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Original Article

COMPARATIVE STUDY OF YIELDS AND TOTAL PHENOLIC AND FLAVONOID CONTENTS OF EXTRACTS OF DIFFERENT EXTRACTION METHODS OF *CLITORIA TERNATEA* (FABACEAE)

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ABSTRACT

Objective: The present study aims at comparison of extraction yields, total phenolic and flavonoid contents of methanolic extracts from various methods of extraction of leaves of *Clitoria ternatea* (Fabaceae).

Methods: Dried leaves were extracted by Maceration, Soxhlation and Microwave Assisted Extraction (MAE) methods and the percentage yields were calculated. Further total phenolic and flavonoid contents were determined for the extracts obtained as above. Each of the experiments were performed in triplicate.

Results: The methanolic extract of MAE was found to show the highest yield (12.22 % w/w) compared to the other methods performed. Total phenolic and flavonoid contents were also determined in the highest amount in MAE (248.29±0.29 mg GAE/gm and 191.64±1.45 mg QE/gm) than soxhlation and maceration.

Conclusion: Microwave assisted extraction method possessed highest extraction yield and total phenolic and total flavonoid contents than the other two extraction methods which makes it economical and effective not only for its increased yield but also for better quality of extract, consuming less time and solvent. Further studies on this may be beneficial to establish this method for regular extraction and isolation of phytoconstituents.

Keywords: *Clitoria ternatea,* MAE, Total phenolic content, Total flavonoid content

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INTRODUCTION

Extraction is an important step in the itinerary of phytochemical processing for the discovery of bioactive constituents from plant materials. Selection of a suitable extraction technique is also important for the standardization of herbal products, as it is utilized in the removal of desirable soluble constituents, leaving out those not required with the aid of the solvents [1].

There are two types of extraction techniques, conventional techniques and recent techniques. Conventional techniques such as; maceration, percolation, infusion, decoction, hot continuous extraction and soxhlet extraction etc. are older and require more time for extraction while the recent techniques like ultrasound-assisted solvent extraction (USE), microwave-assisted solvent extraction (MAE), Accelerated Solvent Extraction (ASE) and Supercritical Fluid Extractions (SFE) are newer extraction techniques which require less time for extraction with less solvent consumption [1, 2]. There is a difference of yield value after extraction between the conventional techniques and the recent techniques [1].

Microwaves are part of the electromagnetic spectrum of light with a range of 300 MHz to 300 GHz and wavelengths of theses waves range from 1 cm to 1m [3]. These waves are made up of two perpendicular oscillating fields which are used as energy and information carriers. First application of microwaves includes its interaction with specific materials, which can absorb a part of its electromagnetic energy and can convert it into heat. Commercial microwaves use 2450 MHz of energy for this purpose which is almost equivalent to 600-700W [4]. Practically, microwaves induce dipole rotation in organic molecules along with heating which causes the destruction of hydrogen bonding. This causes the traffic of ions which results in a heating effect due to increased kinetic energies of ions as well as friction between ions due to their continuous movements and change in directions. Destruction of hydrogen bonding also increases the penetrating efficiency of the solvents into the plant matrix [5, 6]. Microwave Assisted Extraction (MAE) gives more yield value after extraction than the other conventional technique like maceration and soxhlet extraction [1].

Clitoria ternatea (Fabaceae) is a perennial herb which is commonly known as Aparajita [7, 8]. The leaves are pinnate. The deep blue coloured flowers are solitary and very short pedicellate and 4-5 cm long. Pods are 6-12 cm long, 0.7-1.2 mm wide with flat, linear shape. The brown or black in coloured seeds are 4.5-7 mm long and 3-4 mm wide [9, 10]. It is distributed throughout India [4, 5]. The whole plant and seed extracts are used for stomatitis, hematemesis, insomnia, epilepsy, and psychosis, purgative, cathartic. The roots and root bark possess anti-inflammatory, analgesic, antipyretic and diuretic, laxative properties. The leaves are mainly used for the treatment of nostalgia and eruptions. The seeds have purgative, cathartic activity and are useful in visceralgia [10-12].

MATERIALS AND METHODS

Reagents and chemicals

Solvents and reagents are analytical grade which were used in the study and obtained from Merck, Fisher. Different chemicals which used as standards, procured from Sigma Aldrich.

Collection and identification of plant material

Leaves of *Clitoria ternatea* (Fabaceae) were collected from local area of Ashoknagar, West Bengal, India and was identified and authenticated from BSI, Howrah, India.

Extraction

Leaves of *Clitoria ternatea* were collected, shed dried and grinded with mixer grinder. For extraction, grinded leaves were passed through a sieve (Sieve no 10) to get coarse powder.100 gm powder drug and 1:4 ratio of menstruum (methanol) were taken in a different container. Each of the following methods of extractions were performed in triplicate.

