Comparison Of Ultrasound-Assisted And Conventional Solvent Extraction Techniques For Characterization Of Phenolic And Flavonoid Compounds From fresh Leaves Of Alpinia Mutica

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Abstract

Since a few years ago, the traditional methods, such as the Soxhlet and reflux apparatushave been used most frequently to extract plant material. However, these procedures take a long time and a lot of solvent. Today, the innovative extraction method is employed to extract plant material in order to solve this issue. The ultrasonic-assisted extraction process is superior to the traditional method in that it uses less solvent, takes less time, and produces more bioactive phytoconstituents. *Alpinia mutica* (A.M.) is an Indian herb that is a member of the *Zingiberaceae* family and is used as a traditional treatment. The *Zingiberaceae*family's largest genus, Alpinia, contains over 230 herbs that are widely used throughout Asia's distinctive and sub-peculiar regions. In southern India, the plant is said to contain 9 different species of plants. *Alpinia mutica* is a perennial herb that grows in Malaya and the Kingdom of Thailand. It produces horizontal, subterranean stemmed, scented plants. Although cultivation has undergone a few adjustments, the sorted types are scattered throughout northern Malaysia. The northern region of the Malayan foreland is home to a variety of species, despite the fact that there are some agricultural sources that provide alternatives to A.M. Locals utilise these plants to alleviate stomach gas issues, and the fruits are used to relieve edoema. *Alpinia mutica* leaves were extracted using both traditional and ultrasonic methods using a variety of solvents, including petroleum ether, ethyl acetate, methanol, hydroalcohol, and aqueous solvents. When compared to traditional approaches, the results show that the ultrasonic-assisted extraction procedure yields a high yield of phytoconstituent.In order to determine the overall phenolic and flavonoid content of the extracts produced by both processes, further analysis was performed on them. According to the research, the phenolic and flavonoid content of the extract has been noticeably boosted by the use of ultrasonic-assisted extraction procedures. Additionally, the ultrasonic-assisted extraction method for plant extraction proved to be a quick and effective solution.

Key Words: Alpinia mutica, Ultrasonic-assisted extraction technique, Optimization
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INTRODUCTION

Nowadays, pharmaceutical, food, and nutraceutical industries are studying and using more and more traditional medicinal herbs.
Since ancient times, plants have been employed as the main source of disease therapies, and many plants have been shown to have a variety of functions today (1). Since then, all societies around the world have utilised plants, with India having one of the oldest, wealthiest, and most diverse cultures (2). The favourable action of plant drugs in the treatment of more diseases was present in the analysis and standard jurisdiction (3).

Therefore, using novel extraction techniques to get around the issues with traditional methods is necessary in the modern world. In this situation, ultrasonic-assisted extraction (UAE) can increase the extraction efficiency by generating high-purity extracts with little solvent use and a shorter extraction time. The extraction of bioactive compounds from plant materials is a common practise in the UAE (4). UAE is known for its use of green technology because it uses less organic solvent.

The Zingiberaceae family's largest genus, *Alpinia*, contains over 230 herbs that are widely used throughout Asia's distinctive and sub-peculiar regions. In southern India, the plant is said to contain 9 different species (5, 6). *Alpinia mutica* (A.M.) is a perennial herb that grows in Malaya and the Kingdom of Thailand. It produces horizontal, underground-stemmed, aromatic plants. Although cultivation has undergone a few adjustments, the sorted types are scattered throughout northern Malaysia. A number of species are dispersed at the northern part of the Malayan foreland, despite the fact that there are some agricultural sources that provide alternatives to A.M. Locals use these plants to alleviate stomach gas issues, and the fruits are used to relieve edoema (7). The effectiveness of a methylene chloride extract against Bacillus subtilis and Staphylococcus aureus growth was tested in order to determine whether or not it might be used for lipid oxidation. There were many bioactive compounds found in the rhizomes of A.M., including flavokavain B, pinocembrins, 5,6-dehydrokawain, and 1,7-diphenyl-5-hydroxy-6-hepten-3-one. (8).

