EXTRACTION OF POLYPHENOLS FROM GREEN TEA USING MICROWAVE ASSISTED EXTRACTION METHOD
TRÍCH LY POLYPHENOL TỪ TRÀ XANH SỬ DỤNG PHƯƠNG PHÁP TRÍCH CÓ HỖ TRỢ VI SÓNG

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TÓM TẮT

Dịch trích trà xanh được trích từ lá trà xanh sử dụng dung môi trích theo hai phương pháp trích thông thường và phương pháp trích với sự hỗ trợ vi sóng .Một số yếu tố ảnh hưởng như dung môi (rượu-nước), tỉ lệ nguyên liệu/dung môi (1/5-1/15), pH, nhiệt độ trích, thời gian trích và ngâm của hai phương pháp được khảo sát. Ở cùng điều kiện khảo sát, phương pháp trích với sự hỗ trợ vi sóng cho hiệu suất cao với thời gian ngắn hơn (82,6 % trong 360 giây) phương pháp trích ly thông thường, 62,1% trong 180 phút. Dịch trích trà xanh theo phương pháp trích có hỗ trợ vi sóng có hàm lượng polyphenol (36 %) cao hơn phương pháp thông thường. Phương pháp trích có sự hỗ trợ vi sóng hữu hiệu hơn phương pháp trích thông thường về chất lượng, thời gian và chi phí năng lượng.

ABSTRACT

Green tea extract (GTE) was extracted from raw green tea by using solvent extraction with traditional heating method (SETM) and microwave-assisted extraction (MAE method). Several factors such as solvents (alcohol aquaus), material: solvent ratio (1/5-1/15), pH, extraction temperature, immersion and extraction time of both methods were studied. In same conditions, MAE method gave higher yield in much shorter time than SETM, 82.56% in 360 seconds and 62.14% in 180 minutes, respectively. GTE from MAE method had total polyphenols concentration higher (36%) than that of SETM. Consequently, MAE method was found to be much more effective than SETM in quality of GTE, time and energy consumption.

Keywords: Polyphenols, green tea, green tea extract, extraction, microwave assisted extraction.

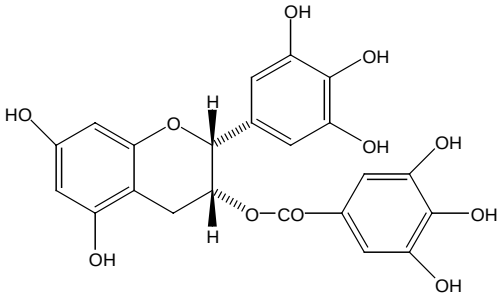
1. INTRODUCTION

Tea (*Camellia sinensis*) is native to the East Asia region. *Camellia Sinensis*, a member of the Theaceae family, is an evergreen, usually picked as young shoots in cultivation. Its first recorded use dates from the fourth century A.D. in China [Tong.V.Hang 1985]. Nowaday, tea with its variety products is one of the most widely consumed beverages in the world. Main tea producers concentrated in equatorial regions. Three biggest tea producers and exporters are China, India and Vietnam.

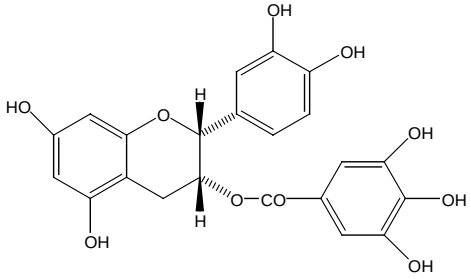
Young shoots tea (green tea) contains many polyphenolic compounds, up to 30-40 percent of the extractable solids of dried green tea leaves, with most of the polyphenols being flavanols more commonly known as catechins. The primary catechins in green tea are epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG) (Fig. 1) [Tong.V Hang 1985], [Fujikia et al 2002], [Hemiway et al 1992].

