



## An Overview on Extraction Techniques of Bioactive Phytochemicals- Plai Plant

Sini Baby\*, Dimal Jose, Agnes K Mathew, Biniya John, Antony Varghese Antony.

Department of Pharmaceutical Chemistry, Nirmala College of Pharmacy, Muvattupuzha, P.O, Ernakulam District, Kerala, 686661, India and affiliated to Kerala University of Health Science, Thrissur, Kerala, India.

### ABSTRACT

Medicinal plants are gaining more importance these days as it can provide better treatment alternative for many common diseases with less side effects. One such herb Plai-Zingiber cassumunar of Zingiberaceae family proved to have many pharmacological actions like anti-inflammatory, anti-microbial, cytotoxic etc. Various extraction methods for its rhizomes are getting more interest as it constitutes numerous Phytochemical constituents to elicit various actions. Any studies on medicinal plants start with their extraction methods and thus different types of extraction methods are available today. Hence this review focuses on comparison of most common extraction methods for various active constituents from rhizomes of Zingiber cassumunar.

### Key words:

Medicinal Plants, Herb Plai, Zingiberaceae, Extractions, Zingiber montanum.

### Article History:

Received On: 19.09.2019,

Revised On: 17.12.2019,

Accepted On: 19.12.2019.

### \*Corresponding Author

Name: Dimal Jose

Email: dimaljv@gmail.com

<http://doi.org/10.37022/WJCMPR.2019.01067>

## INTRODUCTION

For the biological evaluation of herbal plants extraction is found to be one of the first and foremost crucial step. Various modern extraction methods are developed for the enhancement of traditional remedies. To develop various analytical methods which are required to analyze the different constituents found in botanical and herbal preparations or formulation of the sample is of great importance. Modern sample preparation techniques are prevalent and have greater advantages over conventional methods in order to ensure that herbal products of a higher quality are made available to the consumer worldwide. Traditional medicines are being commonly used since ages to improve health care and is used in the prophylaxis and treatment of a number of physical and mental illness. Various healing methods to tackle different health and life-threatening diseases were developed ancient societies. In this article we come across various extraction methods and pharmacological activities of Zingiber cassumunar.

## ZINGIBER CASSUMUNAR

Is a synonym of Zingiber montanum and it is belonging to the Zingiberaceae (ginger) family. It is commonly found in Thailand and is known colloquially as plai consisting of underground rhizomes and is perennial herb. The rhizomes are used in treatment of skin diseases, wound healing, muscles, joint problems, menstrual diseases, inflammations and abscess. Zingiber cassumunar is prevalent not only in Ayurveda medicines but also find use in modern medicines. The species is probably indigenous to India and is also cultivated in south east Asia to a great extent for its medicinal uses. It is a commonly used adulterant and substituted in the world wide trade for Ginger (Zingiber officinale). The plant also finds place in the global compendium of weeds. Just like other Zingiber species Zingiber cassumunar are propagated using seeds and rhizomes and commonly seen to thrive in habitats that are

moist. It considered as a weed are cultivation escape in Puerto rivo and Greater Antilles.

## METHODS OF EXTRACTION

### 1. SEPARATION OF CYTOTOXIC COMPOUNDS

700g of finely ground rhizomes of Zingiber cassumunar was taken and extracted using cold maceration technique. The rhizomes were soaked in non-polar solvent such as petroleum ether at room temperature for 72 hours. The Extraction with Petroleum ether was followed by chloroform, ethyl acetate and methanol. The extraction was repeated thrice so that the non-polar organic compounds, waxes and fats are removed. The removal of solvents was done under reduced pressure and the crude extracts were obtained. This crude extract is separated by vacuum liquid column chromatography and column chromatography by using the polarity of solvent. Various fractions thus obtained are detected by TLC. Crude extract of different concentration is assayed for cytotoxicity<sup>1</sup>.

### 2. EXTRACTION OF ZERUMBONE AND KAEMPFEROL DERIVATIVES

Rhizomes of Zingiber cassumunar were shade dried (1200g) was extracted successfully using 95% ethyl alcohol and its 50% (8L, 2 times each for 72 hrs.) at room temperature. Extract obtained was filtered and evaporated by using a rotary evaporator to obtain 132.5g of dried extract. This was then suspended in water and hexane was used for the extraction (1000ml, 3 times) to obtain a water soluble and hexane fraction. The hexane fraction was subjected to silica gel column chromatography to obtain the first compound. The water-soluble fraction was then subjected to CMC gel CHP20P column chromatography and successively eluted using water,

40%, 70% and 100% methyl alcohol to give second fraction. Out of this fraction, fraction no.5,6 and 7 are mixed and subjected to silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub>: methyl alcohol: water =9:1:0.1) to obtain six fractions. These fractions were further subjected to silica gel column chromatography to extract zerumbone and kaempferol derivatives<sup>2</sup>.

