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Biopolymer (chitosan) encapsulation of natural antioxidants extracted from caffeoyl derivative containing medicinal and aromatic plants: (*Artemisia pallens*, *Ocimum tenuiflorum)*

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ABSTRACT

The use of chitosan (CS) is use for the encapsulation of active components has gained interest in the last years due to its mucous adhesiveness, non-toxicity, biocompatibility and biodegradability. The benefits of encapsulating active agents in such polymer matrix include their protection from the surrounding medium or processing conditions and their controlled release. In this present study Chitosan was employed for the encapsulation of caffeoyl derivatives and medicinal plants (*Artemisia pallens* (Tulsi), *Ocimum tenuiflorum* or *Ocimum sanctum* (Marikolunthu).Encapsulation has been done with the help of ionic gelation method. Chitosan and TPP- (Tripolyphosphate pentasodium) were mainly involved. The active components were added to the TPP solution and this was added dropwise to the chitosan solution with continuous stirring and also in this case, to investigate the anti-oxidant activity of the methonolic extract of caffeoyl derivative and medicinal plants.Anti-oxidant activity of methonolic extract was determined by DPPH -(1, 1-diphenyl 1-2 picryl hydrazyl) free radical scavenging activity. Then the effect of encapsulating systems on the active ompound stability and its release properties was analysed.

Keywords: *Artemisia pallens* (Thulsi), *Ocimum tenuiflorum* or *Ocimum sanctum* (Marikolunthu), Chitosan, Anti-oxidant, TPP- (Tripolyphosphate pentasodium) , DPPH-(1, 1-diphenyl 1-2 picryl hydrazyl).

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INTRODUCTION

Chitosan, a linear amino polysaccharide composed of randomly distributed (1→4) linked D-glucosamine and N-acetyl-D-glucosamine units, is obtained by the deacetylation of chitin, widespread natural polysaccharide found in the exoskeleton of crustaceans such as crab and shrimp [1].This cationic polysaccharide has drawn increasing attention within pharmaceutical and biomedical applications, owing to its abundant availability, unique mucoadhesivity, inherent pharmacological properties, and other beneficial biological properties such as biocompatibility, biodegradability, non-toxicity and low-immunogenicity[2]. The physicochemical and biological properties of chitosan are greatly influenced by its molecular weight and degree of deacetylation. The presence of reactive functional groups in chitosan offers great opportunity for chemical modification, which affords a wide range of derivatives such as quaternized chitosan (N,N,N-trimethyl chitosan; TMC), carboxyalkyl chitosan, thiolated chitosan, sugar-bearing chitosan, bile acid-modified chitosan and cyclodextrin-linked chitosan[3].Chitosan have a number of commercial and possible biomedical uses. It can be used in agriculture as a seed treatment and biopesticide, helping plants to fight off fungal infections. In wine making it can be used as a fining agent, also helping to prevent spoilage. In industry, it can be used in a self-healing polyurethane paint coating. In medicine, it may be useful in bandages to reduce bleeding and as an antibacterial agent; it can also be used to help deliver drugs through the skin. More controversially, chitosan has been asserted to have use in limiting fat absorption, which would make it useful for dieting, but there is evidence against this. Other uses of chitosan that have been researched include use as a soluble dietary fibre. Chitosan is an important additive in the filtration process. Sand filtration apparently can remove up to 50% of the turbidity alone, while the chitosan with sand filtration removes up to 99% turbidity. Chitosan has been used to precipitate caseins from bovine milk and cheese making. Chitosan is also useful in other filtration situations, where one may need to remove suspended particles from a liquid. In combination with bentonite, gelatin, silica gel, or other fining agents, it is used to clarify wine, mead, and beer. Added late in the brewing process, chitosan improves flocculation, and removes yeast cells, fruit particles, and other detritus that cause hazy wine.

Scientific classification of *Ocimum tenuiflorum*

Kingdom; Plantae

(Unranked): Asterids

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum

Species: O. tenuiflorum

In Ayurveda Tulsi (Ocimum sanctum L.) has been well documented for its therapeutic Potentials and described as Dashemani Shwasaharni (antiasthmatic) and antikaphic drugs (Kaphaghna)[4] .Although the traditional medical practitioners in India have been widely using this medicinal plant for management of various disease conditions from ancient time, not much is known about the mode of action of Tulsi, and a rational approach to this traditional medical practice with modern system of medicine is also not available. In last few decades several studies have been carried out by Indian scientists and researchers to suggest the role of essential oils & eugenolin therapeutic potentials of *Ocimum sanctum* L.[5,6]. Eugenol is a phenolic compound and major constituent of essential oils extracted from different parts of Tulsi plant. The therapeutic potential of Tulsi has-been established on the basis of several pharmacological studies carried out with eugenol and steam distilled, petroleum ether and benzene extracts of different parts ofTulsi plant [7,8,9,10,11,12,13,14,15].

