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Preservation of Antioxidant Activity and Polyphenols in *Mentha spicata* L. with the Use of Microencapsulation by Calcium Alginate

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Abstract

Natural polyphenols have scavenging characteristics for radical oxygens species and chelating properties towards proteins. Due to these properties, the polyphenols are used for the treatment of several diseases such as inflammation, cancer or diabetes and are also used additionally in cosmetic formulations and nutraceutical applications. The benefit of encapsulation is less evaporation and degradation of volatile active compounds. It masks unpleasant feelings during eating, such as bitter taste and astringency of polyphenols. However, their limited stability or solubility, often combined with poor bioavailability, have to be resolved to make these compounds more able to answer growing demands in cosmetics, nutrition, and health.

We attempted to develop an efficient encapsulation procedure that can enhance the encapsulation amount of polyphenols and in turn, enhance the antioxidant activity of *Mentha spicata* (Mint or Pudina) for three months at room temperature. Ethanolic extracts containing polyphenols were prepared. Effect of time and temperature on polyphenol release from calcium alginate encapsulated *Mentha spicata* leaf extract was studied. Results show that calcium alginate influenced microencapsulation efficiency, polyphenol content, and stability. The short-term stability of micro-encapsulated beads was studied for three months. It was observed the polyphenol content and antioxidant activity remained stable in encapsulated beads compared to unencapsulated beads. Thus, Calcium alginate encapsulated beads prove to be a promising technique for food supplements / nutraceuticals.

Keywords: *Mentha spicata*, polyphenols, antioxidant activity, encapsulation, calcium alginate

Introduction

Mentha spicata L. belongs to the Lamiaceae family is an aromatic herb that is economical and easily grown in temperature and sub-temperature regions of the world [1, 2]. This plant contains a high amount of secondary metabolites like polyphenolic compounds which exhibits antioxidant properties [3, 4]. Consumption of diets that are rich in polyphenol provides a defence mechanism against the development of diseases like diabetes, cardiovascular disease, delayed aging, cancer, reducing inflammation and neurodegenerative diseases [5]. Polyphenols present in the plants have a very key and vital role against environmental stress [6]. Bioactive constituents such as polyphenol compounds are mainly stable when present in plants. These compounds are sensitive towards the light, pH, temperature,

moisture content and oxygen and are unstable upon extraction and prone to degradation. To address these limitations, microencapsulation has been introduced to retain, preserve and maintain the stability of bioactive constituents within a matrix or a membrane and bypass degradation of bioactive constituents [7, 8]. It protects the unstable encapsulated compound from the external environment by acting as a physical barrier between the wall materials and core [9]. The biopolymers like sodium alginate are used as a coating material which provides efficient protection thereby resulting in high-efficiency values of bioactive constituents like polyphenolic compounds extracted from plants [10]. Microencapsulation by sodium alginate gained attention and attraction in food and pharmaceutical industries as it is responsible for masking bitterness or any off-flavours [11, 12]. Sodium Alginate is unbranched linear heteropolysaccharide (1-4) glycoside linkages that join the monomers of ß-D mannuronic acid and its C-5 epimer and α-1 guluronic acid residues [13]. This polymer uses a microencapsulation technique where bioactive constituents can be entrapped for a longer period of time which increases its application [14]. Sodium alginate matrices increase the shelf-life of the compound encapsulated by decreasing the permeability of oxygen and other molecules [15]. This polymer serves qualities for encapsulation as it is cost-efficient, simple to use, biocompatible, biodegradability [16, 17].

Mentha spicata commonly known as mint or spearmint is often used in Indian cuisine. Mentha genus is characterized by their volatile oils, used as herbal teas and condiments [18]. This work aimed to encapsulate polyphenols of *Mentha spicata* with sodium alginate through extrusion technology to increase their bioavailability and check the stability of polyphenols and their antioxidant activities for three month storage period.

