BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2013. Vol. **10**(1), 383-386

Extraction of Green Tea Leaves: The use of Different Methods, their Optimization and Comparative Evaluation

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(Received: 30 January 2013; accepted: 30 March 2013)

In the present study, green tea leaves were extracted by four different methods viz. traditional maceration, Soxhlet extraction, ultrasonication extraction and microwave irradiation method, using ethanol as solvent. Methods proposed herein were suitably optimized for the purpose of efficient extraction of tea leaves and further, these methods were subjected to comparative evaluation on the basis of results expressed as percentage yield of air-dried tea extract. From results, ultrasonication and microwave irradiation methods were found to be more efficient in extracting green tea leaves as compared to traditional maceration and conventional Soxhlet methods of extraction.

Key words: Tea leaves, Polyphenols, Ultrasonication, Microwave irradiation, Extractive value.

In recent years, green tea (*Camellia sinensis*, belonging to family theaceae) has been recognized as the most popular beverage worldwide, because of its potential health benefits in a variety of ailments, ranging from cancer to common cold. Medical and health care professionals agree that drinking tea being a potent medicine keeps the mind alert and sharp as well as promotes good health and longevity. Accumulating evidence suggest that tea consumption is beneficial for a wide range of clinical illness such as cancer, hypercholesterolemia, artherosclerosis, stroke, Perkinsons' disease, Alzheimers' disease, diabetes, inflammation, microbial diseases, aging etc. (Weinreb *et al*., 2004; Zaveri, 2006). Due to this reason, the consumption of green tea has been expedited by the general as well as patient population, with the inclusion of its decaffeinated extract as a featured ingredient in several marketed nutritional supplements (POLYPHENON E, TEAVIGO™). The beneficial effects of green tea extract are attributed due to the polyphenolic compounds (flavonoids), particularly the catechins, the amount of which has been regarded as an indicator of functional quality of green tea (Graham, 1992). Besides, there are several components present in green tea which includes polysaccharides, vitamin B, C, E, amino acids, especially á-amino butyric acid, proteins, lipids and fluoride. However, tea polyphenols, commonly called catechins, are the leading functional components, which accounts for 30% of the dry weight of green tea leaves (Yang, 1999). The proportion of catechins are more in green tea (unfermented) than in black (extensively fermented) or oolong (partially fermented) tea because of variation in the degree of fermentation of tea leaves after harvest. The catechins present in green tea are commonly called polyphenols. Fresh green tea leaves are very rich in catechins, which include mainly (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), (-)-

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epigallocatechin-3-gallate (EGCG), (+)-catechin, and (+)-gallocatechin (GC). EGCG is the most abundant catechin of green tea, which accounts for at least 65% of the total catechin content (Chu, 1997). Moreover, other compounds of interest include caffeine (3–6%), gallic acid, quercetin, kaempferol, myricetin, caffeic acid and chlorogenic acid etc. (Graham, 1992).

The composition of green tea leaves varies with climate, season, and variety of tea and age of the leaf. Solvent extraction is the most common and efficient method of separating active ingredients from plant materials and therefore, it is extensively used in the process of isolation of phytoconstituents from plant. It includes various classical techniques like maceration, decoction, infusion, enzymatic extraction, and continuous hot (Soxhlet) extraction. These methods work less efficiently in terms of their capacity and time required for extraction. Recently, some advanced techniques have been evolved that were found to be more advantageous and efficient over above mentioned classical techniques of extraction, because of their sophisticated design and improved working performance. These are namely, ultrasound assisted extraction, microwave assisted extraction, microwave-assisted high pressure extraction etc. (Victor *et al*., 1999). Among these, ultrasound and microwave assisted extractions have been successfully applied for the extraction of capsaicinoids from capsicum (Ming-Fei, 2012). Several attempts have also been made towards the development of a suitable method for the isolation of epicatechin compounds (with high yield) from the extract of green tea leaves (Copeland *et al*., 1998; Yoshida *et al*., 1999).

In the present study, extraction of green tea leaves, previously powdered was carried out by four different methods of extraction viz. traditional maceration, Soxhlet extraction, ultrasonication and microwave irradiation using redistilled methanol as solvent. Methods adopted for the extraction of tea leaves were thoroughly optimized in terms of different operational parameters such as extraction time, extraction temperature, irradiation power etc. (Talebi *et al*., 2004; Dabiri *et al.*, 2005). As far as functional quality of tea polyphenol components concerned, all the related parameters associated with different extraction techniques have been suitably optimized. Other parameters, namely the amount of dried sample (100 g) and solvent volume (200 mL), implicating the extraction process was kept constant.

The aim of the present study was to find out the most suitable method/technique (after optimization of different extraction related parameters) for the extraction of green tea leaves using methanol as solvent. In addition, a comparative study was also carried out among different methods of extraction in order to assess their extraction efficiency in terms of percentage yield of air-dried extract obtained from each individual method. Furthermore, the suitability of these instruments for routine extraction work has also been checked out.