Scientific classification of *Artemisia pallens*

Kingdom: Plantae

(unranked):Angiosperms

(Unranked):Eudicots

(Unranked):Asteride

Order:Asterales

Family:Asteraceae

Genus: Artemisia

Species: A.pallens

Artemisia pallens Wall. (Davana) is an aromatic annual small herb belonging to family Compositae. It is distributed in certain parts of South India, especially in Karnataka and Tamil Nadu. *Artemisia pallens* has been ascribed to possess anthelmintic and stomachic properties in indigenous system of medicine [16].

Caffeoyl derivative

Chlorogenic acid (CGA) is a natural chemical compound which is caffeic acid and (-)-quinic acid. It is important intermediate. CGA is an important intermediate in lignin biosynthesis. This compound, long known as an antioxidant may also slow the release of glucose in to the bloodstream after a meal.CGA is marketed under the trade name svetol in nor way and the

United kingdom as a food additive used in caffee, chewing gum and mints, Dried sunflower leaves called intermediately after opening are processed into 98.38% chlorogenic [17].

Antioxidant activity

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources. There is a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants One such method that is currently popular is based upon the use of the stable free radical diphenylpicrylhydrazyl (DPPH) [18].

MATERIALS AND METHODS

Chemically pure reagents were used in this method. After that all other glassware were dried overnight in an oven before use.DPPH-(1, 1-diphenyl 1-2 picryl hydrazyl),TPP-(Tripolyphosphate pentasodium) were purchased from Ponmani, Royal scientific and Biopolymer (Chitosan) was purchased from Pelican Biotech. These chemicals were used without further purification. HPLC studies were helpful for analyzing and identifying caffeoyl derivative compounds present in the plants. Antioxidant activity studies (Free Radical Scavenging Activity) were also analyzed with the help of UV-Visible spectrometer.

Extraction

Air dried and coarsely powdered (10g) *Artemisia pallens* (Tulsi) , *Ocimum tenuiflorum* or Ocimum sanctum (Marikolunthu) herb was taken. Extraction was carried out in a distillation extractor using DCM. The extract was then concentrated to dryness under reduced pressure. The distillation extraction process was carried out until the solvent was found to be colourless. Then the solvent was filtered and distilled off. Final traces of DCM removed under pressure by using rotary vacuum flask evaporator [19].

Encapsulation preparation

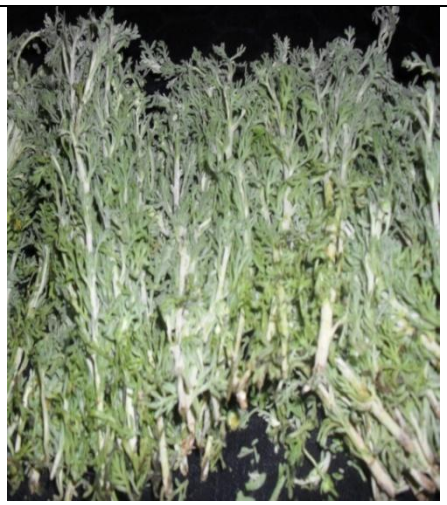
Chitosan and TPP was used to prepare by ionic gelation method**.** Different concentration of Chitosan and TPP were used and then mixed. The concentration of .CS-0.15%,TPP-0.084.The active component was encapsulated in before mixed form of Chitosan and TPP by mixing it with the TPP solution before encapsulation formation with constant stirring[20].

HPLC analysis of caffeoyl derivatives

Qualitative and quantitative HPLC analysis was carried out with an apparatus comprising two 510 pumps, a 680 solvent programmer and a 991 photodiode array detector(water Associates,

Milford, MA,USA).A 25µl of aliquot was injected onto a lichrocart 125-4 superspher RP8-E,5µm column(Merck).The mobile phase consisted of solvent A:H_2O/H_3Po_4 85%(100:0.3V/V) and solvent B:MeCN/H_2O/H_3Po_4 85% (80:20:0.3V/V).Separation was performed by a quadriconcave gradient of B in A at a flow rate of 2ml/min as follows:0-5min,12%-15%B;5-30min,15%-30% B:30-40min,30%-50%B:40-45min,50%-70% B.Under these conditions, standard compounds were correctly separated and eluted at approximate retention times(R_t), as follows:c hlorogenic acid(7.1min),3,5-di-O-Caffeoylquinic acid (32.5min),1,5-di-O-Caffeoyl quinic acid(33.0min),Chicoric acid(33.5min)4,5-di-O-Caffeoyl quinic acid(34.2min).The specificity of the method was verified for each hydroxyl cinnamic constituent using a photodiode array detector to compare their UV Spectra with those of standard to compounds. UV absorption maxima were 217,238 325 nm for chlorogenic acid,218,240 and 328 for 1,5-di-O-Caffeoyl quinic acid,218,240 and 328 for 1,5-di-O-Caffeoyl quinic acid,218,243 and 328 for 1,5-di-O-Caffeoylquinic acid,218,243 and 329 for chicoric acid and 217,241 and 326 for 4,5-di-O-Caffeoylquinic acid. Detection was therefore carried out at 340nm for all compounds linearity and reliability standard deviations of caffeoyl derivatives were <5% linearity correlation coefficient was greater than 0.99(5 points:3 assays).All samples were run in triplicate and quantification was carried out using external standards. The content of each compound was calculated and expressed as g/kg on dry matter (DM)[21].