Materials and Methods

Quercetin standard, Aluminium chloride, Potassium acetate, Ethanol, Methanol, Gallic acid standard, Folin-Ciocalteu's (FC) reagent, Sodium Carbonate, Ascorbic Acid standard, Sulphuric acid, Sodium Phosphate, Ammonium Molybdate, BSA, glucose, Ferrous ammonium sulphate, Hydrogen peroxide, Ferrozine, Ferrous sulphate, Potassium persulfate, Phosphate buffer, Potassium ferrocyanide, Trichloro-acetic acid, Ferric chloride, Sodium hydroxide, were obtained from Sisco Research Laboratory while DPPH powder, Bradford reagent, Anthrone reagent, Phenanthroline, ABTS tablets, was bought from Sigma-Aldrich (USA).

Collection of plant sample: *Mentha spicata* L. was bought from the local market, near Vellore Institute of Technology, Vellore, Tamil Nadu, India. The *Mentha spicata* L. plant was authenticated by Dr T Sekar, Pachaiyappa's College, Chennai, and the Voucher specimen was preserved at the centre (vitcbst201901-04). The sample was stored at 4ºC for further use.

Preparation of mentha spicata extract and phytochemical analysis: *Mentha spicata* (*M. spicata*) extract was prepared by drying the leaves and grinding them to powder form. The powder was dissolved in 70% ethanol, refluxed for 4h and concentrated under vacuum. The resulting powder was further taken up for the estimation of polyphenols, flavonoids and anti-oxidant [19].

Estimation of total polyphenols: Folin-Ciocalteu's (FC) reagent was used to determine total polyphenolic compound and absorbance was noted at 725nm. Gallic acid was taken as standard as it's used as a reference for estimation of polyphenol content in a given extract [19, 20]. Results were expressed as mg/g equivalent of Gallic acid equivalent (GAE).

Estimation of total flavonoids: The flavonoid content was estimated in the plant extracts by using four different reagents like aluminium chloride, potassium acetate, 95% ethanol and 80% methanol [19, 21] and absorbance was noted at 415nm. Quercetin was taken as a standard compound as it is known to belong to

the flavonoid group of polyphenols (Flavon-3-ols). Results were expressed as mg/g equivalent of Quercetin equivalent (QE) [19].

Identification of polyphenol by using RP-HPLC: Polyphenolic compounds present in the extract were determined by RP-HPLC. UV visible detector was used for the separation of bioactive compounds (λ=290nm). HPLC water pumps-1525 binary pump system and C18 column (Waters, 150 × 3.9mm, i.d., 5µm) were used. The solvents (mobile phase) used for separation were 0.1% acetic acid in water and 100% acetonitrile [19]. Gradient elution was carried out for sample and standards at a flow rate of 1mL/min and the eluent was monitored at 290nm. Breeze data (water) system was used for performing data acquisition and processing. The retention time of the sample was compared with the standards.

Estimation of total antioxidant: The determination of Antioxidant activity was done by the phosphomolybdenum method [19] and absorbance was noted at 695nm. Ascorbic acid was taken as a reference compound as it's a polyphenol having better antioxidant potential. Results were expressed as mg/g equivalent of Ascorbic acid equivalent (AAE).

Preparation of alginate beads with m. Spicata extracts: The beads were prepared as per the protocol of Reddy et al., [22] with minor modifications. Alginate beads were prepared by adding sodium alginate, glycerol, xanthan and distilled water with extract and without *M. spicata* extract as control. The solution containing 2% $CaCl_2$ was agitated for 1h and centrifuged at 10000 rpm for 10min at 20°C. The solution was homogenised and the beads were obtained by adding drop by drop of the mixture from the burette into a 1 litre of 2N hydrochloric acid. The collected beads were stored at room temperature. Micro-encapsulation efficiency [23] was calculated accordingly to the formula:

Encapsulation efficiency = (Theoretical weight of the polyphenols/Polyphenols to be encapsulated) × 100%

SEM Analysis: Scanning electron microscopy was performed to determine the structural and morphological features of alginate beads [24]. Before analysis beads were taken, washed with 70% ethanol and was incubated overnight at -80°C and kept for lyophilization for 3-4h and further dried in a hot air oven overnight at 40-45°C. The surface and the internal structure of the beads were analysed using SEM (Carl Zeiss, EVO18) at room temperature and with a 10 kV acceleration voltage. Microparticle's size and size distribution were also performed by analysing SEM photomicrographs**.**

Destabilization of the encapsulated beads: The destabilization of the beads were performed by dissolving the beads in 1ml of distilled water and boiled at various temperatures separately (40°C, 50°C, 60°C, 70°C and 80°C) for different time intervals (1h, 4h and 7h). 1ml of 20% TCA was added to each and centrifuged at 2000 rpm for 20mins. The supernatant was collected and polyphenol content was determined by using the FC method [21].

