Ultrasound-assisted extraction (UAE) of Quercetin from *Thespesia populnea* leaves

Rohini Samadarsi[1]*

[1*]Department of Biotechnology and Biochemical Engineering, Sree Chitra Thirunal College of Engineering, Pappanamcode, Thiruvananthapuram, Kerala, India.

1. Introduction

The search for naturally occurring quercetin has a great interest in industries as well as in scientific research. There was no qualitative and quantitative information on effective isolation on the flavonoid, particularly quercetin content in the leaves of the plant. As flavonoids are one of the major natural product subgroups in all the plants, it is useful to explore their content in the T. populnea leaves. Furthermore, there is a growing interest for naturally derived medications; effective methodology should be developed to extract the bioactive compounds from the leaves [1-5].

The plant materials (leaves of T. populnea) were air- dried with the help of an autoclave (at 60 °C) for one weeks and later it was then ground to uniform powder. The extracts of leaf samples were prepared in a sequential procedure by soaking 100 g of dried powder in 900 ml with solvents like ethanol for 1h. And it was break down with an ultra-sonicator for various time(5min,10min,15min,20min). At the end of respective extraction, the plant extracts were filtered using Whatman filter paper [6-7].

Total flavonoids were determined using the modified aluminium chloride colorimetric method of Chang et al [8]. Briefly, the mixture of 2 mL extract and 0.5 mL of 5% AlCl3 and 0.5 mL of 1 M potassium acetate solution was incubated at room temperature for 15 min. Absolute ethanol was used as a control.

Since plant extracts usually occur as a combination of various type of bioactive compounds or phytochemicals with different polarities, their separation remains a big challenge for the process of identification and characterization of bioactive compounds. It is a common practice in isolation of these bioactive compounds that several different separation techniques such as TLC, column chromatography, flash chromatography, Sephadex chromatography and HPLC, should be used to obtain pure compounds [10].

Regarding the use of nanomaterials in drug delivery, the selection of the nanoparticle is based on the physicochemical features of drugs. The combined use of nanoscience along with bioactive natural compounds is very attractive, and growing very rapidly in recent times. It presents several advantages when it comes to the delivery of natural products for treating cancer and many other diseases [11-16]. Natural compounds have been comprehensively studied in curing diseases owing to their various characteristic activities, such as inducing tumour-suppressing autophagy and acting as antimicrobial agents. The enrichment of their properties, such as bioavailability, targeting and controlled release were made by incorporating nanoparticles. After encapsulation, it showed six-fold increase in bioavailability in comparison to free thymoquinone and thus protects the gastrointestinal stuffs. It also increased the pharmacokinetic characteristics of the natural product resulting in better therapeutic effects [17].

2. Materials and Methods

Quercetin standard was obtained from Sigma-Aldrich, USA. The other solvents were of HPLC grade, were purchased from Merck Private Limited, India Chitosan (minimum 90%), Tripolyphosphate (Technical Grade 85% purity) and were obtained from Sigma-Aldrich Co. (St. Louis, MO). All other reagents were analytical grade purchased from Merck.

2.1. Ultrasound-assisted extraction (UAE) of Quercetin from *Thespesia populnea*

The Quercetin extraction from Thespesia popunea was performed by a method described by (Padmapriya et al., 2012) with major modifications. Microwave-assisted extraction was replaced with UAE using a probe ultra-sonicator apparatus (Samsung Trio, Model CE117ADV; 230 V*50 Hz) in a closed vessel system. Ethanol solvent was used to extract quercetin from Thespesia popunea powder under different UAE conditions. Ultrasonication time (1–20 minutes, with 5-minute interval), Amplitude percentage (1 to 100 %, with 10% interval), duty cycle (0.1 to 0.9 W/s, with 0.1 W/ s interval), ethanol concentration (50–100 %, v/v), Solvent to solute ratio (5:1, 10:1, 15:1, 20:1, 25: 1, and 30:1)) and pre-leaching time (1–30 min, with 5 minute interval) were evaluated for the extraction of Quercetin from Thespesia populnea. UAE extracted a clear solution of Thespesia populnea extract was evaporated using Rotary Vacuum Evaporator (Yamato, Japan), and the inactive components were removed by chloroform and butanol, filtered and separated. The final purified extract was lyophilized (DigitechL yophiliser) and stored. Quercetin was estimated by a method followed by Joubert, 2012. Purified extract was dissolved in DMSO and analysed in a UV–vis spectrophotometer (UV-2310, Tech comp), at the wavelength of 410 nm. The purity of the sample was determined using LCMS analysis (310-MS LC/MS, Triple quadrupole Mass Spectrometer, Agilent Technologies).