Tulsi

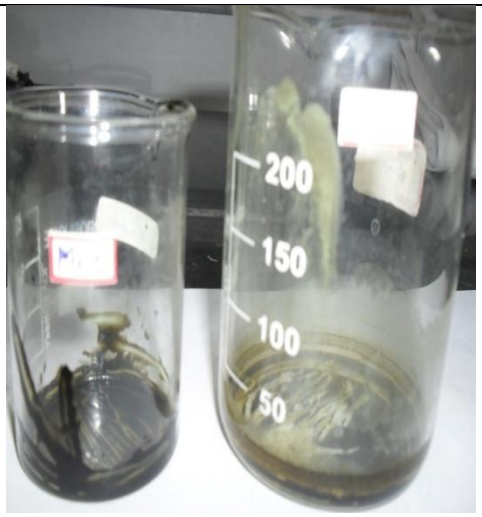


Marikolunthu

Biopolymer(CHITOSAN)

Extraction Distillation Methods

Extraction Distillation Methods



Caffeoyl derivative compounds prepared by the use of tulsi extract and marikolunthu extract

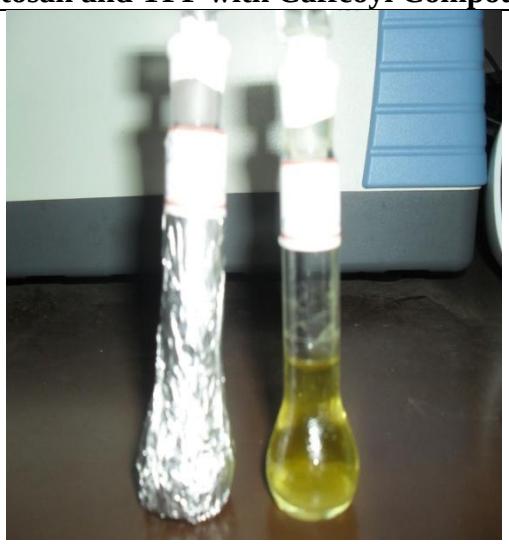
Chitosan Encapsulated With Plants

Marikolunthu chitosan and Tulsi chitosan Derivatives

Collection of Plant Sample

Chitosan and TPP with Caffeoyl Compound

DPPH and MCD+C Derivatives



DPPH and TCD+C Derivatives

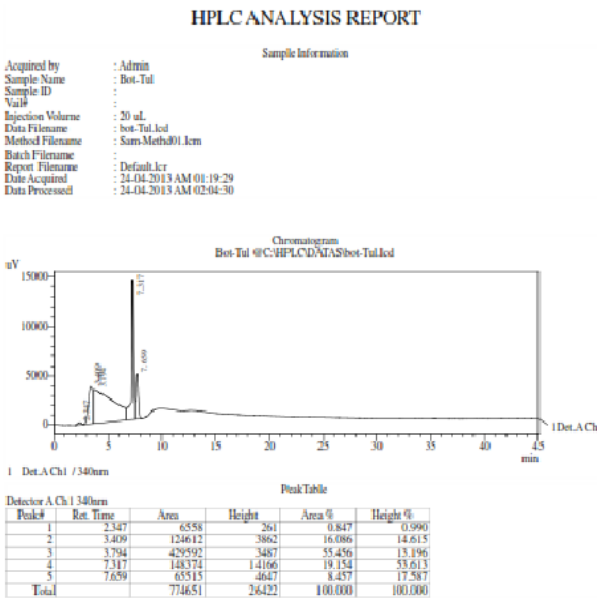
Antioxidant activity (DPPH- free radical scavenging activity)

150µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 516nm for control reading. Different concentrations (100,120,140,160,180,200,240,260µl) were taken by dilution with methanol. Diluted with methanol with up to 3ml.150µl DPPH solution was added to each test tube. Absorbance was taken at 516nm in UV-Visible spectrophotometer after 15min using methanol as a blank. The % reduction and IC_{50} were calculated as follows. The free radical scavenging activity (FRSA) (% antiradical scavenging activity) was calculated using the following equation: % antiradical activity = ((Controlabsorbance - sampleabsorbance) ÷ Controlabsorbance) ×100[19,22] .

