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Nanocapsules of Catechin Rich Extract for Enhanced Antioxidant Potential and *In Vitro* Bioavailability

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ABSTRACT

Catechin is a phytochemical largely present in *Acacia catechu* which is known for its biological potential, but its utilization is greatly limited by its low bioavailability. In the present research work catechin rich extract has been chosen for converting it into nanoformulation by emulsion solvent evaporation technique with minimized dosing employing Eudragit L 100 as a polymer for enhancing its bioavailability. Catechin entrapment efficiency of NF1, NF2 and NF3 nanoformulations were found to be 75 %, 62 % and 40 % respectively with decreased entrapment with an increased polymer concentration. SEM characterization of nanoparticles revealed the occurrence of both asymmetrical as well as aggregated particles in nano-meter range (100 nm to 400 nm).The nanoformulations were subjected for antioxidant activity by DPPH method and *in vitro* bioavailability by *in vitro* digestion method. Nano formulations displayed higher antioxidant activity (85.64 % - 92. 47 %) compared to Curcumin (80.58 %, used as standard for comparison) and pure extract (83.08 %). *In vitro* bioavailability of total polyphenols and Catechin was higher in acidic pH compared to alkaline pH for all the nanoformulations with a maximum bioavailability of 45 to 59 percentage respectively in an acidic media as shown by NF1.Thus, the findings proved significant enhancement in the antioxidant activity and *in vitro* bioavailability of catechin nanonization of active catechin rich extract using biodegradable polymer Eudragit L 100 in the form of nanocapsules.

INTRODUCTION

Catechin is a secondary metabolite of herbal source which belongs to a group of flavanoids that has diverse health benefits in mankind. They are basically polyphenolic compounds important for their antioxidant activities (JinZe *et al*., 2004). The potential sources of catechin include apples, oranges, pears, black grapes, blackberries, cherries, raspberries, red wine and dark chocolate. Commercially, *Acacia catechu* being the richest source of catechin is widely used. Flavanoids, a phytoconstituent present in catechin seem to have numerous therapeutic effects due to their inherent potential of various biochemical activities involved in inhibiting many enzymatic pathways and enzymes (Vanna SannaImtiaz *et al*., 2013), especially catechin possess anti-proliferative, antimicrobial, anti-inflammation, and antioxidant properties. It also improves blood flow and has potential benefits in cardiac health (Gupta and Ramteke, 2011; Schroeter *et al*., 2006; Cesar and Patricia, 2011). Chemoprevention using herbal based products or plant secondary metabolites are essentially important incost effective treatment of cancer therapy and mitigating the side effects of conventional cancer therapies. Bioavailability of the drug is a vital parameter for assessing the pharmacokinetics *in vitro* due to antioxidant activity and capacity to interact with cell signaling proteins and membrane proteins of Catechin (Kohri *et al*., 2001). The *in vivo* absorption studies proved that high quantity of oral dosage of catechin is required which equals to 100 folds more than intravenous dosage (Tasnima *et al*., 2013).

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There are only few clinical studies carried out on humans, the main reason being its very low bioavailability which might be due to rapid elimination from liver by biliary excretion (Agrawal, 2011) when administered *in vivo* (Zhu *et al*., 2000). Decreased bioavailability of catechin was found to be prominent in most of the *in vivo* research works, but the reason for such low bioavailability is still not clear.

Hence, the prevailing problems of low bioavailability, poor absorption and high first pass effect drives the need to develop strategies for optimizing the dosage of catechin for maximum therapeutic activity dueto high absorption and higher bioavailability. It is therefore essential to make the molecule reach cells effectively and stay in cells for prolonged time to execute therapeutic activity.

