ENCAPSULATION AND ANTIOXIDANT ACTIVITY OF BIOACTIVE COMPONENTS EXTRACTED FROM TERMINALIA ARJUNA BARK WITH CHITOSAN

Hafiz Talha Akhtar[1*], Muhammad Irslan Ilyas[1], Muhammad Hassan[3], Muhammad Ayaz Ashraf[2], Ziad Ahmad[2], Muhammad Alyas Habib[3]

[1]Faculty of Sciences, Department of Chemistry, The University of Lahore

[2]Faculty of Sciences, International Islamic University Islamabad

[2]Faculty of Sciences, Government boys Degree College Takht bhai

[3]University of Management Technology

ABSTRACT

Plants have potent antioxidant properties, protecting against free radicals. Various products have been developed from vegetables to harness their antioxidant benefits, guarding the human body from diseases like brain and diabetes issues. Foods with antioxidant activities shield the body from oxidative damage. Microencapsulation is a method used to preserve antioxidants, ensuring controlled release and masking taste. It involves using core material to safeguard active components. In this study, freeze-drying was employed for encapsulation. Antioxidant properties were evaluated using UV-visible spectroscopy, FTIR, SEM, and DPPH techniques, comparing non-encapsulated and encapsulated extracts. Encapsulation was confirmed, showing higher catalytic activity in the encapsulated extract. The antioxidant activity was studied under different scavenging conditions. Microcapsule systems made of Chitosan and STPP proved effective in distributing, storing, and delivering bioactive materials. Terminalia Arjuna bark extract can now be utilized more widely in food and medicine based on this study.

Keyword. Encapsulation, Antioxidant activity, Bioactive components, Terminalia arjuna bark, Chitosan, Natural extracts Phytochemicals, Controlled release, Drug delivery, Polymeric nanoparticles,

INTRODUCTION

Recent years have seen successful development and application of micro- and Nano encapsulation technologies in the food business for conserving bioactive ingredients and ensuring targeted delivery [1]. Encapsulation protects chemically delicate bioactive compounds from deterioration under adverse external conditions while controlling their release [2]. Microencapsulation revolutionized industries with diverse applications in chemicals, pharmaceuticals, cosmetics, printing, creating self-healing composites, disinfectant textiles, and defense materials [3]. Various enclosing agents form distinct adhesive layers for the manipulation and preservation of core materials with differing affinities to water, such as lipids, gums, nucleic acids, and complex carbohydrates [4]. Maltodextrins used in probiotic microencapsulation via spray drying maintain benefits despite reduced survival [5]. Maltodextrins: limited emulsification, weak volatile retention; often co-used for food ingredient encapsulation [6]. GA is a cost-effective, reactive heteropolysaccharide used with gelatin to stabilize emulsions, control moisture, and inhibit oxidation, characterized by low viscosity, easy dissolution, and forming a white solid solution [7]. Cyclodextrins optimize pharmaceuticals via encapsulation for enhanced absorption, dissolution, stabilization, and bioavailability [8] Research aims to utilize cyclodextrin Nano sponges to enhance essential oil solubility, stability, and antimicrobial properties in food packaging, particularly with slow Cardamom oil release [9]. Chitosan, derived from crab shells, is a biodegradable polymer enhancing drug delivery through complex formation in biomedical uses such as wound dressings and microcapsules; it dissolves in pH 5.9 buffer and bases despite limited water solubility [10]. The University of Wisconsin's pharmacy department developed an air suspension coating technique using a distribution plate, control panel, coating chamber, and film coating nozzle to suspend particles in an air stream within the container [11]. Applying polymer solution in recirculating flow to coat core particles, and then drying with air stream to achieve desired thickness [11]. Spray-drying in food industry: Fractionates mixture, evaporates solvent with hot air, cyclone collects encapsulated lactose, whey protein, enhancing efficiency [12]. Freeze-drying efficiently encapsulates solid compounds with higher sorption capacity and antioxidant activity compared to spray-drying [13].Utilized various natural sources and