RESULTS AND DISCUSSION

HPLC analysis results

Polyphenolic compounds were analyzed with the help of HPLC analysis. Different peaks were



HPLC ANALYSIS REPORT

Sample Information

Acquired by	: Admin
Sample Name	: Bot-Tul
Sample ID	:
Vial#	:
Injection Volume	: 20 uL
Data Filename	: bot-Tul.lcd
Method Filename	: Sam-Meth01.lcm
Batch Filename	:
Report Filename	: Default.lcr
Date Acquired	: 24-04-2013 AM 01:19:29
Data Processed	: 24-04-2013 AM 02:04:30

Chromatogram

Bot-Tul @C:\HPLC\D\ATA\bot-Tul.lcd

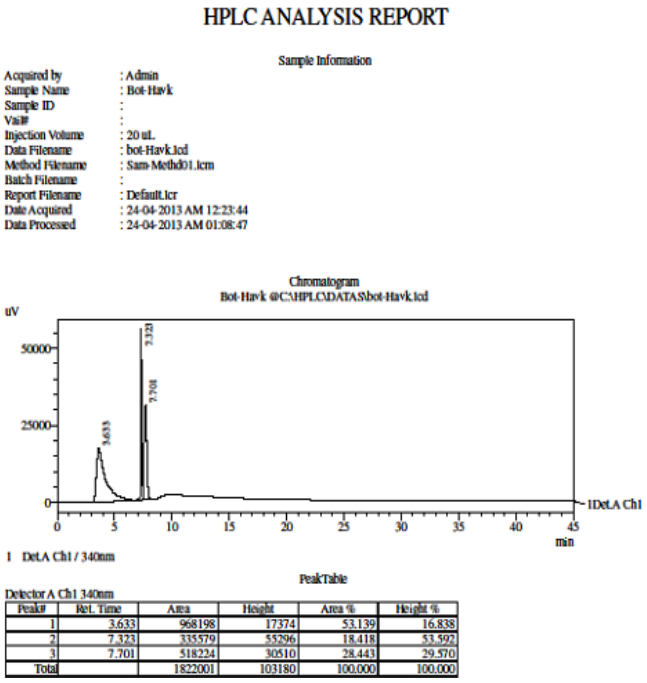
1 Det.A Ch1 / 340nm

PeakTable

Detector A Ch 1 340nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.347	6558	261	0.847	0.990
2	3.409	124612	3862	16.086	14.615
3	3.794	429592	3487	55.456	13.196
4	7.317	148374	14166	19.154	53.613
5	7.659	65515	4647	8.457	17.587
Total		774651	26422	100.000	100.000

Tulsi plant HPLC analysis report



HPLC ANALYSIS REPORT

Sample Information

Acquired by	: Admin
Sample Name	: Bot-Havk
Sample ID	:
Vial#	:
Injection Volume	: 20 uL
Data Filename	: bot-Havk.lcd
Method Filename	: Sam-Meth01.lcm
Batch Filename	:
Report Filename	: Default.lcr
Date Acquired	: 24-04-2013 AM 12:23:44
Data Processed	: 24-04-2013 AM 01:08:47

Chromatogram

Bot-Havk @C:\HPLC\DATA\bot-Havk.lcd

1 DetA Ch1 / 340nm

PeakTable

Detector A Ch1 340nm

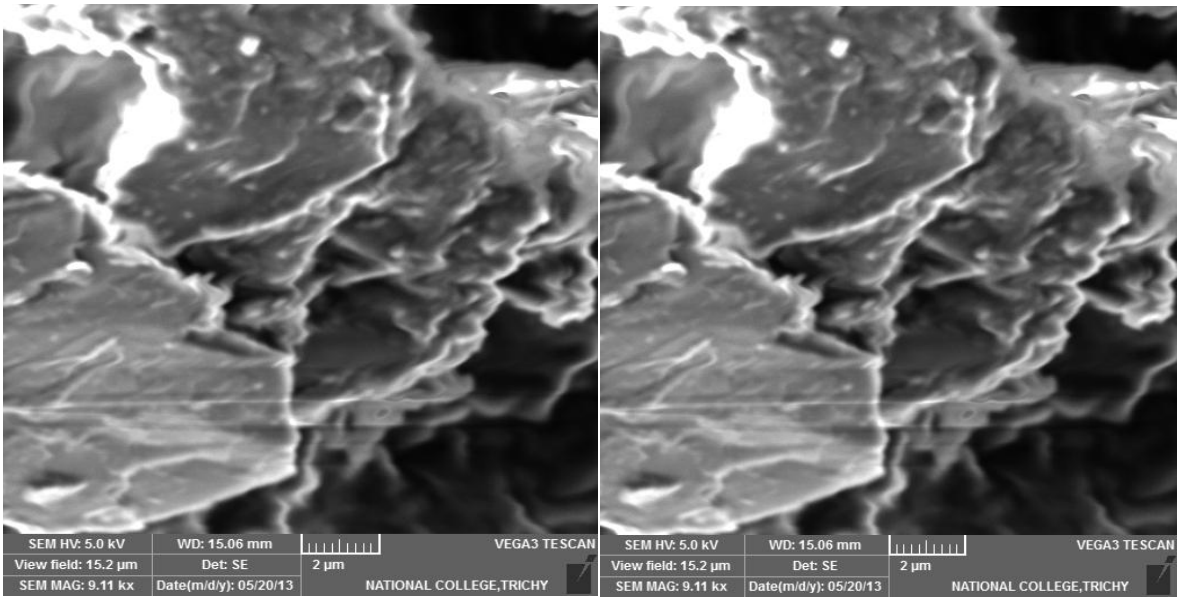
Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.633	968198	17374	53.139	16.838
2	7.323	335579	55296	18.418	53.592
3	7.701	518224	30510	28.443	29.570
Total		1822001	103180	100.000	100.000

