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ORIGINAL ARTICLE **OPEN ACCESS**

Formulation and Characterization *Tagetes erecta* Nanosuspension with Enhanced Antihyperlipdemic Potential

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ABSTRACT
Nanosuspension of Tagetes erecta was evaluated for its anti-hyperlipidemic activity and antioxidant with enhanced solubility and permeability with curcumin. Tagetes erecta (NS-TE) methanolic extract and Curcumin (isolated from rhizomes of Curcuma longa) was used to prepared the nanosuspension by sonication method. Nanosuspension of Tagetes erecta flower extract (NS-TE) and Tagetes erecta flower extract with curcumin (NS-CTE) was prepared and evaluated for particle size, entrapment efficiency, zeta potential, SEM, UV spectroscopy, FTIR spectroscopy. Average particle size of NS-TE and NS-CTE was found to be 138.3 nm and 100.9 nm, entrapment efficiency 56.32 (NS-TE), 89.6(NS-CTE), zeta potential of NS-TE and NS-CTE was -17mV and -18.9 mV respectively. SEM studies of NS-TE and NS-CTE revealed the spherical shape of particle and smooth surface of formulation. The □ max for NS-TE was found to 250 nm and calibration curve showed a linear curve indicating a response that obeys Beer Lamberts law. FTIR spectroscopy indicated the presence of functional groups like amide, amines, aldehyde, nitro compounds & carboxylic acids. NS-TE and NS-CTE was evaluated for their in vitro antioxidant with nitric acid and hydroxyl radical scavenging assay and in-vivo anti Hyperlipidemic with PTU induced hyperlipidemia. NS-TE and NS-CTE showed the prominent antioxidant capability and also showed significant anti-hyperlipidemic activity at dose of 100 mg/kg bd. wt.
Key words: Nanosuspension, *Tagetes erecta*, Propyl thiouracil (PTU), Curcumin

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INTRODUCTION

Hyperlipidemia is a medical term for abnormally high levels of fats (lipids) in the blood. The two major types of lipids found in the blood are triglycerides and cholesterol [1]. Hyperlipidemia is considered one of the major risk factors causing cardiovascular diseases (CVDs). CVDs accounts for one third of total deaths around the world, it is believed that CVDs will turn out to be the main cause of death and disability worldwide [2]. According to Centers for Disease Control data from a survey of 1,492 physicians, provide ambulatory care in non-government settings, hyperlipidemia is second in the list of the 10 most common chronic conditions that were seen [3]. Dyslipidemia can be induced by diet management, i.e., high fat diet and triton X-100 induced hyperlipidemia model. Dyslipidemia can be controlled with triton X-100, without change/ manipulation in diet, by blocking the endogenic synthesis of lipid levels or by decreasing fat absorption from guts.

Nanotechnology refers to an emerging field of science that includes synthesis and development of various Nano materials particle size range from 1-100 nm [4]. Two approaches are generally applied in preparing Nano suspension as top-down and bottom-down approach. Top-down approach: This is based on the principle of breaking down large drug particles to smaller particles that are in Nano range. Bottom-up approach: It is based on the principle of first dissolving the drug molecules in a solvent and building them up to Nano-sized particles [5]. Curcumin, obtained from rhizomes of *Curcuma longa*, is used as bio-enhancer for anti-microbial agents and anti-cancer drug [6]. Epigallocatechin-3-gallate due to low oral bioavailability, a need arises to improve its membrane permeability and transporter-mediated intestinal efflux. Therefore, an attempt was made to enhance permeability and bioavailability of EGCG using curcumin to treat hyperlipidemia [7].

In the present study, dried methanolic extract of *Tagetes erecta* was used to formulate the nanosuspension of *Tagetes erecta* (NSTE) and nanosuspension of *Tagetes erecta* with curcumin (NS-CTE).

NSTE& NS-CTE are characterized and screened for hyperlipidaemia with enhanced permeability and solubility.

MATERIAL AND METHODS

Collection, Identification and Extraction of *Tagetes erecta*

The flower heads of *Tagetes erecta* were collected from local market near Mehindipatnam, Hyderabad, Telangana (November 2019). The crude plant material is authenticated by P. Suresh babu,(Voucher Specimen TEP-5) Govt Degree College, Hyderabad, and Telangana.Flower heads of *Tagetes erecta* were dried under shade, coarsely powdered and was subjected to soxhlation with methanol. Crude methanolic extractof *Tagetes erecta* (ME-TE)was dried and stored for further use [8].

