RECENT ADVANCES IN EXTRACTION OF CLINACHANTHUS NUTANS: A REVIEW

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Abstract. Interest in the development of extraction of phytochemicals compounds from Clinacanthus nutans has increased in recent years due to its potential applications in food, chemical, and pharmaceutical industries. The quality and yield of the phytochemicals are greatly influenced by the extraction method employed as well as other factors. This paper provides an up-to-date overview of traditional (maceration, infusion, and soxhlet), with modern methods (pressurized hot water extraction, supercritical fluid extraction, ultrasound-assisted extraction, and microwave-assisted extraction) to extract phytochemicals compounds from C. nutans. Problems associated with conventional extraction methods have led to a demand for alternatives and indeed several new extraction methods have been developed and refined in the last two decades that can efficiently extract a great variety of valuable bioactive compounds.

1. Introduction

Recently, there is a growing consumer interest towards medicinal plants since it is perceived to be healthier and free from side effects unlike synthetic drugs. The majority of the world population still rely on plants and their extracts for primary health care and needs. Medicinal plants as herbal remedies maintain their use for therapeutic effect despite tremendous advances in modern medicine. The high efficacy, low cost, and low toxicity of medicinal plants have popularized them in the treatment of diseases even in modern times [1]. Clinacanthus nutans belongs to the Acanthaceae herbal family and grows exceedingly well in equatorial regions, especially in Southeast-Asia. It is traditionally used as a remedy for herpes, snake and insect bites, and inflammation [2]. Many studies have shown C. nutans extracts to have anti-inflammatory, anti-microbial, and anti-viral activity against herpes simplex virus (HSV) and varicella- zoster (VZV) [3]. C. nutans extracts also contain natural antioxidants that prevent oxidative damage caused by free radicals in the body [4]. Natural antioxidants are preferable to artificial antioxidants as they are non-toxic and are more easily decomposed by the body. The leaves of C. nutans are rich in natural antioxidants; phenolic and flavonoid compounds [5], that scavenge free radicals by way of acting as reducing agents, hydrogen donors, or oxygen singlet quenchers [6].
There exist numerous studies on the extraction of these bioactive compounds from plants. Conventional methods like maceration, infusion, and ordinary reflux have been the industry staple for many decades but they suffer from the disadvantages of being time- and solvent- consuming. More recently, modern methods such as supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), microwave assisted extraction (MAE), and pressurized liquid extraction (PLE) have been developed and they have higher efficiencies, lower solvent consumption, and give better yields of phytochemicals compared to conventional methods [7]. The current literature body lacks a comprehensive study on the various methods to selectively extract phytochemicals from *C. nutans*. Therefore, the present mini review aims to provide an up-to-date technical discussion on current extraction techniques and their advantages and disadvantages, as well as an overview on the employment of cutting edge green extraction methods and the yield and quality of phytochemicals compound extracted from *C. nutans* using these methods.

2. Extraction methods of *C. nutans*

The choice of extraction method is important if one is to get a high quality and yield of bioactive compounds from *C. nutans*. Extraction methods can be classified into two groups; traditional or conventional extraction methods, and modern extraction methods. Conventional extraction methods include soxhlet, infusion, and maceration among others. A general overview has been provided by Khoo et al [8] on the extraction of phytochemicals compounds from *C. nutans* using conventional extraction methods. Conventional extraction methods often involve lengthy extraction times and excessive use of expensive organic solvents which are also bad for the environment. For example, to treat 20 g of plant material, the amount of solvent required is at least 250 mL while the extraction time may exceed 24 hrs [9]. Furthermore, toxics fumes are emitted during the extraction process as it is generally carried out at the effervescence point of the solvent used [10].

Previous studies have investigated the soxhlet extraction of phytochemicals from *C. nutans* using different solvents such as methanol, chloroform, ethanol, and hexane. Depending on the solvent used, *C. nutans* extracts are found to contain stigmasterol, C-glycosyl flavones, β- sitosterol, lupeol, vitexin, iso vitexin, betulin, shafto side, iso mollupentin, orientin, iso orientin, sulphur containing- glucosides, glycolglycerolipids, a mixture of-nine-cerebrosides, 7-O-β- glucopyran-oside, and mono acylmono galactosyl glycerol [11]. The yield for phytochemicals extracts from *C. nutans* using conventional methods is shown in Table 1.