compounds, including Argentinian red wine components, onion peel, beetroot, and lime waste, employing hesperidin for bitterness reduction and α-lacto globulin for gastrointestinal safeguarding, to encapsulate sour cherry flavonoids for enhanced bioavailability and functionality [14]. Ongoing research enhances encapsulation techniques (lipid nanoparticles, coacervation, cyclodextrin insertion complex) for food, preserving nutrients with flavor stability under intellectual property safeguards [15]. Microencapsulated insect pheromones disrupt mating and stabilize for bio-rational crop protection [16]. Active films in food and pharmaceuticals enhance treatment stability, bioavailability, and targeted drug delivery through covalent or electrostatic bonding for improved disease management [17].Combined anionic ulna and cationic CS polymers create stable bimolecular structures promoting osteoblast growth, valuable for biomedical scaffolds [18]. Food encapsulation enhances stability, masks flavors, and preserves nutrition via oxidation protection in storage [19].Current research aims to enhance processed meals, probiotics, and encapsulated delivery of bioactive for controlled release and protection [20]. Plants have historically provided essential sustenance, medicine, and diverse bioactive compounds for human well-being [21].Study explores bioactive elements, antioxidants, and therapeutic potential of Terminalia arjuna extracts from Pakistani arjuna plant [22].Terminalia arjuna bark powder long-term use reduces oxidative stress, enhances endogenous antioxidants, and prevents cardiac issues in ischemic heart disease [23]. Plant-based diet provides the bulk of dietary antioxidants, offering diverse biological and chemical properties [24].Past synthetic antioxidants (BHA, BHT) in food additives face safety concerns, driving focus towards natural alternatives [25]. Flavonoids, along with vitamin C and other phenolic compounds, stand out as widely recognized natural antioxidants derived from plants [26]. Antioxidants such as phenolic acids, polyphenols, and flavonoids work to neutralize free radicals, providing protection against degenerative illnesses by inhibiting oxidative processes [27].Antioxidants halt chain reactions by neutralizing free radicals, with plant-based ones playing a significant role in human health alongside diverse animal and plant antioxidant systems [28].Rising popularity of natural antioxidants for countering free radicals' impact on the body and preserving dietary components, surpassing synthetic sources [29].

MATERIAL AND METHOD

Chemicals

The bark of Terminalia Arjuna was utilized in the experiment. Methanol with a molecular weight of 32.04g/mol, chitosan with a molecular weight of 1526.5g/mol, sodium tripolyphosphate with a molecular weight of 367.864g/mol, acetic acid with a molecular weight of 60.052g/mol, distilled water with a molecular weight of 18.04g/mol, sodium hydroxide with a molecular weight of 40g/mol, and hydrochloric acid with a molecular weight of 36.5g/mol were employed.

Creating an extract from Terminalia Arjuna bark involves the following steps.

The bark of Terminalia Arjuna plant family of Combretaceae was taken from Bagh e Jinnah park Lahore Pakistan. The bark was washed and dried under the shadow For a week after drying bark was grind in a grinder and made the extract by dissolving 15g of grind dried bark powder in absolute alcohol .Kept it in a closed container for 3 days to prepare the extract. Then filter the solution through Whitman's paper no 1.The juicy mass of extract was dried in Rotary evaporator and kept in bottles at room temperature until use.

Encapsulation of extract

Throughout the investigation, 1% Chitosan dissolved in 0.1 percent acetic acid was utilized at a concentration of around 0.1 percent w/v. The nanoparticles were made by adding Terminalia Arjuna extract (T.A.E) to a high molecular weight Chitosan solution and stirring it magnetically. A solution of TPP at a concentration of 1 percent w/v in distilled water was gradually introduced in droplets to the Chitosan T.A.E solution following a 5-minute delay after the addition of T.A.E, pH 4.0 at room temperature, under magnetic stirring (700 rpm for 3 hours). Centrifugation at 4000rpm for 30 minutes separated the formed nanoparticles, which were then freezing dried and lyophilized.

