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Ultrasonication Extraction Techniques for a New Approach for Development of Pharmacognostical and Phytochemical Screening of *Syzygium aromaticum*

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Abstract

Herbal therapy is becoming increasingly popular as a safe and effective treatment option for a variety of medical problems. Herbs are frequently chosen since they are natural and do not contain hazardous chemicals. Clove (*Syzygium aromaticum*) is a natural spice with antibacterial and antioxidant qualities that is used as a medicine and a preservative. Clove is being used as a larvicidal agent to treat dengue fever, which is one of the most significant health issues in tropical nations. In the present study, various standardisation procedure has been used to evaluate the extracts (Maceration and ultrasonication process) of the plant. The result showed that total ash value was found to be as 4%w/w. Furthermore, swelling index was found to be as zero indicating absence of mucilage in the sample. Phytochemical screening showed that alkaloids are present in drug in higher amount. Also, it indicated the presence of tannins and glycosides. These data can be used to make pharmaceutical preparations from the clove.

Keywords: Clove, Ultrasonication, ash value, remedies, bitterness value

Introduction

Herbal remedies are the favoured treatment choice for a variety of common diseases in virtually all areas of India due to its traditional values, fewer known adverse effects, ease of availability, cost, and other factors (1, 2). Spices are produced, consumed, and exported in significant quantities in India. Out of the eighty-six spices grown worldwide, India produces over fifty (3). Many leaves (mint, coriander), bulbs (garlic) and buds (clove) have been used as food preservatives and traditional remedies from ancient times in addition to flavouring agents in meals (4). One of the most common spices is clove (*Syzygium aromaticum*). Cloves are the dried flower buds of the evergreen tree *Syzygium aromaticum*, belonging to family Myrtaceae (5). It is used to treat dyspepsia and stomach irritations. It is fragrant, carminative, and stimulating. Clove buds and essential oils have been recognised to have antibacterial and antioxidant effects for a long time (6). Clove oil is widely used to flavour a variety of foods, including meats, sausages, baked goods, confectionary, chocolates, table sauces, pickles, and so on. Its antibacterial, antiseptic, and antibiotic qualities make it useful in medicine (7).

Extraction is a phrase used in the pharmaceutical industry to describe the separation of medicinally active parts of plants using the right solvent. Impure liquids, semisolids, or powders are collected from the plant area, from which the pure form of active substances is removed using conventional techniques. Before moving on to biological testing, this procedure entails extracting and determining the quality and an amount of bioactive components (8-11).

Ultrasound assisted extraction is a stimulating approach for producing high-value

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compounds, and it will help to raise the value of some food by-products once they are employed as a source of natural chemicals. The most significant advantages will be simpler extraction, which saves energy, and the usage of moderate temperature, which is useful for heat-sensitive chemicals. Many technique factors must be considered for effective use of ultrasound-assisted extraction, the most important of which are the supersonic power, frequency, extraction temperature, reactor parameters, and the solvent-sample interaction. The first extraction, which is the most profitable quantity, is finished within the first few minutes. A rate equation and a defined method characterization area unit were required to optimise this approach, which had previously been lacking. (12-14).

Material and Methods

Authentication

The flowe buds was procured from local market of Sonipat and authenticated by department of botany, MDU Rohtak.

Preparation of Extract

Maceration

In a weighing bottle, 20 g of coarsely powdered drug was shifted to a dry 1000 ml conical flask. The solvent (methanol) was poured to the delivery mark in a 500 mL graduated flask. The flask was corked and left for 18 hours, shaking often. After that, it was filtered and placed in a thin porcelain plate. It was then dried on a water bath. Then stored in a desiccator so that it can be use further. The percentage w/w of extractable material was determined (15).

Ultrasonication Assisted Extraction

In this investigation, ultrasonic-assisted extraction was used. The extraction procedure was carried out with methanol as the solvent. The ultrasonic effect was created with a ultra-sonicator (Model-TU60W, 20 kHz). A water bath was put underneath the extraction set-up to regulate the temperature. The ultrasonic probe was dipped directly into the sample-holding solution. To aid in the extraction process, the ultrasonic device may generate cavitation with a bubble implosion effect (16).