3. Results and Discussions

3.1. Effect of Ethanol concentration for the UAE extraction of Quercetin from *Thespesia populnea*

The commonly used extraction solvents are methanol, acetone, ethanol, and water. However, due to the toxicity of methanol to human beings and the environment, water, a nontoxic and inexpensive solvent has widely been applied for extraction of bioactive compounds, but it has lower extraction efficiency. Aqueous ethanol was reported as a good solvent for the extraction of higher molecular weight polyphenols like Quercetin

in various studies [1-5], hence ethanol was chosen for quercetin extraction. The extraction efficiency is significantly reduced using pure solvent, which can be overcome when a mixture of water and solvent was used. It was observed that the extraction efficiency ranged from 60 to 100 % for various plant materials [6]. Thus, aqueous ethanol was used for the present work. The effect of concentration of ethanol on quercetin yield was observed, and other parameters were fixed as follows, the solvent to solute ratio, 10:1 (ml/g), pre-leaching time 30 minutes, sonication time of 20 minutes, duty cycle 0.8 W/s and amplitude % of 80 %. In the present study, the extraction technique is performed selectively to extract polyphenols. Only polyphenol present in Thespesia populnea plant material is Quercetin. Other polymeric polyphenols and polymers would require an extraction solvent with high water content to be extracted [20-23]. Quercetin extraction efficiency is greater when the mixture of solvent and water is used. To selectively screen quercetin ethanol water mixture was used. The higher yield was observed when the ethanol water mixture was in a 3:1 ratio. The results are shown in Fig. 1. When the concentration of ethanol increased, the quercetin yield increased significantly up to 0.553 ± 0.01 mg/g ($P < 0.005$) at 75% ethanol concentration, followed by a decrease in quercetin yield with further increase in ethanol concentration. Maximum quercetin yield of 0.553 ± 0.01 mg/g was obtained at 75% ethanol concentration; hence75% ethanol concentration was maintained for the subsequent experiments.

