frontiers in Energy Research

ORIGINAL RESEARCH
published: 27 May 2020
doi: 10.3389/fenrg.2020.00090

Efficient Extraction of Bioenergy From *Cinnamomum camphora* Leaves

Zanpei Zhang†, Xuanxuan Wu†, Yong Lai, Ximei Li, Dangquan Zhang and Yuanyuan Chen**

College of Forestry, Henan Agricultural University, Zhengzhou, China

Cinnamomum camphora is an evergreen tree native to China and distributed to some Asian countries. Its leaves are rich in bioactive compounds and their pharmacological effects are well-known. A potent method to extract and preserve the active biological substances is crucial for latter application in bioenergy and as chemical raw material. In this study, the metabolite profiles of *C. camphora* were comprehensively analyzed by using FT-IR and GC-MS after extracted by three type of solvent: ethanol, benzene, and acetone. The identified chemical compounds were then classified into different functional groups based on previous study. Our result proposes the potential application of *C. camphora* into biomedicine, bioenergy, and chemical raw materials industry.

Keywords: renewable biomass, *Cinnamomum camphora* leaves, extract, bioactive components, bioenergy

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****Correspondence:***
Dangquan Zhang
zhangdangquan@163.com
Yuanyuan Chen
cyuan091@163.com

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Bioenergy and Biofuels, a section of the journal Frontiers in Energy Research

Received: *10 November 2019*
Accepted: *28 April 2020*
Published: *27 May 2020*

Citation:
Zhang Z, Wu X, Lai Y, Li X, Zhang D and Chen Y (2020) Efficient Extraction of Bioenergy From Cinnamomum camphora Leaves. Front. Energy Res. 8:90. doi: 10.3389/fenrg.2020.00090

INTRODUCTION

Increment of human population has huge impact to energy consumption that mainly depends on non-renewable energy such as fossil fuel, oil, coal, and natural gas to sustain the human need. To date, only 11% of energy is generated from renewable energy (https://www.iea.org/weo2018/) due to several shortages such as efficiency and resources. Therefore, researcher started to explore resource like *Cinnamomum camphora* as alternative to fossil fuel.

Cinnamomum camphora (Lauraceae) is commonly known as camphor tree, are widely distributed in subtropical zones, including southeastern China and northeastern Australia (Yakefu et al., 2018). *C. camphora* is famous for its ornamental, economic, and medicinal value (Chen and Dai, 2015). In particular, *C. camphora* contain oil gland cells that can be used to extract camphor oil (Afrin et al., 2019). The essential oil has antifungal activity (Sattar et al., 1991), insecticidal and insect repellent properties (Liu L. et al., 2018), antioxidant (Fu et al., 2016), anti-aging, anti-bacterial, and anti-inflammatory effects (Chen C. et al., 2018; He et al., 2018; Chen et al., 2019). The bark is used in treating limb ulcer and the fruit of *C. camphora* has properties to relieve fever, treat common flu, and dysentery (Satyal et al., 2013). The root of *C. camphora* promote blood circulation, improving rheumatism, treating stomach diseases, and bruises (Jiang et al., 2016; Chen S. et al., 2018; Liu X. et al., 2019).

The application of camphor oil is not limited to medical application but also as flavors, soap, painting materials, mineral processing, plastics polymer, explosives, and as preservative (Guo et al., 2016). The leaf powder of *C. camphora* was reported to absorb and remove dye (Wang et al., 2014), copper (II) ions (Chen et al., 2010a), and Pb (II) (Chen et al., 2010b) from aqueous solution. The broth of *C. camphora* represents new green material in palladium nanoparticles formation to replace synthetic nanoparticles (Yang et al., 2010).

To tackle the problem of resources, supply in application as renewable energy, the large-scale production of *C. camphora* has been established via *in vitro* micropropagation technique (Shi et al., 2016). To fully utilize the resources of *C. camphora,* the biosynthesis pathway of secondary metabolomics production is also well explored (Chen et al., 2017). Volatile constituents from *C. camphora* have been detected and identified such as camphor, eucalyptol, linalool, nerolidol, limonene, β-pinene, -terpineol, and other volatile substances (Yang et al., 2016; Xu Y. et al., 2018). In particular, the biosynthesis pathway of secondary metabolomics production is also well explored (Chen et al., 2017). The constituent of oil composition was also reported (Jiang et al., 2016; Liu Z. et al., 2018). However, there is no complete list of metabolite profile available for different solvent types and hence it is difficult to suggest the optimal extraction method for *C. camphora.* As we know different solvent type has affinity to different type of metabolites and results to the extraction of different functional groups, we tested three types of solvents and listed the metabolite profile from the three solvents.