Formulation of Nano-suspension of *Tagetes erecta* and *Tagetes erecta* with Curcumin

Nanosuspension of *Tagetes erecta* was prepared by the homogenization method. ME-TE(0.15g) was dissolved in methanol by sonication for 10 min, and polyvinyl alcohol (1.5%) was mixed. 0.1% chitosan solution and phosphoric acid was added to maintain the pH 5.4. Chitosan solution was mixed with extract solution dropwise with sonication (Probe sonicator-HD 2070, Source –Bandelin Sonopuls, Germany) for 30 min. The orange opalescent nanosuspension of *Tagetes erecta*a (NS-TE) was formed[9].

ME-TE (0.15g) and 25 mg of Curcumin is sonicated for 10 min, and polyvinyl alcohol (1.5%) was mixed. 0.1% chitosan solution and phosphoric acid was added to maintain the pH 5.4. Chitosan solution was mixed with extract solution drop wise with sonication (Probe sonicator-HD 2070, Source – BandelinSonopuls, Germany) for 30 min. The yellow opalescent nanosuspension of *Tagetes erecta* a (NS-CTE) was formed.

Characterization of Nano-suspension

Determination of Particle size and Zeta potential

The NS-TE and NS-CTE was characterized for its particle size, PDI (particle homogeneity in the dispersion), and zeta potential using Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK). The particle size was measured by Particle size analyzer (Nanotrac wave, Model: - W 3275, Microtrac, USA) using dynamic light scattering technique [10].

Entrapment Efficiency

The entrapment efficiency of NS-TE and NS-CTE was determined by sonicating NS-TE and NS-CTE at 20,000 rpm for 20 min [11]. Aliquots of above both solutions (0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL and 0.5mL) were taken and diluted up to 10 mL. Resultant concentrations were centrifuged at 1500 RPM for 20 min. The aliquot of the supernatant was measured by UV/visible spectrophotometry at 250 nm for NS-TE and at 535 nm for NS-CTE [12].

Entrapment efficiency (%) was calculated using this formula:

% Entrapment efficiency = Total amount of drug − Free drug in supernatant × 100 / Total amount of drug.

Morphological Analysis by Scanning Electron Microscopy (SEM)

SEM was performed at high magnifications to check surface morphological characteristics and particle size of NS-TE & NS-CTE with a scanning electron microscope (Hitachi-S 3400N) at an acceleration voltage of 15.0 kV. SEM analysis of NS-TE & NS-CTE was done in the Central Analytical Facility-University College of Technology, Osmania University, Hyderabad, and Telangana, India.

Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR was run for NS-TE, NS-CTE, and crude extract of *Tagetes erecta* for detecting functional groups and characterizing covalent bonding information on IR affinity 1 spectrophotometer (Shimadzu, Japan) and absorption bands were recorded and expressed in cm^{-1} [13].

In-vitro Antioxidant assay of NS-TE and NS-CTE

NS-TE & NS-CTE was subjected to *in vitro* anti-oxidant assay with Hydroxyl radical & Nitric oxide scavenging assay [14].

Experimental Animals

Wistar rats (170-200 g) were procured from Jeeva Life sciences, Uppal, Hyderabad. The experimental protocol of present study was approved by the IAEC (Institutional Animal Ethical Committee Reg. No. 1175/PO/Re/S/08/CPCSEA) of CPCSEA (Committee for control and supervision of experimentation on animals).

In-vivo Anti Hyperlipidemic activity

Propylthiouracil induced Hyperlipidemia Model

Wistar rats were randomized into 5 groups containing 6 animals each [15]. Group-I served as control group, Group-II served as disease control group treated with PTU (10 mg/kg bd. wt), Group-III served as hyperlipidemia rats treated with NS-TE (100 mg/kg *p.o*), Group-IV hyperlipidaemic rats treated with NS-CTE (100 mg/kg *p.o. T.e +* 25 mg/ kg *p.o*Curcumin), Group-V hyperlipidaemic rats treated with Simavastatin (10 mg/kg bd. wt) [16].

