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Effect of Microencapsulation by Calcium Alginate on the Anti-Oxidant Properties of *Swietenia macrophylla* Polyphenols

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Abstract

Polyphenols are the secondary metabolites that possess scavenging properties towards radical oxygen species. Polyphenols have antioxidant properties which are in demand because of their therapeutic uses in several diseases like diabetes, cancer, inflammatory, cardiovascular diseases etc. The problem arises with the lack of long-term stability of the polyphenols when exposed to heat and light. It masks the bitter and astringent taste, which limits their use. Nonetheless, the restricted stability and/or solubility needs to be addressed to make these polyphenols capable to answer the increasing demand for food supplements/ Nutraceuticals. We attempted to develop an encapsulation procedure using calcium alginate beads, which retain the anti-oxidant properties and polyphenol content of *Swietenia macrophylla* (Sky fruit). Effect of time and temperature on polyphenol release from calcium alginate encapsulated *Swietenia macrophylla* was studied. Results show that the calcium alginate influenced micro-encapsulation efficiency, polyphenol content and stability. The short-term stability of the microbeads was studied for three months. Due to the excellent polyphenol stability, the encapsulated beads showed stable anti-oxidant activity compared to unencapsulated beads. Thus, the results revealed that calcium alginate beads prove to be a promising technique for food supplements.

Keywords: *Swietenia macrophylla*, anti-oxidant activity, polyphenols, microencapsulation, calcium alginate

Introduction

Plants contain beneficial phytochemicals which supplement the human body's needs by acting as natural antioxidants [1]. These anti-oxidants control and reduce the oxidative injury in foods, thereby delaying/inhibiting the oxidation, and hence leading to increased shelf-life and quality of these foods [2]. Polyphenolic compounds are the major secondary metabolites found in many plants and show anti-oxidant properties [3]. Polyphenols are known for their therapeutic properties and consumption of plant polyphenols rich food protects against cancer, delaying aging, diabetes mellitus, osteoporosis, cardiovascular diseases, reducing inflammation and neurodegenerative diseases [4, 5]. Polyphenols are generally stable in plants but once extracted from the plants, they are prone to degradation. The instability of these polyphenols during the processing of food, distribution and/or storage, or in the digestive tract restricts the activity and the promising health advantages of polyphenols. Polyphenols are sensitive towards temperature, oxygen, light, pH, moisture content etc. [6]. Degradation of natural anti-oxidants may hamper the efficacy of application of these polyphenols in nutraceutical and pharmaceutical applications and also produces deleterious effects. To address these limitations and to retain polyphenol content and their anti-oxidant stability, microencapsulation has been used as

a reliable technique to bypass the degradation of bioactive compounds, protecting them from adverse environmental effects [7]. Microencapsulation is a process that helps to retain volatile compounds or bioactive material inside a protective layer that prevents it from the external environment [8]. Additionally, it masks the astringency and bitterness that most of the polyphenolic compounds display [9]. Biopolymers like sodium alginate are reported to provide an effective barrier if used for encapsulation, thereby resulting in increasing the encapsulation efficiency of polyphenols from plant extracts [10]. It is a naturally occurring widely used polymer, a biocompatible, nontoxic, biodegradable hydrophilic polymer that has been widely used to encapsulate various formulations, including plant extracts prevents the degradation of the compounds [11].

Swietenia macrophylla (Family Meliaceae), also known as "Sky fruit", is a medicinal plant found in most of the southeast Asian countries [12]. This plant contains different bioactive compounds such as polyphenols, which exhibit activities like antimicrobial, anti-oxidant, anti-inflammatory, anticancer, hypo-lipidemic and antitumor properties [13]. This study is the first to explore the efficiency of encapsulated *S. macrophylla* extract with alginate beads for polyphenols stability and their anti-oxidant properties over a 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, sulphuric acid, sodium phosphate, ammonium molybdate, BSA, glucose, ferrous ammonium sulphate, hydrogen peroxide, ferrozine, ferrous sulphate, potassium persulfate, phosphate buffer, potassium ferrocyanide, trichloroacetic acid, ferric chloride, sodium hydroxide, were obtained from Sisco Research Laboratory while DPPH powder, phenanthroline, ABTS tablets were bought from Sigma-Aldrich (USA).