RESULTS AND DISCUSSION

UV-visible characterization

One of the most crucial methods for determining the colloidal stability of an extract encapsulated in aqueous solution is UV-Vis spectroscopy. At room temperature, the reaction was monitored using a spectro-photometer with a 1 nm resolution. By analyzing the extract with a UV-Vis spectrophotometer, the extraction's creation and stabilization were tracked. The greatest absorbance of the encapsulated extract is reported to occur between 200 and 800 nm. UV-visible spectroscopy was used

to characterize the encapsulating Terminalia Arjuna bark extract, chitosan, and sodium Tripolyphosphate. After dilution with deionized water and a deionized water blank, the absorption spectra were determined in the 200 to 800 nm region. Chitosan has a spectral response T1 of roughly 250 nm. STPP's T3 absorption spectra are in the 300nm range. The TAE's T4 absorption spectrum, which ranges from around 250 nm to 550 nm, reveals the various components that are contained inside the extract. The presence of several components that are not chemically coupled with one another is shown by the optical absorption in the image of T5, which is approximately ranging from 200 nm to 550 nm. Chitosan and the extract interact physically, proving that the extract has been encapsulated [30].

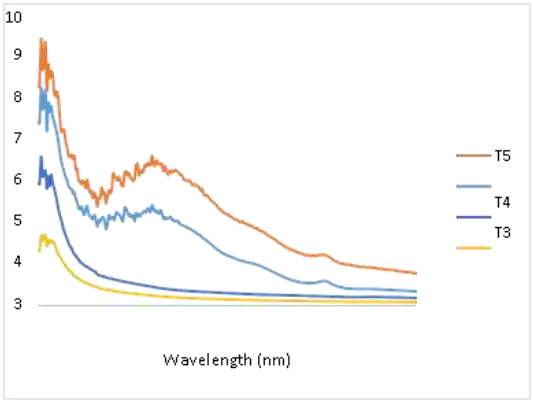


Figure 1.1 Uv-vis spectra of chitosan, T.A extract, STPP, STPP and chitosan, encapsulated extract

FTIR Characterization

Chitosan nanoparticles loaded with Terminalia Arjuna bark extract (TAE) were subjected to chemical characterization using Fourier-transform infrared (FTIR) analysis (CSNPs). The FTIR spectra in Figure 1.2 depict distinctive functional groups within the compounds' structures: CS, STPP, TAE, CSNPs, and TAE-loaded CSNPs. Key peaks were observed, such as the O-H stretching peak at 3352, aromatic C-H stretching at 2973, N-H deformation at 1638, NO2 stretching at 1380, C-F stretching at 1066, C-S stretching at 1044, C-H deformation at 876, alongside other characteristic peaks. CS powder exhibited evident peaks at 3333 (OH and NH2 stretching vibrations), 2930 (C-H bond vibrations in alkanes), 1637 (C=O bond vibrations in amide I molecules), 1555 (general OH groups bending), 1412 and 1278 (symmetric stretching of C-O-C bonds) [31]. Crosslinking of CS polymer with STPP molecules influenced amide group peaks. STPP displayed peaks at 3340 (O-H group stretching), 1637 (carbon double bond stretching), 1044 (carbon single bond stretching), and 894 (Beta-1-4 glycoside linkage stretching). Comparing FTIR spectra of chitosan, STPP, and CSNPs revealed shifts in peaks, e.g., the -NH2 group stretching peak at 3333 cm-1 in CS shifted to 3410 cm-1 in CSNPs due to STPP presence. Peaks related to amide I's C=O stretching in CS (1655 and 1598 cm-1) shifted to 1646 and 1550 cm-1 in CSNPs, suggesting involvement of STPP polymeric phosphate groups and CS amine groups [32]. The FTIR spectra of CSNPs lacked the 1168 cm-1 broad band associated with TTP's -COOH groups, indicating complete cross-linking of chitosan with STPP. Analysis of TAE functional groups revealed absorptions attributed to tannic acid, elegiac acid, and Gallic acid. The broadband between 3333 and 3450 cm-1, responsible for N-H and O-H bands, indicated the presence of these compounds. Bands at 2930 and 1637 cm-1 corresponded to carbon, C-H, and C=O groups. The weaker bands at 1550, 1412, and 1238 cm-1 indicated vibrations of the aliphatic ring in TAE, while C-O stretching of carboxylic acid was evident at 1030 cm-1. The FTIR analysis determined the mode of TAE entrapment within CSNPs. Comparing CSNPs and TAE-loaded CSNPs showed no spectral changes, suggesting physical entrapment. The increased strength of C-H stretching bands in the presence of TAE indicated successful incorporation into CSNPs, aligning with previous research findings [33].