Standardisation

Standardisation of *Syzygium aromaticum* was carried out for various parameters such as crude fibre content, ash values, bitterness value, loss on drying, extractive value and foaming index were evaluated according to WHO guidelines (15, 17, 18).

Ash Values

Ash is the residue left after the crude drug has been incinerated. The inorganic salt naturally existing in the drug and sticking to it is represented by the ash residue produced. It fluctuates within certain limits depending on the soils. Inorganic particles may also be intentionally introduced for the aim of adulteration. As a result, assessing the ash value provides the foundation for establishing the identity and cleanliness of any medication, as well as providing information about its adulteration/contamination with inorganic materials. As a result, ash values are valuable in identifying the quality and purity of medicinal drugs (8).

Total Ash

On completion of incineration of powdered extract(2g) at a temperature not more than 450°C, a residue is left which is known as total ash. The percentage of ash was evaluated on air dried drug basis (8) (Figure 1).

Water Soluble Ash

Water soluble ash is unambiguously recommended for drugs which are probable to be exhausted with water. The total ash was boiled with water (25 ml) for 5 min. In a crucible, insoluble material was collected and then washed with hot water. Further it was ignited at 450°C. The weight of insoluble

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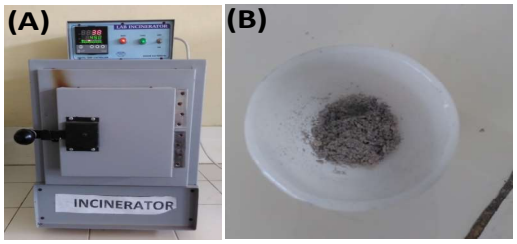

Fig 1. (A) Incinerator (B) Total ash of Powdered Drug of *Syzygium Aromaticum*

substance was deducted from weight of ash. Limit given in I.P. for water soluble ash is not less than 1.7% w/w (8).

Acid Insoluble Ash

Acid insoluble ash was calculated as per the procedure given in I.P. (8).

Sulphated Ash

Powdered extract (2g) was placed in a properly weighed crucible and gently ignited until the substance was completely burned. After cooling, with 1ml sulphuric acid, residue was wetted, heat slowly until no white vapours are released, then ignite at 800°C ± 25°C until black particles are no longer visible. Before adding a few drops of sulphuric acid and heating, the crucible was cooled. Ignited as previously, cooled, and weighed. The technique was continued until the difference between two subsequent weighing was less than 0.5 mg (8).

Extractive Values

It is one of the imperative parameters for evaluation of a crude drug and helps in determination of polarity of chemical constituents. It can be achieved by two processes of extraction:

Cold Maceration

In a weighing bottle, 4 g of coarsely powdered drug was shifted to a dry 250 ml conical flask. The solvent was poured to the delivery mark in a 100 mL graduated flask. The flask was corked and left for 18 hours, shaking often. After that, it was filtered and placed in a thin porcelain plate. It was then dried on a water bath before being stored in a desiccator. The percentage w/w of extractable material was determined (15).

Loss on Drying

The presence of excessive water in medicinal plant material may lead to deterioration through microbial and bacterial growth or enzyme mediated hydrolysis. There should be limit of water content for every plant material. It can be determined by weighing about 2 g of powdered drug into a weighed thin and flat porcelain dish. At 100°C, the material was dried until two consecutive weighing do not fluctuate by more than 0.5 mg. It was cooled in desiccator and weighed (15).

Foaming Index

Saponins containing drugs give persistent foam. A plant extract/ material should be evaluated for foaming index to check its capability to form foam. Foaming index was calculated according to WHO guidelines 2011.

The height of foam was measured by means of equation (i):

$$Foaming\ Index = \frac{1000}{a} \quad \text{..... (i)}$$

Where a is the volume in ml of filtrate in test tube showing 1cm foam height (15).

Crude Fibre Content

Crude fibre content is the deposit of resistant tissues which can be achieved after giving treatment to powdered drug with dilute acid followed by dilute alkali. It is an important tool for detection of adulteration in the drug. Crude fibre content was evaluated (17).