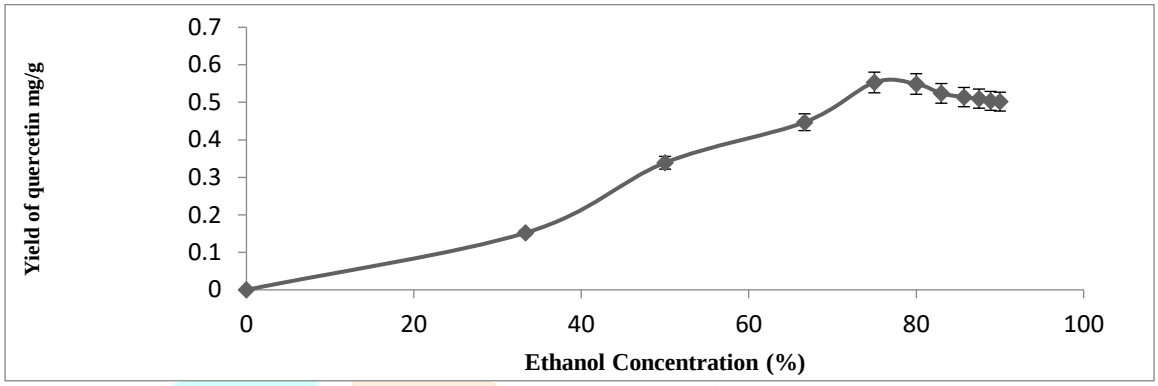

Fig. 1 Effect of ethanol concentration on the yield of quercetin; Each value represents the mean ± S.D of three replicates; X-axis denotes ethanol concentration (%) and Y-axis denotes Yield of Quercetin (mg/g). Greyline = QUERCETIN Yield. Bars represent mean ± standard errors (n = 3). The data values are statistically significant ($p < 0.05$).

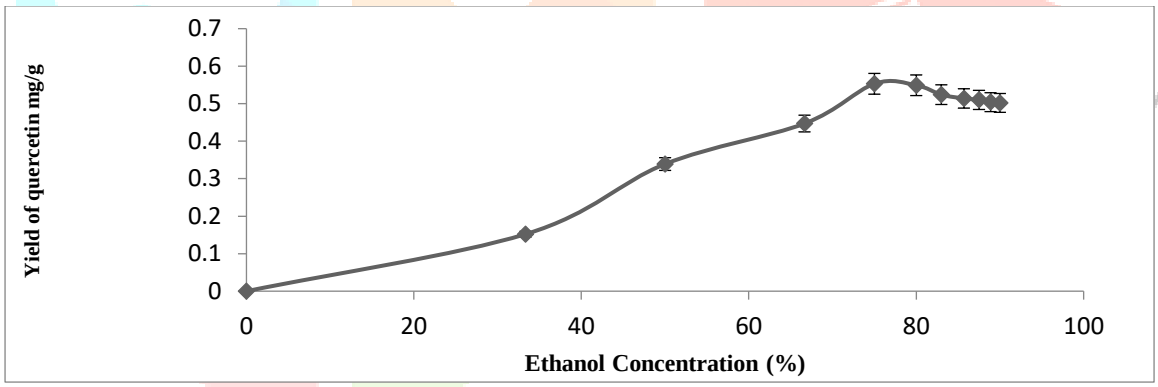

Fig. 1 Effect of ethanol concentration on the yield of quercetin; Each value represents the mean ± S.D of three replicates; X-axis denotes ethanol concentration (%) and Y-axis denotes Yield of Quercetin (mg/g). Greyline = QUERCETIN Yield. Bars represent mean ± standard errors (n = 3). The data values are statistically significant ($p < 0.05$).

3.2. Effect of Pre-leaching time on quercetin Yield

Organic solvents with water were reported to have a desirable impact on the extraction efficiency of polyphenols. When *Thespesia populnea* powder was kept in 75 % ethanol solvent for 20 minutes pre leaching time, it enhances the diffusion of soluble compounds from the plant matrix. It was observed that the presence of water in organic solvents led to enhanced penetration of the extractant in matrix molecules. This subsequently promotes ultrasonic treatment and imparts a positive impact on overall efficiency and extraction time using organic solvents alone [18-20]. Pre leaching also gives an increase in the moisture content which results in higher ultrasound penetration, which in turn the cell more efficiently to release the solutes, thereby improving the extraction yield [21-23]. Hence optimizing the pre leaching time would be useful in achieving the optimal extraction of the quercetin. From the results of the present study, it was observed that the yield of quercetin yield increased with the increase in pre-leaching time from 5 to 20 minutes (Fig. 2). When the pre-leaching time increased from 25 minutes to 50 minutes, the quercetin yield was almost unchanged. The maximum yield of 0.683 ± 0.01mg/g($P < 0.005$) (Fig. 4.4.2.1) of quercetin was obtained at 20 minutes pre-leaching time, hence for further experiments pre-leaching time was maintained at 20 minutes.