Collection of Plant sample: *Swietenia macrophylla* was bought from the local market near Vellore Institute of Technology, Vellore, Tamil Nadu, India. The *Swietenia macrophylla* plant was authenticated by Dr. T Sekar, Pachaiyappa's College, Chennai, and the Voucher specimen was preserved at the center (vit201901-03). The sample was stored at 4ºC for further use.

Preparation of swietenia macrophylla extract and phytochemical analysis: *Swietenia macrophylla* (*S. macrophylla*) extract was prepared by grinding it to powder form. The powder was dissolved in 70% ethanol, refluxed for 4h and concentrated under vacuum with rotavapor (Buchi). The resulting powder was further taken up to estimate polyphenols, flavonoids and anti-oxidant [14].

Estimation of total polyphenols: The determination of *S. macrophylla* extract's total polyphenolic content was done by using Folin-Ciocalteu's (FC) reagent, and absorbance was noted at 725nm [14]. The compound used as a standard was Gallic acid (GAE).

Estimation of total flavonoids: Total flavonoid content in *S. macrophylla* extract was determined using aluminium chloride, potassium acetate, 95% ethanol, and 80% methanol [14, 15] and absorbance was noted at 415nm. Quercetin (QE) was taken as a standard compound.

Identification of polyphenol by using RP-HPLC: Polyphenolic compounds present in *S. macrophylla* extract were determined by RP-HPLC and UV visible detector (λ=290nm). HPLC water pumps-1525 binary pump system, and C18 column (Waters, 150 × 3.9mm, i.d., 5µm) was used. Gradient elution was of sample and standards was carried out with solvents 0.1% acetic acid in water and acetonitrile [14] at 1mL/min flow rate and the eluent was monitored at 290nm. Breeze data (waters) system was used for performing data acquisition and processing, and the retention time of the sample was compared with the standards.

Estimation of total antioxidant: The determination of *S. macrophylla* extract's antioxidant activity was done by phosphomolybdenum method [14] and absorbance was noted at 695nm, with ascorbic acid as standard compound.

Preparation of alginate beads with S. macrophylla extract: The alginate beads were made

as per the protocol of Reddy et al., [16], with minimum modifications. For preparing the encapsulation beads, distilled water was mixed with glycerol, sodium alginate and xanthan with and without *S. macrophylla* extract as control. The prepared solution with 2% $CaCl_2$ was agitated for 1h at 10000rpm it was centrifuged for 10 min at 20°C. The mixture was homogenized and dropped into 1 litre of 2N hydrochloric acid from a burette to obtain the beads. Microencapsulation efficiency [17] was calculated accordingly to the formula:

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

SEM analysis: To learn about features of alginate beads like morphology and structure, Scanning Electron Microscopy or SEM was performed. Before analysis beads were taken, washed with 70% ethanol and was incubated overnight at -80°C and kept for lyophilization for 3-4 h and further air-dried in an oven overnight at 40-45°C [18]. The surface and the bead's internal structure were analyzed using SEM (Carl Zeiss, EVO/18 Research) at room temperature. The beads were attached to the specimen aluminium holder and bombarded with gold particles for 45min. The SEM images were obtained at a magnification of 86X and 90X at ambient temperature and an acceleration voltage of 10kV, working distance of 12mm and 12.5mm, respectively. Microparticle's size and size distribution were also performed by analyzing SEM photomicrographs.

Destabilization of the encapsulated beads: The destabilization of the alginate beads of *S. macrophylla* extract was performed by dissolving the beads in 1ml of distilled water and boiled at 40ºC, 50ºC, 60ºC, 70ºC and 80ºC separately at different time intervals (1h, 4h and 7h). To each of the test tubes, 20% TCA was added and centrifuged for 20min at 2000rpm. The supernatant was collected and polyphenol content was estimated with the FC method.

Stability study of polyphenols of encapsulated beads of S. macrophylla extract

Estimation of total polyphenols: Total polyphenolic compound was estimated by using Folin-Ciocalteu's reagent. The absorbance was noted at 725nm [14, 19] with encapsulated and unencapsulated extracts of *S. macrophylla* with gallic acid as standard.

***Estimation of total antioxidan*t:** The determination of Anti-oxidant activity was done by phosphomolybdenum method [14] and absorbance was noted at 695nm with encapsulated and unencapsulated extracts of *S. macrophylla* with ascorbic acid as standard.