Current research practices employs nanotechnology approaches to improve the solubility, bioavailability and bioefficacy as it allows the use of biodegradable, non-toxic nanoparticles having higher surface to volume ratio to attach or encapsulate natural plant products (McNeil, 2005). Nanophytomedicines prepared from active standardized extracts are used as nanophytomedicines for improved efficacy and bioavailability. Thus minimizing the side effects and toxicity of administered drugs (Alakh, 2013). Due to the advantages of nanophytomedicines and many potential health benefits of catechin (an active phytocompound), the pure and rich extract of catechin was investigated in the present study for effective management of the associated pharmacokinetic problems. The research intent was to investigate the role of nanotechnology with a pH-sensitive, biodegradable and non-toxic polymer Eudragit L 100 for efficient encapsulation and protection of the drug from an acidic degradation for sustained drug release from nanocapsulesin the alkaline environment with an enhanced bioavailability and bio-efficiency of catechin.

MATERIALS AND METHODS

Catechin rich extract (CRE) was a gift sample from Green Chem Herbal Pvt. Ltd., Bangalore, India. Sodium Lauryl Sulphate was procured from HiMedia, India. Eudragit L 100. All other chemicals and solvents used for experiment were of analytical/HPLC grade obtained from Merck, Mumbai, India. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), Curcumin were purchased from HiMedia. Gallic acid, Folin- Calcaceu reagent (FC), Pepsin 1:3000 were procured from Nice Chemicals, Cochin, India. All other chemicals and solvents used are of analytical/HPLC grade obtained from Merck, Mumbai, India.

Development of Nanoformulations

Nanoformulations were developed by emulsion solvent evaporation technique (JaiPrakash *et al*., 2013) with some modifications and the standardized nanoformulation ingredients and their quantity is given in Table 1. Nanoformulation was prepared by solvent evaporation technique using CRE as a pure compound extract, Eudragit L 100 as polymer, SLS as detergent, lactose as diluent and methanol as a solvent (Figure 1). The resultant catechin nanoparticles were then encapsulated within the polymer by solvent evaporation technique and the nanoformulation was converted into multiple unit nanocapusles as oral drug delivery system.

Catechin entrapment efficiency

Catechin entrapment efficiency in nanoparticles was determined as follows: 100 mg of formulation sample was transferred into a flask containing phosphate buffer pH 6.8 to get a dispersion in which the catechin has high solubility and polymer least solubility. The dispersion was centrifuged at 12000 rpm for 15 minutes using Eltec centrifuge. The concentration of catechin in the supernatant was determined by UV–Visible spectrophotometry at 276 nm after suitable dilution. The drug entrapment efficiency was calculated formula:

$$\%Drug\ entrapment = \frac{\text{Initial amount of drug} - \text{amount of drug in the supernatant X 100}}{\text{Ititial amount of drug}}$$

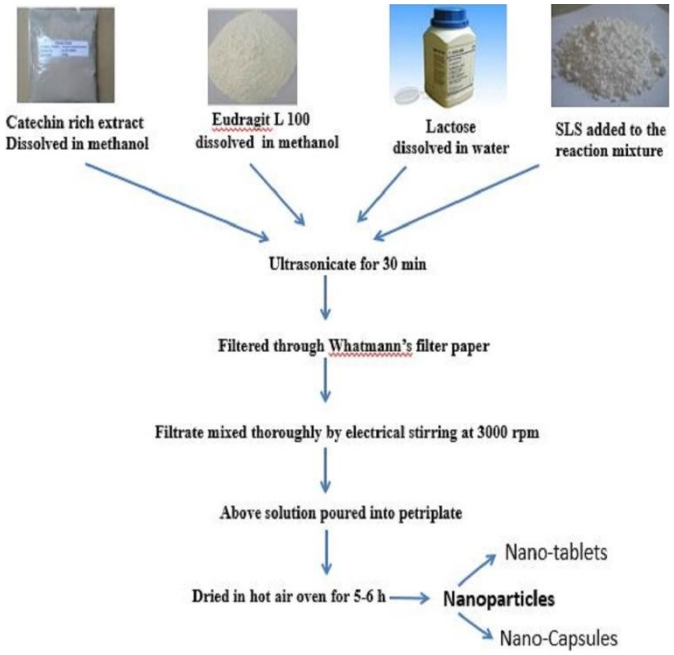

Fig. 1: Schematic representation of preparation of nanoparticles of catechin by emulsion solvent evaporation technique

Table 1: Standardized nanoformulation ingredients with quantity.