Stability study of polyphenols of encapsulated beads of M. spicata extract

Estimation of total polyphenols: The determination of total polyphenolic compound was done by using Folin-Ciocalteu's (FC) reagent and absorbance was noted at 725nm [19] with encapsulated and unencapsulated extracts with Gallic acid as standard.

Estimation of total antioxidant: The determination of Antioxidant activity was done by the phosphomolybdenum method [19] and absorbance was noted at 695nm with encapsulated and unencapsulated extracts. Ascorbic acid was taken as a standard compound.

Stability of antioxidant activity encapsulated beads of m. Spicata extract

DPPH Radical scavenging assay: The antioxidant activity of the plant extract was determined by their ability to scavenge

DPPH stable radical [25, 26]. The assay was determined by measuring the declination in the absorbance. 0.2mM DPPH with encapsulated and unencapsulated extracts and incubated for 15 min in dark conditions. Absorbance was measured at 517nm in a spectrophotometer with ascorbic acid used as a standard reference compound.

The percentage of free radical scavenging activity was calculated as follow:

Radical scavenging (%) = $(A_C - A_S / A_C) \times 100$

Wherein, A_S is the absorbance with the test sample and A_C is the absorbance of control.

ABTS radical Scavenging activity: ABTS radical cation decolourization assay determines free radical scavenging activity [27, 28]. Mixing ABTS solution with 2.45mM potassium persulfate and incubated for 12-16h 7mM ABTS reagent was prepared. ABTS reagent was diluted with methanol to measure the absorbance at 734nm. Unencapsulated and encapsulated Extracts were mixed with diluted ABTS reagent and incubated for 15min in dark conditions and absorbance was measured with ascorbic acid used as a standard reference compound .

The percentage of free radical scavenging activity was calculated as follow: Radical scavenging (%) = $(A_C - A_S / A_C) \times 100$

Wherein, A_S is the absorbance with the test sample and A_C is the absorbance of control.

H_2O_2 scavenging activity: The hydrogen peroxide scavenging activity of the extract was carried out by the standard protocol with some slight modifications [19]. Unencapsulated and encapsulated extracts (1mg/ml) were mixed with 1mM ferrous ammonium sulphate and 5mM hydrogen peroxide and incubated for 5 min in dark conditions. 1mM Phenanthroline was added to it and incubated for 10min at room temperature. Absorbance was measured at 510 nm with ascorbic acid used as a standard reference compound.

The percentage of H_2O_2 scavenging activity was calculated as follow:

Radical scavenging (%) =$(A_C - A_S / A_C) \times 100$

Wherein, A_S is the absorbance with the test sample and A_C is the absorbance of control

Metal chelating activity: The determination of the Metal chelating activity of the plant extract was carried out according to the standard protocol [19]. Unencapsulated and encapsulated extracts (1mg/ml) were mixed with 0.1mM ferrous sulphate, 0.25mM ferrozine and 80% methanol and incubated for 10min in dark conditions. Absorbance was measured at 562nm in a spectrophotometer and ascorbic acid as a reference compound.

The metal chelating activity was calculated as follow:

Metal chelating activity (%) = $(A_C - A_S / A_C) \times 100$

Wherein, A_S is the absorbance with the test sample and A_C is the absorbance of control.

Statistical analysis: Statistical analysis between groups was performed with Two-way ANOVA followed by the Bonferroni method for independent observation using Graph Pad Prism 6 at $p \leq 0.05$. Each experiment was performed in triplicates.

Results and Discussion

Phytochemical analysis of the plant extract and identification of polyphenols by RP-HPLC

M. spicata extract was estimated for its polyphenol, flavonoid and antioxidant content. The polyphenol, flavonoid and antioxidant content were 70.14 ± 2.5mg of GAE /g, 67.3 ± 2.1mg of QE /g and 56.89 ± 1.9mg of AAE /g respectively.