Recently green tea as a healthy beverage has attracted science attention for its anticancer and antioxidation activities [Weisburger 2000]. Polyphenols in green tea are believed as good free radical scavengers. Several clinical studies found them to be active in cancer prevention in several ways and polyphenols have been recently recognized as functionally active molecules, possessing antioxidant, anticancer, antimutagenic

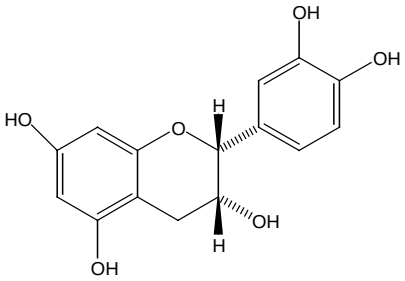
properties, as well as exerting protective effects against cardiovascular and other diseases [Brown, 1999], [Aldini et al 2003], [Gupta et al 2002], [Jun Cai et al 2002], [Azam et al 2004].



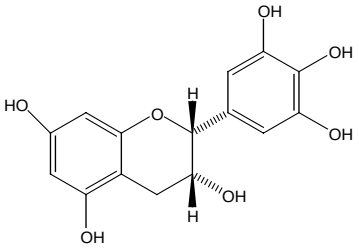
Epigalogatechin –3- gallate (EGCG)



Epicatechin –3- gallate (ECG)



Epicatechin (EG)



Epigalocatechin (EGC)

Figure 1. Catechins in green tea extracts

Vietnam is one of biggest tea producers and exporters in the world, however main export products is raw material or traditional products such as black tea, oolong tea and green tea. Green tea extract is still new in Vietnam market and also new in research and application.

Green tea extract was prepared using several different techniques, including solvent extraction, soxhlet extraction; microwave assisted extraction, solid-phase extraction, and supercritical fluid extraction. Each technique has its own advantages and problems, in small-scale produce, microwave method seems to be a reasonable method that helps reduce time and save energy for producer.

In this article, several factors involving in microwave-assisted extraction were studied and compare to those of solvent extraction using traditional heating method.

2. MATERIALS AND METHODS

Reagents

Fresh green tea (picked as shoots with two or three leaves) was purchased from Ladotea (Lam Dong Vietnam). Folin - Ciocalteau agent (Merck), citrate - phosphate pH buffer (Merck), ethanol and other chemical in AP grade

Green tea extraction method

Microwave assisted extraction (MAE): Fresh green tea was collected as shoot on fields. 100g of fresh green tea were cut to 1 - 1.5mm size then immerse in solvents (1:5 to 1:15 g/ml) for a certain time (0 - 90 minutes). Then it was transferred to flask, adjusted pH, and brewed in microwave oven (450W) (times: 300- 420s), radiation is done at regular intervals (30s interval) to keep temperature not rise above 70^{o}C. After that, the infusion was let cool down to room temperature, filtered to separate solid and concentrated by rotary vacuum evaporation to 50ml. Final infusion was stored in refrigerator at 4^{o}C.

Solvent extraction using traditional heating method (SETH): Extraction procedure was carried out similar to MAE, except extraction time was 180 minutes.

Total polyphenols determination

Total polyphenols concentrated (TPC) in infusion were spectrophotometrically determined measuring absorption after using Folin-Ciocalteau reagent, using Jenway 6505 UV-VIS Spectrophotometer at 700 nm. GTE composition was identified by TLC.

3. RESULTS AND DISCUSSION

Effect of extraction time

Fig 2. show that when using MAE extraction yield increases with extraction time and get the highest at 360 seconds, 82.56%, compare to 61,14% of that of SETH (highest yields in 180 minutes extraction). Increasing extraction time in MAE did not help increase yield. In some experiments, when increasing extraction time above 420 seconds, extraction yield dropped below 50%, could be due to effect of long radiation of microwave to polyphenols molecules.