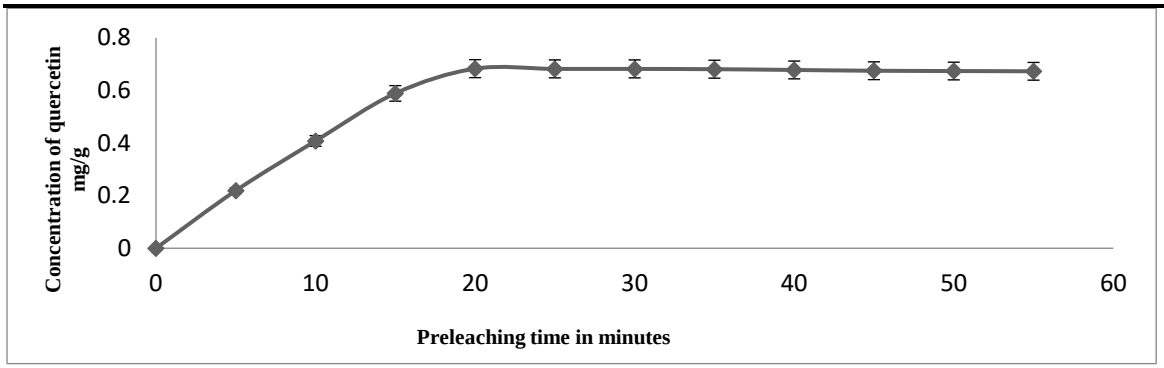

Fig. 2. Effect of pre-leaching time on the yield of quercetin, each value represents the mean ± S.D of three replicates; X-axis denotes Pre-leaching time (minutes) and Y-axis denotes Yield of Quercetin (mg/g). Greyline = QUERCETIN Yield. Bars represent mean ± standard errors (n = 3). The data values are statistically significant ($p < 0.05$)

3.3. Effect of Liquid-to-Solid Ratio on the quercetin Yield

The effect of liquid-to-solid ratio on the quercetin yield was explored, and other experimental parameters were fixed as follows, the ethanol concentration, 75%; and pre-leaching time, 20 min. The results are summarized in Fig. 4.4.3.1. When the solvent to solute ratio increased from 4:1 to 10:1, the quercetin yield increased with the increase of the solvent to the solute ratio (Fig. 3). When the solvent to solute ratio increased from 10:1 to 16:1, the quercetin yield was almost unchanged (Fig. 4.4.3.1). The maximum quercetin yield was obtained at a solvent to solute ratio of 10:1, a yield of 0.717 ± 0.01 mg/g ($P < 0.005$) (Fig. 3). The large solvent to solute ratio can dissolve components more effectively, resulting in an extraction yield enhancement. However, this can lead to solvent wastage. On the contrary, a small solvent to solute ratio decreases the extraction yield. Therefore, the solvent to solute ratio is a significant parameter in determining the yield [22]. From the result, it could be concluded that the quercetin yield increased significantly when the solvent to solute ratio increased from 4:1 to 10:1. After 10:1, the quercetin yield almost unchanged, therefore, a solvent to the solute ratio of 10:1 was used in the subsequent experiments.

