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ULTRASONICAÇÃO DE *CUCUMIS MELO* VERSUS PERCOLAÇÃO: POLIFENOIS, FLAVONÓIDES TOTAIS E EXTRATO DE BETA-CAROTENO COMO CORANTES NATURAIS

ULTRASONICATION OF *CUCUMIS MELO* VERSUS PERCOLATION: POLY PHENOL, TOTAL FLAVONOID, AND BETA–CAROTENE EXTRACT AS NATURAL DYES

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RESUMO

Os corantes naturais são pronunciados como uma alternativa interessante aos corantes fabricados. Assim, a demanda atrativa para desenvolver as técnicas de extração dos melhores ingredientes de corantes sólidos e líquidos para corantes naturais a partir de materiais de frutos e sua aplicação na vida humana tem sido cultivada. A extração é processada usando solventes ou usando técnicas modernas como ultrassom e micro-ondas. A extração atual de corante natural de *Cucumis melo* foi realizada usando a técnica de percolado como método de extração de modelo, bem como a aplicação de ultrassom. Na técnica de ultrassonografia, as amostras foram extraídas com metanol em banho ultrassônico de limpeza por sonicação indireta para obtenção de extratos ultrassônicos. A determinação da estrutura dos ingredientes ativos foi realizada por espectroscopia de UV. O corante, de cor intensa, extraído do *Cucumis melo* foi comprovado como sendo β-caroteno, um incremento bem-sucedido de 12,5% de rendimento foi relatado para o processo de extração ultrassônica. O β-caroteno como carotenoide, que possui propriedades antioxidantes, é o principal carotenoide do melão. De acordo com seus benefícios para a saúde, o teor de β-caroteno nos frutos é de grande interesse para a indústria alimentícia e para os criadores de melão. Além disso, a determinação do conteúdo total de flavonoides fenólicos e totais para a percolação e extratos ultrassônicos foi alcançada com sucesso. Os teores totais de fenólicos e flavonoides dos extratos foram determinados pelo método de Folin-Ciocalteu e cloreto de alumínio, respectivamente. o método de extração por percolação apresentou 176,0 $mg \cdot g^{-1}$ como o menor conteúdo fenólico total comparado ao método de extração ultrassônica (183,2 mg g^{-1}). O teor de flavonoides totais da polpa de *Cucumis melo* variou de 59,6 e 71,4 mg CE g^{-1}, por percolação e extrato ultrassônico, respectivamente.

Palavras-chave: *Cucumis melo*, extração sonoquímica, corante β-caroteno, polifenol, flavonoides totais

ABSTRACT

Natural dyes are pronounced as an interesting alternative to fabricated dyes. Accordingly, the attractive demand to develop the extraction techniques of the best ingredients of solid and liquid for natural dyes colorants from fruits materials and their application in human life have been grown. Extraction is processed using solvents or using modern techniques like ultrasonication and microwave. Current extraction of natural colorant from *Cucumis melo* has been performed using percolate technique as a model extraction method as well as applying ultrasonication. In the ultrasonication technique, samples were extracted with methanol in an ultrasonic cleaning bath by indirect sonication to yield ultrasonic extracts. Determination of the structure of active ingredients was performed conducted by UV spectroscopy. The dye extracted from *Cucumis melo* of intense color was proven

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to be β-carotene, a successful increment by 12.5% yield has been reported for the ultrasonic extraction process. β-carotene as a cortenoid which has antioxidant properties is the major carotenoid in cantaloupe. According to its health benefits, the β-carotene content in fruits is of great interest to the food industry and to melon breeders. Moreover, the determination of total phenolic and total flavonoid contents for the percolation and ultrasonic extracts have been successfully attained. The total phenolic and flavonoid contents of the extracts were determined using the Folin–Ciocalteu and aluminum chloride method, respectively. the percolation extraction method had 176.0 mg g^{-1} as the least total phenolic contents compared to ultrasonic extraction method (183.2 $mg{\cdot}g^{-1}$). The total flavonoid content of *Cucumis melo* flesh was varied from 59.6, and 71.4 mg CE g^{-1} possessed by percolation and ultrasonic extracts, respectively.