Nanoformulation ingredients	Quantity (mg)		
	NF1	NF2	NF3
CRE	100	100	100
Eudragit L 100	100	200	300
SLS	5	5	5
Lactose (aqueous solution)	10	10	10
Methanol (ml)	30	30	30
CRE : Eudragit L 100 ratio	1:1	1:2	1:3

Scanning Electron Microscopy for surface morphology

The surface morphology and particle size of the nanoparticles was determined by Scanning Electron Microscopy (SEM) (Zeiss Ultra 55, Germany) (Hu Daode *et al*., 2012). In the

analysis, the samples were firstly attached to a small piece of electro-conductive silicon chip, coated with gold using a vacuum sputter coater.

Antioxidant Activity

Antioxidant activity of the nanoformulations (NF1, NF2 and NF3), CRE and curcumin (well known antioxidant compound) was carried using DPPH method. Free radical scavenging potentials of the extracts were tested against a methanolic solution of DPPH. Antioxidants reacts with DPPH and converts it intoα,α-diphenyl-β-picryl hydrazine. The extent or intensity of discoloration indicates the scavenging potential of the antioxidant extract. The changes in the absorbance produced at 517 nm has been used as a measure of antioxidant activity. Methanolic solution of DPPH (100µM) was prepared by dissolving 39.4 mg of DPPH in one liter of analytical grade methanol.

Preparation of the extract solutions

Aliquots of extracts (CRE, NF1, NF2 and NF3) and standard (curcumin) were taken in different concentrations such as 1 ppm, 2.5 ppm, 5 ppm, 7.5 ppm, 10 ppm, 20 ppm and 25 ppm in different test tubes. To these extracts 2 ml of methanolic solution of DPPH was added, shaken well and the mixture was allowed to stand at room temperature for about 20 minutes. The blank was also prepared as above without extract. The readings were noted at 517 nm. The absorbance of blank was first noted at 517 nm using a UV spectrophotometer (UV – 1600). The changes in absorbance of samples were measured. Scavenging activity was expressed as the inhibition percentage calculated using the following formula,

$$\% \text{ Anti radical activity} = \frac{\text{Control abs X Sample abs X 100}}{\text{Control abs}}$$

Each trial was performed in triplicate and the deviation of absorbance value was recorded (Jaiprakash R*et al.*, 2009 andFayaz Mohamed *et al*., 2005).

***In vitro* Bioavailability Studies**

Determination of Total Polyphenols

An *in vitro* measurement was done by mimicking the conditions of gastric (pH 1.35) and intestinal fluids (pH 7.5) to determine the total polyphenols and catechin bioavailability. NF1, NF2 and NF3 (0.5 g) were mixed separately with 2 % pepsin solution (2 g of pepsin dissolved in 100 ml distilled water). The pH of the mixture was adjusted to 1.35 with analytical grade 1N HCl so that the final volume obtained was 25 ml. The solution was incubated in 25 ml conical flask at 37 °C in a metabolic shaker water bath for 30 min time interval. At the end of incubation, the contents were centrifuged at 3000 rpm for 15 min and the supernatant filtered through Whatman's filter paper of. 0.45 µ size. Soluble polyphenols and catechin was determined in aliquots of filtrate at pH 1.35.In another aliquot, pH was adjusted to 7.5 with 1 N NaOH and incubated at 37 °C for 30 min interval in a water bath as mentioned above for pH 1.35. At the end of incubation period, the flask contents was filtered and the filtrate was used to determine soluble polyphenols and catechin. The absorbance was measured at 725 nm and concentration of total polyphenols in the samples was obtained from standard gallic acid curve.

Determination of Catechin

Catechin in the samples were determined similar to total polyphenols with absorbance measured at 276 nm and concentration of catechin in the samples was obtained from standard curve of catechin for 1N HCl and 1N NaOH.