MATERIALS AND METHODS

Extraction of Leave Sample by Different Types of Solvents

An amount of 500 g of *C. camphora* leaves were cleaned, dried, and crushed into fine powder. The powder is subsequently sieved through a 200 mesh, the sample was divided into three parts and soaked with three types of solvents: ethanol, acetone, and benzene with ratio of material to liquid 1:15 (Xu K. et al., 2018; **Figure 1**). The mixture was boiled in water baths for 4 h and the temperature were set to the boiling point of each solvent. After removal from water bath, the solution were filtered by pump filter and then dried up using rotary evaporator. The solvent was collected once it reach approximately amount of 20 mL (Kumar and Kumari, 2019).

FT-IR Analysis

Solid Sample

After 200 mesh screening, the solid powder was taken as the sample. 0.5–2 mg of sieved powder was mixed with an appropriate amount of potassium bromide. It was then ground into fine and uniform particles in a mortar. A sample of this powder was taken and pressed to flatten the sample into an evenly transparent sheet. This sheet was then dried in an oven.

Liquid Sample

Solvents of ethanol, acetone, and benzene were pipetted out by using manual pipette and dropped evenly in the center of pure potassium bromide thin films. The film was then oven dried before it was measured at 4,000–400 cm^{-1} by Fourier transform infrared spectroscopy (Shimadzu, IR affinity-1) (Wu et al., 2019).

Gas Chromatography-Tandem Mass Spectrometry

GC-MS Determination

The program was set as initial temperature at 5°C without retention, increased and remained from 13 to 18°C, and then

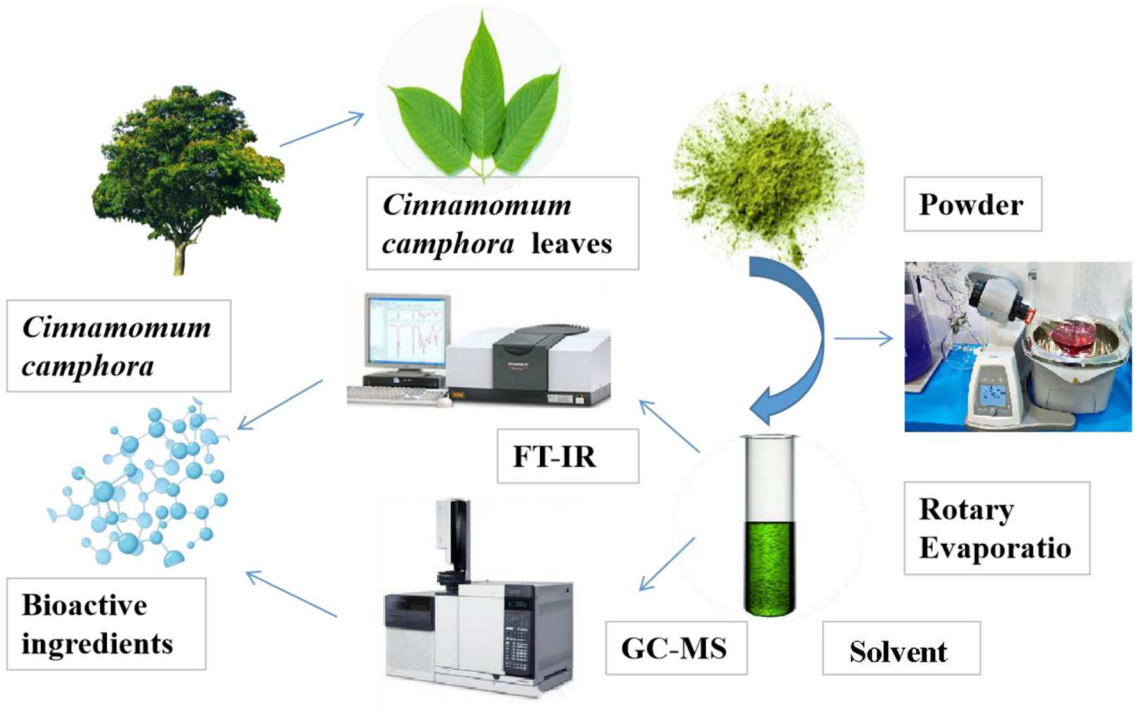

FIGURE 1 | The preparation process of *C. camphora* leaves.