Maceration

Leaves of *Clitoria ternatea* extract was produced by extraction using the maceration method. Required quantity of powder drug and menstruum (methanol) were taken as mentioned above and kept in a closed container for 72 h at room temperature with occasional stirring. Finally, it was filtered and dried at reduced pressure [3].

Soxhlation

For Soxhlation extraction method, the required quantity of powder drug and menstruum (methanol) were taken in the flask as mentioned above. The thimble was clogged with cotton in order to avoid the transfer of sample particles to the distillation flask. The entire process repeatedly continues until the drug was completely extracted, a point when a solvent flowing from the extraction chamber does not leave any residue behind and become totally colourless. The methanolic extract was filtered and concentrated on rota evaporator to give the methanolic extract. Percentage yield of extract was calculated [1, 3].

Microwave-assisted extraction

For Microwave Assisted Extraction (MAE), required quantity of powder drug and menstruum (methanol) were taken in the flask and shaken well and kept for some time. The flask is treated for the microwave process in the microwave oven. Extraction temperature was set at 3 min and irradiation power set at 480 W. After the extraction completed, the conical flask was taken out from the oven. Sufficient quantity of solvent was added to make a solution and then filtered. Concentration of extract was then carried out on a water bath and calculated the percentage yield of extract (%w/w) [1].

Determination of total phenolic content

The total phenolic content was determined using the spectroscopic method. The reaction mixture was prepared by mixing 1 ml of plant extracts (1 mg/ml), 1 ml of 10% Folin-Ciocalteu's reagent dissolved in 13 ml of deionized water, followed by the addition of 5 ml of 7% Na2CO3 solution. The mixture was mixed thoroughly and kept in the dark at room temperature for 2 h. The blank solution was also prepared. The absorbance was recorded using a spectrometer at 760 nm. All the analysis was repeated three times and the mean value of absorbance was obtained. Total phenolic content was determined by extrapolating the calibration line which was construed by gallic acid solution. The TPC was expressed as gallic acid equivalent (mg GAE) per gram of the dried sample [13-15].

Determination of total flavonoid content

The total flavonoid content of the crude extract was determined by the aluminium chloride colorimetric method. In brief, 50 µl of crude extract (1 mg/ml ethanol) were made up to 1 ml with methanol, mixed with 4 ml of distilled water and then 0.3 ml of 5% $NaNO_2$ solution; 0.3 ml of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 ml of 1 mol/l NaOH solution were added, and the final volume of the mixture was brought to 10 ml with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per g dry weight [13, 16].

RESULTS AND DISCUSSION

The yields of the different extraction methods are given in table 1 and graphically represented by fig. 1, which reveal that the extractive values of MAE were found to be much higher compared to the other two methods and the time taken by MAE (3 min) is much lower than that of maceration (72 h) and soxhlation (3 h). Therefore, MAE was found to be the most efficient extraction method in terms of yield and time when compared to the other conventional methods performed.

In analytical chemistry, it has been observed that microwave extraction results in greater yields of phytoconstituents such as flavonoids than other novel extraction techniques, such as ultrasonic extraction [17]. In addition, this method is further proved to be an economical substitute for routinely used homogenization and vortexing extraction techniques [18].

Table 1: Yield of different extraction methods

Extraction method	Extractive value (%w/w)	Extraction time
Maceration	7.42±0.08	72 h
Soxhlation	10.29±0.16	3 h
MAE	12.22±0.11	3 min

Values are expressed as mean±SD, n = 3

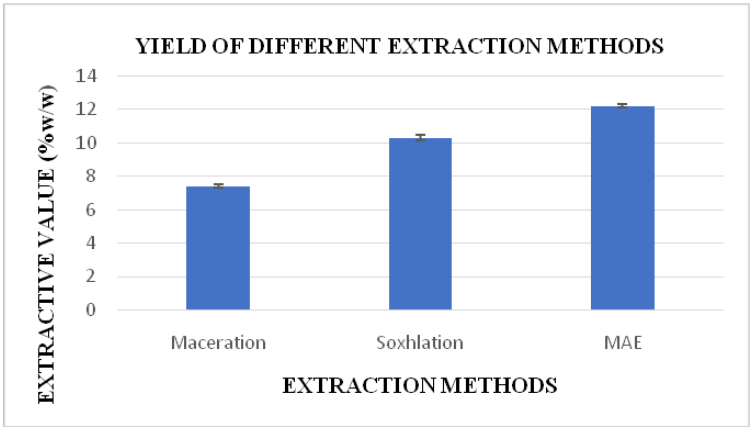

Fig. 1: Yield of different extraction methods

Total phenolic content and total flavonoid content

The total phenolic content was determined by using the Folin-Ciocalteu method. Gallic acid was used as standard and the value was expressed as mg gallic acid equivalence per gram (mg GAE/g). The total content of phenols of *Clitoria ternatea* was determined from the regression equation of the calibration curve (y= 0.0051x+0.1524, R^2= 0.9463). The total phenolic content of leaves of *Clitoria ternatea* by different extraction methods was found to be in the range of163.38±0.59 to 248.29±0.29 mg of GAE/g powder weight. The MAE exhibited the highest total phenolic content (248.29±0.29 mg GAE/g), followed by Soxhlation (208.68±0.96 mg GAE/g) and Maceration (163.38±0.59 mg GAE/g).