Group-II to Group-V animals were treated PTU (10 mg/kg bd. wt) for 8 days and on 8thday treated with cholesterol (400 mg/kg bd. wt), and, blood was withdrawn by retro-orbital plexus 6 h post treatment for estimation of lipid levels.

Statistical Analysis

Statistical data analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The results were expressed as mean ± SEM using Graph Pad Prism version 8.0 for Windows [17].

RESULTS AND DISCUSSION

NS-TE and NS-CTE was formulated with biodegradable polymer, chitosan by homogenization method, characterizedfor its particle size, particle size distribution, zeta potential, and morphological features with SEM, and evaluated for its anti Hyperlipidemic activity.

Particle size & zeta potential NS-TE

Particle size ofNS-TE (Fig 1) and NS-CTE (Fig 2) was found to be 138.3 nm and 100.9 nm respectively. Zeta potential ofNS-TE (Fig 1) and NS-CTE (Fig 2) was found to be -17.1 mV and -18.9 mV respectively. Zeta potential shows the charge on the particle surface which indicates the physical stability of the dispersed systems. A high zeta potential will confer stability of the preparation that will resist aggregation [18].

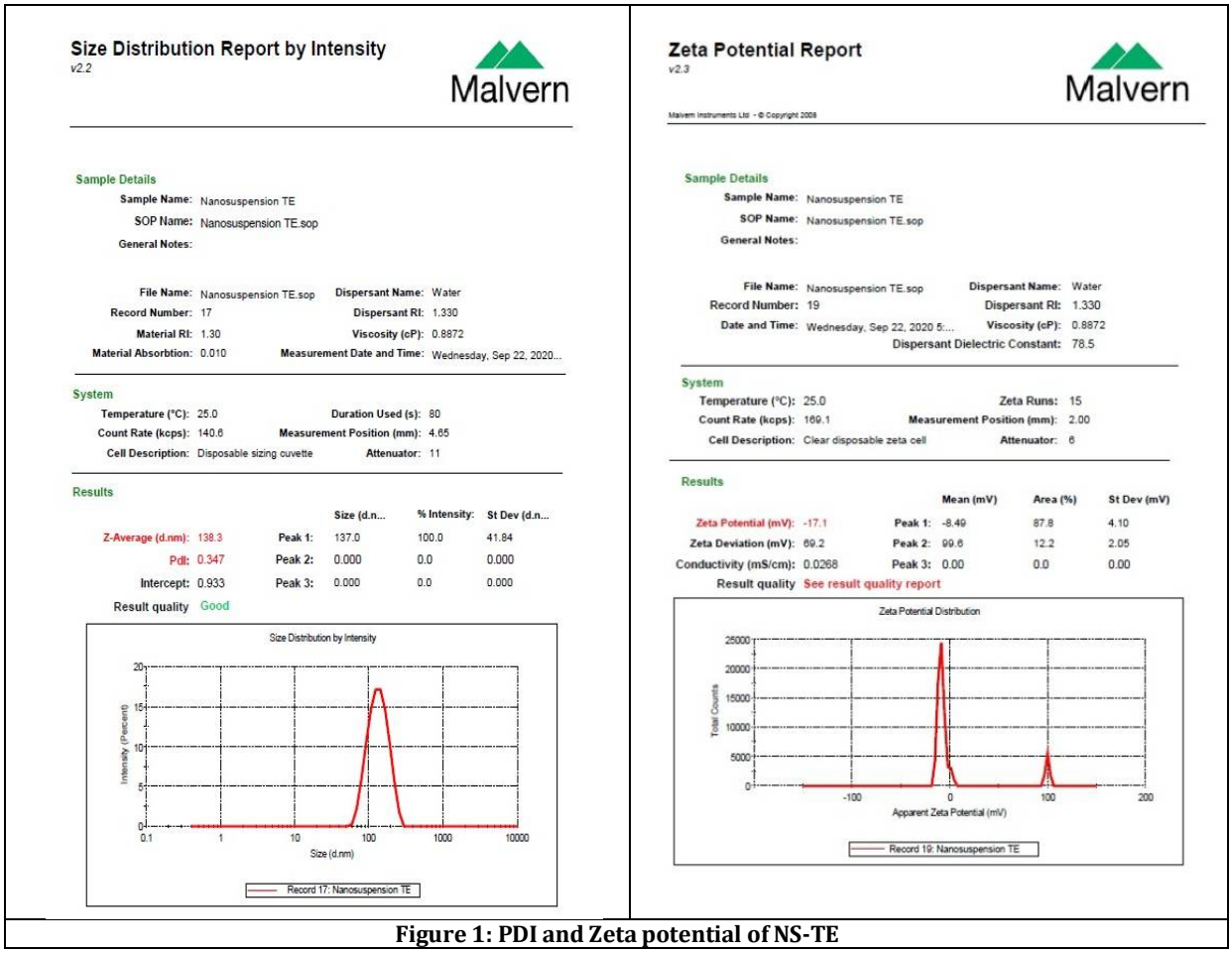

Figure 1: PDI and Zeta potential of NS-TE

Figure 2. PDI and Zeta potential of NS-CTE

Scanning Electron Microscopy

NS-TE(Fig 3)and NS-CTE(Fig 4) were sputter coated with gold/palladium for 120 s at 14 mA under argon atmosphere for secondary electron emissive SEM (Hitachi-S 3400N) at an acceleration voltage of 15.0 kV. Scanning electron images of surface morphology of NS-TE and NS-CTE revealed their smooth texture of particles. Images showed that most of the particles were having spherical shape and smooth shape and topology. Average particles size of NS-TE and NS-CTE was found 77.2 ± 7.11 nm and 49.96 ± 2.57 nm [19].

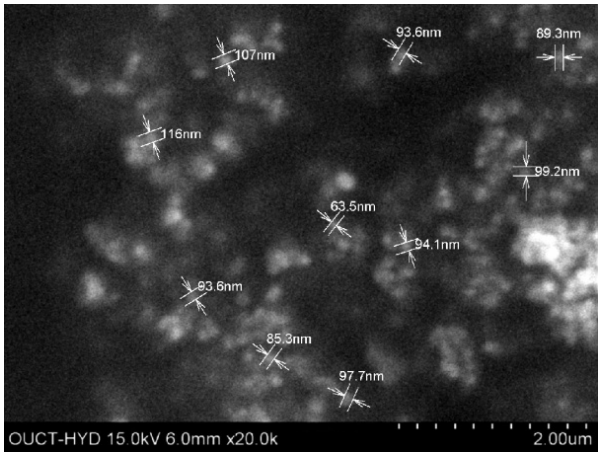

Figure 3: SEM image of NS-TE

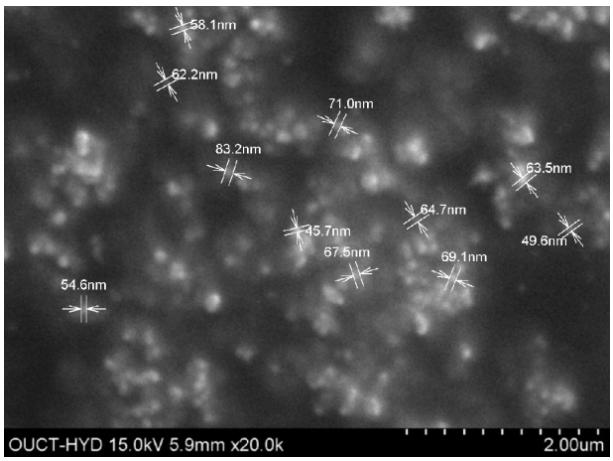

Figure 4: SEM image NS-CTE

Entrapment efficiency of NS-TE and NS-CTE

NS-TE (50 mg) and NS-CTE(50 mg) were dissolved in 100 mL of phosphate buffer pH 6.8 and the drug amount was determined by U.V analysis. Entrapment efficiency of NS-TE (Fig 5)and NS-CTE(Fig 6) was found to be 54.3% and 65.52% respectively.