Swelling Index

The volume occupied by the plant material in millilitre (mL). It provides idea about the mucilage content of the drug. It can be calculated by taking the

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Table 1. Serial Dilution for the Initial Test

Tube No.	Sq (ml)	Safe Drinking Water	Quinine Hydrochloride in 10 ml of Solution (°C) (mg)
1	4.2	5.8	0.042
2	4.4	5.6	0.044
3	4.6	5.4	0.046
4	4.8	5.2	0.048
5	5.0	5.0	0.050
6	5.2	4.8	0.052
7	5.4	4.6	0.054
8	5.6	4.4	0.056
9	5.8	4.2	0.058
Sq- Stock solution of quinine hydrochloride			

powdered drug material in a 25 ml stoppered cylinder. Further, water was added up to 25 mL volume mark. Shaken occasionally during 23 h and then kept aside for one h. The swollen drug material's volume was calculated (17).

Bitterness Value

The bitterness value was determined through standard guidelines issued by WHO in 2011. The various compositions that were prepared by serial dilution method for initial and second test are prepared 0.042-0.058 mg/10mL and 1-10 mL respectively then bitterness value was calculated according to equation (ii):

$$Bitterness\ value\ in\ units\ (per\ g) = 2000 \times CA \times B$$

…….. (ii)

Where, A = the quantity of material in mg/mL of St; B = the volume of St in mL/10mL of dilution threshold bitter concentration; C = the quantity of quinine HCL R in mg/10mL of the dilution of threshold bitter concentration (15).

Qualitative Phytochemical Screening: Detection of alkaloids, carbohydrates, proteins, flavonoids, resins, organic acids, volatile oils, steroids, tannins etc (17).

Table 2. Serial Dilution for the Second Test

Tube No.	St (ml)	Safe Drinking Water
1	1	9
2	2	8
3	3	7
4	4	6
5	5	5
6	6	4
7	7	3
8	8	2
9	9	1
10	10	-
St = stock solution (herbal material being examined)		

Result and Discussion

Morphological Evaluation of Syzygium Aromaticum

Colour- Dark Brown

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Odour- Pungent

Taste- Spicy and pungent taste

Microscopy

In the microscopy, the epidermis, oil glands, collumela, clusters of calcium oxalate crystals, vascular bundles, and cortex are depicted. However, starch was absent.

Physical Evaluation

Ash Values

The *Syzygium aromaticum* had a low ash level (Total ash 4%, water-soluble ash 0.2 %, acid-insoluble ash 0.0041%, and sulphated ash 0.025 % w/w), suggesting that there were less organic compounds and foreign organic materials present as impurities.

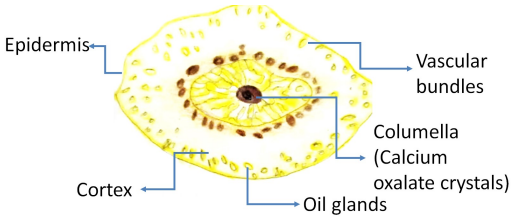

Fig 2. Microscopy of Clove

Table 3. Ash Values of Rhizomes of *Syzygium aromaticum* Linn

Ash	Values
Total ash	4% w/w
Water- soluble ash	0.2 %w/w
Acid-insoluble ash	0.0041%w/w
Sulphated ash	0.025%w/w

Extractive Values

In evaluation of crude drugs, the extractive value plays an important role. Further, it helps in understanding the chemical components of the drugs that was increased using ulta-sonicatiton in comparision to cold maceration. The extraction values were found to be petroleum ether (10.5 %w/w), ethanolic (10.8%w/w), hydroalcoholic (8.5% w/w), and water (14.4%w/w).

Loss on Drying

Loss on drying gives idea about moisture content present in the drug. Loss on drying was found to as 10%w/w.

Foaming Index

Foaming index was found to as 0.0085%w/w indicating that saponins may be present in minute amount.

Swelling Index

Swelling index was found to be zero indicating that there is absence of mucilage content in the dried buds of clove.

Crude Fibre Content

Crude fibre content was found to be 10.5%w/w.

Bitterness Value

Bitterness value was found to be 7.5.