Through RP-HPLC, it was observed that the extract contained ascorbic acid, gallic acid, naringin, taxifolin, myricetin, luteolin, quercetin, naringenin and kaempferol and pelargonidin

chloride was compared with the retention time of standards (Table 1). The results indicate that the *M. spicata* extract is very rich in polyphenol content with the presence of polyphenols such as quercetin, myricetin, naringenin etc. Quercetin and myricetin are known for their antioxidant activities and their presence in the *M. spicata* extract may contribute to antioxidant activity [29].

Encapsulation of M. spicata and SEM analysis

The diameter of encapsulated beads of *M. spicata* was 2.2 ± 0.3mm. The Encapsulation efficiency and encapsulation yield were calculated as 79± 2.7% and 8.95 ± 1.2 % respectively. The results indicate there was no significant ($p<0.05$) loss of extracts for *M. spicata* during the microencapsulation procedure and successfully extracts were encapsulated. Higher encapsulation efficiency was obtained in this study indicates that there is a possibility of the stability of polyphenols and their properties may be maintained. It can be inferred that the encapsulation of *M. spicata* extract with alginate can maintain the stability of the polyphenols with better efficiency. Our results corroborate another study wherein *Ilex paraguariensis* extract encapsulated with alginate was able to maintain 85% of its antioxidant potential [30].

The SEM images were obtained at 86X and 90X magnifications, respectively at ambient temperature and an acceleration voltage of 10kV, working distance of 12mm and 12.5mm, respectively. The shape and surface morphology of encapsulated and unencapsulated beads were evaluated by SEM analysis. It was observed that through this extrusion method the difference between shape and surface morphology was observed in encapsulated and unencapsulated beads. The unencapsulated beads had rough morphology with heterogenous distribution compared to encapsulated beads. The SEM images of encapsulated beads showed that the extracts of *M. spicata* L. was dispersed in the polymeric matrix in a homogenous manner and were of dense gel structures which are mostly due to polyphenol loaded into the beads. These morphological observations were confirmed as observed in the SEM images (Figure 1).

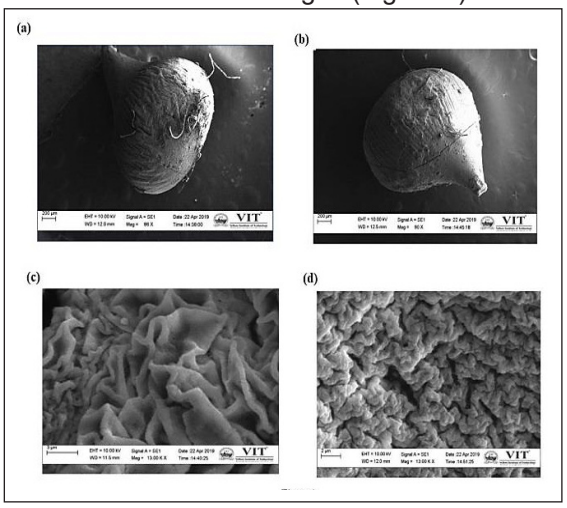

Figure 1: SEM microphotographs of (a) control bead (b) encapsulated bead (c) surface morphology of control bead and (d) surface morphology of encapsulated bead of *Mentha spicata.*

Effect of temperature and time on the release of polyphenols from alginate encapsulated M. spicata extract

Encapsulated beads of the dried ethanolic extract of *M. spicata* showed a gradual increase in polyphenol content as the temperature and duration increased. It was observed that after 60°C the release of polyphenols decreased as the time increased. Most of the polyphenolic compounds were released when the beads were incubated at 60°C for 4h in case of encapsulated *M. spicata* extract (Figure 2). A study showed that alginate beads heated at 56°C was able to destabilize the beads releasing the extract [31]. Heat is known to rupture the alginate beads by destabilizing the bonds formed between the extract and matrix. It suggests that the proper selection of time and temperature is a crucial step for maintaining the stability and amount of polyphenols in the beads [22].