In one study, soxhlet extraction was carried out on 3 g of dry *C. nutans* using 80 mL of absolute ethanol for 8 hrs. Then, the solvent was removed using a rotary evaporator at 45°C and the product was transferred into an amber glass bottle, weighed, and stored in a freezer at -7°C until use. The moisture content was 12.17% of the dry weight and the yield of *C. nutans* extract obtained was 21.28% [13]. Tiew et al [14] extracted flavonoids from *C. nutans* using the maceration method and got a yield of 0.05 ± 0.002 mg quercetin equivalent (QE)/g of dried plant material total flavonoids content (TFC). They had used methanol as the solvent and ran the process for 3 days. Furthermore obtained 0.22 ± 0.006 mg QE/g of dried plant material TFC also with the maceration technique but using a different solvent. The difference in TFC yield is mainly due to the choice of solvent used and therefore it is imperative that a suitable solvent is chosen when developing a protocol for the extraction of phytochemicals. Additionally, TFC will degrade to some degree during the extraction process and this must also be taken into account. Although the number of studies using this method to extract phytochemicals from *C. nutans* is significant, fact remains that the method is plagued by low yields, high organic solvent consumption, and low extraction efficiency [15].
Table 1. The yield of phytochemicals from *C. nutans* using conventional extraction methods.

| Extraction Parameters | Method         | Plant Part    | Solvent         | Temperature (°C) | Time (h) | Amount of solvent (mL) | Phytochemical Compounds                      | Reference |
|-----------------------|----------------|---------------|-----------------|------------------|----------|------------------------|----------------------------------------------|-----------|
|                       | Maceration     | Leaves        | Methanol        | 30               | 48       | 50                     | Total phenolic content, total flavonoid content, antioxidants. Analysed using GC-MS | [12]      |
|                       | Infusion       | Whole plant   | Methanol: water | 40               | 6        | 150                    | DPPH and ferric reducing antioxidants. Analysed using (FRAP) assays | [2]       |
|                       | Soxhlet extraction | Leaves and stems | Ethanol | 40 | 6 | 100 | Phytosterols, phenols contents, flavonoids contents, and sitosterol | [12] |

3. Novel extraction techniques

The major disadvantages of conventional extraction techniques are that they are time consuming, require a lot of expensive solvents, and have low extraction efficiency due to the thermal degradation of phytochemicals. To tackle these issues, a whole host of new extraction techniques have been developed in recent years [15]. Some of the most promising advanced extraction techniques are discussed below.

3.1. Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is one of the new methods developed to extract phytochemicals compounds from plants [16]. Carbon dioxide (CO₂) is a typical solvent in the SFE process and it is non-toxic, non-flammable and safe enough to use even in food applications. The CO₂ gas is brought to the supercritical region mainly through the use of high pressures and mild heating where it then behaves like both a liquid and a gas. This fluid can then be used to extract thermo-labile compounds because its critical temperature is low, at only 31°C. In addition, the low boiling point of carbon dioxide at atmospheric pressure means the CO₂ can then be easily removed without leaving any unwanted residue [16]. Another advantage of using SFE is that the CO₂ fluid can be optimised for the extraction process by adjusting its temperature and pressure. Since supercritical CO₂ is a non-polar solvent, using it in tandem with amphipathic solvents such as ethanol can improve its ability to extract polar compounds [17].

Mustapa et al [15] used supercritical CO₂ at a pressure of 350 bars and temperature of 60°C to obtain 0.83 ± 0.10 mg sitosterol and 1.35 ± 0.12 mg phytosterols after 120 min. The SFE process was found to be especially unfavourable for the extraction of chlorophyll and phenols due to the non-polar nature.
of CO\textsubscript{2}. The slow rate of extraction and the solutes being tightly bound to the plant matrix also contribute to the low extraction efficiency of phytochemicals from \textit{C. nutans} using SPE.

3.2. Ultrasound-assisted extraction (UAE)

The UAE method uses ultrasonic waves to destroy the walls of the plant cells so that the phytochemical compounds within can be extracted [18]. Temperature, pressure, and extraction time are all factors that affect UAE efficiency. The UAE method is relatively easy, versatile, and low in cost compared to most other methods. Baharuddin et al [7] successfully extracted phenols and flavonoids from \textit{C. nutans} leaves using 28 kHz frequency wave. He found the optimum temperature to be 55 °C and at higher temperatures, the phytochemicals will degrade. The maximum yield was obtained after 25 min at this temperature. Despite its utility, speed (5–30 min for one sample), high yield, and relative low cost, this method suffers from many problems, namely the lack of uniformity in the distribution of ultrasound energy and decline of power with time. Filtration is also required after the extraction step making the process labour intensive [19].