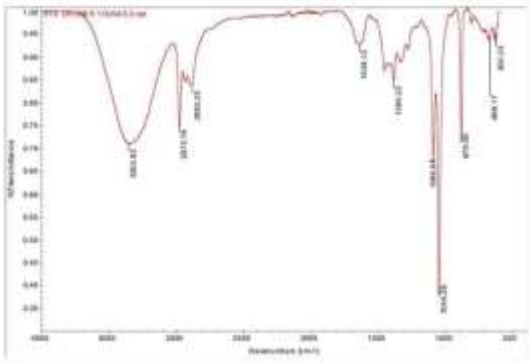
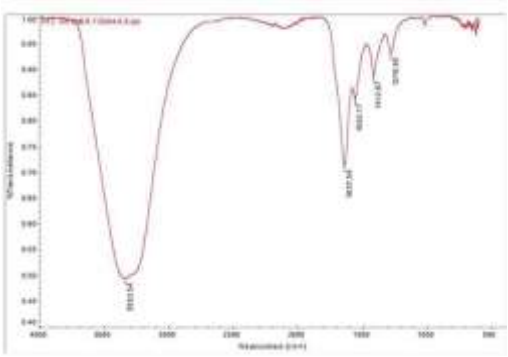
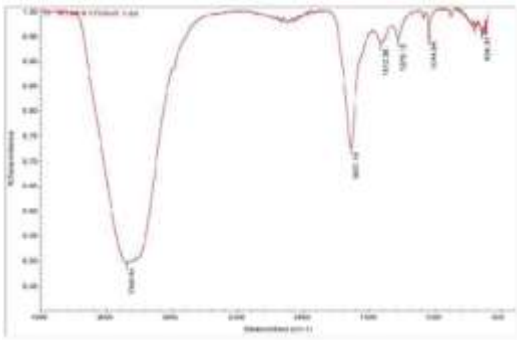


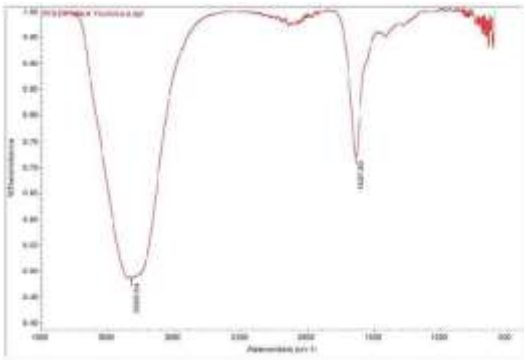
Figure1.2 FTIR spectra of Terminalia Arjuna bark extract



Graph 1.3 illustrates the FTIR spectra associated with Chitosan.



Graph 1.4 illustrates the FTIR spectra associated with sodium tripolyphosphate.



Graph 1.5 Portrays the FTIR spectra concerning the encapsulated extract derived from Terminalia Arjuna bark.