Mrikolunthu plant HPLC analysis report

confirmed by this method. In this present work 3 different compounds were discussed. The study was aimed at caffeoyl derivative compounds. This particular compound is present in Asteraceae family. Peaks ranges of Chlorogenic acid-3.633,3,5-Dicaffeoyl Quinic Acid-7.323,1,5-Dicaffeoyl Quinic Acid-7.701.(%) Percentage of chlorogenic acid-53.19%,3,5-DCQA-18.418%,1,5-DCQA-28.443%.Peaks ranges of Lamiaceae family, Chlorogenic acid-3.794,3,5-Dicaffeoyl Quinic Acid-7.317,1,5- Dicaffeoyl Quinic Acid-7.659. Percentage of chlorogenicacid-55.456%,3,5-DCQA-19.154%,1,5-DCQA-8.459.Compare to two different

families the percentage of chlorogenic acid is high in Lamiaceae family. And then large amount of chlorogenic acid is present in both families.

SEM analysis

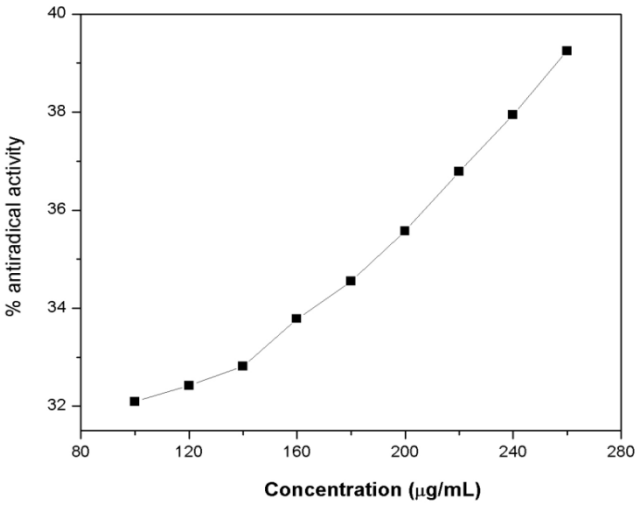


Chitosan encapsulated with Tulsi Chitosan encapsulated with Marikolunthu

SEM analysis results

Chitosan encapsulated with plant SEM images were taken. The shape of chitosan is spherical and elongated. Both plants have wave like structure. Encapsulation images were not clear in shape. Because this study was aimed caffeoyl derivative compound encapsulating with chitosan. this particular compound is not powder formed. If the sample is not powder like formed means SEM images were not clear.

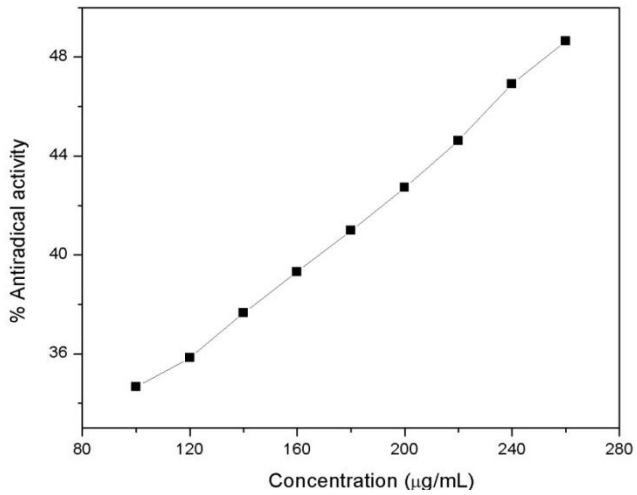
Antioxidant activity (DPPH method) results