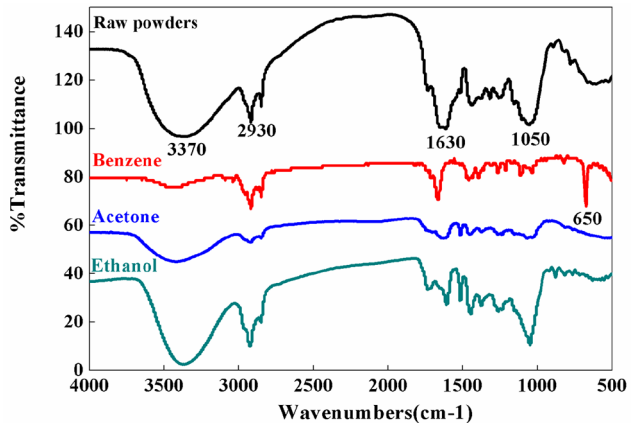

FIGURE 2 | The FTIR results of the raw powder and three solvents from *C. camphora* leaves.

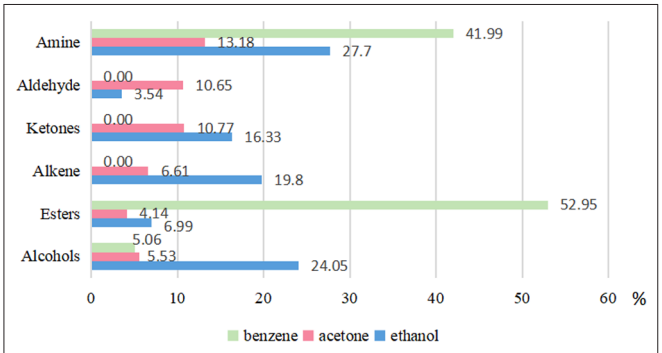

FIGURE 3 | Content percentage of three solvents from *C. camphora* leaves.

rose to 30°C for 5 min. The inlet temperature was set to 28°C with column flow of 1.0 mL/min, and split ratio was 20:1. A fused silica HP-5ms capillary GC column (30 m × 0.25 mm × 0.25 μm) was used. Mass spectrometry proceeded under the following conditions: ionization mode was EI, electron energy of 70 eV, ion source temperature of 23°C, scanning starting point of 30–60°C (Liu Z. et al., 2019; Ma et al., 2019).

Data Processing

The raw MS spectra data were first normalized by peak area and based line corrected. The chemicals composition detected were then identified by in house databased program and were grouped into similar functional groups based on previous study.

RESULTS

Characteristics of Solid and Liquid Extracts of *C. camphora*

The FTIR result of the fine powder sample show peaks and variation observed in the regions of 3,730–3,050 cm^{-1}, 3,050–2,500 cm^{-1}, 2,000–1,500 cm^{-1}, and 1,500–500 cm^{-1} (**Figure 2**). According to the infrared functional group comparison table, there is a strong absorption peak in the region of 1,500–500 cm^{-1} represents to ether bond, which can be identified as aliphatic ether and aromatic ether. The region observed at 3,050–2,500 cm^{-1} represents mainly olefins, alkynes, and aromatic compounds, resulted by the stretching vibration absorption of unsaturated carbon C-H.

Benzene solvent causing a sharp peak observed at 650 cm^{-1}. The presence of organic halides could be determined due to the expansion and contraction of aliphatic C-Br. In the 2,000–1,500 cm^{-1} region, there are olefins due to the vibrations of the bonds of unsaturated carbon, which are consistent with the compounds predicted in the same interval of raw powder sample. In the acetone solvent, the region around 1,500–500 cm^{-1} may represent alcohol, phenol, aromatic ether, and ester. Finally, the solvents of ethanol may contain functional group of olefins and amines as shown in the region 1,500–500 cm^{-1} and aromatic ethers as observed at region 1,050 cm^{-1}.