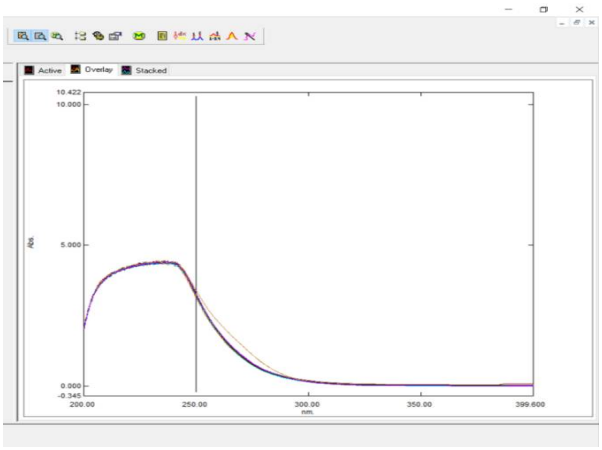

Figure 5: Entrapment efficiency of NS-TE

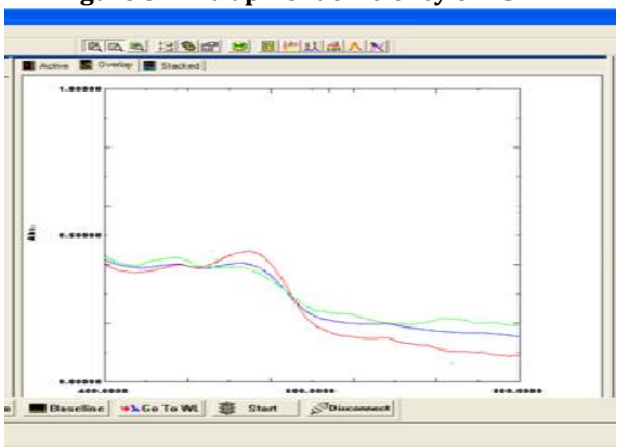

Figure 6:Entrapment efficiency of NS-CTE.

UV- VIS Spectral Analysis of NS-TE and NS-CTE

NS-TE and NS-CTEwas centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filtered paper. The sample is diluted to 1: 10 same solvents. Resultant solution was scanned at wavelength ranging from 200 to 600 nm using Shimadzu UV-1800, Mumbai. Spectra and characteristic peaks were detected and UV- VIS were recorded [20](Table 1, Fig 7 and Fig 8).

Table 1: Calibration of NS-TE and NS-CTE

Concentration (mg/mL)	Absorbance λ_{max}=250 nm	Absorbance λ_{max}=535 nm
0	0	0
2	0.108	0.118
4	0.134	0.168
6	0.186	0.241
8	0.248	0.298
10	0.287	0.376
12	0.328	0.439