Keywords: *Cucumis melo, Sonochemical extraction, β-carotene dye, Polyphenol, Total flavonoid.*

الملخص

ازداد الطلب على الأصباغ الطبيعية كبديل أساسي للأصباغ المصنعة، مما ترتب علية الاحتياج إلى تطوير تقنيات الاستخلاص للمكونات الصلبة والسائلة المنتفاه من الفاكهه وتطبيقاتها في الحياه البشرية. حيث تتم المعالجة والاستخلاص باستخدام المذيبات العضوية، وكذلك استخدام تقنيات حديثة كأشعة الموجات فوق الصوتية وأشعة الميكروويف. في هذا السياق، تمت عملية استخلاص صبغة البيتا-كاروتين من الشمام بإستخدام طريقة الإستخلاص بالترشيح كنموذج منهجي للإستخلاص. علاوة على ذلك، تمت عملية الإستخلاص عن طريق تطبيق تقنية أشعة الموجات فوق الصوتية. في هذه الطريقة الأخيرة استخلصت العينات بإستخدام الميثانول كمذيب عن طريق التعريض الغير مباشر لأشعة الموجات فوق الصوتية. وقد تم اثبات بنية المكونات النشطة بواسطة التحليل الطيفي للأشعة فوق البنفسجية، والذى أثبت أن الصبغة المستخلصة ذات اللون الكثيف هي صبغة البيتا-كاروتين. حيث أدى استخدام تقنية الموجات الصوتية إلى التحسين في الاستخلاص وزيادة نسبة الناتج بنسبة 12.5% متفوقا على الاستخلاص بالترشيح. مما ترتب علية اعتبار صبغة البيتا-كاروتين، ككاروتينويد مضاد للأكسدة، هو الكاروتينويد الغالب في فاكهة الشمام. وبالنظر في الفوائد الصحية للفواكه الطبيعية والغنية بصبغة البيتا-كاروتين جعلها ذات اهتمام عظيم في مجال الصناعات الغذائية والأغذية القائمة على الشمام. وفي هذا العمل تم أيضا تعيين محتوى البولي فينول والفلافونيدات في المستخلصات التى تم الحصول عليها. حيث وجد أن طريقة الإستخلاص بالترشيح أنتجت نسبة البولي فينول الأقل (176.0 مجم لكل جرام) مقارنة بتقنية الموجات فوق الصوتية التى أعطت 183.2 مجم لكل جرام. وتدرج المحتوى الكلي للفلافونيدات ما بين 59.6 – 71.4 مجم لكل جرام لمستخلصي الإستخلاص بالترشيح ومستخلص الموجات فوق الصوتية على التوالي.

الكلمات المفتاحية: *الشمام، الاستخلاص بالأشعة فوق الصوتية، بيتا-كاروتين، البولي فينول، الفلافونيدات.*

1. INTRODUCTION

Nowadays, the consumer preference for natural products instead of artificial ones and this has acquired an important role in the food industry, at least in part, due to nutritional supplementation and healthy properties correlated with them. For instance, *Cucumis melo* that belongs to the *Cucurbitaceae* family, the fruit is known locally in the Kingdom of Saudi Arabia as "Shamam" and it is widely known as a result of its calyces have been widely used to prepare cold beverages in many of the world's tropical and subtropical countries as well as in phytotherapeutic applications (Amaro *et al.*, 2012; Neocleous *et al.*, 2017; Daniel *et al.*, 2011; Melo *et al.*, 2008; Gonda *et al.*, 2010; Ismail *et al.*, 2009; Ismail *et al.*, 2011; Kourkoutas *et al.*, 2006). Many biological activities have been extensively reported, such as antihypertensive (Naito *et al.*, 2005) cardioprotective agents (Napier *et al.*, 2014; Paris *et al.*, 2012), hepatoprotective (Sebastian *et al.*, 2010), an inhibitor against porcine pancreatic amylase (Sebastian *et al.*, 2010; Vouldoukis *et al.*, 2008), sedative (Lin *et al.*, 2007), and antioxidant capacity (Ali *et al.*, 2005; Hansawasdi *et al.*, 2000; Amos *et al.*, 2003). Moreover, *Cucumis melo* has gained an important spot in the soft drinks market, and commercial preparations of *Cucumis melo* extract are forthwith marketed as supplements due to their supposed plausible health benefits and as a colorant to compensate some synthetic dyes.