RESULTS AND DISCUSSIONS

Catechin extract entrapment efficiency

Catechin extract loading capacity of catechin was determined spectrophotometrically in a phosphate buffer 6.8 by sonication and solvent dilution method. Catechin extract entrapment efficiency of NF1, NF2 and NF3 nanoparticles were found to be 75 %, 62 % and 40 % respectively with decreased entrapment efficiency with an increased polymer concentration. With the increase in drug load, a more porous polymeric matrix structure may be formed with a more number of channels and pores, through which the catechin could easily diffuse to the outer phase thereby decreasing the content of drug inside the polymeric shell. Additionally, with an increased drug concentration inside the polymer exists an osmotic pressure differential between the outer and inner aqueous phase facilitating the catechin to escape from the inner core.

Determination of Surface Morphology and Particle Size by Scanning Electron Microscopy

The monodispersed particle size distribution of catechin loaded nanoparticles as determined by SEM is shown in Figure 2. It was evident from SEM image (Figure 2) that nano-formulations were found to be a blend of asymmetric as well as aggregated particles with particle size ranging from 100-400 nm. The outer surface was found to be highly porous and smooth, facilitating a better release of entrapped molecule from the nanoformulations (Figure 3).

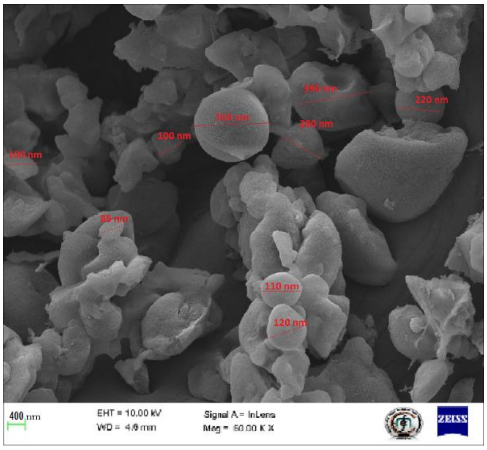

Fig.2: SEM image of CRE nanoformulation showing the distribution of nanoparticles with the average particle size ranging between 100-400 nm (n=3)

Table 2: Free radical scavenging activity of nanoformulations of catechin rich extract against a methanolic solution of DPPH.

Concentration (ppm)	Antioxidant activity* (% of free radical inhibition)				
	Curcumin	CRE	NF1	NF2	NF3
1	55.05 ± 0.050	64.69 ± 9.710	88.09 ± 3.120	33.31 ± 8.365	30.83 ± 18.810
2.5	80.58 ± 5.610	83.03 ± 2.730	91.78 ± 0.715	63.75 ± 3.602	52.94 ± 27.060
5	80.56 ± 4.060	76.96 ± 0.450	91.73 ± 1.115	85.64 ± 4.970	66.78 ± 20.955
7.5	79.23 ± 1.960	74.012 ± 3.255	92.47 ± 0.410	85.05 ± 5.889	74.93 ± 17.275
10	73.46 ± 1.340	65.98 ± 6.135	92.27 ± 1.065	82.65 ± 7.660	79.20 ± 11.925
20	69.72 ± 1.705	58.41 ± 9.625	92.37 ± 0.820	84.82 ± 8.090	90.69 ± 2.825
25	61.02 ± 7.775	60.44 ± 8.488	90.13 ± 1.410	80.37 ± 7.570	91.89 ± 1.290

*CRE: catechin rich extract: NF1: nanoformulation having CRE & polymer ratio 1:1; NF2:1:2; and NF3: 1:3 (mean ± S.D., n=3).

Antioxidant Activity

The antioxidant activity of curcumin (a well-known antioxidant compound), catechin rich extract, NF1, NF2 and NF3 are presented in Table 2. Nanoformulations showed higher percentage inhibition compared to curcumin and pure catechin rich extract and measured at various concentrations such as 2.5, 5, 7.5, 10, 20 and 25 ppm as shown in figure3.