METHODS AND MATERIALS:

Collection and authentication of plant material

Fresh *Alpinia mutica*plant was collected in summer seasons (May–Jun) from local areas of Hyderabad, Telangana, India. These parts were botanically authenticated by P.V. Prasanna, Scientist "G" and Ho O, Botanical Survey of India, Deccan Regional Center, Hyderabad, Telangana, India. A sample voucher (BSI / DRC/2020-21/ Identification / Tech /305) has been provided by the Department of Pharmacognosy, Geethanjali College of Pharmacy, Hyderabad, India. This authentication voucher will be used for the future reference purposes. All the reagents used in our current study of analytical grade.

Conventional Method:

The plant parts were gathered, air dried at room temperature in the shade, and then pulverised into a coarse powder. The powdered plant components were extracted in a Soxhlet apparatus using petroleum. ether, ethyl acetate, methanol, hydroalcohol, and aqueous solvents, in that order, to get the desired extracts. By distillation, the solvent was eliminated, and a rotary evaporator was used to concentrate it under vacuum. Desiccators were used to store all of the dried extracts until future usage (9).

Ultrasound assisted extraction method

A 250 mL Erlenmeyer conical flask was filled with 20 mL of water and 1 g of dry, pulverised plant material to create the extracts. The ultrasonic bath was run for 30 minutes at 60 °C with a maximum input power of 240 W and a frequency of 35 kHz. After incubation, the produced extracts were filtered and kept at 4°C without the addition of any preservatives until analysis (10).

Phytochemical quantification of plant extracts

Estimation of total phenolic content

Principle

The phenolic content was measured using the Folin-Ciocalteu method. In colorimetric assays of polyphenolic and phenolic antioxidants, phosphor tungstate and phosphomolybdate are combined to form the FolinCiocalteu reagent. It aids in determining the amount of the necessary material that must be tested in order to prevent the reagent from oxidising. FolinCiocalteu reagent was used to oxidise the diluted sample extract, and sodium carbonate was added to the reaction mixture to neutralise it. After 30 minutes, the blue colour solution's absorbance at 765 nm was measured.

Preparation of Sample:

Alpinia mutica leaves weighing 1 g were individually extracted using petroleum ether, ethyl acetate, methanol, hydroalcohol and aqueous solvents; A volumetric flask's contents were brought up to 50 ml after the solution had been filtered. Exactly 1 millilitre of the sample was mixed with 10 millilitres of sterile water in a test tube. FolinCiocalteu's reagents should then be added, and the mixture should be allowed to sit for 5 minutes at room temperature. 20% (w/v) sodium carbonate is added (4ml). Distilled water was used to increase the solution's volume by 25ml. After thoroughly stirring, the mixture was left at room temperature for 30 minutes. By using a UV spectrophotometer, the solution's absorbance was measured at 765nm (11). There were three copies of each result. Gallic acid standard curve was used to determine the sample's quantification.

The Gallic acid standard curve was shown in Figure 1and the extract total phenolic content was shown in **Table 2.**

Calculation:

C= c × V /M

C-Total Phenolic content

c-Gallic acid conc. achieved by standard curve (mg/ml)

V-Extract Volume (ml)

M-Extract Mass (g)

Assessment of the Potential Flavonoid Content of Plant Extracts

Principle

The modified procedure for the aluminium chloride colorimetric test that Woisky and Salatino described. The complex was formed as a consequence of interactions between aluminium chloride and either the C-4 position of the keto group or the C-3 and C-5 positions of the hydroxyl groups of flavonols and flavones (acid stable). The orthodihydroxyl (acid labile compound) of flavanoids can also be formed by aluminium chloride.