The intense red pigments in the yellow calyces of *Cucumis melo* are beta-carotene (Chen *et al.*, 2004; Zhang *et al.*, 2004). Differentiation found on beta-carotene are related by the number of hydroxyl groups, the nature, position, and number of sugars (*e.g.,* D-glucose, D-galactose, *etc.*) attached to the molecule and the number of aliphatic acids (*e.g.*, acetic, malic, and malonic acids, *etc.*) or aromatic acids (*e.g.*, caffeic, *p*-coumaric or ferulic acid) linked to sugars in the molecule(Singh *et al.*, 2016). Among eighteen known naturally occurring a glyconesare common in higher plants, they are named as pelargonidin (Pg), peonidin (Pn), cyaniding (Cy), malvidin (Mv), petunidin (Pt) and delphinidin (Dp) (Bhuyan *et al.*, 2004; Bednar *et al.*, 2003; Ichiyanagi 2000). The glycosides of the three non-methylated anthocyanidins (Cy, DpandPg) are the most prevalent in nature (Calvo 2008), and their structures were elucidated using spectroscopic and chemical transformation methods (Arráez-Román *et al.*, 2009; Carrasco *et al.*, 2006).

Beta-carotene pigments are compelled for the red to purple colors of several fruits, and they are very useful in taxonomic studies to scrutinize their obvious importance to the color quality of fresh and processed fruits and vegetables (Bednar *et al*., 2005).

The main aim of the present work was to develop a green and efficient method for the selective extraction, separation, and identification using spectroscopic methods in order to determine qualitatively beta-carotene in *Cucumis melo*. Nowadays people try to avoid synthetic materials, that could affect health and instead use the natural ones in both food decoration and pharmaceutics. Accordingly many types of research are going on to find new attractive natural extracts from vegetables or fruits. Such coloring ingredients are natural phenolic compounds, generally characterized by especial structure formula that contains more than one aromatic ingredients(Kong *et al*., 2003). These colored ingredients; which include specified core structures, with some other substituent radicals such as aromatic rings, alkyl groups, OH, OR, are present in many plants, with various complicated structural formulae such as flavones, coumarins, xanthones, carotenoids of high terpenes and anthraquinones.

The above findings encouraged us to extract the *β*-carotene as natural dyes from *Cucumis melo* using percolation method in comparison to the ultrasonication process. Additionally, investigating its chemical composition and exploring the total phenolic and total flavonoid contents was the second target.

2. MATERIALS AND METHODS

1. Chemicals

Ferrozine, Trichloroacetic acid (TCA) and Chemical reagents were purchased from Sigma Chemicals Co. (St Louis, MO, USA). Quercetin, Gallic acid, EDTA, Potassium ferricyanide, and Aluminium chloride were purchased from Merck (Darmstadt, Germany). All other chemicals and reagents used were of the highest commercially available purity.

2. Plant material preparation

The fresh fruit Cucumis melo were collected from Jappar (40.0kg North of Al-khurma Province), Taif, KSA. The fruit collected was further shelled, and dried organs were ground to a reduced fine powder using Thomas-Willey Milling Machine.

3-Extraction process

a- Percolation method

24.0 g of homogenized *Cucumis melo* powder dry parts was mixed with one liter of methanol for 48 hours with magnetic stirring at room temperature. The solution was filtered off, the filtrate was mixed with 40.0 g Amberlite XAD-2 (pore size 9 nm, particles size 0.3-1.2 mm) and stirred in a magnetic stirrer for 40 minutes at room temperature, in order to absorb enough dyes quantity. The Amberlite particles were then packed into a glass column (64 cm × 42 cm) and the column was washed with deionized water until a colorless solution was obtained. The carotenoids remained absorbed on the column was eluted with one liter of methanol (70% v/v) and acetic acid (1% v/v). The intense red solution was concentrated to dryness in a rotary evaporator under reduced pressure at 27℃. During the last step, 4 ml of water were added to dissolve the extract, which was then passed through a 5 ㎜ membrane filter and keep before the analysis by using spectroscopic methods.