3.3. Microwave assisted extraction (MAE)

Microwaves are used to heat up the water content within the plant cell until it boils into steam. When it does, the water expands, rupturing the cell from within to release the phytochemicals. The process is quick, requires only a small amount of solvent, and is energy efficient. The MAE process is influenced by factors such as solvent type, compound type, and dielectric constants of the plant material [20]. The advantages of using the MAE process to extract phytochemicals from \textit{C. nutans} include high extraction efficiency, low solvent consumption, good antioxidant-activity of the extracts, and short extraction times [21].

Ismail & Kasim [2], had used 80% methanol as the solvent and microwave heating at frequency of 2.45 GHz for 20 minutes. Theye found the TPC and TFC content to be 15 mg GAE/g DM and 15 mg Que/g DM respectively and they contribute to an antioxidant activity of 80%. Mustapa et al [15], considered the MAE to be a green process because it is safe, quick, easy to use, result in high extraction yields and can work with or without the use of solvent. Despite its good qualities, the high energy involved with the use of microwaves causes the oil itself to oxidise.

3.4. Pressurized hot water extraction techniques

PHWE, also known as pressurized solvent extraction (PSE) or accelerated solvent extraction (ASE) uses high temperatures and pressures to improve the extraction of phytochemicals. The high pressure is used to maintain the solvent in the liquid phase even when heated above its boiling point. The process needs only a small amount of solvent, but still requires labour intensive washing and filtration after the extraction step [22]. Extraction efficiency can be improved by selecting the appropriate solvent or mixture, pressure (usually 3-20 MPa) [23], temperature (usually 100-200 °C), and extraction time. High temperature leads to an increase in the conductivity of the water as well as a decrease in surface tension and thus an increase in propagation of phytochemical molecules through the water. This method can be used to extract non-polar, moderately polar and polar compounds [24]. A brief summary of the extraction yields of phytochemicals from \textit{C. nutans} using conventional extraction methods shown in Table 2.

Ismail & Kasim [2], studied the extraction of TPC, TFC, DPPH scavenging activity of different parts of \textit{C. nutans} (stem, leaves, and mixture of both stem and leaves) by using PHWE technique with extraction condition at temperature of 121°C, pressure of 4 bars and extracted for 20 min. When compared to the control (maceration using water at 60°C), PHWE show a very significant effect. The use of water as solvent can be exploited with the used of pressurized hot water extractor. Leaves and
mixture of *C. nutans* contain high TPC and TFC but lower DPPH activity. However, the stem of *C. nutans* has high DPPH activity but lower TPC and TFC.

**Table 2**: A brief summary of the extraction yield of phytochemicals from *C. nutans* using novel extraction methods.

| Extraction Parameters | Method                        | Part          | Types of Solvent | Temperature (°C) | Time (min) | Volume of solvent (mL) | Phytochemicals compounds                                                                 | Reference |
|-----------------------|-------------------------------|---------------|------------------|------------------|------------|------------------------|-----------------------------------------------------------------------------------------|-----------|
|                       | Pressurized hot water extraction | Leaves        | Water            | 121              | 20         | 20                     | TPC, TFC, and DPPH antioxidant activity                                                  | [2]       |
|                       | Supercritical fluid extraction | Leaves-stems  | Ethanol–water    | 60               | 120        | 50                     | Chlorophyll, TPC, TFC, and phytosterols                                                   | [15]      |
|                       | Ultrasound assisted extraction | Leaves-stems  | Water            | 35               | 5-30       | 45                     | Phenolic, flavonoid contents, and vitamin C                                              | [21]      |
|                       | Microwave assisted extraction | Leaves        | Ethanol–water    | 186              | 5-20       | 14                     | Phytosterols, TPC, TFC, and sitosterol                                                    | [15]      |

4. **Comparison of conventional and novel extraction methods**

In general, conventional extraction methods have been used extensively and are rather effective in extracting compounds from plant. However, the main disadvantages of these methods are the need of large amounts of solvents and low extraction rates. On the other hand, modern methods can extract phytochemical compounds from *C. nutans* at a significantly higher rate. Mustapa et al. [15], investigated and optimised modern methods for the extraction of phytochemicals compounds from *C. nutans*. In terms of extraction time, UAE and MAE were found to be the quickest, only requiring 5 and 10 min respectively but the extraction yield was lower than that obtained using PHWE and the extracts needed to be purified which is labour intensive. SFE resulted in the lowest level yield of polar phytochemicals because the supercritical carbon dioxide solvent used is non-polar and cannot extract polar compounds such as chlorophylls and polyphenol [25]. After taking into consideration the several modern extraction methods discussed above in terms of extraction time, temperature, and the ability to carry out sequential extractions using different solvents, it can be concluded that the PHWE method is the best method to use as it results in the highest yield of phytochemical compounds in the shortest time. The factors that influence the yield of phytochemicals extracted through the PHWE method are the temperature and pressure used, and the number of cycles and its duration [22].