Scanning electron microscopy:

The tribological and size dispersion of CSNPs, TAE-loaded Chitosan, and STPP were examined using SEM. Figure displays the findings. SEM micrographs of both encapsulated extracts revealed spherical shape and homogeneous size distribution. Particle size diameter and distribution were measured using Image, open-source image software programmed. After employing manual mode to measure particle length in the images, the SEM image scale was modified to match the indicated scale-bar value of 200 micrometers. The graphic displays the particle size as measured in several graphs of various vales that display the sample's constituent parts. The results of the extraction of mean diameter, minimum diameter, and standard deviation from SEM images. Compared to chitosan that had been loaded with TAE, the normal size of the blank chitin was lower. Malvern Zetasizer lively particle size quantification results were supported by SEM observations, which showed that adding TAE to chitosan raises mean particle size radius. Furthermore, it was observed that the dimensions of nanoparticles appear to be reduced in scanning electron microscope (SEM) images when contrasting the size information obtained from SEM images with measurements obtained using the Malvern Zetasizer.

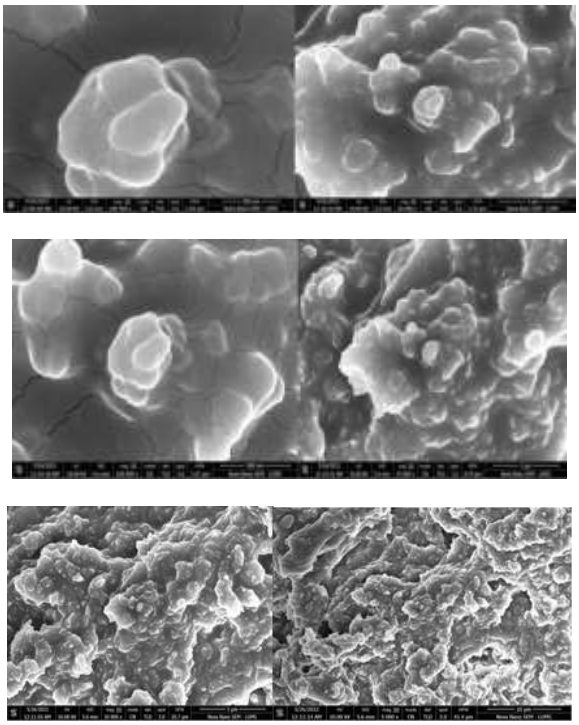


Figure 1.6 SEM Spectra of encapsulated Terminalia Arjuna bark extract

Activity

Antioxidant activity and total phenolic content were noted. The findings showed that the majority of the antioxidant activity was caused by the thermo labile components found in herbs and their interactions. Solutions were taken in different beakers at different PH vale and the percentage of contents was different at different PH value. In graph phenolic contents was

maximum at PH value of 2.At PH 7.4 the phenolic contents were in average amount and At maximum PH value of 10 these were low.

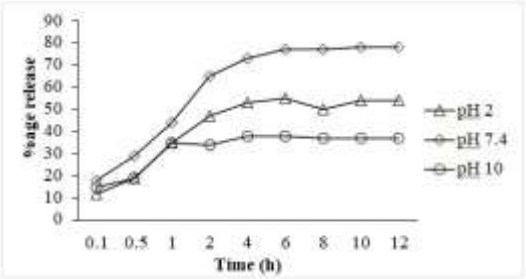
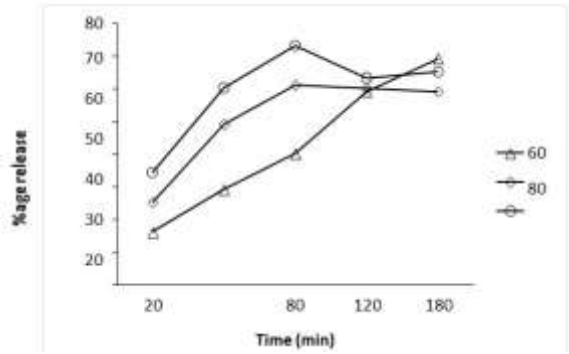


Illustration 1.6 depicts the percentage liberation of overall phenolic content from the encapsulated substance under varying pH conditions.