Stability of antioxidant activity encapsulated beads of s. macrophylla extract

DPPH Radical scavenging assay: The plant extract's antioxidant activity was determined by its ability to scavenge DPPH radicals [20, 21]. The test was performed by measuring the decline in the absorbance of DPPH with encapsulated and unencapsulated extracts of *S. macrophylla*. DPPH with and without the extract was incubated for 15min in the dark and absorbance was noted at 517nm, with ascorbic acid used as a standard. DPPH scavenging activity percentage was calculated as mentioned:

DPPH radical scavenging (%) = $(A_{control} - A_{sample}/ A_{control}) \times 100$

Wherein, A_{sample} is test sample absorbance and $A_{control}$ is control's absorbance.

ABTS radical scavenging activity: ABTS radical decolorization test determines free radical scavenging activity [22, 23]. ABTS was mixed with 2.45mM potassium persulfate and incubated for 12-16h for preparation of ABTS reagent. ABTS reagent was diluted with methanol to measure the absorbance at 734nm (Control). Unencapsulated and encapsulated extracts were mixed with diluted ABTS reagent and it was incubated for 15 min in the dark condition and by taking ascorbic acid as standard the absorbance was measured.

ABTS scavenging activity percentage was calculated as mentioned:

Radical scavenging (%) = $(A_{control} - A_{sample}/ A_{control}) \times 100$

Wherein, A_{sample} is test sample absorbance and $A_{control}$ is control's absorbance.

H_2O_2 scavenging activity: Hydrogen peroxide scavenging assay of the *S. macrophylla* extract was carried out according to the protocol [14]. Unencapsulated and encapsulated extracts were mixed with 1mM ferrous ammonium sulphate and 5mM hydrogen peroxide and incubated for 5 min in dark conditions. To this, 1mM phenanthroline was added and it was incubated at room temperature for 10 min. Solution without extracts served as control and ascorbic acid served as standard with absorbance measured at 510nm.

H_2O_2 scavenging activity percentage was calculated as mentioned:

$$\text{Radical scavenging (\%)} = (A_{control} - A_{sample} / A_{control}) \times 100$$

Wherein, A_{sample} is test sample absorbance and $A_{control}$ is control's absorbance.

Metal chelating activity: The determination of metal chelating assay of plant extract was carried out according to the protocol [14] wherein unencapsulated and encapsulated extracts were mixed with 0.1mM ferrous sulphate, 0.25mM ferrozine and 80% methanol and incubated for 10min in dark condition with ascorbic acid as standard and absorbance were measured at 562nm. Solution without the extracts served as control

The metal chelating activity was calculated as follow:

$$\text{Metal chelating activity (\%)} = (A_{control} - A_{sample} / A_{control}) \times 100$$

Wherein, A_{sample} is test sample absorbance and $A_{control}$ is control's absorbance.

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 S. macrophylla and identification of polyphenols by RP-HPLC

S. macrophylla extract was estimated for its polyphenol, flavonoid and antioxidant content. Polyphenol, flavonoid and anti-oxidant content was 59.2 ± 2.4mg of GAE/g, 51.7 ± 2.2mg of QE/g and 35.10 ± 1.9 mg of AAE/g respectively. The total polyphenol content in ethanolic extract was more in *S. macrophylla,* which suggests that the alcoholic solution increases the extraction efficiency and total phenolic content [24].

In RP-HPLC analysis sharp peaks were obtained at 290nm. Identification of polyphenols was performed by comparing retention time in the extract against standards. These peaks confirm the presence of ascorbic acid, gallic acid, catechin, naringenin, myricetin, naringin, luteolin (Table 1). In this study, it was observed that the ethanolic extracts of *S. macrophylla* showed the maximum anti-oxidant activity which corroborates with recent studies that have proved that polyphenol compounds are known to contribute vastly to the ability to scavenge phosphomolybdate complex [25]. The presence of different polyphenols like myricetin, luteolin, gallic acid, catechin may contribute to the antioxidant potential of the extract [14].

Encapsulation of S. macrophylla and SEM analysis

The diameter of the microcapsules of the *S. macrophylla* was 2.01 ± 0.1mm and the findings indicated that the average efficiency of the *S. macrophylla* after encapsulation was 61.67%, Also, the encapsulation yield of *S. macrophylla* extract after encapsulation and 6.87 ±0.9%. Higher encapsulation efficiency correlates with the better stability of polyphenols. Previous reports have suggested that encapsulation of plant extracts with alginate was able to maintain the anti-oxidant property [26]. The encapsulation of *S. macrophylla* extract with alginate may increase polyphenols' stability as the encapsulation efficiency is higher, as observed in this study.