Preparation of sample:

With the help of petroleum ether, ethyl acetate, methanol, hydroalcohol and water, the leaves of *Alpinia mutica*, weighing 1 gm, were separately extracted. The volume of the filtered solution was raised to 50 ml in a volumetric flask. In order to get this data, we utilised 0.6 mL of a sample solution that contained 2% aluminium chloride (0.6mL). The resulting mixture was then incubated for a further hour at room temperature. The absorbance of the reaction mixture was then determined by using a UV spectrophotometer set at 420 nm (12,13). There were three copies of each result. The sample was measured in accordance with the quercetin standard curve. Figure 2 depicts the quercetin standard curve; **Table 3** shows the total amount of flavonoids found in the extract.

Calculation

C= c × V/M

C - Total Flavonoid content

c-Quercetin conc. achieved by standard curve (mg/ml)

V-Extract Volume (ml)

M-Extract Mass (g)

RESULTS:

Extraction

In comparison to Ultra sound assisted extraction, there was an increase in the yield of the various plant extracts from *Alpinia mutica.* The outcomes suggest that the UAE is superior to the traditional extraction approach.

Table 1: Colour, consistency and % yield of *Alpinia muticaExtracts* Sample AM

Plant Extract	Color observed in day Light	Consistency	Conventional Method Yield (%w/w)	Ultrasound assisted extraction (% w/w)
Methanolic extract	Dark green	Semisolid	11.56	15.45
Ethyl acetate extract	Light green	Semisolid	6.8	12.46
Hydro alcoholic extract	Dark green	Semisolid	6.91	13.42
Petroleum ether extract	Light green	Semisolid	2.98	6.45
Aqueous extract	Light green	Semisolid	8.98	13.56

Table 2: Total phenolic contents of *Alpinia mutica*

S. No	Test sample (*Alpinia mutica*)	Conventional extraction total phenolic content in mg/g equivalent of Gallic acid	USE total phenolic content in mg/g equivalent of Gallic acid
1	Methanolic extract	2.07±1.86	4.24±1.83
2	Ethyl acetate extract	1.11±0.52	1.45±0.87
3	Hydro alcoholic extract	1.18±0.17	2.01±0.44
4	Petroleum ether extract	2.35±0.34	2.71±0.26
5	Aqueous extract	2.5±0.098	2.71±0.26

Estimation of Total Phenolic contents

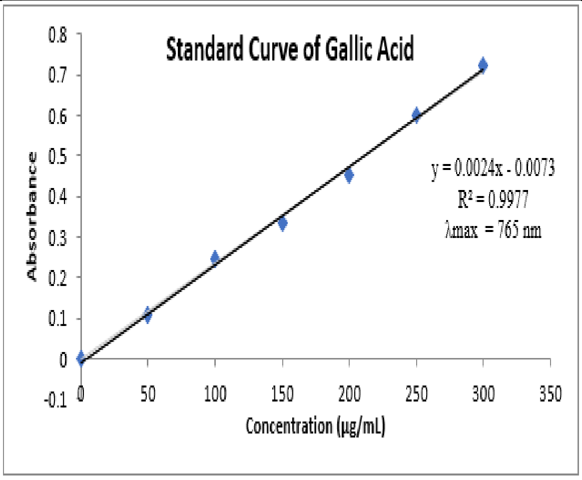

Figure 1: Standard Curve of Gallic acid.

S. No	Test sample (*Alpinia mutica)*	Conventional extraction total phenolic content in mg/g equivalent of Quercetin	USE total phenolic content in mg/g equivalent of Quercetin
1	Methanolic extract	2.55±1.00	3.50 ± 1.34
2	Ethyl acetate extract	0.94±0.22	1.05±0.10
3	Hydro alcoholic extract	0.96±0.16	1.49±0.71
4	Petroleum ether extract	1.05±0.10	1.31±0.28
5	Aqueous extract	1.09±0.27	1.31±0.38