At different temperature percentage release of total phenolic content from encapsulates was measured. Solutions were taken in different beakers at different temperature and total phenolic contents were observed. At 60 C the percentage release of phenolic content was maximum. At 80 C the percentage release of phenolic content was moderate. At 100 C the percentage release of phenolic content was minimum.



Conclusion:

The study's findings also shown that chitosan and STPP-based microcapsules might be used to entrap and transport the active molecules of Terminalia Arjuna through the digestive system. High encapsulation efficiency and release qualities may be found in microcapsules created by freeze drying extract with Chitosan and STPP as wall components. The encapsulation matrices under investigation demonstrated a protective effect against oxidation throughout a 25-day period of room temperature storage. Microcapsules were successful in delivering 86.4 percent of the encapsulated bioactive components to the gastrointestinal environment. The findings suggest that the microcapsule systems made of Chitosan and STPP may be used to distribute, store, and carry bioactive materials. The bioactive components of Terminalia Arjuna bark extract can now be used more often in both food and medicine, according to this study.

Reference

1. Atay, E., et al., *Development and characterization of chitosan/gelatin electrosprayed microparticles as food grade delivery vehicles for anthocyanin extracts.* Food hydrocolloids, 2018. **77**: p. 699-710.
2. Papoutsis, K., et al., *Encapsulation of citrus by-product extracts by spray-drying and freeze-drying using combinations of maltodextrin with soybean protein and ι-carrageenan.* Foods, 2018. **7**(7): p. 115.
3. Dubey, M., S. Bhadauria, and B. Kushwah, *Green synthesis of nanosilver particles from extract of Eucalyptus hybrida (safeda) leaf.* Dig J Nanomater Biostruct, 2009. **4**(3): p. 537-543.
4. Tan, C. and H. Zhang, *Two-dimensional transition metal dichalcogenide nanosheet-based composites.* Chemical Society Reviews, 2015. **44**(9): p. 2713-2731.
5. Suryabhan, P., K. Lohith, and K. Anu-Appaiah, *Sucrose and sorbitol supplementation on maltodextrin encapsulation enhance the potential probiotic yeast survival by spray drying.* Lwt, 2019. **107**: p. 243-248.
6. Šturm, L., et al., *Encapsulation of non-dewaxed propolis by freeze-drying and spray-drying using gum Arabic, maltodextrin and inulin as coating materials.* Food and Bioproducts Processing, 2019. **116**: p. 196-211.
7. Shaddel, R., et al., *Use of gelatin and gum Arabic for encapsulation of black raspberry anthocyanins by complex coacervation.* International journal of biological macromolecules, 2018. **107**: p. 1800-1810.