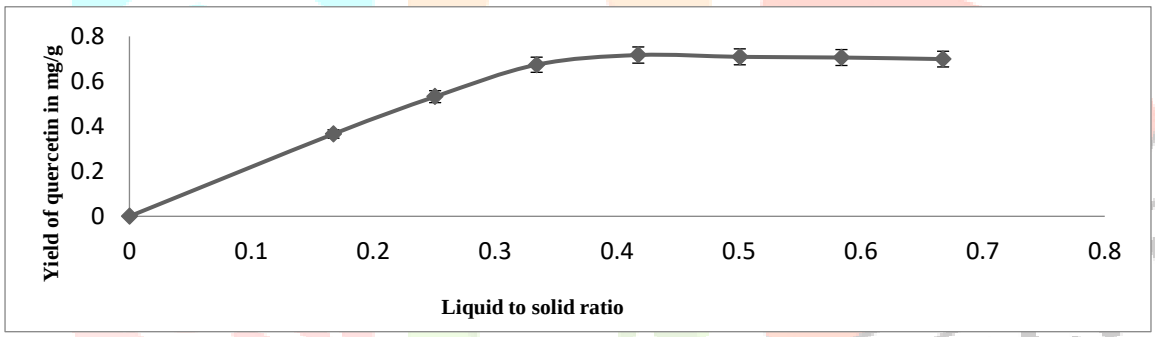

Fig. 3 Effect of liquid to solid ratio on the yield of quercetin; Each value represents the mean ± S.D of three replicates; the X-axis denotes Liquid (ethanol solvent) to solid ratio (*Thespesia populnea* powder) and Y-axis denotes Yield of Quercetin (mg/g).Greyline = quercetin Yield. Bars represent mean ± standard errors (n = 3). The data values are statistically significant ($p < 0.05$)

3.4. Effect of ultrasonication time on the Yield of QUERCETIN

As the ultrasonication time increased from 0 to 10 minutes, the yield of quercetin was enhanced (Fig 4). Further increase in ultrasonication time led to a decrease in quercetin yield (Fig 4). The maximum yield of quercetin, at an ultrasonication time of 5 minutes was 0.88 ± 0.01 mg/g ($P < 0.05$) of *Thespesia populnea* powder. In the present study, a slight declining trend in Quercetin yield from *Thespesia populnea* during elevated ultrasonication extraction time was observed. This might be due to slight thermal degradation of phenolic compounds at elevated temperatures during longer extraction times [23]

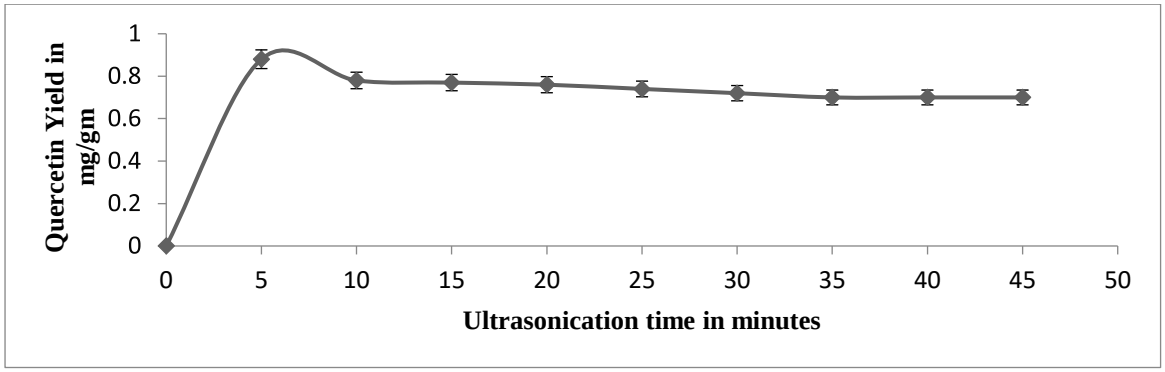

Fig 4 Effect of ultrasonication time on quercetin yield. Each value represents the mean ± S.D of three replicates; the X-axis denotes ultrasonication time (minutes) and Y-axis denotes Yield of Quercetin (mg/g).Greyline = quercetin Yield. Bars represent mean± standard errors (n = 3). The data values are statistically significant ($p < 0.05$).

4.6. Quercetin extraction from *Thespesia populnea*

The chromatographic profile of standard Quercetin, as well as UAE, extracted Quercetin from *Thespesia populnea* is depicted in Fig. 5. The retention time, 10 min of the extract was well in conformance with the standard, confirming the presence of Quercetin in *Thespesia populnea* extract.