Tulsi + Chitosan derivatives

Calculation of Tulsi + Chitosan derivatives

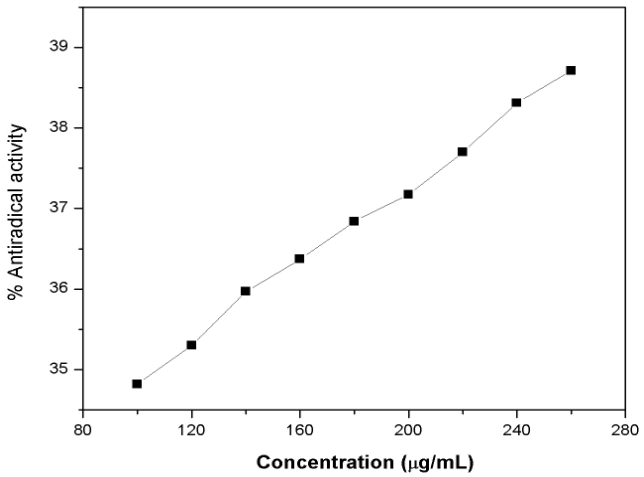
T+CD extracts	
Y=mx+c	
y	Y=0.04577X+26.781
Intercept	26.781
Slope	0.04577
IC50 Value(µg/ml)	503.61



Tulsi Extract derivative

Calculation of Tulsi Extract derivative

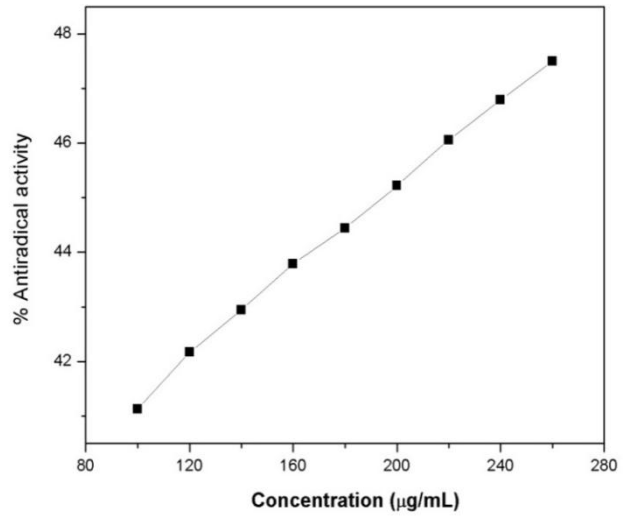
Tulsi extracts	
Y=mx+c	
y	Y=0.08882X+25.26928
Intercept	25.26928
Slope	0.08882
IC50 Value(µg/ml)	278.78



Marikolunthu+Chitosan derivatives

Calculation of Marikolunthu+Chitosan derivatives

MCD+C extracts	
y = mx + c	
y	Y=0.02404x+32.47139
Intercept	32.47139
Slope	0.02404
IC50 Value(µg/ml)	727.27



Marikolunthu Extract derivative

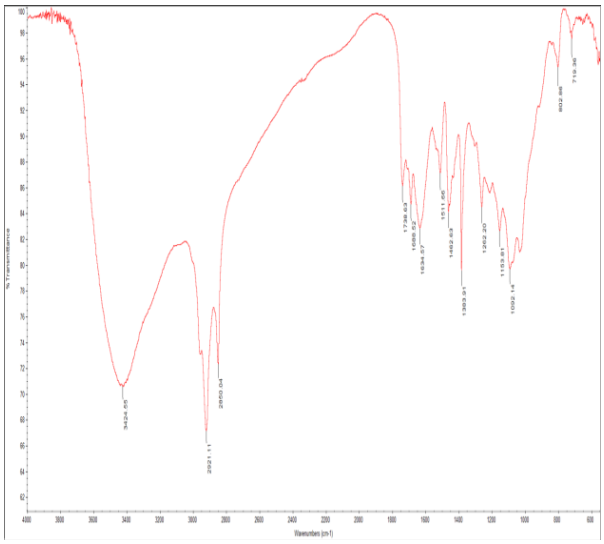
Calculation of Marikolunthu Extract derivative

Marikolunthu extracts	
Y=mx+c	
y	Y=0.03917X+37.397
Intercept	37.397
Slope	0.03917
IC50 Value(µg/ml)	330.30

Antioxidant activity (DPPH method) results

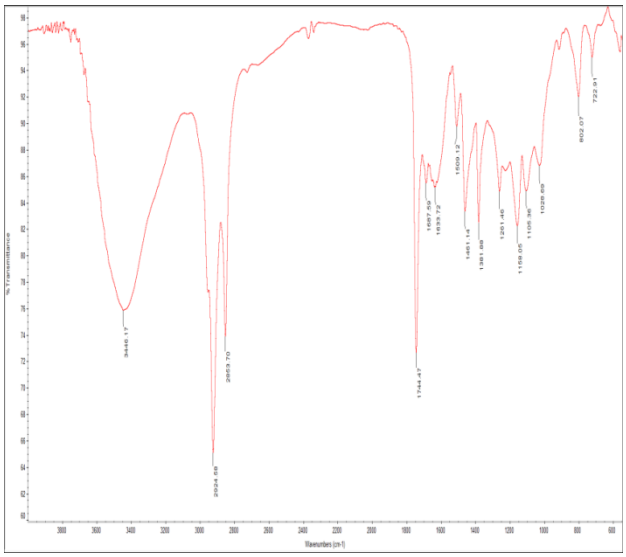
Application of this study is to evaluate the control release of free radical scavenging activity.(Ic50) value is calculated. which means the amount sample required for