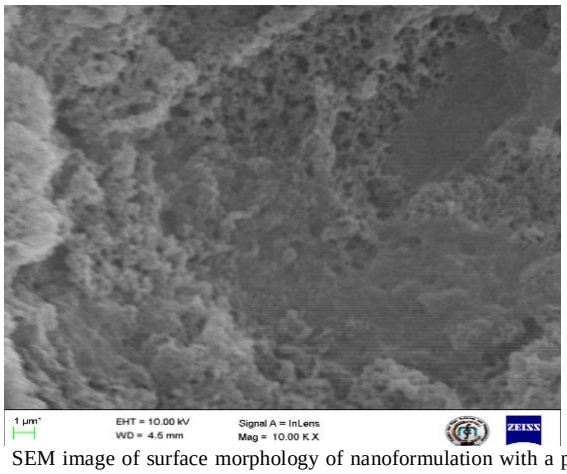

Fig. 3: SEM image of surface morphology of nanoformulation with a porous outer surface.

Antioxidant activity or the free radical scavenging activity of the standard extract (curcumin), pure catechin extract and nanoformulations are given in Table 2. The antioxidant activity was measured for various stock solutions having concentrations such as 1 ppm, 2 ppm, 5 ppm, 7.5 ppm, 10 ppm, 25 ppm and 25 ppm. Standard antioxidant used for comparison was curcumin and the scavenging activity of this was compared with the pure compound extract and nanoformulations (Figure 4). NF1 showed similar range of antioxidant activity for all concentrations whereas, NF2 and NF3 showed increase in antioxidant level with an increase in concentrations (Figure 4). Catechin rich extract showed higher percentage inhibition of 83.03 % compared to curcumin (80.58 %).

Also the nanoformulations had higher percentage inhibition compared to curcumin and the catechin extract with a trend of decrease of antioxidant activity with increase in polymer concentration. Order of percentage inhibition is as follows,

Curcumin (80.58 %) <CRE (83.03 %)< NF2 (85.64 %) < NF3 (91.89 %) < NF1 (92.47 %). Thus, the particle size reduction and nanoformulation process technically enhances antioxidant activity of the pure catechin extract.

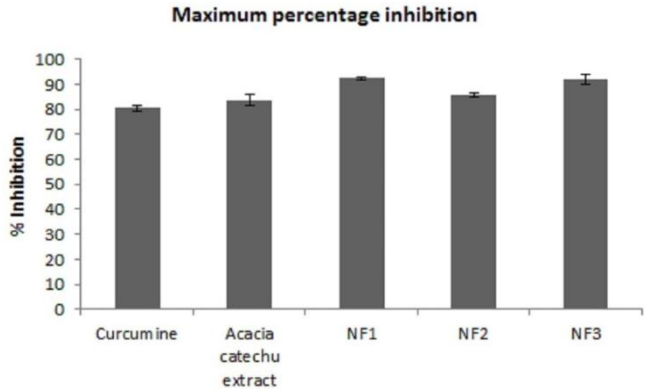

Fig. 4: Comparative Antioxidant or the free radical scavenging activity of the standard (curcumin), pure catechin extract and nanoformulationsNF1 (1:1 Drug and polymer ration), NF2 (1:2 Drug and polymer ration)and NF3 (1:3 Drug and polymer ratio).

***In vitro* Bioavailability studies**

The *in vitro* bioavailability of polyphenols in aqueous solution of 1N HCl at pH 1.35 and aqueous solution of 1N NaOH at pH 7.5 is shown in the Table 3.

Table 3: *In vitro* bioavailability of total polyphenols and catechin in aqueous solution at 1N HCl and 1N NaOH in mcg/ml.