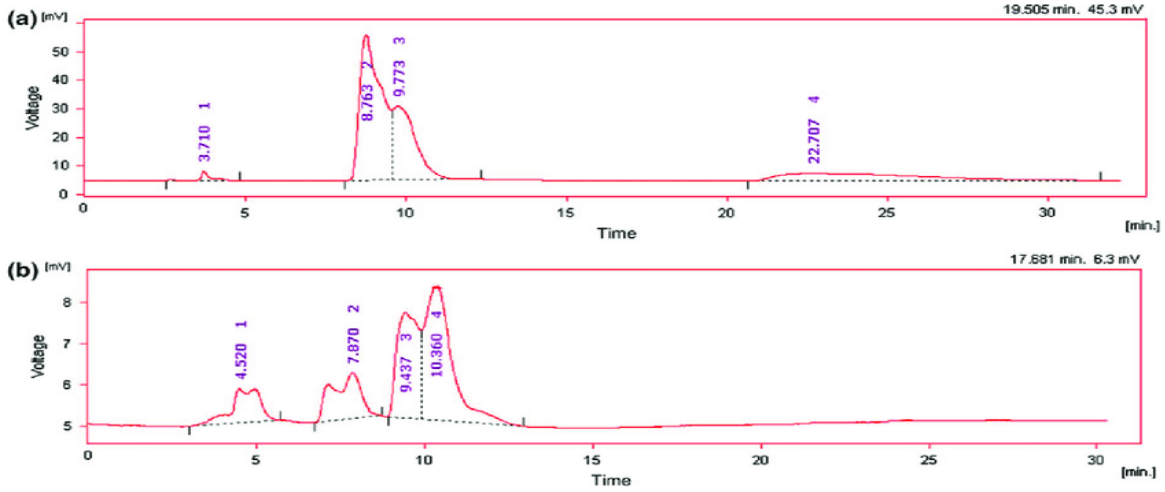

Fig. 5. Chromatographic profile (HPLC) (a) Quercetin standard and (b) UAE extracted Quercetin from *Thespesia populnea*

Conclusion

Increasing the relevance of nutraceuticals on the overall food market has increased the search for new types of biomolecules with high nutritive benefits. Quercetin is one such molecule with numerous therapeutic properties having potential in the food industry as a nutritional supplement. In the present study, the ultrasonic-assisted extraction of Quercetin from *Thespesia populnea* was optimized. The results showed that sonication time and liquid to solid ration had significant roles in the extraction of Quercetin. The best combination, to obtain a yield of UAE is an effective and feasible method for the extraction of Quercetin from *Thespesia populnea*.

References

Hwang MK, Song NR, Kang NJ, Lee KW, Lee HJ. Activation of phosphatidylinositol 3-kinase is required for tumor necrosis factorα-induced upregulation of matrix metalloproteinase-9: Its direct inhibition by quercetin. The international journal of biochemistry & cell biology. 2009;41(7):1592- 1600