### 3. ETHANOLIC EXTRACTION

The rhizomes were frozen and 1 gm of this was ground with liquid N<sub>2</sub> using mortar and pestle, 5ml of 95% ethanol solution was added and then homogenized. This was then collected and centrifuged for 10 minutes at 6000 rpm till the supernatant becomes clear. The supernatant from the centrifuge tube was decanted and kept at -20°C and kept in capped bottle and can be used for antioxidant activity assay<sup>3-6</sup>.

### 4. MICROWAVE EXTRACTION FOR ESSENTIAL OIL

100gm of rhizome that were frozen were cut into small pieces and homogenized 800ml of distilled water was added and distilled in microwave oven for 60minutes. As polar solvents were found to absorb microwave energy more readily than non-polar solvents. Distilled water was used as the solvent. The oil obtained was store in a capped light brown bottle and placed in a refrigerator at 20°C to reduce the exposure to oxygen, light and heat<sup>6-7</sup>.

### 5. SOXHLET EXTRACTION

For the Phytochemical and pharmacognostical evaluation of Zingiber cassumunar, rhizomes were subjected to Soxhlet extraction using various solvents like n-hexane, dichloroform, chloroform, acetone, ethanol and water<sup>7</sup>.

### 6. EXTRACTION FOR TOXICITY STUDIES

The rhizomes of Zingiber cassumunar were cut and essential oil was removed using steam distillation. The residue of the plant was dried at 60°C and then filtered and concentrated using the rotary evaporation to give the ethanolic Zingiber cassumunar extract with a yield of 9.1%. The extract was then used for granular preparation. Safety evaluation including acute and chronic toxicity studies in rat of Zingiber cassumunar rhizome extract were carried out with these granules prepared<sup>8</sup>.

### 7. CLEVANGER EXTRACTION

For the extraction of volatile oil, fresh rhizomes were washed, then removed the outermost shell and sliced into small pieces. Then it is steam distilled in Clevenger type apparatus for 6 hours. Antioxidant activity and active compounds were then analyzed<sup>9-10</sup>.

### 8. HEXANE EXTRACTION

Fresh rhizomes were sliced and shade dried. 300gm of dried rhizomes were extracted with hexane 3 times at room temperature by maceration for 3days. Then it is filtered and filtrate was evaporated at 40°C by rotary evaporator. The extractor obtained is preserved at 4°C protected from light until it was taken out to carry out Phytochemical analysis by GC-MS. A uterine relaxant compound is isolated<sup>10</sup>.

### 9. MACERATION

Aqueous extract of Zingiber cassumunar was prepared by extracting 75g of dried Zingiber

cassumunar leaves with water by maceration and was then evaporated at 60°C to get the extracted product in semisolid consistency. In the same way, ethanolic extract was also prepared by using 20 % ethanol instead of water. The crude extract is then analyzed for inhibitor effect on pancreatic lipase activity<sup>11</sup>.

### 10. SAPONIN EXTRACT

The saponin extract was prepared by modifying a previously reported method. The sample were refluxed with methanol: dichloromethane (1:1) for 30 minutes. Flavonoid, alkaloids and all other unwanted compounds other than saponins were removed by refluxing the dry extract using 4 portion of ethyl acetate: chloroform (1:1). The ethyl acetate: chloroform solvent was removed and methanol was added to the residue which was then subjected evaporation at 40°C to yield required saponin extract. This extract is then evaluated for anti-obesity activity<sup>11</sup>.

### 11. PRESSURIZED LIQUID AND SUPER HEATED WATER EXTRACTION

Zingiber cassumunar has been successfully extracted by pressurized liquid extraction (PLE) using ethanol or methanol and superheated water extraction (SWE). This method requires optimum condition like 1 ml/min flow rate and 5-minute static time. PLE & SWE performed at 100°C for 5 minute and 140°C for 20 minutes respectively. By this method high amount of terpine-4-ol and 1-(3,4-dimethoxy phenyl) butadiene (DMPBD) were obtained which may also possess anti-inflammatory action<sup>12</sup>.

### 12. METHANOL EXTRACT AND ITS FRACTIONS FOR ANTI-INFLAMMATORY ACTION

Fresh rhizomes of Zingiber cassumunar were sliced and refluxed for 6 hours in 70% methanol for three times. The extract obtained is filtered and evaporated under vacuum. This extract is treated with ether, extracted with water three times and evaporated. This fraction obtained is treated with n-hexane extracted using methanol for three times. All the three fractions show anti-inflammatory activity. From n-hexane soluble fraction two oily compounds and crystal that is zerumbone are isolated<sup>13</sup>.

### 13. SOLVENT FREE MICROWAVE EXTRACTION (SFME)

In this extraction in order to get high yield of essential oil, microwave is equipped with Clevenger apparatus. Here sliced fresh rhizomes (200g) are taken in microwave chamber and to which Clevenger apparatus and condenser are assembled. Microwave power and irradiation time are adjusted to have maximum essential oil. It is dehydrated by anhydrous sodium sulphate and stored at 4°C<sup>14</sup>.