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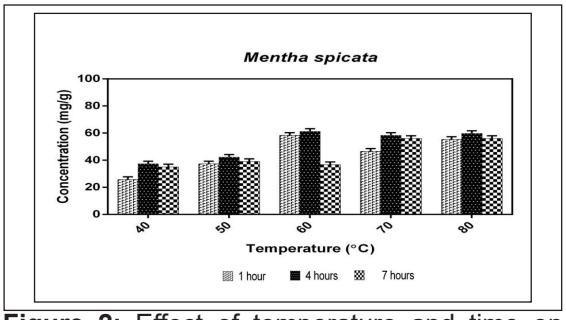

Figure 2: Effect of temperature and time on polyphenol release from calcium alginate encapsulated ethanolic extracts of *Mentha spicata.*

Polyphenol and antioxidant stability in encapsulated alginate beads during three months of storage

We observed that the encapsulated *M. spicata* extract showed a polyphenol content of 58.28mg/g polyphenol equivalent of gallic acid at day 1 (Figure 3a). Polyphenol content was studied for three months and it was found out that there was stability with an average mean value of 58.26mg/g of GAE. However, the unencapsulated *M. spicata* extract showed a polyphenol content of 70.14mg/g of GAE at day1. The polyphenol content of these

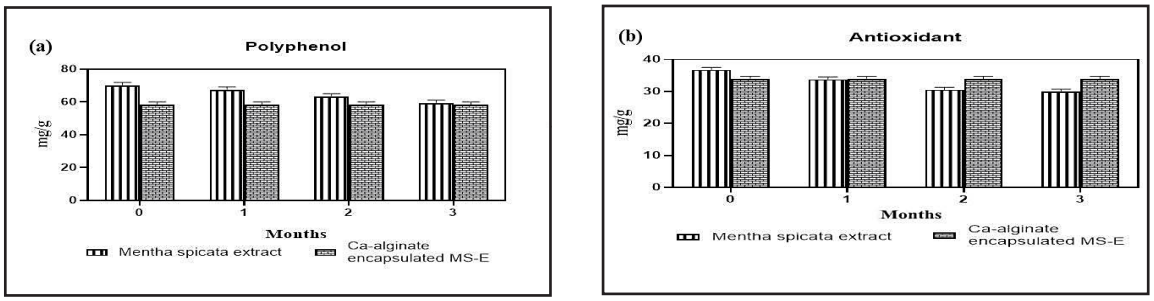

Figure 3: Stability of (a) Polyphenol content and (b) Anti-oxidant content in unencapsulated and encapsulated *Mentha spicata* extract for three months of storage.

