecane, 2,6,10-trimethyl-		0.74
20	24.15	Dodecane, 2,6,11-trimethyl-		0.36
21	27.983	Tetradecane, 2,6,10-trimethyl-		0.56
22	28.488	3-Methyl-2-butenoic acid, tridec-2-ynyl ester		5.06
23	45.36	.gamma.-Sitosterol	4.27	9.01
24	18.3	4,11,11-trimethyl-8-methylene-, [1R-(1R*,4Z,9S*)]-	0.12	
25	27.677	Neophytadiene	2.45	
26	28.182	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.19	

3.2 Volatility Characteristics of SCC

There are three stages of rapid loss during the 90°C -386°C analysis period. (Figure 2). The first is from 30°C to 90°C, and the amount at 90°C is about 5839.52 μg. The mass loss caused by this stage is due to the evaporation of water and a small amount of volatile substances. The next stage is between 225°C and 386°C, reaching 3397.1 μg, or 43.82%. Obvious peaks can be observed in the curve between 90°C and 600°C. The rate reached a maximum at 366°C, indicating that the SCC mass loss was the fastest at this time, and the rate was 0.415% / °C. From 386°C, the quality begins to enter the slow loss period, and after 600°C, the pyrolysis is completed. The mass reduced to 2448.1 μg, with the loss of 59.6%. The DTG curve shows the range of mass loss rates for the three stages. There are three temperature turning points during the period. The three time points of SCC quality change significantly due to the rapid pyrolysis of macromolecules into small molecules. The study of this curve provides data for the heat treatment of SCC.

3.3 Changes in chemical composition and residue of SCC

Through the infrared spectroscopy, the infrared spectroscopy method was used to detect the change of the groups in SCC and its extracted residue[28]. As shown in Figure 3, the overall trend of the infrared absorption peak of SCC and the extracted residue did not change significantly. As shown in Figure 3, the infrared spectrum of pecan shells shows that there are 13 strong absorption peaks. First, there are two strong absorption peaks near 750 cm^{-1} to 850 cm^{-1}, which may be vibrations of aromatic compounds Peak, there are two absorption peaks near 1000 cm^{-1} to 1150 cm^{-1}, which may be alcohol species, There are three peaks near 1200 cm^{-1} to 1400 cm^{-1} wavelength, possibly C-OH Bond, ether, C = O bond or N-containing compound[13,29]. There are two strong absorption peaks near the wavelength of 1400 cm^{-1} to 1550 m^{-1}, indicating that there may be hydrocarbon CH bonds and carbonyl C = O bond aldehydes, and two of them are about 1600 cm^{-1} to 1700 cm^{-1} Strong absorption peaks, possibly carbonyl C = O bonds, aldehydes, ketones, lipids or organic carboxylic acids, anhydrides and salts, have an absorption peak near 2900 cm^{-1}, possibly due to hydrocarbon-CH3 vibrations Peak, 3300 cm^{-1} around the emergence of an absorption peak, may be organic carboxylic acid, anhydride and salts, or amide substances.

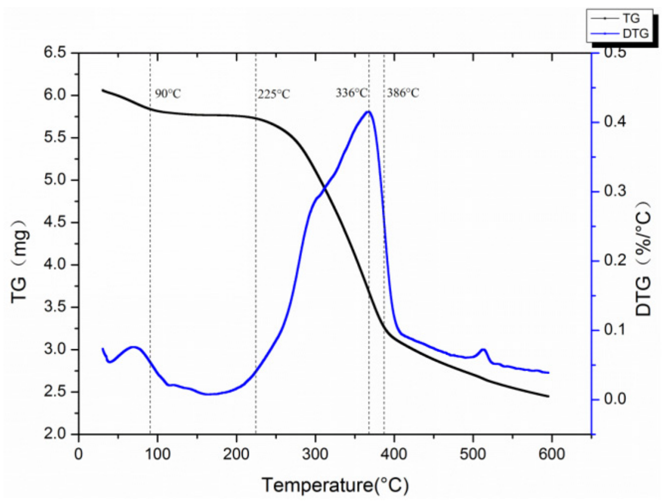


Figure 2: Thermogravimetric curve of SCC.