b- Ultrasonically assisted extraction

Cucumis melo powder dry parts were extracted with methanol in an ultrasonic cleaning bath over a one hour by indirect sonication at a frequency of 100 kHz and a temperature of 25±3°C to yield ultrasonic extract. The extract was then separated from the sample residue by filtration. The resultant extract was concentrated in a rotary evaporator until a crude solid extract was obtained which was freeze-dried for complete solvent removal and used as ultrasonic (US) extract (Tantipaibulvut *et al*., 2018).

4- **Determination of total flavonoid content**

Determination of flavonoid was carried out using a colorimetric aluminum chloride method (Ghasemi *et al*., 2009). 0.5 mL solution of the methanol extracts were mixed separately with 1.5 mL of methanol, 10 % aluminum chloride (100 µL), 1M potassium acetate (100 µL), and 2.8 mL of distilled water. Afterward, the mixture was left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 417 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). Total flavonoid contents were calculated as quercetin equivalent from a calibration curve. A calibration

curve was designed for the calculation of total flavonoid contents as quercetin equivalent using prepared quercetin solutions at concentrations 12.5 to 100 mg mL^{-1} in methanol.

5- Determination of total phenol content

Measurement of total phenolic content was performed via a colorimetric method using Folin-Ciocalteu reagent (Rabiei *et al*., 2012). The extract samples (0.5 mL) were mixed with Folin Ciocalteu reagent (5 mL, 1:10 diluted with distilled water) for 5 min and then aqueous Na_2CO_3 (4 mL, 1M) were added. after incubation for 2h at room temperature, the absorbance of the reaction mixture was measured at 760 nm. The standard curve was furnished using gallic acid as a standard reference; 0, 50, 100, 150, 200, and 250 mg mL^{-1} solutions of gallic acid in aqueous methanol (50%). The results were expressed as milligram gallic acid equivalent (mg GAE)/g of extract.

6- Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times.

3. RESULTS AND DISCUSSION:

β-carotene is a deeply-colored, red-orange pigment founded in plants. It is an organic compound and chemically is classified as a hydrocarbon and specifically as a terpenoid (isoprenoid), reflecting its derivation from isoprene units. It is biosynthesized from geranyl pyrophosphate (Bednar *et al*., 2005) and it was a member of the carotenes, which are tetraterpenes, synthesized biochemically from eight isoprene units and thus having 40 carbons as shown in figure 1. Among this general class of carotenes, *β*-carotene is distinguished by having beta-rings at both ends of the molecule (Ghasemi *et al*., 2009; Hou *et al*., 2007). Separation of *β*-carotene from fruits appeared in carotenoids is commonly isolated via column chromatography. The extraction of *β*-carotene from a mixture of the other carotenoids is based on the polarity of a compound. *β*-carotene is a non-polar compound, so it is separated with a non-polar solvent such as hexane (Gradinaru *et al*., 2002). Being highly conjugated, it is deeply colored, and as a hydrocarbon lacking functional groups, it is very lipophilic (Prior *et al*., 1998; Tsuda *et al*., 2002).

Choo *et al*., (2008) reported different methods for extraction of *β*-carotene from palm oil (Almahy *et al*., 2015), it involves selective adsorption of carotenoids from alkyl esters in an open column. High recovery of carotenoids (> 90 %) could be obtained, and the column could be used over 50 times without any loss of activity. At the same time in addition to extracted *β*-carotene from palm oil Ooi *et al.,*(2004) reported different methods that involve the extraction of *β*-carotene from *Dunaliella salina*.
The results obtained from the UV-Visible spectrophotometric analysis of natural dye obtained from *Cucumis melo* showed that the maximum absorbance for the sample was at a wavelength of 452 nm, which is characteristic of *β*-carotene expressed as total carotenoids as shown in figure 2. The absorbance values for natural dye extract obtained by percolation and ultrasound were shown in figure 2 and table 1. The results indicate that there is about 12.5% improvement in the % yield of extract due to the use of ultrasound as compared to the percolation process.