5. **Factors effecting extraction methods**
Although most modern extraction techniques are quick, reliable, and highly efficient, there are several factors that influence phytochemical extraction efficiencies regardless of the method used. The solvent choice is the most important factor to consider in the extraction step and in general, the solvent used will depend on the desired phytochemical content to be extracted from the plant. There are several studies that report high recovery of phytochemicals using solvents provided that the other experiment parameters are optimized for the sample. Usually, pure solvents only result in low extraction efficiencies and 40 to 60% methanol, ethanol or acetonitrile with the remainder being water is necessary to achieve high recoveries. Also, due to environmental and economic concerns, it is preferable to use a lesser toxic solvent like ethanol or methanol instead of acetonitrile. The use of acids (especially HCl) in the extraction solvent should be avoided since there are evidence that indicate certain phytochemicals in C. nutans degrade under acidic conditions [7].

In non-conventional methods, both polar and non-polar solvents have been used. Extraction efficiency also depends on the solubility of the desired phytochemicals in the extraction solvent. In general, the like dissolves like principle applies and polar solvents will solubilize non-polar phytochemicals while non-polar solvents will solubilize non-polar phytochemicals. Lowly polar solvents have been reported to successfully extract flavonoids aglycones and an addition of a polar solvent allows the extraction of flavonoids glycosides and anthocyanins. Benzene, chloroform, ether, and ethyl acetate are typical solvents for the extraction of lowly polar compounds such as isoflavones, flavonones, dihydroflavonols, flavones, and flavanols whose chemical structures are very methylated [26]. Very polar flavonoids glycosides like hydroxylateflavones, flavonoles, biflavonyls, and chaliounes have been conventionally extracted using acetone, alcohols, and water. In fact, water is a typical solvent to use in the PHWE method to extract phenolic compounds [27].

In the MAE method, the extraction of quercetins and rutin is also affected by the concentration of solvent used. Mlyuka et al [28] observed that phytochemical yield increases with increase in ethanol concentration from 30% to 50% and then decreases with dilution of the ethanol. The same study also reported that the pH of the solvent affects extraction efficiency. Solvent volume is another important factor to consider and generally the PHWE method is advantageous in this aspect as the amount of solvent required is relatively low [25]. Because a high pressure is used, even large volumes of solvent can be heated up to the desired temperature quickly [25].

Extraction time is another important parameter to consider in the extraction of the phytochemicals from C. nutans. Conventional methods tend to require lengthy extraction times (2–24 hours) which will also degrade the phytochemicals during the extraction process. For this reason, the PHWE method is preferable because since higher temperatures can be achieved in the pressurized vessel, the extraction time can be kept short.

Temperature can greatly impact extraction efficiency as it affects the solubility of major phytochemical compounds, the average rate of diffusion of analytes through the solvent, and the proportion of the phytochemicals extracted. Some phytochemicals will degrade at high temperatures and therefore the selection of an appropriate temperature is important, especially if the extraction time is long. According to a study by [29], the yield of phenolic and flavonoids, and their DPPH scavenging activity when using PHWE technique was observed to increase with temperature from 100 until 180°C, however at above 180°C, the extract is expected to scorch. The increase of the extracted compound is believed due to the amplified mass transfer of the compound with the water temperature. At higher temperature, the compound degraded or completely decomposed due to thermal activity and/or Maillard reaction [30].

6. Conclusion
C. nutans extracts have great potential in medicinal research and therefore, efficient and commercially viable methods to extract them are well developed. This mini review presents a brief comparison between the conventional and non-conventional methods to extract phytochemicals from C. nutans. Major advantages of the non-conventional techniques include short extraction time, low solvent consumption, low generation of toxic pollutants and high extraction yields. The right selection of method results in good yield and quality of the extracts. There is always room for improvement in developing modern extraction method to cater to the demanding needs of commercial applications that use plant derived products. Improvement of existing extraction methods enhances extraction efficiency, yields, quality, and the eco-friendliness of the process.

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8. References

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