The shape of the control alginate beads (without extracts), beads encapsulated with *S. macrophylla* extract into the alginate matrix was evaluated by SEM analysis. The SEM photographs of the control beads (without *S. macrophylla* extract) were compared with encap-

sulated beads and showed a difference in the shape and surface morphology for the extrusion method. The smoothness of the beads was increased when the extracts were encapsulated with *S. macrophylla* extract. It was also observed that the beads had a rigid exterior, which may contribute to the prevention of the degradation of the polyphenol contents. It was observed that the encapsulated beads had a non-porous surface, whereas the control (without extracts) beads had a porous surface (Figure 1a, 1b). This result corroborates with another study that the system is a polymeric matrix system by extrusion method [27].

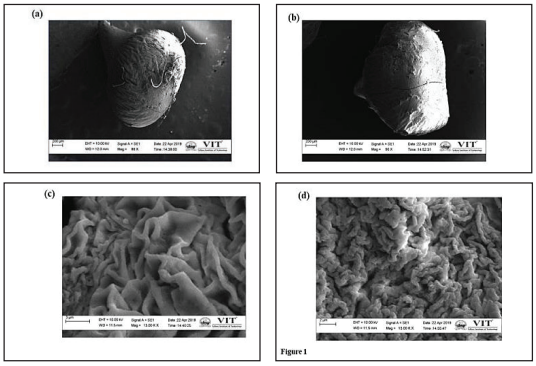


Figure 1: SEM microphotographs of (a) control bead (b) encapsulated bead (c) surface morphology of control bead and (d) surface morphology of encapsulated bead of *Swietenia macrophylla.*

Effect of temperature and time on the release of polyphenols from alginate encapsulated s. Macrophylla extract

To check the impact of time and temperature of incubation on the polyphenol release from the encapsulated alginate beads, they were heated at different temperatures and at several periods. There was a gradual increase in temperature and incubation time in the polyphenol release of the encapsulated beads of ethanolic extract of *S. macrophylla* till 60°C for 4h. After 60°C, the release of polyphenols decreased with high temperature and incubation time (Figure 2). There was a gradual increase up to 60°C due to the proper digestion of the calcium alginate, which released the contents that were encapsulated [16, 28]. After 60°C, the polyphenol content decreased due to the polyphenols' degradation, suggesting that the proper selection of temperature and time are the key factors in the assurance of the polyphenols stability and amount in the beads. The results corroborate with Reddy et al., that the temperature showed a remarkable impact on the total polyphenol content of extracts that are encapsulated [16].

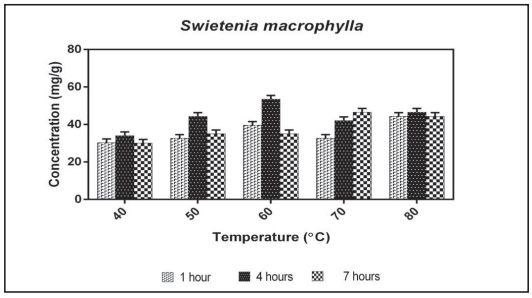


Figure 2: Effect of temperature and time on polyphenol release from calcium alginate encapsulated ethanolic extracts of *Swietenia macrophylla.*

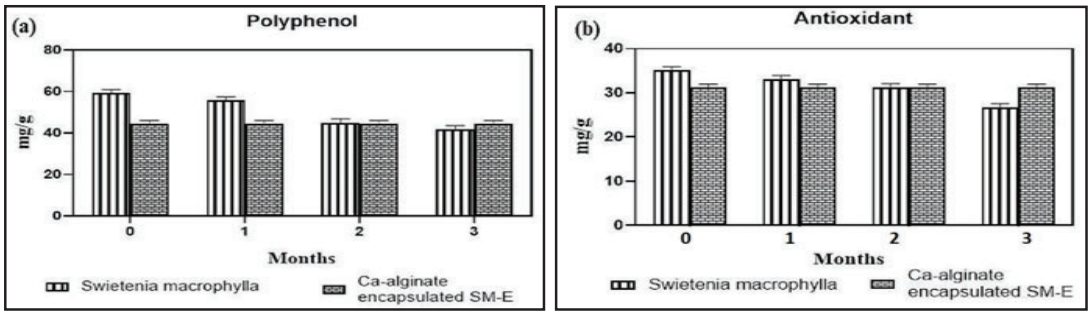
Polyphenol and antioxidant stability in encapsulated alginate beads during three months of storage