Table one and figures 2 and 3 revealed that there was a significant improvement in the % yield of coloring matter extract obtained from ultrasound superior to percolation. The difference in the enhancement in extraction yield with ultrasound could be due to the different degree of binding of coloring matter attached to plant cell membranes. Moreover, another important factor is chemical constituents present in plant material responsible for the color (chromophore group) and their solubility nature.

Total phenolic content

Polyphenols are decisive components in fruit tissues. Phenolic compounds are a class of antioxidant compounds, they are acting as free radical terminators (Shahidi and Wanasundara 1992). It was reported that the antioxidant activities in the plant are resonated to total phenolic content (Chang *et al*., 2001). The plausible explanation is laying on their redox properties, which permits them to act as reducing agents. They are hydrogen donors as well as oxygen quenchers.

Total phenolic content of percolation and ultrasonic extracts were determined by extrapolation of the gallic acid calibration curve ($y = 0.08x-0.010$, $R^2 = 0.9934$) and expressed in milligrams of gallic acid (mg GAE g^{-1}), Figure 4. The Folin-Ciocalteu phenol reagent assay is used widely for a crude estimation of the amount of phenolic compounds exist in an extract. This method is based on the reducing power of the

phenolic hydroxyl groups, which react with the Folin-Ciocalteu phenol reagent to form chromogens that can be distinguished spectrophotometrically at 760 nm. Table one and figure five showed that the percolation extraction method had lower total phenolic contents (176.0 mg g^{-1}) than ultrasonic extraction method (183.2 mg g^{-1}).

Total flavonoids content

Flavonoids, which have the basic skeleton of diphenylpropanes (C6 - C3 - C6) with different oxidations of the central pyran ring, are widely distributed in the plant kingdom and constitute about half of the 8,000 or so explored phenols (Heim *et al*., 2002). Flavonoids are the molecules responsible for the color of fruit and flowers. As the products of secondary metabolism in plants, they have an attractive interest to the pharmaceutical and food industries owing to their exploited antioxidant activity (Paniwnyk *et al*., 2001). They are proved to be the most interesting antioxidants as they are almost the commonly and widely distributed group of plant phenolic compound (Yanishlieva-Maslarova, 2001).

Using this method, flavonoids with some distinct chemical structure can react with Al^{3+} and furnished a red complex, which provides the superlative absorption at 510 nm. We, therefore, can only roughly evaluate the amount of flavonoids present in an extract by using the spectrophotometric analysis method. Flavonoids form a pervasive group of polyphenolic materials customarily originated by plants. Flavonoids, in other words, are of great interest for their bioactivities, which are basically related to their anti-oxidative properties (Cote *et al*., 2010). It has been recognized that flavonoids show antioxidant activity and their influences on human nutrition and health are appreciable. At the current study, total flavonoids content of percolation and ultrasonic extracts were determined by extrapolation of the catechin calibration curve ($y = 0.002x-0.027$, $R^2 = 0.9951$) and expressed in milligrams of Catechin (CE)/gram (Fig. 6). In this study, aluminum chloride colorimetric was provided as a specific explorer of the typical structure of flavonoids. From table one and figure five, it was envisioned that the total flavonoid content of *Cucumis melo* flesh was varied from 59.6 and 71.4 mg CE g^{-1} percolation and ultrasonic extracts, respectively. The ultrasonic extract was superior, and it has a higher amount of total flavonoid content.

4. CONCLUSIONS

Natural dyes provide an environmentally safe privilege for the coloring of food and other materials. It was concluded that the application of ultrasound could increase the extraction yield percent of *β*-carotene compared to percolation. The reason for the improvement could be due to better leaching of natural dye material from plant cell membranes and mass transfer to solvent reinforced by acoustic cavitation administered by ultrasound. Assorted process parameters such as solvent system, temperature, ultrasound power, amount of dye material, etc., are interesting for our study as future work. Additionally, the total flavonoid and poly phenolic contents are interestingly influenced by the technique of extraction applied.