These results show that the infrared spectra of the ethanol as solvent are similar to those of raw powder, indicating that ethanol is a better solvent for extracting bioactive components from *C. camphora* leaves than benzene and acetone.

Volatile Components of the Three Extracts

A total of 120 compounds were detected from the acetone solvent by GC-MS (**Appendix Figure 1**, **Appendix Table 1**). Amongst them, 94 were identified and group into functional groups of amines (13.18%), ketone (10.77%), alkenes (6.61%), alcohol (5.53%), and esters (4.14%) (**Figure 3**). There are 58 of chemical compound were detected in ethanol solvent, in which 48 are organic compounds (**Appendix Figure 2**, **Appendix Table 2**). These compounds were classified into amines group (27.70%), Tetrazol-5-amine and N-(3, 4-dimethoxybenzyl) (25.44%), alcohols (24.05%), alkenes (19.80%), ketones (16.33%), and esters (6.99%). In benzene solvent, only 12 chemical compounds were detected and 4 substances were identified and classified into esters (52.95%), amines (41.99%), and alcohols (5.06%) (**Appendix Figure 3**, **Appendix Table 3**).

The results showed that the three solvents for *C. camphora* leaves have outstanding functional properties that serve as chemical raw materials for different green application including cosmetics, spices, food additives, chemicals raw materials, and biomedicine. The percentage of the bioactive components detected are highest in benzene solvent (41.99%), followed by ethanol solvent (33.53%), and acetone solvent (28.23%). For cosmetics and food additives application, the best extraction method is ethanol as solvent which enables recovery of 2 and 10.44% of active compounds, respectively. The functional content of spices was highest when acetone as solvent (15.51%) (**Figure 4**).

CONCLUSION

In order to enhance the application value of *C. camphora* leaves, the effects of different extraction methods on the bioactive substances in *C. camphora* leaves were investigated. Three common solutions of ethanol, acetone, and benzene were used to extract the substances in *C. camphora* leaves. There were significant differences in the types and proportions of bioactive

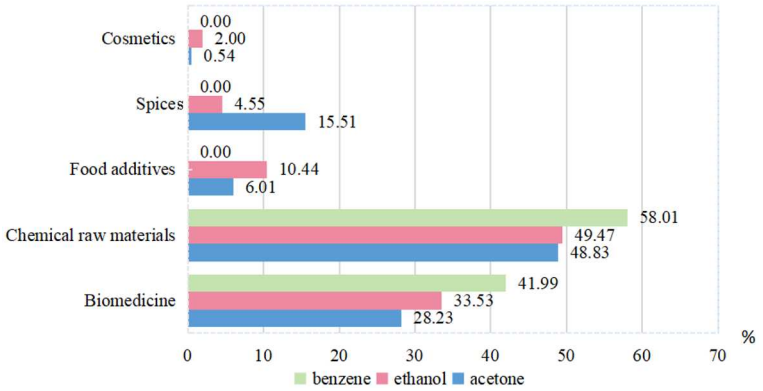

FIGURE 4 | Potential application based on thefunctional groups constitute of three solvents from *C. camphora* leaves.

compounds obtained by the three extraction methods. The FT-IR results showed that ethanol can effectively extract the bioactive compounds in *C. camphora* leaves. The GC-MS results indicated that more amines and alcohols were obtained from *C. camphora* using ethanol as solvent. The amines can be used in cosmetics and biomedicine, and the alcohols can be widely used in industrial fields. Aldehyde, ketones, alkene, and esters in ethanol solvent can also be applied in the fields of chemical raw materials, biomedicine, bioenergy, and spices.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

DZ contributed to the integrity of the entire research, research concepts, and research design. YC contributed to the literature research, manuscript editing, and critically revision. ZZ and XW did the experimental studies, data acquisition, data analysis, and manuscript preparation. YL and XL gave guidance and participated in the experiments.

FUNDING

This project was supported by the talent project (DZ) of Henan Agricultural University, China, and the Project of Henan Provincial Science Research, China (192102110174).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenrg.2020.00090/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.