## CONCLUSION

Extraction methods have equal importance in the study of medicinal plants. It may affect the phytochemical constituents and pharmacological action of final extract. It can be concluded that each extraction method of Zingiber cassumunar is used to determine its various activities as well as different phytochemical constituents.

## CONFLICT OF INTEREST

None

**ACKNOWLEDGMENT**

We extend our gratitude to our management Rev.Fr. Jos Mathai Mailadath and HOD, Prof Dr. Badmanaban. R for the immense guidance and support.

**REFERENCES**

1. Zulkhairi AM, Aspollah SM, Lian EG, Bustamam AA. Phytochemicals and cytotoxic studies of Zingiber cassumunarRoxb. *J. Trop. Agric. and Fd. Sc.* 2017;45(2):187-197.
2. Hassan M, Adhikari-Devkota A, Imai T, Devkota HP. Zerumbone and Kaempferol Derivatives from the Rhizomes of Zingiber montanum (J. Koenig) Link ex A. *Dietr. from Bangladesh. Separations.* 2019 Jun;6(2):31.
3. Suksaeree J, Charoenchai L, Madaka F, Monton C, Sakunpak A, Charoonratana T, Pichayakorn W. Zingiber cassumunar blended patches for skin application: Formulation, physicochemical properties, and in vitro studies. *Asian journal of pharmaceutical sciences.* 2015 Jul 1;10(4):341-349.
4. Risnawati E, Ainurofiq A, Wartono MW. Study of antibacterial activity and identification of the most active fraction from ethanol extraction of Zingiber cassumunarRoxb. rhizomes by vacuum liquid chromatography. *Journal of Chemical and Pharmaceutical Research.* 2014;6 (9):101-107.
5. Nurkhasanah N, Santoso RD, Fauziah R. The immunomodulatory effect of Zingiber cassumunar ethanolic extract on phagocytic activity, nitrit oxide and reaxtive oxygen intermediate secretions of macrophage in mice. *IOP Conf. Series: Materials Science and Engineering.* 2017; 259:1-7.
6. Bua-in S, Paisooksantivatana Y. Essential oil and antioxidant activity of Cassumunar ginger (Zingiberaceae: Zingiber montanum (Koenig) Link ex Dietr.) collected from various parts of Thailand. *Kasetsart J (Nat Sci).* 2009; 43:467-475.
7. Koparde A, Magdum C. Phytochemical studies and pharmacognostical evaluation of Zingiber cassumunarRoxb. *Asian J. Pharm. Clin. Res.* 2017; 10:129-135.
8. Koontongkaew S, Poachanukoon O, Sireeratawong S, Dechatiwongse Na Ayudhya T, Khonsung P, Jaijoy K, Soawakontha R, Chanchai M. Safety evaluation of Zingiber cassumunarRoxb. rhizome extract: acute and chronic toxicity studies in rats. *International scholarly research notices.* 2014; 2014:1-2.
9. Kamazeri TS, Samah OA, Taher M, Susanti D, Qaralleh H. Antimicrobial activity and essential oils of Curcuma aeruginosa, Curcuma mangga, and Zingiber cassumunar from Malaysia. *Asian Pacific journal of tropical medicine.* 2012 Mar 1;5 (3):202-209.
10. Sukatta U, Rugthaworn P, Punjee P, Chidchenchey S, Keeratinijakal V. Chemical composition and physical properties of oil from Plai (Zingiber cassumunarRoxb.) obtained by hydro distillation and hexane extraction. *Kasetsart J (Nat Sci).* 2009;43(5):212-217.
11. Iswantini D, Silitonga RF, Martatilofa E, Darusman LK. Zingiber cassumunar, Guazumaulmifolia, and Murrayapaniculata extracts as antiobesity: in vitro inhibitory effect on pancreatic lipase activity. *Hayati Journal of Biosciences.* 2011 Mar 1;18 (1):6-10.
12. Chienthavorn O, Poonsukcharoen T, Pathrakorn T. Pressurized liquid and superheated water extraction of active constituents from Zingiber cassumunarRoxb. rhizome. *Separation Science and Technology.* 2011 Feb 25; 46 (4):616-624.
13. Ozaki Y, Kawahara N, Harada M. Anti-inflammatory effect of Zingiber cassumunarRoxb. and its active principles. *Chemical and Pharmaceutical Bulletin.* 1991 Sep 25;39(9):2353-2356.
14. Yingngam B, Brantner A. Boosting the essential oil yield from the rhizomes of cassumunar ginger by an eco-friendly solvent-free microwave extraction combined with central composite design. *Journal of Essential Oil Research.* 2018 Nov 2; 30 (6):409-420.