50%inhibition of free radicals. Tulsi extracts=278.78µg/ml, TCD+C=503.61 µg/ml, Marikolunthu extracts=330.30 µg/ml, MCD+C=727.27 µg/ml. Compare to normal plant, chitosan encapsulated plants free radical scavenging activity is low. chitosan encapsulated with plant compared to two different families control release of free radical scavenging activity is high in Lamiaceae family.



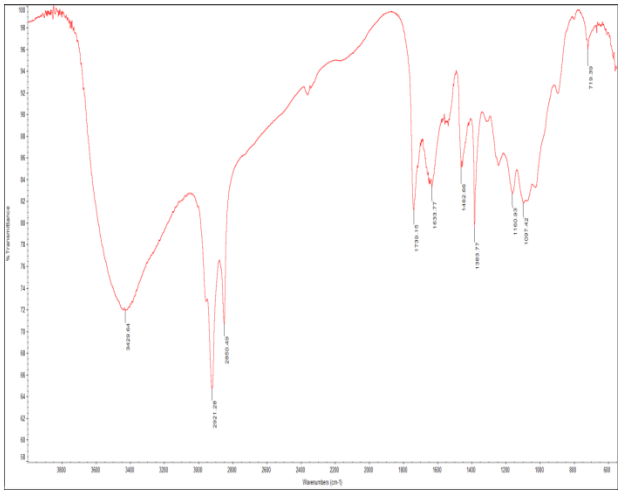
Functional groups	FT-IR absorption frequencies(υ cm^{-1})
OH	3424
C-H	2921
C-H	2850
C=O	1738, 1688
C=C	1634, 1511, 1462
C-F	1383, 1262, 1153, 1092

FT-IR Absorption frequencies of functional groups Tulsi+Chitosan derivatives



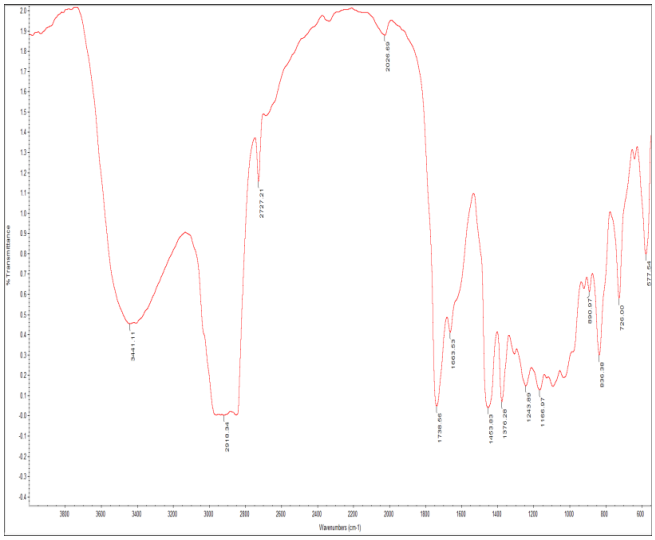
Functional groups	FT-IR absorption frequencies(υ cm^{-1})
OH	3446
C-H	2924,1744
C-H	1744
C=O	1687
C=C	1633, 1509, 1461
C-F	1381

FT-IR Absorption frequencies of functional groups of Tulsi Extract derivative



Functional groups	FT-IR absorption frequencies(υ cm^{-1})
O-H	3429
C-H	2921, 2850
C=O	1739, 1633
C=C	1462
C-F	1383, 1160, 1097

FT-IR Absorption frequencies of functional group of Marikolunthu + Chitosan derivatives



Functional groups	FT-IR absorption frequencies(υ cm^{-1})
O-H	3441
C-H	2918, 2727
C=O	1738
C=C	1663
C-F	1376, 1243, 1166

FT-IR Absorption frequencies of functional groups of Marikolunthu Extract derivative

FT-IR analysis results

FT-IR is mainly used for the identification of functional groups. FT-IR absorbance frequencies were calculated by the use of different wavelength. These two families high amount of OH group is present in normal plant. FT-IR absorbance frequencies of Lamiaceae family-3446 and Asteraceae family-3441.Other functional groups C=O, C-F absorbance frequencies are high in Chitosan encapsulated plants. FT-IR absorbance frequencies of Lamiaceae family-C=O-1738,C-F-1383 and Asteraceae family C=0, 1739,C-F-1383.