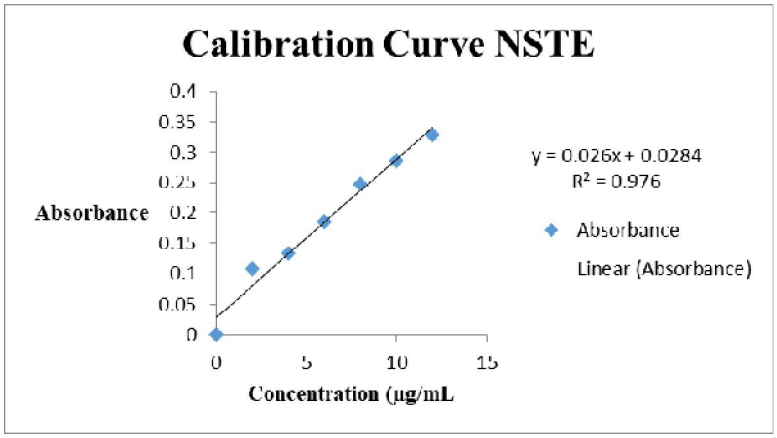

Figure 7: Calibration curve of NS-TE

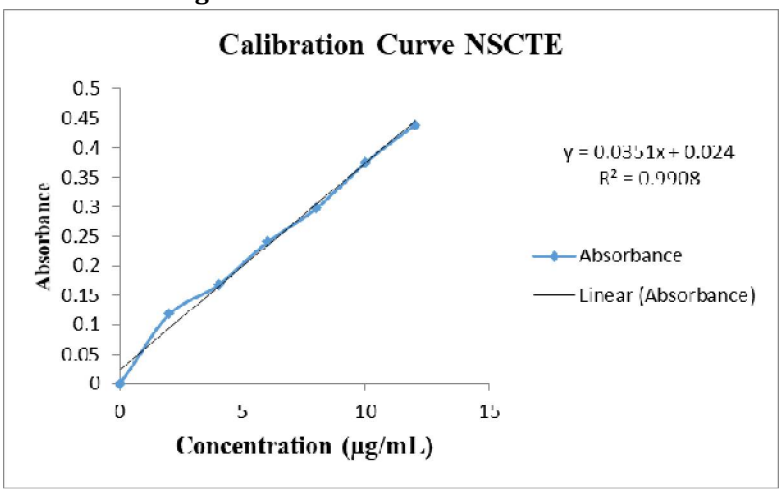

Figure 8: Calibration curve of NS-TE

***In vitro* Drug release studies of NS-TE & NS-CTE:**

The percentage drug release of NS-TE & NS-CTE (at 37 ± 0.5°C) was carried out in phosphate buffer solution pH 6.8 [21] and was reported in Table 2. NS-TE showed complete dissolution of 55.6138 % in 120 mins & NS-CTE showed dissolution of 89.69 % in 120 mins indicating that the NS-CTE has improved solubility and bioavailability of *Tagetes erecta* extract(Fig 9).

Table 2: Percentage Drug release of NSTE & NSCTE

Time	% Release of NS-TE	% Release of NS-CTE
0	0	0
5	4.8076	1.234286
15	10.19231	14.81143
30	15.69231	34.35429
45	22.34615	55.33714
60	32.38462	72.93714
90	43.76923	82.365
120	55.61538	89.69143

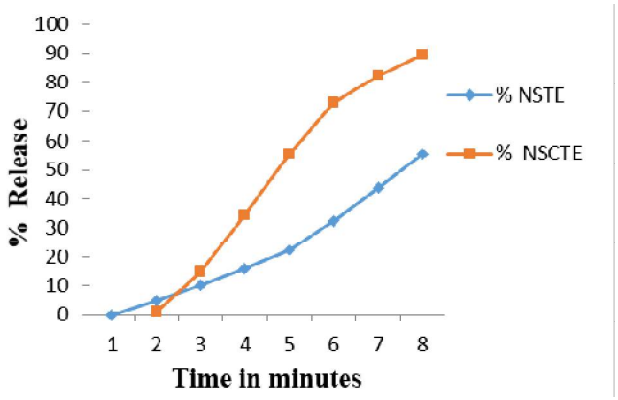

Figure 9:*In vitro* release profile of formulation NSTE & NSCTE in Phosphate buffer solution pH 6.8

Fourier transform infrared spectroscopy:

FTIR spectroscopy of NS-TE and NS-CTE shows the sharp characteristics peak at 3305.7 cm^{-1} NH (1^0 & 2^0 amines),2951.12cm^{-1} CH (Alkane), 2703.5cm^{-1} H-C=O:C-H (Aldehyde), 1642.1cm^{-1}N-H (1^0 Amine)1323.5cm^{-1} N-O (Nitro Compounds), 1247.01 cm^{-1} C-O (Carboxylic acid),1151.8cm^{-1} C-N (Aliphatic Amines) function groups. The prominent peaks representing NS-TE, NS-CTEand extract appear in the spectra; did not show any significant shifting in the position of the absorption peak. Overlay of *Tagetes erecta* extract, NS-TE & NS-CTE was performed, indicates that all the functional groups present in the *Tagetes erecta* extract were also present in the NS-TE & NS-CTE (Fig 10, Fig 11, Fig 12).