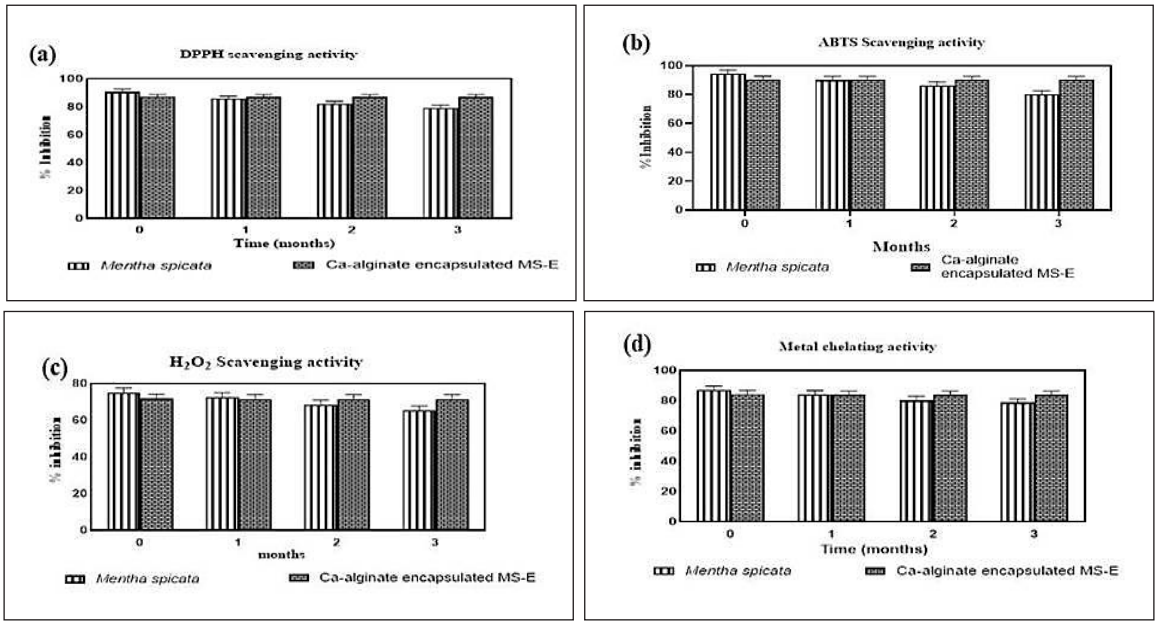

Figure 4: Antioxidant stability in unencapsulated extract and encapsulated *Mentha spicata* extract during three months study by (a) DPPH (b) ABTS (c) H_2O_2 and (d) Metal chelating activity

unencapsulated extracts was evaluated and it was found that there was instability in the polyphenol content during the three months. At the end of three months, the polyphenol content was decreased to 59.3mg/g of GAE. The total antioxidant content in the unencapsulated and encapsulated *M. spicata* extract at day 1 were 39.33 and 33.99mg/g of AAE respectively (Figure 3b). The antioxidant activity was evaluated and found to be stable in the case of encapsulated beads for a period of three months as compared to unencapsulated extract. The stability of polyphenols in encapsulated beads may be maintained due to the conjugation of the hydroxyl group of polyphenols with the matrix of alginate beads. During destabilization, this bond may be broken thereby releasing the hydroxyl groups resulting in an increased value of total polyphenols and anti-oxidants [32].

Table 1: HPLC analysis of an ethanolic extract of *Mentha spicata* compared with the standards.

Standards	Retention Time (min)	Class	Methanolic sample of *M. spicata* L.
Ascorbic Acid	2.32	Vitamin C	+
Gallic Acid	2.85	Hydroxybenzoic acid	+
Catechin	4.59	Flavon(ol)es	-
Naringin	6.90	Flavanon(ol)es	+
Taxifolin	7.10	Flavanon(ol)es	+
Pelargonidin Chloride	7.47	Anthocyanidin	+
Myricetin	8.35	Flavon(ol)es	+
Luteolin	9.23	Flavon(ol)es	+
Quercetin	9.97	Flavon(ol)es	+
Naringenin	10.32	Flavanon(ol)es	+
Kaempferol	10.42	Flavon(ol)es	+

All experiments done in Triplicates

Antioxidant potential in encapsulated alginate beads during three months of storage

The percentage inhibition values of the antioxidant activity by DPPH method, ABTS method, Hydrogen peroxide scavenging method, Metal chelating method in the encapsulated *M. spicata* at day 1 was 87%, 93.1%, 71.64% and 84.31% respectively. The antioxidant activity was evaluated every month for three months and it was found that encapsulated extract maintained the antioxidant activity i.e., 86.8% (DPPH), 92.3 % (ABTS), 71.3% (H_2O_2) and 83.8% (Metal chelating) even after 3 months while there was a decrease in the activity of unencapsulated extract (Figure 4). The ability of the extract to scavenge free radicals be it ABTS, DPPH, H_2O_2 has been reported to be due to the presence of polyphenolic compounds. In the case of metal chelating activity, the ferrozine forms a chelating complex with the ferrous ion leading to a decrease in metal chelating potential [19]. It can be inferred that the polyphenols in *M spicata* extract may chelate with the ferrous complex before ferrozine leading to better chelating activity. The encapsulated beads which have maintained the polyphenols and anti-oxidant content have better potential to scavenge the free radicals and metal chelating property compared to unencapsulated extract.

Conclusion

This study reports that it is possible to encapsulate plant extracts using ionic gelation.

Encapsulated beads obtained from extrusion exhibited a more uniform, homogeneous morphology compared to control beads. This method is a multipurpose encapsulation method that can create a novel nutraceutical product appropriate for various applications in the food processing industries [33]. Results from this study indicate that alginate is suitable for encapsulation of polyphenols of the *M. spicata* L. The polyphenolic content and the antioxidant activity were found to be stable in the alginate beads for a period of three months. Further studies are needed to check the stability of polyphenols and anti-oxidant for a longer period of time. As time and temperature play a vital role in the release of the polyphenols antioxidant capacity. So, future studies should be carried out to check the bioavailability in animal models. This encapsulation method could be used for the incorporation of bioactive compounds which can be used as a nutraceutical or in the food industry.