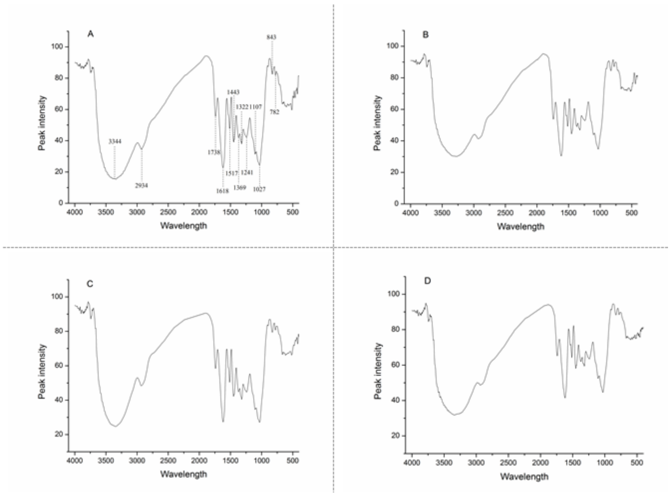


Figure 3: A, B, C and D are infrared spectra of SCC and its three organic extractives. They represent three extraction solvents: ethanol, petroleum ether and benzene/ethanol.

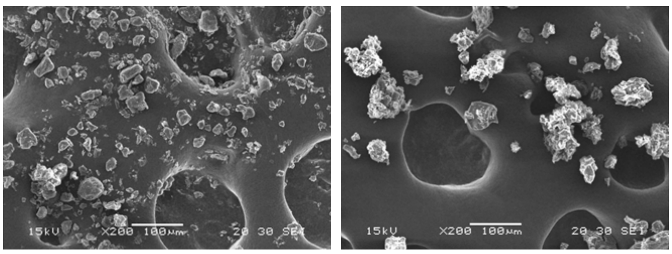
3.4 Micromorphology Characteristics of Extracts from SCC

Scanning Electron Microscopy (SEM) is a method of observing samples by micromorphology[30-32]. It is an indication of the sample being displayed by secondary electronic signal imaging. The sample was irradiated by an electron beam. The effects of electron beam and sample generation. The secondary electrons can feed back the surface of the sample and image it at a point to obtain the image. We used the pecan shell and its three types of extraction residues in × 200 and × 2000 multiple of multi-site scanning, and selected the representative of the overall micro-morphology of the site to observe the camera. Each sample in the SEM × 200 and the SEM × 2000 were selected to produce two representatives of the scanning photos, as shown in Figure 4. A more detailed observation of the surface morphology of pecan powder was obtained by selecting a particle for a variety of magnification observations at 200 times, 1000 times, 2000 times, 3500 times, 8000 times, and 12000 times, see Figure 6.

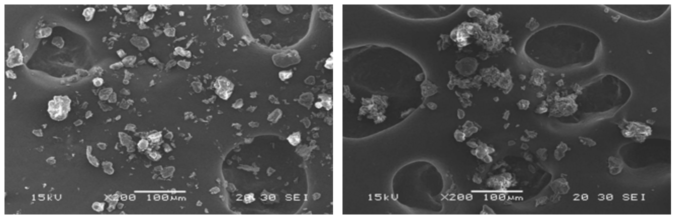
The SEM × 200 times of the SCC showed a variety of different micro-morphologies, see Figure 4. The morphology has an overall hole-like structure, the surface had spherical, ellipsoid, and block shapes. The structural differences were more obvious, with a rough surface granular material. The overall pore structure left only a few large particles with a more angular granular material during the ethanol, the phenylethyl alcohol, and the petroleum ether reagent extraction.

The SEM × 2000 times of the SCC showed that the powder surface was smoother, mostly oval, see Figure 5. The surface of SCC powder was no longer smooth after the ethanol extraction, where it became a layered, angular, oval granular material.

Figure 6 showed that when a particle on SCC powder was selected for a variety of multiple observations, the surface particles observed at 3500 times were the clearest. From magnifications between 8000 and 12000 times, there was poor pecan conductivity, and the equipment was unable to product quality photos. The photos were pixilated and specific shapes on the particle surface cannot be observed.