MATERIALS AND METHODS

Materials

Commercial tea powder was purchased from the local distributor of Dibrugarh, town, Dibrugarh, Assam, India. Ethanol was procured from *Sd* Fine Chemicals Ltd., Mumbai, India. Soxhlet apparatus (500 mL) and domestic appliances of ultrasonic bath and microwave oven were used.

Defatting of tea powder

Coarsely powdered air-dried leaves of green tea (250 g) was placed in a glass- stoppered conical flask, macerated with sufficient quantity of petroleum ether shaking frequently, and allowed to stand for over two nights. The completion of defatting process was confirmed by performing spot test on ordinary filter paper. The solvent from defatted tea powder was then removed by filtration and dried. Dried tea powder was stored in an air-tight container until subjected for further extraction process.

Maceration

About 100 g of tea powder, defatted previously was macerated with 200 mL of ethanol in an Erlenmeyer flask for 6, 12, 24, 48 hours respectively.

Soxhlet extraction

100 g of defatted green tea powder was subjected to Soxhlet extraction using 200 mL of ethanol in an Erlenmeyer flask as solvent. The extraction process was carried out at a temperature ranging from 60-70ºC until the tea powder gets completely exhausted.

Ultrasonic extraction

In this method, 100 g of defatting tea powder was transferred in an Erlenmeyer flask and 200 mL ethanol was added into it. The flask was then placed in a clean ultrasonic bath (PCI India, 50MHz) for facilitating extraction process. In order to evaluate the effect of time on extraction process, extraction (sonication) was carried out for 10, 20, 30 and 40 minutes at room temperature.

Microwave extraction

Microwave extraction was carried out on a Samsung microwave oven (GW73BD, 950 watt). 100 g tea powder, defatted previously was transferred in Erlenmeyer flasks containing 200 mL ethanol. The instrument was set at a temperature of 60ºC and glass beads were added cautiously into the flask to avoid bumping. Extractions were performed for 10, 20, 30 and 40 minutes at a constant power supply. To avoid unwanted rising in temperature, extraction process was continued in a pulse manner with 5 minutes rest for 10 minutes operation.

Calculation of percentage yield

After completion of extraction process, extracts obtained from each method were filtered separately through Whatman No. 1 filter paper and filtrate was collected. Filtrate was concentrated by removal of solvent in a rotary evaporator ($60^{O}C$), transferred into a flat bottom dish and dried at $105^{O}C$ for about 3 hours. Extract was then cooled immediately in a desiccator for about 1 hour and weighed. The content of extractable matter was calculated in % w/w of air dried material.

RESULTS AND DISCUSSION

In the present study, green tea leaves (100 g) were extracted by four different proposed methods using ethanol (200 mL) as solvent. Results depicted in table 1 clearly indicate that all the four methods were found to be efficient in extracting maximum yield of dried extract (expressed on air-dried basis) from powdered tea leaves for a different time interval. Ultrasonication and microwave irradiation techniques showed better yield of dried extract (% extractable matter) than maceration and Soxhlet extraction methods. Results reveal that ultrasonication and microwave irradiation extracted tea powder with maximum yield of about 13.3% in about 40 minutes, whereas in Soxhlet and maceration, this yield was obtained after 4 and 48 hours of extraction, respectively. However, it could be assumed that ultrasonication and microwave irradiation are more powerful extraction tools as compared to other two conventional methods, because of easy penetration of solvent molecules into plant material followed by enhancing the rate of extraction in these two methods. Ultrasonicator works on ultrasound and microwave oven on microwaves radiation that offers a very rapid delivery of radiant energy into the solvent and thereby tracing sample cells directly. As a result, plant cell walls get disrupted and extraction process is thus facilitated even more (Sharma *et al*., 2008). Further, extraction can efficiently be carried out at room temperature in ultrasonicaton process; which is surely encourageous for the extraction of plant materials that contain thermolabile principles (Wang and Keit, 2000). In this way, it is more advantageous over other conventional extraction methods. This study also proves the suitability of the utrasonication and microwave irradiation as a rapid and efficient extraction process for the extraction of biologically important phytoconstituents from tea leaves avoiding unnecessary tedious clean-up or derivatisation steps.

Table 1. Extractive values of powdered tea leaves obtained by different extraction methods

Method of extraction	Colour of extract	% Yield (w/w)*				
		4 h	6 h	12 h	24 h	48 h
Maceration	Dark brown	-	5.53	6.09	10.34	13.36
Soxhlation	Dark brown	13.30	-	-	-	-
		5 min	10 min	20 min	30 min	40 min
MW irradiation	Dark brown	-	3.14	7.01	13.41	13.39
Ultrasonication	Dark brown	-	2.14	6.03	13.24	13.35

*mean of three replicates

However, an attempt has been made, in this study, to assess the extraction efficiency of different extraction processes considering optimization of different extraction parameters, and also find out their suitability as well as selectivity towards extraction of green tea leaves with high yield and possible separation of active ingredients without any chemical/biological deactivation. Ultrasonication and microwave extraction has been shown to be efficient methods for the extraction of tea leaves and hence can be adopted, in large scale, for the extraction and/or isolation of bioactive components from crude drugs.

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