The total flavonoid content was determined by the aluminium chloride colorimetric method. Quercetin was used as the standard and the value was expressed as mg Quercetin equivalence per gram (mg QE/g) [11, 20]. The total content of flavonoids of *Clitoria ternatea* was determined from the regression equation of the calibration curve (y= 0.0062x+0.0728, R2= 0.9878) and expressed as milligrams of Quercetin equivalents (QE). The MAE exhibited the highest total flavonoid content (191.64±1.45 mg QE/g), followed by Soxhlation (180.08±0.73 mg QE/g) and Maceration (126.1±0.56 mg QE/g).

The value of total phenolic and total flavonoid contents in extracts of *C. ternatea* leaves obtained by different extraction methods is shown in table 2 and graphically represented by fig. 2.

Table 2: Total phenolic and total flavonoid contents in extracts of *C. ternatea* leaves obtained by different extraction methods

Extraction method	Total phenolic contents (mg GAE/gm)	Total flavonoid contents (mg QE/gm)
Maceration	163.38±0.59	126.1±0.56
Soxhlation	208.68±0.96	180.08±0.73
MAE	248.29±0.29	191.64±1.45

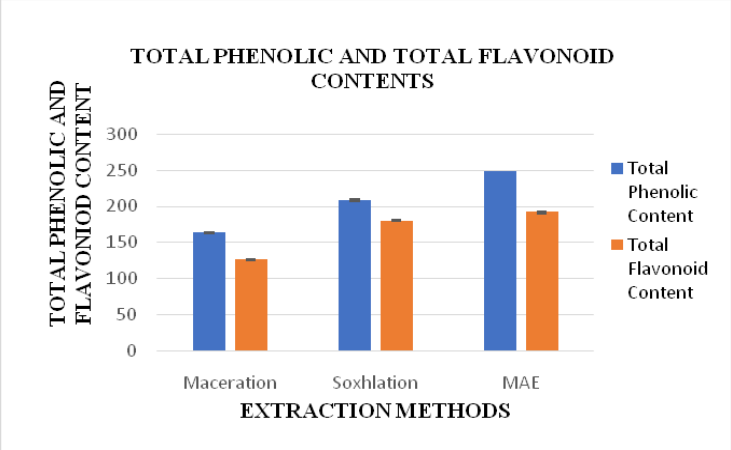

Fig. 2: Total phenolic and flavonoid contents. (Total phenolic content is expressed as mg GAE/gm and total flavonoid contents is expressed as mg QE/gm)

It was observed that conventional methods of extraction possess less phenolic and flavonoid content than other advanced methods of extraction like MAE. Plant extracts which contain phenolic compounds exert free radical scavenging and antioxidant properties due to the presence of hydroxyl groups in their chemical structure [13, 16, 19]. In the aluminium chloride colorimetric assay, aluminium chloride acts as a strong oxidizing agent. The flavonoids form stable complexes with this, where keto groups and highly reactive hydroxyl groups are mainly involved in the reaction mechanism [13, 21, 22].

The present study reveals the strong presence of phenols and flavonoids in the leaves of *Clitoria ternatea* when extracted with methanol in all the methods of extraction. Presence of these secondary metabolites are proved to be related with antioxidant and antiradical properties [13, 19, 21-24]. This can even be correlated with our previous study on leaves of *Clitoria ternatea,* which was reported to possess similar property [10].

CONCLUSION

In this present study, the microwave-assisted extraction method is proved to be more efficient than the other two conventional methods of extraction and thus can be used as a promising tool for extraction of *Clitoria ternatea* because of high yield and fast extraction ability with less consumption of solvent and time as well. This method may be preferred not only for its increased yield but also for better quality, as it producedmoreamounts of secondary metabolites. Further studies on isolation, characterization and high throughput screening of different phytochemical entities of *Clitoria ternatea* extracted by this exaction method may be encouraging to establish its place as an economical and alternative option replacing the conventional methods of extraction.

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AUTHORS CONTRIBUTIONS

For preparation of the article, each author has contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest associated with this study.

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