2. **DeSimone J.M**. Practical Approaches to Green Solvents. *Science.* 2002;297:799–803. doi: 10.1126/science.1069622.
3. **Moran M.J., Zogorski J.S.**, Squillace P.J. Chlorinated Solvents in Groundwater of the United States. *Environ. Sci. Technol.* 2007;41:74–81. doi: 10.1021/es061553y.
4. **Ikeda M.** Solvents in urine as exposure markers. *Toxicol. Lett.* 1999;108:99–106. doi: 10.1016/S0378-4274(99)00078-8.
5. **De Santi V., Cardellini F., Brinchi L., Germani R**. Novel Brønsted acidic deep eutectic solvent as reaction media for esterification of carboxylic acid with alcohols. *Tetrahedron Lett.* 2012;53:5151–5155. doi: 10.1016/j.tetlet.2012.07.063.
6. **Brinchi L., Germani R., Braccalenti E., Spreti N., Tiecco M., Savelli G**. Accelerated decarboxylation of 6-nitrobenzisoxazole-3-carboxylate in imidazolium-based ionic liquids and surfactant ionic liquids. *J. Colloid Interface Sci.* 2010;348:137–145. doi: 10.1016/j.jcis.2010.04.029.
7. **Gutfinger** T. Polyphenols in olive oils. *J. Am. Oil Chem. Soc.* 1981;58:966–968. doi: 10.1007/BF02659771
8. **Pérez-Gregorio R.M., García-Falcón M.S., Simal-Gándara J., Rodrigues A.S., Almeida D.P.** Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest. *J. Food Compos. Anal.* 2010;23:592–598. doi: 10.1016/j.jfca.2009.08.013.
9. **Rhodes M.J., Price K.R.** Analytical problems in the study of flavonoid compounds in onions. Food Chem. 1996;57:113–117. doi: 10.1016/0308-8146(96)00147-1.
10. **Su J., Fu P., Shen Y., Zhang C., Liang M., Liu R., Li H., Zhang W**. Simultaneous analysis of flavonoids from Hypericum japonicum Thunb.ex Murray (Hypericaceae) by HPLC-DAD–ESI/MS. J. Pharm. Biomed. Anal. 2008;46:342–348. doi: 10.1016/j.jpba.2007.10.032.
11. 78. Wilm M. Principles of Electrospray Ionization. Mol. Cell. Proteom. 2011;10:M111.009407. doi: 10.1074/mcp.M111.009407.
12. **S. G. Dmitrienko, V. A. Kudrinskaya, and V. V. Apyari,** "Methods of extraction, preconcentration, and determination of quercetin," *Journal of Analytical Chemistry*. 2012, doi: 10.1134/S106193481204003X.
13. **C. C. Chang, M. H. Yang, H. M. Wen, and J. C. Chern,** "Estimation of total flavonoid content in propolis by two complementary colometric methods," *J. Food Drug Anal.*, 2002.
14. **E. Joubert, M. Manley, and M. Botha,** "Evaluation of spectrophotometric methods for screening of green rooibos (Aspalathus linearis) and green honeybush (Cyclopia genistoides) extracts for high levels of bio-active compounds," *Phytochem. Anal.*, vol. 19, no. 2, pp. 169–178, 2008, doi: 10.1002/pca.1033.
15. **S. Sasidharan, Y. Chen, D. Saravanan, K. M. Sundram, and L. Yoga Latha,** "Extraction, isolation and characterization of bioactive compounds from plants' extracts," *African J. Tradit. Complement. Altern. Med.*, 2011.
16. **D. M. Byler and H. Susi,** "Examination of the secondary structure of proteins by deconvolved FTIR spectra," *Biopolymers*, 1986, doi: 10.1002/bip.360250307.
17. **C. Berthomieu and R. Hienerwadel,** "Fourier transform infrared (FTIR) spectroscopy," *Photosynthesis Research*. 2009, doi:

10.1007/s11120-009-9439-x.

18. **A. Alyamani and O. M.,** "FE-SEM Characterization of Some Nanomaterial," in *Scanning Electron Microscopy*, 2012.
19. **M. A. Augustin and Y. Hemar,** "Nano- and micro-structured assemblies for encapsulation of food ingredients," *Chem. Soc. Rev.*, 2009, doi: 10.1039/b801739p.
20. **K. G. H. Desai and H. J. Park,** "Recent developments in microencapsulation of food ingredients," *Drying Technology*. 2005, doi: 10.1081/DRT-200063478.
21. **M. Rinaudo,** "Chitin and chitosan: Properties and applications," *Progress in Polymer Science (Oxford)*. 2006, doi: 10.1016/j.progpolymsci.2006.06.001.
22. **W. L. Teng, E. Khor, T. K. Tan, L. Y. Lim, and S. C. Tan,** "Concurrent production of chitin from shrimp shells and fungi," *Carbohydr. Res.*, 2001, doi: 10.1016/S0008-6215(01)00084-2.
23. **A. Agnihotri, N. N. Mallikarjuna, and T. M. Aminabhavi**, "Recent advances on chitosan-based micro- and nanoparticles in drug delivery," *Journal of Controlled Release*. 2004, doi: 10.1016/j.jconrel.2004.08.010