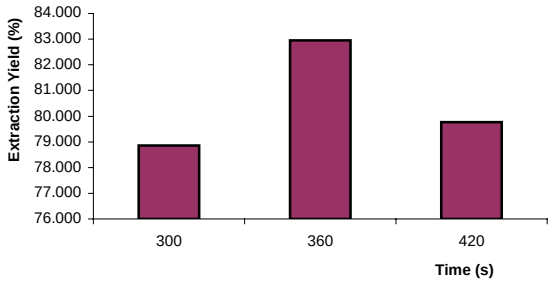


Figure 2. Effect of time to TPC

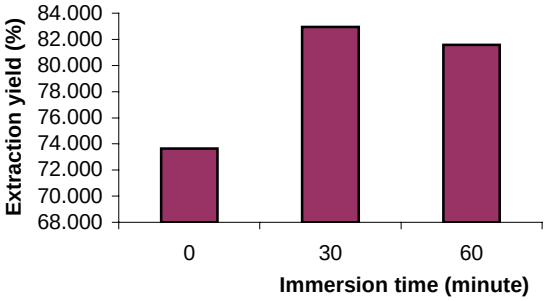


Figure 3. Effect of immersion time to TPC

Effect of immersion time

Immersion before extracting help increase extraction yield, but not significant. Immersion gives time to solvent to complete absorbed into material. Yield from 73,64% when not immersion went to 82,56% when immersion in 30 minutes. Fig 3. shows that immersion time doubles (60 minutes), extraction yield slightly decreased to 81.59%. In this factor, SETH showed the same change trend, although with lower yield.

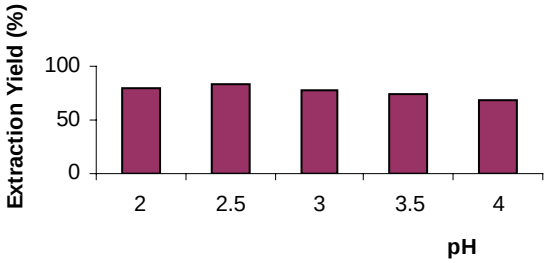


Figure 4. Effect of pH to TPC

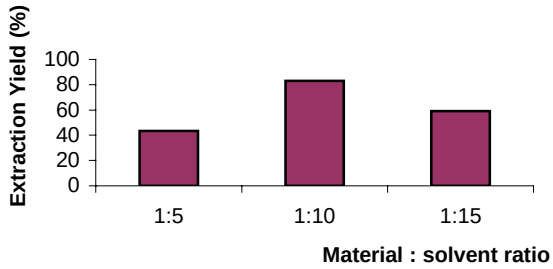


Figure 5. Effect of material: solvent ratio to TPC

Effect of pH

Polyphenols are strong antioxidants, so they are easily oxidated in high pH. Extracting is usually carried out at acid pH because of high antioxidant activities in neutral and base pH. The lower pH is, the better polyphenols are protected, and the higher yield gets. In Fig 4, highest yield at pH 2.5 (82.56%), slightly lower at 2 (79.09%), but continuously decreased above pH 2.5, at pH 6, only 52,50%. The results were similar in SETH with highest yield is 74.57% at pH 3.

Effect of material: solvent ratio

Polyphenols in material go into solvents by diffusion, so extraction yield increase with material: solvent ratio. In Fig. 5, ratio 1:10 gave highest yield (82,56%), 1:5 only 43.28%. But when increasing ratio to 1:15, yield downed to 59,18%, because more time need to heat a large volume of solvents (extraction time in MAE is only 360 seconds). In SETH, ratio lower than 1:5 gave very low yield, material: solvent ratio from 1:5 to 1:10 had not get significant effect to extraction yield because of long extraction time (180 minutes).

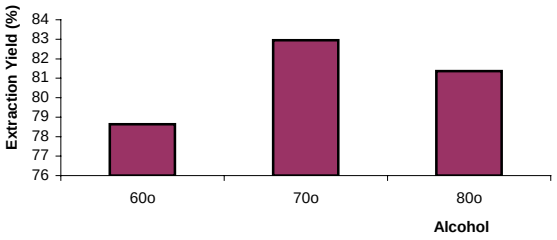


Figure 6. Effect of solvents to TPC

Effect of solvents

The most effective solvent in this experiment was ethanol 70^o. Extraction yield when using ethanol 70^o was 82.56%, higher than using ethanol 80^o (81,36%) and ethanol 60^o (78.64%). However, these changes did not give much effect to extraction procedure.

4. CONCLUSION

Compare to solvent extraction using traditional heating method, in solvents, immersion time, pH, material: solvent ratio factors, microwave assisted extraction gave similar changing trend, but with higher yield (36% average). In time consumption, MAE is superior to SETM with extraction time only 360 seconds (6 minutes- and 36 minutes total, including immersion time), while extraction time in SETM is 180 minutes – 210 minutes total. Consequently, MAE is suitable method to extract green tea for further investigation.

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