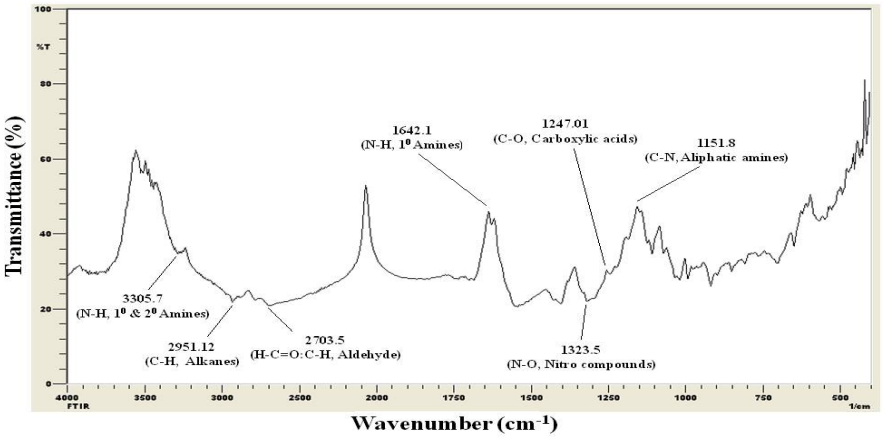

Figure 10: IR spectra of NS-TE

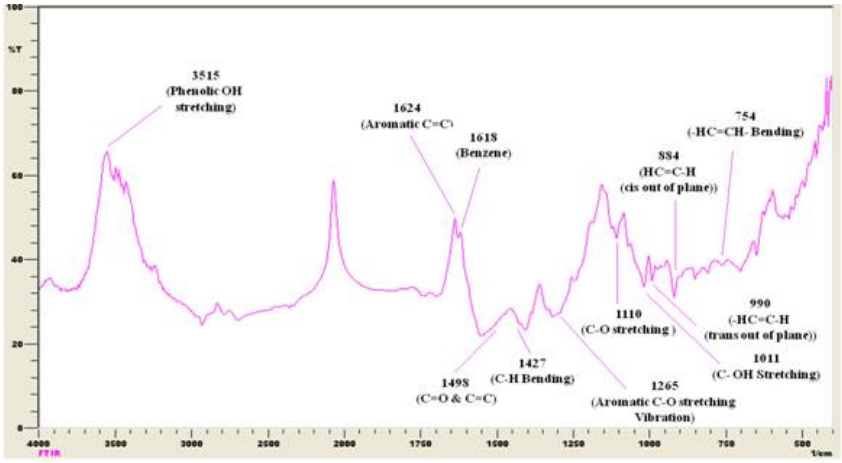

Figure 11: IR spectra of NS-CTE

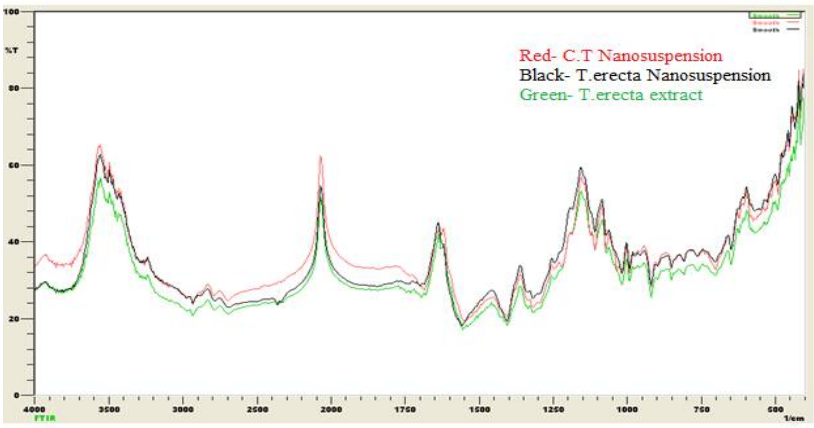

Figure 12: Overlay spectra of *T. erecta* extract, NS-TE & NS-CTE

***In-vitro* antioxidant assay of NS-TE & NS-CTE**

In hydroxyl radical scavenging assay and nitric acid radical scavenging assay, IC_{50} value for NS-TE, NS-CTE and standard drug (ascorbic acid) was found to be 41.02, 36.05 & 29.41; 32.1, 26.8, and 25.5 µg/mL respectively, which showed prominent anti-oxidant potential of NS-TE and NS-CTE(Table 3). NS-CTE has showed enhanced anti-oxidant potential; might be due to curcumin which acts as permeability enhancer.