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Conflict of Interest

None

References

1. Al-fartosi, K.G., Radi, H. and Al-rekabi, E.A. (2014) Lipid profile of diabetic male rats treated with phenolic compounds of leaves extracts from *Mentha longifolia* and *Mentha spicata*. Int J Pharm Biol Sci. 3: 26–31.
2. Zaidi, S. and Dahiya, P. (2015) In vitro antimicrobial activity, phytochemical analysis and total phenolic content of essential oil from *Mentha spicata* and *Mentha piperita*. Int Food Res J. 22: 2440–2445.
3. Kanatt, S.R., Chander, R. and Sharma, A. (2005) Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. Food Chem. 100: 451–458.
4. Bimakr, M., Rahman, R.A., Taip, F.S., Ganjloo, A., Salleh, L.M., Selamat, J., Hamid, H. and Zaidul, I.S.M. (2011) Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves. Food Bioprod Process. 89: 67–72.
5. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G. and Lightfoot, D.A. (2017) Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 6: 42–65.
6. Ames, B.N., Shigenaga, M.K. and Hagen, T.M. (1993) Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A. 90: 7915–7922.
7. Arriola, N.D.A, Medeiros, P.M.D, Prudencio, E.S., Müller, C.M.O and Amboni, R.D.D.C (2015) Encapsulation of aqueous leaf extract of *Stevia rebaudiana* Bertoni with sodium alginate and its impact on phenolic content. Food Biosci. 13: 32–40.
8. Li, M., Rouaud, O. and Poncelet, D. (2008) Microencapsulation by solvent evaporation: State of the art for process engineering approaches. Int J Pharm. 363: 26–39.
9. Bastos, L.P.H., dos Santos, C.H.C., de Carvalho, M.G. and Garcia-Rojas, E.E. (2020) Encapsulation of the black pepper (*Piper nigrum* L.) essential oil by lactoferrin-sodium alginate complex coacervates: Structural characterization and simulated gastrointestinal conditions. Food Chem. 316: 126345-126352.
10. Cordoba, A.L, Deladino, L. and Martino, M. (2013) Effect of starch filler on calcium-alginate hydrogels loaded with yerba mate antioxidants. Carbohydr Polym 95: 315–

323.

11. Pravinata, L.C. and Murray, B.S. (2019) Encapsulation of water-insoluble polyphenols and β-carotene in Ca-alginate microgel particles produced by the Leeds Jet Homogenizer. Colloids Surfaces A Physicochem Eng Asp. 561: 147–154.

12. Belscak-Cvitanovic, A., Stojanovic, R., Manojlovic, V. et al. (2011) Encapsulation of polyphenolic antioxidants from medicinal plant extracts in alginate-chitosan system enhanced with ascorbic acid by electrostatic extrusion. Food Res Int. 44: 1094–1101.

13. Apoorva, A., Rameshbabu, A.P., Dasgupta, S., Dhara, S. and Padmavati, M. (2020). Novel pH-sensitive alginate hydrogel delivery system reinforced with *gum tragacanth* for intestinal targeting of nutraceuticals. Int J Biol Macromol. 147: 675–687.

14. Champagne, C.P. and Fustier, P. (2007). Microencapsulation for the improved delivery of bioactive compounds into foods. Curr Opin Biotechnol. 18: 184–190.

15. Brezoiu, A.M., Matei, C., Deaconu, M., Stanciuc, A.M., Trifan, A., Gaspar-Pintiliescu, A. and Berger, D. (2019). Polyphenols extract from grape pomace. Characterization and valorisation through encapsulation into mesoporous silica-type matrices. Food Chem Toxicol. 133: 110787-110798.

16. Desai, K.G.H. and Park, H.J. (2005). Encapsulation of vitamin C in tripolyphosphate cross-linked chitosan microspheres by spray drying. J Microencapsul. 22:179–192.

17. Fang, Y.P., Tsai, Y.H., Wu, P.C. and Huang, Y.B. (2008) Comparison of 5-aminolevulinic acid-encapsulated liposome versus ethosome for skin delivery for photodynamic therapy. Int J Pharm. 356:144–152.