Phytochemical Investigation

Phytochemical screening of petroleum ether, alcoholic, hydroalcoholic, and aqueous extracts obtained using different extraction techniques such as maceration (MC) and ultrasonication (US). The vaious

Table 4. Extractive Values of dried *Syzygium aromaticum*

S.No.	Extract	Extractive Values	
		Cold Maceration	Ultra- sonication
1.	Petroleum Ether (40-60˚C)	4.25%w/w	10.5 %w/w
2.	Ethanolic	6.75%w/w	10.8%w/w
3.	Hydroalcoholic	5%w/w	8.5% w/w
4.	Water	12.5%w/w	14.4%w/w

Phytochemical Screening of *Syzygium Aromaticum*

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phytoconstituents carbohydrates, flavonoids, glycosides, alkaloids, tannins, amino acids and proteins were present.

Table 5. Phytochemical Screening Results of Different Extracts of *Syzygium aromaticum*

S. No.	Test	Petroleum Ether (40-60˚C)		Alcoholic		Hydroalcoholic		Aqueous	
		MC	US	MC	US	MC	US	MC	US
1.	Molisch's test	+	+	+	+	+	+	+	+
2.	Benedict's test	+	+	+	+	+	+	+	+
3.	Fehling's test	+	+	+	+	+	+	+	+
4.	Pentose sugar test	+	+	+	+	+	+	+	+
5.	Tollen's phloroglucinol test	+	+	+	+	+	+	+	+
6.	Iodine test	+	+	+	+	+	+	+	+
7.	Legal's test	+	+	+	+	+	+	+	+
8.	Keller–Killiani test	+++	+++	++	++	++	++	++	++
9.	Foam test	+	+	+	+	+	+	+	+
10.	Cyanogenetic glycosides	+	+	+	+	+	+	+	+
11.	Hager's test	+++	+++	+++	+++	+++	+++	+++	+++
12.	Mayer's test	+++	+++	+++	+++	+++	+++	+++	+++
13.	Wagner's test	+++	+++	+++	+++	+++	+++	+++	+++
14.	Tannic acid test	+++	+++	+++	+++	+++	+++	+++	+++
15.	Dragendroff's test	+++	+++	+++	+++	+++	+++	+++	+++
16.	Salkowski reaction	-	-	-	-	-	-	-	-
17.	5% $FeCl_3$ solution	++	++	++	++	++	++	++	++

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18.	Lead acetate solution	++	+ +	++	+ +	+ +	++	+ +	+ +
19.	Acetic acid solution	++	+ +	++	+ +	+ +	++	+ +	+ +
20.	Dilute HNO_3	++	+ +	++	+ +	+ +	++	+ +	+ +
21.	Bromine Water	++	+ +	++	+ +	+ +	++	+ +	+ +
22.	Biuret Test	+	+	+	+	+	+	+	+
23.	Protein containing sulphur	+	+	+	+	+	+	+	+
24.	Precipitation test								
	a. 5% $HgCl_2$	+	+	+	+	+	+	+	+
	b. 5% CuSO4	+	+	+	+	+	+	+	+
	c. 5% lead test	+	+	+	+	+	+	+	+
25.	Ninhydrin test	+	+	+	+	+	+	+	+
26.	Cysteine	+	+	+	+	+	+	+	+
27.	Sulphuric acid test	+	+	+	+	+	+	+	+
28.	Lead acetate test	+	+	+	+	+	+	+	+

Conclusion

The different standardisation characteristics calculated in this study might aid in the botanical identification and standardisation of drugs in unrefined form. On the basis of its phytochemistry, the original plant material may also be investigated for its pharmacological and phytochemical potential. Short extraction times, minimal solvent consumption, low hazardous pollution generation, and high extraction yields were shown to be major advantages of innovative or non-conventional extraction techniques. We can use new techniques to cut down on time. Using the novel extraction of method, like ultra-sonication, the yield can be increased as the cell burst out during the process of ultrasonication. High frequency of ultrasound accommodates in the bursting of cell and penetration of solvent in the drug thus increasing the yield. Furthermore, the extraction time is lesser as compared to the conventional methods of extraction. We can utilise the novel methods to increase the yield, lesser solvent consumption and lesser time.

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