5. ACKNOWLEDGMENTS:

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Table 1. *Total phenolic and Flavonoids contents of different fruit extracts*

Extraction method	Yield (%)	Total phenolic content (mg g^{-1})	Total flavonoid contents (mg g^{-1})
Percolation	34.2	176.0 ± 4.6	59.6 ± 1.3
Ultrasonication	39.7	183.2 ± 5.4	71.4 ± 3.2

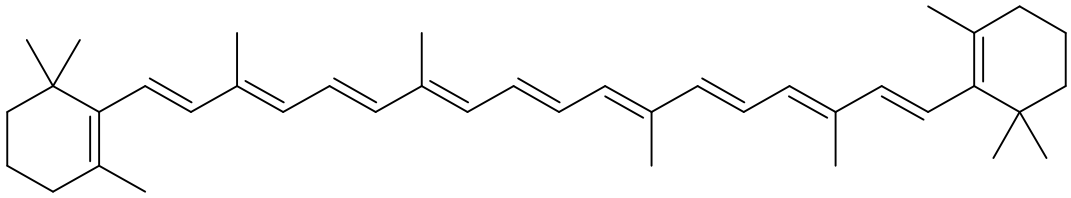

Figure 1. *Betacarotene (1,3,3-trimethyl-2-[3,7,12,16-tetramethyl-18-(2,6,6-trimethyl cyclohex-1-en-1-yl) octadeca-1,3,5,7,9,11,13,15,17-nonaen-1-yl] cyclohex-1-ene)*