The polyphenol content of the encapsulated ethanolic extract was found to be 44.58mg/g polyphenols equivalent of gallic acid at day 1. The polyphenol content of these extracts for every month was evaluated for a time period of three months, and stability was observed with an average mean value of 44.28mg/g of GAE. In the case of unencapsulated *S. macrophylla*, polyphenol content was 59.2mg/g of GAE at day1. There was a decrease in polyphenol amount in the unencapsulated extract as at the end of three months, it was 41.74mg/g of GAE (Figure3a).

The anti-oxidant activity in the encapsulated extract at day 1 was 31.73 mg/g of AAE and by the end of three months study period, the anti-oxidant content was 31.23mg/g of AAE.

However, in the unencapsulated extract, the anti-oxidant content was 35.95mg/g of AAE on day 1 and by the end of three months study period, it decreased to 27.8mg/g of AAE (Figure 3b).

These results are in accordance with the reports of Montoro *et al*., 2012, that over a period of time, polyphenols, flavonoids and anthocyanins are precarious or unstable by nature. The concentrates can't be utilized following three months of their preparation [29]. Polyphenol stability may be due to the bond formed between the matrix of the alginate beads and the hydroxyl group of polyphenols. This bond may have disintegrated due to heat during destabilization, releasing the polyphenols and thereby maintaining polyphenols' stability [30].



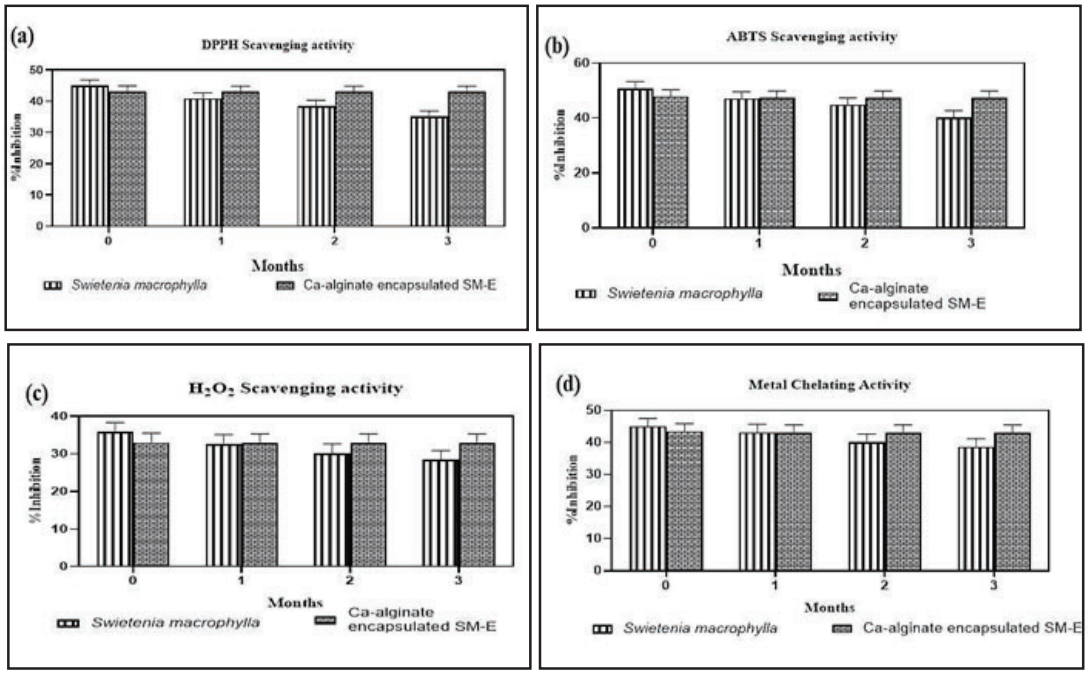


Figure 3: Stability of (a) Polyphenol content and (b) Anti-oxidant content in unencapsulated and encapsulated *Swietenia macrophylla* extract for a period of three months of storage.