8. Munin, A. and F. Edwards-Lévy, *Encapsulation of natural polyphenolic compounds; a review.* Pharmaceutics, 2011. **3**(4): p. 793-829.
9. Simionato, I., et al., *Encapsulation of cinnamon oil in cyclodextrin nanosponges and their potential use for antimicrobial food packaging.* Food and chemical toxicology, 2019. **132**: p. 110647.
10. Hassani, A., et al., *Review on micro-encapsulation with Chitosan for pharmaceutical applications.* MOJ Curr. Res. Rev, 2018. **1**: p. 77-84.
11. Naeem, M.Y. and S. Ugur, *Nutritional content and health benefits of eggplant.* Turkish Journal of Agriculture-Food Science and Technology, 2019. **7**: p. 31-36.
12. Rezvankhah, A., Z. Emam-Djomeh, and G. Askari, *Encapsulation and delivery of bioactive compounds using spray and freeze-drying techniques: A review.* Drying Technology, 2020. **38**(1-2): p. 235-258.
13. Pasrija, D., et al., *Microencapsulation of green tea polyphenols and its effect on incorporated bread quality.* LWT-Food Science and Technology, 2015. **64**(1): p. 289-296.
14. K Maulik, S. and C. K Katiyar, *Terminalia arjuna in cardiovascular diseases: making the transition from traditional to modern medicine in India.* Current Pharmaceutical Biotechnology, 2010. **11**(8): p. 855-860.
15. Devlin, B. and N. Risch, *A comparison of linkage disequilibrium measures for fine-scale mapping.* Genomics, 1995. **29**(2): p. 311-322.
16. Petrelli, N.J., et al., *Clinical Cancer Advances 2009: major research advances in cancer treatment, prevention, and screening-a report from the American Society of Clinical Oncology.* Journal of Clinical Oncology, 2009. **27**(35): p. 6052.
17. Brundage, M., et al., *Toward trustworthy AI development: mechanisms for supporting verifiable claims.* arXiv preprint arXiv:2004.07213, 2020.
18. Sun, T., et al., *Engineered nanoparticles for drug delivery in cancer therapy.* Nanomaterials and Neoplasms, 2021: p. 31-142.
19. F. Gibbs, S.K., Inteaz Alli, Catherine N. Mulligan, Bernard, *Encapsulation in the food industry: a review.* International journal of food sciences and nutrition, 1999. **50**(3): p. 213-224.
20. Timilsena, Y.P., M.A. Haque, and B. Adhikari, *Encapsulation in the food industry: A brief historical overview to recent developments.* Food and Nutrition Sciences, 2020. **11**(6): p. 481-508.
21. Noore, S., et al., *Novel bioactive extraction and nano-encapsulation.* Encyclopedia, 2021. **1**(3): p. 632-664.
22. Shahid Chatha, S., et al., *Bioactive components and antioxidant properties of Terminalia arjuna L.* Extracts. J Food Process Technol, 2014. **5**(298): p. 2.
23. Karthikeyan, K., et al., *Cardioprotective effect of the alcoholic extract of Terminalia arjuna bark in an in vivo model of myocardial ischemic reperfusion injury.* Life sciences, 2003. **73**(21): p. 2727-2739.
24. Scalbert, A., I.T. Johnson, and M. Saltmarsh, *Polyphenols: antioxidants and beyond.* The American journal of clinical nutrition, 2005. **81**(1): p. 215S-217S.
25. Iqbal, S. and M. Bhanger, *Effect of season and production location on antioxidant activity of Moringa oleifera leaves grown in Pakistan.* Journal of food Composition and Analysis, 2006. **19**(6-7): p. 544-551.
26. Landrault, N., et al., *Antioxidant capacities and phenolics levels of French wines from different varieties and vintages.* Journal of Agricultural and

Food chemistry, 2001. **49**(7): p. 3341-3348.
27. Jothy, S.L., Z. Zuraini, and S. Sasidharan, *Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitiory activities of Cassia fistula seeds extract.* Journal of Medicinal Plants Research, 2011. **5**(10): p. 1941-1947.
28. Tilak, J.C., S. Adhikari, and T.P. Devasagayam, *Antioxidant properties of Plumbago zeylanica, an Indian medicinal plant and its active ingredient, plumbagin.* Redox report, 2004. **9**(4): p. 219-227.
29. Shahriar, M., et al., *Evaluation of in vitro antioxidant activity of bark extracts of Terminalia arjuna.* Journal of Medicinal Plants Research, 2012. **6**(39): p. 5286-5298.
30. Bhat, G., et al. *NTIRE 2022 burst super-resolution challenge*. in *Proceedings of the IEEE/CVF Conference on Computer Vision and Pattern Recognition*. 2022.
31. Yao, C.-l., et al., *Simultaneous quantitation of five Panax notoginseng saponins by multi heart-cutting two-dimensional liquid chromatography: Method development and application to the quality control of eight Notoginseng containing Chinese patent medicines.* Journal of Chromatography A, 2015. **1402**: p. 71-81.
32. Cui, H., et al., *Biodegradable zein active film containing chitosan nanoparticle encapsulated with pomegranate peel extract for food packaging.* Food Packaging and Shelf Life, 2020. **24**: p. 100511.
33. Hadidi, M., et al., *Chitosan nanoparticles loaded with clove essential oil: Characterization, antioxidant and antibacterial activities.* Carbohydrate polymers, 2020. **236**: p. 116075.