18. Dorman, H.J.D., Kos M.B.¸ Kahlos A.K., Holm Y and Hiltunen A.R. (2003) Antioxidant Properties and Composition of Aqueous Extracts from Mentha Species, Hybrids, Varieties, and Cultivars. J. Agric. Food Chem. 51: 4563−4569.

19. Babu, S.N., Govindarajan, S., Vijayalakshmi, M.A. and Noor, A. (2019) Evaluation of In vitro anti-diabetic and anti-oxidant activities and preliminary phytochemical screening of gel, epidermis and flower extract of *Aloe vera*. Res J Pharm Technol. 12: 1761–1768.

20. Bandara, U.Y., Witharana, C. and Soysa, P. (2020) Extraction, Total phenol Content, Flavonoid content, Free Radical Scavenging Capacity and phytochemical screening of the Parts of Sri Lankan Pomegranate (*Punica granatum* L.) Fruit. Curr Trends Biotechnol Pharm., 14: 70-80.

21. Chang, C.C., Yang, M.H., Wen, H.M. and Chern, J.C. (2002) Estimation of total flavonoid content in *propolis* by two complementary colometric methods. J Food Drug Anal. 10: 178–182.

22. Reddy, B.C., Noor, A., Sarada, N.C. and Vijayalakshmi, M.A. (2011) Antioxidant properties of *Cordyline terminalis* (L.) Kunth and *Myristica fragrans* Houtt. encapsulated separately into casein beads. Curr Sci. 101: 416–420.

23. Koupantsis, T., Pavlidou, E. and Paraskevopoulou, A. (2016) Glycerol and tannic acid as applied in the preparation of milk proteins - CMC complex coavervates for flavour encapsulation. Food Hydrocoll. 57: 62–71.

24. Chan, E.S., Wong, S.L., Lee, P.P., Lee, J.S., Ti TB, Zhang, Z., Poncelet, D., Ravindra, P., Phan, S.H. and Yim, Z.H. (2011) Effects of starch filler on the physical properties of lyophilized calcium-alginate beads and the viability of encapsulated cells. Carbohydr Polym. 83: 225–232.

25. Hossain, M. A. and Shah, M.D. (2011) A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant *Merremia borneensis*. Arab J Chem. 8: 66–71.

26. Mhlongo, N.Y., Naidu, K.S.B., Himakar, R.K., Sershen, Cheriti, A. and Govender. P. (2018) Phytochemical screening,antioxidant and antimicrobial efficacy of *Protorhus longifolia* (Bernh. Ex C. krauss) Engl. (Anacardiaceae) seed extracts. Curr Trends Biotechnol Pharm. 12: 128-138.

27. Zheleva-Dimitrova, D., Nedialkov, P. and Kitanov, G. (2010) Radical scavenging and antioxidant activities of methanolic extracts from *Hypericum* species growing in Bulgaria. Pharmacogn Mag. 6: 74–8.

28. Patnala, H., Ramana G. V. and Babu H. B. (2021) Evaluation of Nutritive, Non-Nutritive Contents and Antioxidant Activity of Polyherbal Formulations. Curr Trends Biotechnol Pharm., 15: 124-132.

29. Csepregi, K., Neugart, S., Schreiner, M. and Hideg, É. (2016) Comparative evaluation of total antioxidant capacities of plant polyphenols. Molecules. 21: 1–17.

30. Munin, A. and Edwards-Levy, F. (2011) Encapsulation of natural polyphenolic compounds; a review. Pharmaceutics. 3: 793–829.

31. Kim, S., Jeong, C. Cho, S. and Kim, S.B. (2019) Effects of thermal treatment on the physical properties of edible calcium alginate gel beads: Response surface methodological approach. Foods. 8: 578–591.

32. Quiroz, J.Q., Velazquez, V., Corrales-Garcia, L.L., Torres, J.D., Delgado, E., Ciro, G. and Rojas, J. (2020) Use of plant proteins as microencapsulating agents of bioactive compounds extracted from annatto seeds (*Bixa orellana* L.). Antioxidants. 9: 4–6.

33. Dehkharghanian, M., Lacroix, M. and Vijayalakshmi, M.A. (2009) Antioxidant properties of green tea polyphenols encapsulated in caseinate beads. Dairy Sci Technol. 89: 485–499.