Table 1: HPLC analysis of ethanolic extract of *S. macrophylla* compared with the standards

Standards	Retention Time (min)	Class	Methanolic sample of *S. macrophylla*
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

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

Antioxidant potential in encapsulated alginate beads during three months of storage

The percentage inhibition values of the antioxidant activity by DPPH, ABTS, H_2O_2 scavenging method and metal chelating method in the encapsulated extracts at day 1 was 43.12%, 46.6%, 35.4%, 43.4%, respectively. For every month over three months, the anti-oxidant activity was analysed and it was found out that the encapsulated extracts showed a stable pattern for anti-oxidant activity i.e., 43.04% (DPPH), 46.3% (ABTS), 33% (H_2O_2), 43.4% (Metal chelating) respectively (Figure 4). Encapsulated ethanolic extracts showed significant protection of anti-oxidant activity compared to unencapsulated extracts. The protective effect in alginate encapsulated beads can be responsible due to the amphiphilic properties of phenolic compounds [31]. Also, it can be assumed that an increase in the number of hydroxyl groups in phenols can enhance the inhibition of oxidation, thereby enhancing the hydrogen donor ability [31]. The better anti-oxidant potential of the encapsulated *S. macrophylla* extract suggests that encapsulated beads have maintained the polyphenol content which has better potential to scavenge the free radicals and metal chelating property compared to unencapsulated extract.

Conclusion

From this study, it can be concluded that the ethanolic extract of *S. macrophylla* yielded a higher amount of polyphenols and exhibited better antioxidant activity. Encapsulated beads obtained from the extrusion method exhibited a more uniform, homogeneous morphology than control beads [32, 33]. No significant change in the color of the encapsulated beads was observed when stored at room temperature over a period of three months. Also, the polyphenolic content and the antioxidant activity were found to be stable in the alginate beads at nontoxic levels. Therefore, it can be said that alginate is suitable for encapsulation of polyphenols of the *S. macrophylla* extract. As time and temperature are important characteristics in the release

of the polyphenols and anti-oxidant capacity, future studies need to be carried out for a longer period of time and check the bioavailability in the animal models. Encapsulation method with alginate may help in the formulation of a novel nutraceutical product appropriate for the various applications in the industries.

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Conflict of Interest

None

References

1. Ames, B.N., Shigenaga, M.K. and Hagen T.M. (1993) Oxidants, anti-oxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA, 90: 7915–7922.
2. Tewari, R., Gupta, M., Ahmad, F., Rout, P.K., Misra, L., Patwardhan, A. and Vasudeva, R. (2017) Extraction, quantification and anti-oxidant activities of flavonoids, polyphenols and pinitol from wild and cultivated *Saraca asoca* bark using RP-HPLC-PDA-RI method. Ind. Crops Prod.,103: 73–80.
3. Swallah, M.S., Sun, H., Affoh, R., Fu, H. and Yu, H. (2020). Anti-oxidant potential overviews of Secondary Metabolites (Polyphenols) in Fruits. Int. J. Food Sci, 2020: 9081686.
4. 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.
5. Mojzer, E.B., Hrncic, M.K., Skerget, M., Knez, Z. and Bren, U. (2016) Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects. Molecules, 21: 901-939.
6. Abaee, A., Mohammadian, M. and Jafari, S.M. (2017) Whey and soy protein-based hydrogels and nano-hydrogels as bioactive delivery systems. Trends Food Sci. Technol., 70: 69–81.
7. Jain, A., Thakur, D., Ghoshal, G., Katare, O.P. and Shivhare, U.S. (2015). Microencapsulation by Complex Coacervation Using Whey Protein Isolates and Gum Acacia: An Approach to Preserve the Functionality and Controlled Release of β-Carotene. Food Bioproc. Technol., 28: 1635–1644.
8. Yang, M., Liang, Z., Wang, L., Qi, M., Luo, Z. and Li, L. (2020) Microen-capsulation Delivery System in Food Industry - Challenge and the Way Forward. Adv. Polym. Technol., 2020: 1–14.
9. Macías-Cortes, E., Gallegos-Infante, J.A., Rocha-Guzmán, N.E., Moreno-Jimenez, M.R., Medina-Torres, L. and González-Laredo, R.F. (2019) Microen-capsulation of phenolic compounds: Technologies and novel polymers. Rev Mex Ing Quim, 19: 491–521.
10. Bastos, L.P.H., Vicente, J., Correa dos Santos, C.H., Geraldo de Carvalho, M. and Garcia-Rojas E.E. (2020) Encap-sulation of black pepper (*Piper nigrum* L.) essential oil with gelatin and sodium alginate by complex coacervation. Food Hydrocoll., 102: 105605-105613.
11. Mahmoud, M., Abdallah, N.A., El-Shafei, K., Tawfik, N.F. and El-Sayed, H.S. (2020) Survivability of alginate-microencapsulated *Lactobacillus plantarum* during storage, simulated food processing and gastrointestinal conditions. Heliyon, 6: e03541.
12. Balijepalli, M.K., Suppaiah, V., Chin, A.M., Buru, A.S., Sagineedu, S.R. and Pichika M.R. (2015) Acute oral toxicity studies of Swietenia macrophylla seeds in sprague dawley rats. Pharmacogn. Res., 7: 38–44.