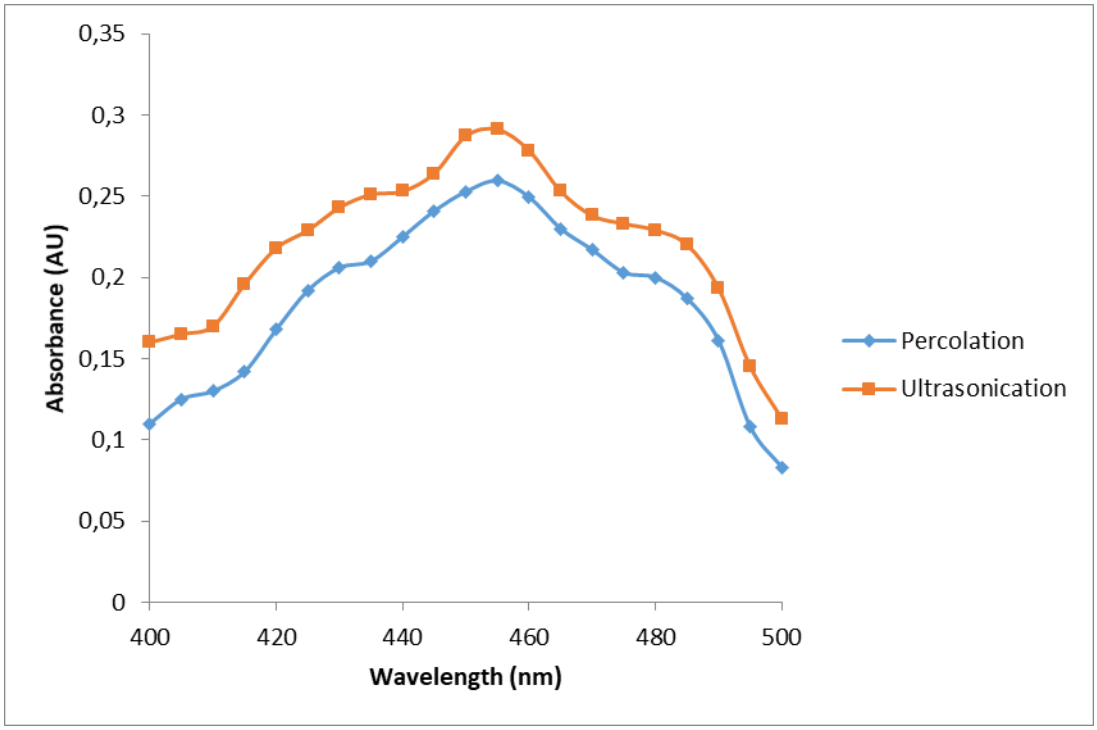

Figure 2. *Wavelength absorbance curve as obtained from the spectrophotometer*

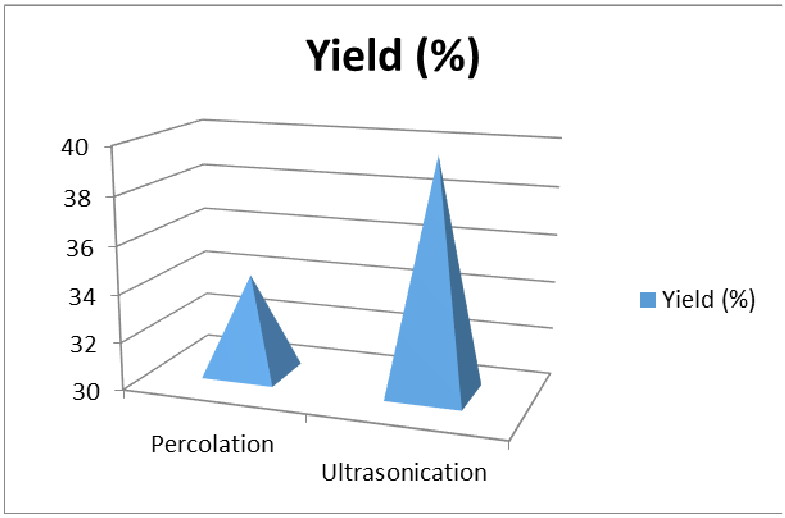

Figure 3. *Yield percent for the percolation and ultrasonic extracts.*

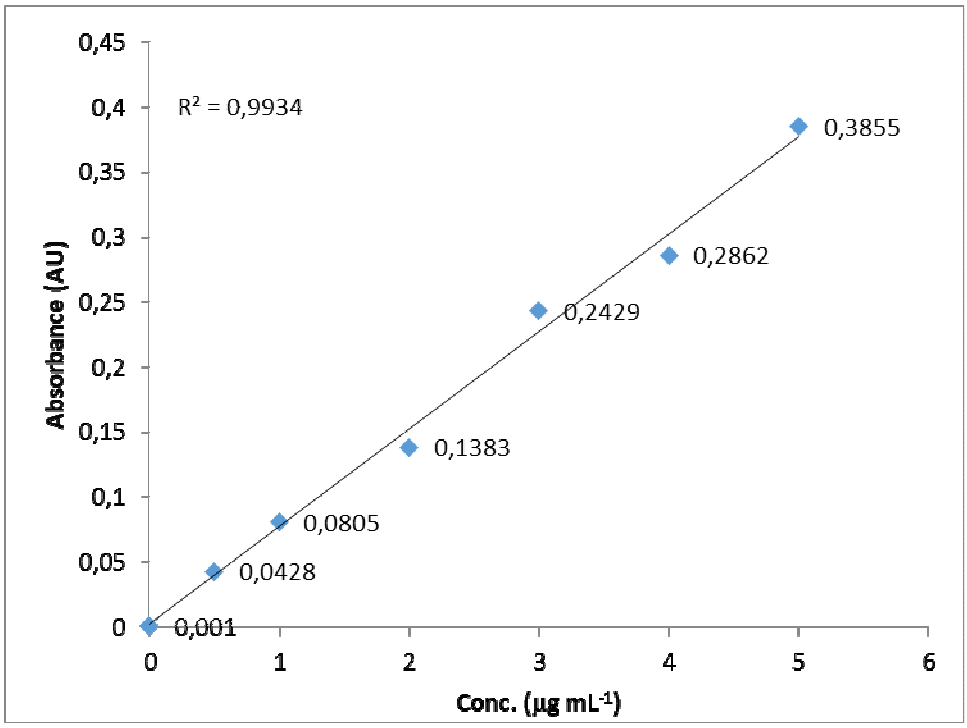