Table 3: *In vitro* hydroxyl radical and nitric oxide radical scavenging assay of NS-TE and NS-CTE

S. No.	Test compound	Antioxidant assay	IC_{50} value
1	Ascorbic assay (standard)	Hydroxyl radical scavenging assay	29.41
		Nitric oxide radical scavenging assay	25.5
2	NS-TE	Hydroxyl radical scavenging assay	41.02
		Nitric oxide radical scavenging assay	36.05
3	NS-CTE	Hydroxyl radical scavenging assay	32.1
		Nitric oxide radical scavenging assay	26.8

***In vivo* Anti hyperlipidemic activity of NS-TE & NS-CTE with Propyl Thiouracil Induced Rat Models:**

NS-TE and NS-CTE were screened for its antihyperlipidemic activity in propylthiouracil induced rat models. PTU elevated the serum lipid and lipoprotein levels in experimental animals. NS-TE andNS-CTE significantly reduced lipid parameters. NS-TE showed significant decreasing in TC (P<0.01), TG (P<0.01), LDL (P<0.01) and VLDL (P<0.01) and induction of HDL (P<0.01) while comparing with the normal control group. NS-CTE showed significant decreasing in TC (P<0.001), TG (P<0.01), LDL (P<0.001) and VLDL (P<0.01) and induction of HDL (P<0.01) when results were compared to Disease control animals. Simvastatin (10 mg/kg bd.wt) significant reduced all determined lipid parameters TC (P<0.001), TG (P<0.01), LDL (P<0.001) and VLDL (P<0.001) and improvedHDL (P<0.001) which is compared with disease control (Table 4).

Table 4: *In-vivo* antihyperlipidemic activity of NS-TE & NS-CTE in Propylthiouracil induced rat model.

Treatment	Lipid Profile (mg/dL)					
	HDL	LDL	VLDL	Total Cholesterol	Triglycerides	Total Glucose
Normal Control	36.11 ± 0.35	41.68±0.25	21.58±0.44	54.916 ± 0.474	105 ± 0.47	151.83± 0.58
Disease Control	17.133 ±0.317	72.66 ± 0.557	51.33 ± 0.494	134.75 ± 0.666	255.88 ± 0.610	162.25 ± 0.572
NS-TE 100 mg/kg bd.wt)	23.166 ±0.401^{*a}	52.35 ±0.727^{**b}	37.3 ±0.984^{**b}	137.33 ± 0.133^{*b}	191.75± 0.259^{**b}	143.38 ± 0.365^{*a}
NS-CTE 100 mg/kg bd.wt)	25.5 ± 0.816^{a}	42.6 ± 0.39^{*a}	34.433± 0.1642441^{b}	175.78± 0.423^{**a}	175.78± 0.423^{**a}	135.28 ± 0.3260^{*a}
Standard Simvasatatin 10 mg/ Kg)	26.61 ±0.619^{*a}	22.15 ±0.276^{**a}	26.85 ±0.519^{**b}	76.65 ±0.942^{*a}	135.86 ±0.592^{*b}	146.73 ±0.6849^{*a}

Values are expressed as Mean ± SEM, (n=6). Statistical analysis was performed by using One way ANOVA followed by Dunnett's test (* = P<0.01, ** = P< 0.001) when results were compared to control group, (a= P<0.0001, b= P<0.001) when results were compared to Disease control.

There was a significant reduction in plasma, and hepatic lipid profiles along with elevation in plasma HDL in NS-CTE treated rats as compared to hyperlipidemic rats, thus indicating the enhanced permeability of curcumin [22]. Epidemiological studies have shown that a higher level of HDL in plasma reduces the risk of coronary artery disease. Flavonoids are reported to increase HDL concentration and decrease LDL and VLDL levels in hypercholesteremic rats [23]. Improved antihyperlipidemic potential of NS-CTE may be due to binding of P-gp present on the epithelial cells of small intestine, results in drug efflux from the intestine to the systemic circulation and hence more amount of drug is available to show therapeutic activity [24]. NS-TE and NS-CTE also showed prominent antioxidant capability for hydroxyl radical and nitric oxide radicals, may contribute to anti-hyperlipidemic action of NS-TE and NS-CTE.It can be concluded that Nanosuspension of *Tagetes erecta* with curcumin has a significant lipid lowering potential and also possess curative properties in conditions of hyperlipidemia and related disorders because the penetration ability of extract was increased by Curcumin[25,26].

CONCLUSION

Nanosuspension of *Tagetes erecta*with curcumin has showed enhanced antihyperlipidemic activity might be a novel approach and can be used in treatment of dyslipidemia.

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