13. Moghadamtousi, S.Z., Goh, B.H., Chan, C.K., Shabab, T. and Kadir, H.A. (2013) Biological activities and phytochemicals of *Swietenia macro-phylla* King. Molecules, 18: 10465–10483.

14. 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.

15. 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.

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

17. 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.

18. Chan, E.S., Wong, S.L., Lee, P.P., Lee, J.S., Ti, T.B., 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.

19. Chauhan, A. P., Patel, M. D., Patel, D. J. and Singh, K. N. (2021) Extraction, Screening, and Characterization of Bioactive Compounds from *Moringa oleifera*: Extends Life-span of *Caenorhabditis elegans*. Curr Trends Biotechnol Pharm., 15: 282-298.

20. Hossain, M.A. and Shah, M.D. (2015) A study on the total phenols content and anti-oxidant activity of essential oil and different solvent extracts of endemic plant *Merremia borneensis*. Arab J Chem, 8:66–71.

21. 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.

22. Zheleva-Dimitrova, D., Nedialkov, P. and Kitanov, G. (2010) Radical scavenging and anti-oxidant activities of methanolic extracts from Hypericum species growing in Bulgaria. Pharmacogn. Mag., 6:74–78.

23. 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.

24. Perva-Uzunalic, A., Skerget, M., Knez, Z., Weinreich, B., Otto, F. and Grüner, S. (2006) Extraction of active ingredients from green tea (*Camellia sinensis*): Extraction efficiency of major catechins and caffeine. Food Chem., 96:597–605.

25. Saeed, N., Khan, M.R. and Shabbir, M. (2012) Anti-oxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern. Med., 12: 221-133.

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

27. Mandal, S., Senthil Kumar, S., Krishnamoorthy, B. and Basu, S.K. (2010) Development and evaluation of calcium alginate beads prepared by sequential and simultaneous methods. Braz. J. Pharm. Sci., 46: 785–793.

28. Vimala, K., Yallapu, M.M., Varaprasad, K., Reddy, N.N., Ravindra, S., Naidu, N.S. and Raju, K.M. (2011) Fabrication of Curcumin Encapsulated Chitosan-PVA Silver Nanocomposite Films for Improved Antimicrobial Activity. J Biomater. Nanobiotechnol., 02:55–64.

29. Sebastia, N., Soriano, J.M., Barquinero, J.F., Villaescusa, J.I., Almonacid, M., Cervera, J., Such, E., Silla, M.A. and Montoro, A. (2012) In vitro cytogenetic and genotoxic effects of curcumin on human peripheral blood lymphocytes. Food Chem Toxicol., 50: 3229–3233.

30. 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.

31. Surveswaran, S., Cai, Y.Z., Xing, J., Corke, H. and Sun, M. (2010) Anti-oxidant properties and principal phenolic phytochemicals of Indian medicinal plants from Asclepiadoideae and Periplocoideae. Nat. Prod. Res., 24: 206–221.

32. Madene, A., Jacquot, M., Scher, J. and Desobry, S. (2006) Flavour encap-sulation and controlled release - A review. Int. J Food Sci. Technol., 41:1–21.

33. Dong. Q.Y., Chen, M.Y., Xin, Y., Qin, X.Y., Cheng Z, Shi, L.E. and Tang, Z.X. (2013) Alginate-based and protein-based materials for probiotics encapsulation: A review. Int J Food Sci. Technol., 48: 1339–1351.