Nanoformulation code	Media	Concentration of polyphenols (mcg/ml)	Concentration of catechin (mcg/ml)
NF1	**1N HCl**	45.84 ± 6.514	59.42 ± 9.094
NF2		45.24 ± 7.330	43.42 ± 3.887
NF3		41.07± 8.285	39.05 ± 3.617
NF1	**1N NaOH**	46.31 ± 5.474	26.58 ± 3.786
NF2		43.45 ± 6.536	17.35 ± 0.966
NF3		38.12 ± 10.321	12.4 ± 1.866

NF1: nanoformulation having CRE & polymer ratio 1:1; NF2: 1:2; and NF3: 1:3, (mean ± S.D., n=3).

NF1 showed higher bioavailability compared to NF2 and NF3. This might be due to higher drug content and lower polymer concentration. It was also observed that the bioavailability was slightly higher in acidic pH (pH 1.35) compared to alkaline pH (pH 7.5) for all the nanoformulations (Figure 5). Similarly, *in vitro* bioavailability of catechin in aqueous solution of 1N HCl at pH 1.35 and aqueous solution of 1N NaOH at pH 7.5 was determined at λ_{max} of 276 nm (Table 4).

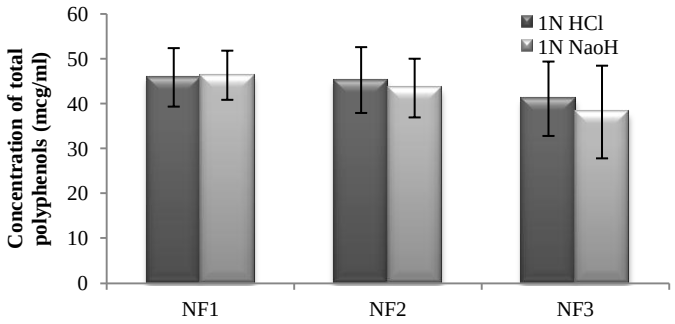

Fig.5: *In vitro* bioavailability of total polyphenols in nanoformulations in two different pH condition: 1N HCl and 1N NaOH.

NF1 showed higher bioavailability compared to NF2 and NF3 in both acidic and alkaline conditions. This might be due to higher drug content and lower polymer concentration. Also, it was observed that the bioavailability was higher in acidic pH (pH 1.35) compared to alkaline pH (pH 7.5) for all the nanoformulations. There is also a decreasing trend of catechin content as observed for NF1, NF2 and NF3, respectively (Figure 6). From this we can conclude that, the *in vitro* bioavailability is inversely proportional to polymer concentration.

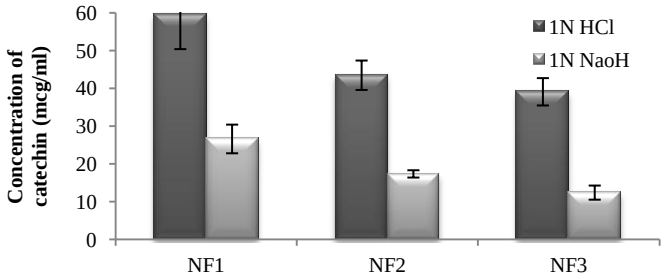

Fig. 6: *In vitro* bioavailability of catechin in nanoformulations in two different pH condition: 1N HCl and 1N NaOH.

CONCLUSIONS

In the present work, nanoformulation of CRE using Eudragit L 100 was developed by emulsion solvent evaporation technique. SEM images showed uniformly distributed nanoparticles with spherical surface morphology and particles in the size range of 100 nm to 200 nm. Nanoformulations evaluated for their antioxidant activity using DPPH method showed higher percentage inhibition compared to curcumin and CRE. Bioavailability of total polyphenols and catechin was higher in acidic pH (pH 1.35) compared to alkaline pH (pH 7.5) for all the nanoformulations. Also, the *in vitro* bioavailability of polyphenols and catechin is inversely proportional to polymer concentration. Thus, the particle size reduction and nanoformulation process found to be highly effective in enhancing the antioxidant activity and *in vitro* bioavailability of CRE.

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Conflict of Interests: There are no conflicts of interest.

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