Figure 4. *Gallic acid calibration curve of absorbance vs. concentration*

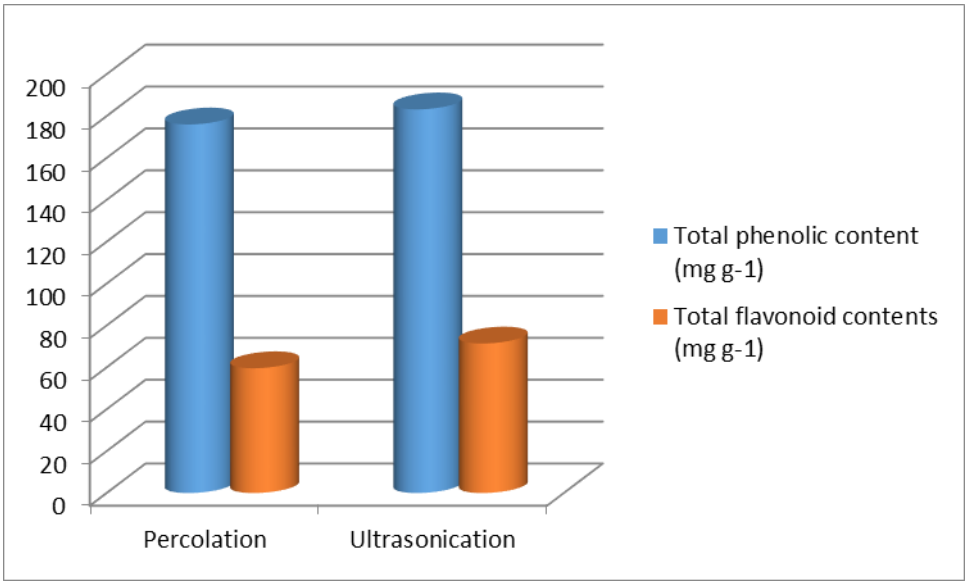

Figure 5. *Total phenolic and total flavonoid contents of different extracts.*

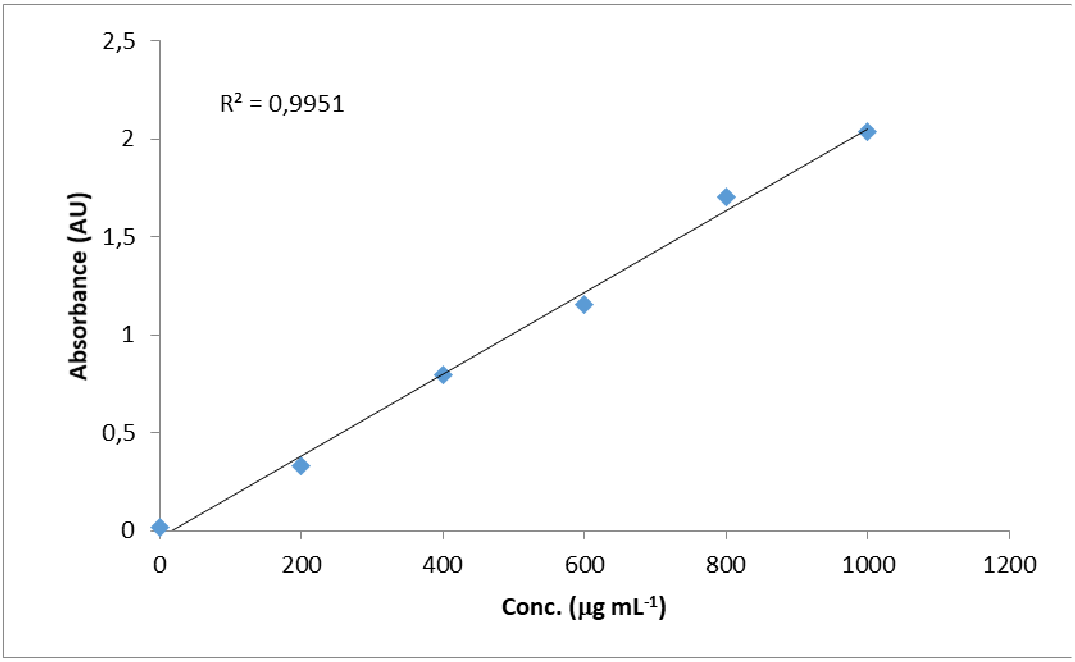

Figure 6. *Catechin calibration curve of absorbance vs. concentration.*