CONCLUSION

Application was carried out by DPPH method (Antioxidant activity).Finally the present study was concluded that Ic50 (Inhibitory Concentration) value (The amount of sample required for

50% inhibition of free radicals) less amount were present in the chitosan encapsulated plants. Normal plants value is high.

REFERENCES

1. Kingston.DGI. Modern natural products drug discovery and its relevance to biodiversity conservation. J Nat Prod. 2011;74:496-511.
2. Kumar. MN, Muzzarelli RA, Muzzarelli C, Sashiwa H., A.J. Domb, Chitosan chemistry and pharmaceutical perspectives, Chem. Rev. 2004;104:6017–6084.
3. Felt .O, P. Buri, R. Gurny, Chitosan: a unique polysaccharide for drug delivery, Drug. Dev. Ind. Pharm. 1998;24: 979–993.
4. Sirkar NN. Pharmacological basis of Ayurvedic therapeutics. In: Cultivation and utilization of medicinal plants. Editors: Atal CK and Kapoor BM(Published by PID CSIR) 1989.
5. Sen P. Therapeutic potentials of Tulsi: from experience to facts. Drugs News & Views 1993; 1(2):15–21.
6. Rajeshwari. S, Ocimum sanctum. The Indian home remedy. In: Current Medical Scene, March-April 1992 (Edited and published .Cipla Ltd., Bombay Central, Bombay).
7. Khanna N, Bhatia J. Action of Ocimum sanctum (Tulsi) in mice: possible mechanism involved. J Ethnopharmacology 2003; 88(2–3): 293–296.
8. Gupta SK, Prakash J, Srivastava S. Validation of claim of Tulsi, Ocimum sanctum Linn as a medicinal plant. Indian J Experimental Biology 2002; 40(7): 765–773.
9. Reghunandana R, Sood S, Reghunandana V, Mehta RM, Singh GP. Effect of Ocimum sanctum Linn (Tulsi) extract on testicular function. Indian J Medical Research 1995; 49(4): 83–87.
10. Bhargava KP, Singh N. Anti stress activity of Ocimum sanctum Linn. Indian J Medical Res 1981; 73: 443–451.
11. Nagarajun S, Jain HC, Aulakh GS. Indigenous plants used in the control of Diabetes. In: Cultivation and utilization of medicinal plants. Editors: Atal CK and Kapoor BM (Published by PID CSJR) 1989, p. 584.
12. Mnadal S, Das DN, Dey K, et. al. Ocimum sanctum Linn - A study on gastric ulceration and gastric secretion in rats. Indian J Physiol Pharmacol 1993; 37: 91–92.
13. Sarkar A, Pandey DN, Pant MC. A report on the effect of Ocimum sanctum (Tulsi) leaves and seeds on blood and urinary uric acid, urea and urine volume in normal albino

rabbits. Indian J Physiol Pharmacology 1990; 34: 61–62.

14. Sethi J, Sood S, Seth S, Thakur A. Protective effect of Tulsi (Ocimum sanctum) on lipid peroxidation in stress induced by anemic hypoxia in rabbits. Indian J Physiol Pharmacology 2003; 47(1): 115–119.
15. Sarkar A, Pandey DN, Pant MC. Changes in the blood lipid profile level after administration of Ocimum sanctum (Tulsi) leaves in the normal albino rabbits. Indian J Physiology Pharmacology 1994; 38(4): 311–312.
16. Anonymous, "Wealth of India – Raw Materials", C.S.I.R.; New Delhi, Vol. **I,** 122 (1956).
17. Boerjan, Wout; Ralph, John, Baucher, Marie (2003). "Lignin biosynthesis". Rev. Plant Biol.54:519,doi:10.1146/annurev.arplant.54.031902.134938. PMID 14503002.
18. Schwarz. K, Bertelsen, G., Nissen, L.R., Gardner, P.T., Heinonen, M.I., Hopia, A., Huynh-Ba, T.,Lambelet, P., McPhail, D., Skibsted, L.H. and Tijburg, L. Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. Eur. Food Res. Technol 2011; 212: 319-328.
19. Sanja SD. Characterization and evaluation of antioxidant activity of portulaca oleracea. Int J Pharm Pharma Sci 2009;1(1).
20. Fernandez-Urrusuna, RD, Calvo P, vila-Jato JL, Alonso, M.J. Development of a freeze dried formulation of insulin-loaded chitosan nanoparticles intended for nasal administrations T P Pharma Sci 1999; 5: 429-436.
21. Fraisse .D, A. Carnat, D. Viala, P. Pradel, J. M. Besle, J. B. Coulon, C. Felgines and J. L. Lamaison, "Polyphenolic Composition of a Natural Pasture. Variations Related to the Period of Harvesting," Journal of the Science of Food and Agriculture, 2007; 87(13): 2427-2435.
22. Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant properties of the ayurvedic formulation triphala and its constituents. Int J Pharmacogn 1997; 35: 313-17.