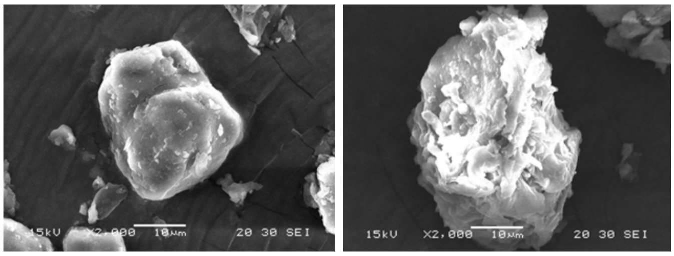


SEM×200 the powder of SCC: SEM×200 Ethanol extraction residue of SCC:

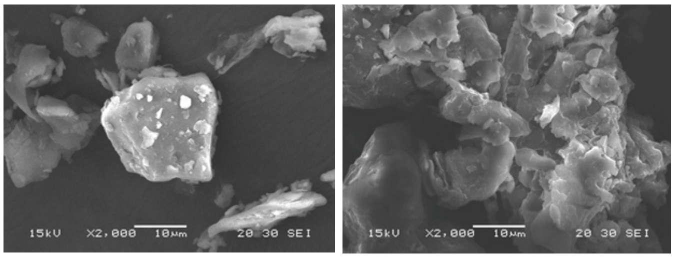


SEM×200 Petroleum ether extraction residue of SCC: SEM×200 Phenylethanol extraction residue of SCC:

Figure 4: Electron microscopy of SCC under SEM×200.

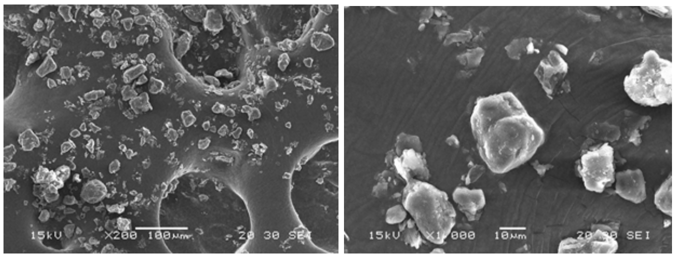


SEM×2000 the powder of SCC: SEM×2000 Ethanol extraction residue of SCC:

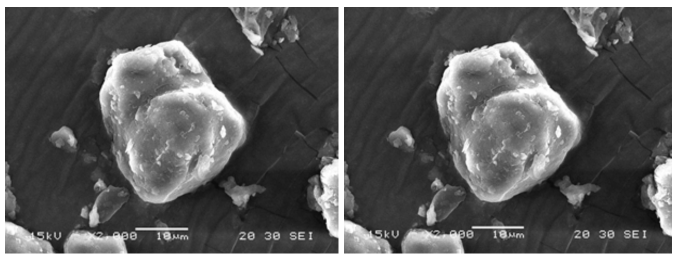


SEM×2000 Petroleum ether extraction residue of SCC: SEM×2000 Phenylethanol extraction residue of SCC:

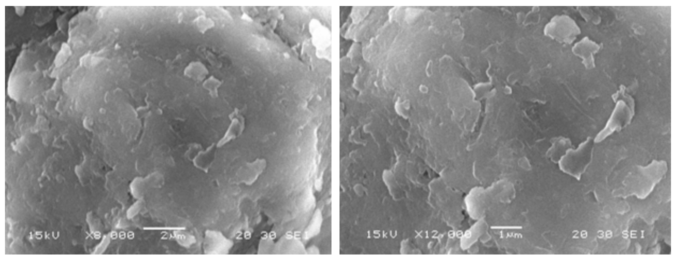
Figure 5: Electron microscopy of SCC under SEM×2000.



SEM×200 the powder of SCC: SEM×1000 the powder of SCC:



SEM×2000 the powder of SCC: SEM×3500 the powder of SCC:



SEM×8000 the powder of SCC: SEM×12000 the powder of SCC:

Figure 6: Electron microscopy of SCC under different conditions.