Table 3: Total flavonoids contents of *Alpinia mutica*

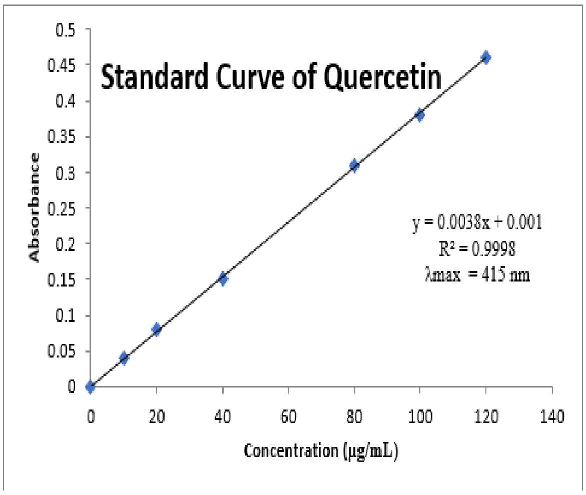

Figure 2: Standard Curve of Quercetin.

CONCLUSION:

According to the research, USE approaches considerably increased the yield and percentage of phytoconstituents, such as phenolic and flavonoid content, in the plant extracts of *Alpinia mutica* as compared to traditional extraction methods.

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REFERENCES

1.Kakkar.S. and Bais. S. "A review on protocatechuic acid and its pharmacological potential," *ISRN Pharmacology*, vol. 2014, Article ID 952943, 9 pages, 2014.
2.V. Tandon, B. Kapoor, and B. M. Gupta, "Herbal drug research in India: a trend analysis using IJP as a marker (1995–August 2003)," *Indian Journal of Pharmacology*, vol. 36, no. 2, pp. 99–100, 2004.
3.Steven D. Ehrlich; NMD, *Solutions Acupuncture, a Private Practice Specializing in Complementary and Alternative Medicine*, Steven D. Ehrlich, NMD, Healthcare Network, Phoenix, Ariz, USA, 2009.
4.Gadjalova, A.V. and Mihaylova, D. Sp, Ultrasound-assisted extraction of medicinal plants and evaluation of their biological activity, Food Research 3 (5): 530 - 536 (October 2019).
5.John Kress. W, A.Z. Liu, M. Newman and Q.J. Li, *The molecular phylogeny of Alpinia (Zingiberaceae): a complex and polyphyletic genus of gingers*. Am. J. Bot., 92, 167–178 (2005).
6.Sabu .M, *Zingiberaceae and Costaceae of South India*., Indian Association for Angiosperm Taxonomy, Calicut (2006).
7.Halijah Ibrahim, YasodhaSivasothy, Devi RosmySyamsir, Noor HasimaNagoor, Natasha Jamil, and KhalijahAwang, Essential Oil Composition and Antimicrobial Activities of Two Closely Related Species, *Alpinia mutica*Roxb. And *Alpinia latilabris*Ridl., from Peninsular Malaysia, *The Scientific World Journal*, Volume 2014, Article ID 430831.
8.Sirat, H.M.; Rahman, A.A.; Itokawa, H.; Morita, H. Constituents of the rhizomes of two *Alpinia* species of Malaysia. *Planta Med.* 1996, *62*, 188-189.
9.Motaleb M.A. (2011). Selected Medicinal Plants of Chittagong Hill Tracts. 1-128.
10.Choudhary N, Bijjem KR V., Kalia AN. Antiepileptic potential of flavonoids fraction from the leaves of *Anisomelesmalabarica*. Journal of Ethnopharmacology. 2011; 135:238–242.
11.Hannah Han, Shusheng Wang, Milan Rakita, Yanting Wang, Qingyou Han, Qin Xu, Effect of

Ultrasound-Assisted Extraction of Phenolic Compounds on the Characteristics of Walnut Shells, Food and Nutrition Sciences, 2018, 9, 1034-1045.

12.Siddiqui N, Rauf A, Latif A, et al. Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (Nepeta bracteataBenth). Journal of Taibah University Medical Sciences. 2017; 1-4

13.Chandra S, Khan S, Avula B, et al. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. Evidence-based Complementary and Alternative Medicine. 2014;1-9.