4. CONCLUSION AND DISCUSSION

It is known from experiments that the ethanol extract of SCC is mainly alcohol, the petroleum ether extract is mainly an alkane and an organic acid, and the benzene/ethanol extract is mainly an ester. The 1, 6, 10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E) could be used for the preparation of roses, lilacs, and other flavors. Neroli can identify new carcinogenesis inhibitors in the large intestine. The structure of the nerolidol causes it to have an effect on the synthesis of the protein. This is not confirmed. The United States allows the use of naproxen as a flavoring agent. Isothiol can improve the anti-proliferative effect of doxorubicin in colorectal cancer cell lines[33-35]. Vitamin E could be used to prevent the occurrence of myopia and play a role in preventing atherosclerosis. Vitamin E could inhibit the free radical reaction in vivo, inhibit the formation of carcinogenic substances, reduce the activity of mutagenic substances, and enhance the expression of tumor suppressor base PS, suggesting that vitamin E has anti-cancer effects[36-38]. It was determined which gamma. -Sitosterol were therapeutic targets by studying the ligand γ-sitosterol docking have several different target proteins. Previous literature suggested that it could be docked with various diabetes-related targets. The gamma-sitosterol could be developed into an effective antidiabetic drug[25]. The extracts were analyzed by GC/MS, and the comet assay of MTT revealed the phytochemicals that can be utilized in the genus Lagerstroemia. γ-sitosterol makes it an important component, with contents ranging between 14.70-34.44%. The extracts, except for the L. speciosa ethanol extract, showed a high percentages of cell viability. The IC50 value of the ethanol L. speciosa extract (0.24 mg/mL) predicted an LD50 of 811.78 mg/kg, placing it in the toxic chemicals. The evaluation showed that the four tested species had DNA damage during induction. According to previous studies, γ-sitosterol has antihyperglycemic activity through insulin secretion. People should be careful about their toxicity[39]. Within the Phytol, the acetate extraction was large, but there was no previous analysis of such substances. The Heptacosane extracted more, but lacks studies that do not pertain to its discovery in tobacco. Squalene could be used as an adjuvant therapy, where it could improve the state of hypoxia within cardiovascular and cerebrovascular disease. It could also be used for hypercholesterolemia and chemotherapy, as well as chemotherapy-induced leukopenia. Epidemiological and laboratory studies have shown that olive oil has a therapeutic and preventive effect on cancer compared to other vegetable oils. Among them, squalene may be part of the beneficial effect of olive oil[40]. The chemopreventive effect of squalene on colonic abnormal crypt foci (ACF) was found in this study. The effect of squalene on serum cholesterol levels in mice revealed that sulindac inhibited the development of ACF. At the same time, the diversity of the crypts is also reduced. Inhibition of total ACF induction and crypt diversity was > 47% ($P < 0.001$). 1% squalene has no effect on cholesterol levels. Squalene can inhibit colonic ACF formation and crypt diversity. It is consistent with the idea that squalene has an effect of treating colon cancer[26, 41]. In the future, we could improve the extraction efficiency of squalene by optimizing the extraction technology, thus improving the utilization value of the SCC.

The thermogravimetric curve of the SCC showed that there three key temperature turning points throughout the total weight loss. The pyrolysis rate of the pecans changed at these temperature points. The three temperature points (90℃, 225℃, and 386℃) could be used as the theoretical basis for the pyrolysis analysis of the SCC.

The results showed that the SCC sample contained aromatic compounds, alkanes, ethers, organic carboxylates, and organic compounds. The results demonstrated that the SCC contained aromatic compounds, alkanes, ethers, organic carboxylates Acids, anhydrides and salts, esters and alcohols, and amides.

By comparing the echocardiography before and after the extraction of SCC, it was found that although the surface particles were reduced after extraction, the entire pore structure did not change. There were some small particles aggregated together to form coarse particles with a coarse surface. The overall structure of the SCC was hard, suggesting it could be used as a grinding material.

This study showed that SCC were highly available. The SCC contained vitamin E, gamma. -Sitosterol, squalene, and other substances that could be applied for medical use. There were other available materials such as naproxen, organic acids, and alkaloids. The observations of the electron microscopy showed that the SCC powder was perforated with many coarse and small granular substances. The SCC was hard and could be studied by using a more refined electron microscope. The SCC could be thermally cracked, by the liquid phase analysis and other methods, in order to utilize the pecan shell for maximized waste usage.

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