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FORMULATION OF *EICHHORNIA CRASSIPES* DERIVED LUTEIN: COCONUT OIL MICRO-EMULSION FOR SUSTAINED OPHTHALMIC DRUG DELIVERY

Thiruvengadasamy R. Poorani, Venupriya Vellingiria, V. Sudarshana Deepa* and Azeez N. Abdula

Nano-Bio Translational Research Laboratory, Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam, Erode - 638401, Tamil Nadu, India.

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Correspondence to Author:

Dr. V. Sudarshana Deepa


Assistant Professor,
Nano-Bio Translational
Research Laboratory,
Department of Biotechnology,
Bannari Amman Institute of
Technology, Sathyamangalam,
Erode - 638401, Tamil Nadu, India.

E-mail: sudarshanadeepav@bitsathy.ac.in

ABSTRACT: Lutein is a lipid soluble, oxygenated derivative of hydrocarbon carotenoid, from xanthophyll family which is naturally present in egg yolk, vegetables and several dark green plants. In human eyes, Lutein and zeaxanthin are the only two carotenoids present in the macula region to filter the harmful ultra violet rays. They protect the eye from oxidative damage associated with diseases such as cancer, cataracts and age related macular degeneration (AMD). Treating AMD is expensive and also prone to after effects of the treatment. Hence a natural low cost alternate has been investigated in this research article. *Eichhornia crassipes*, an aquatic perennial herb weed belongs to pontederiaceae family a dark green-leaf was taken as source for the lutein extraction as it has carotenoids in abundance. Lutein was identified, isolated and purified from crude leaf extract and was analysed by GC-MS, TLC and HPLC using hexane as mobile phase. To improve the bioavailability and stability of lutein, it was micro-emulsified with coconut oil using high energy sonication method. The oil in water (O/W) lutein micro emulsion (MEL) was synthesized and analyzed for their particle size, zeta potential, entrapment efficiency, antioxidant activity, *in-vivo* (dialysis membrane) and *ex-vivo* (goat's eye cornea) lutein release features by franz diffusion cell. The drug release studies showed the gradual and controlled release of lutein in MEL formulation than macro emulsion (EL) can effectively treat the eye related diseases. The effective sustained release of lutein from micro emulsion can be attributed to the kinetic instability of the droplets.

INTRODUCTION: Common weeds gain more attention over the medicinal plants for the rich source of carotenoids that provide potential nutraceutical and pharmaceutical advantages to human health¹. Lutein is a known yellow coloured lipid soluble oxygenated derivative of hydrocarbon carotenoids, are rich in green leafy plants.

Lutein acts as a strong antioxidant and blue light absorptive properties which provides protective role for various ocular disease especially AMD (Age related Macular Degeneration) and cataracts². Its optimal amount of dietary intake is necessary to avoid the risk of eye diseases^{3, 4}. But its bioavailability is low because it is lipid soluble and completely insoluble in water. Lutein is photosensitive and is easily oxidized leading to a further decrease in bioavailability as well as its activity⁵. Bioavailability and stability was improved by incorporating the lipid-soluble bioactive compound lutein into micro-emulsion to optimize its sustained nutrition delivery⁶.

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Micro emulsions are the dispersion of a liquid as droplets into an immiscible liquid phase as micro droplets. These are thermally stable, clear isotropic solutions. They are used to entrap and deliver the water insoluble bio-active compounds with controlled release profile. In this study, sub-micron sized particles were synthesized using high energy sonicator with Tween 80 as surfactant. Micro droplets were prepared kinetically unstable in the interest to release the lutein at uniform phase.

Eichhornia crassipes (water hyacinth) an aquatic macrophyte and a monocotyledon belong to the family pontederiaceae with violet and yellow flowers was utilized as economical source of lutein by solvent extraction technique. Due to its adaptability for growth in harsh environmental conditions it has high degree of invasion ⁷. *Eichhornia crassipes* is rich in nutritionally essential compounds and secondary metabolites like phenolics, flavanoids, alkanoids, terpenoids, sterols and some metabolites. Methanolic extract of *Eichhornia crassipes* has high antimicrobial activities and its aqueous extract has excellent DPPH scavenging activity which predicts its antioxidant activity. Since it has antioxidant activity it is notorious that it has highly efficient anti-tumour activity. Also this plant has the ability to act as the wound healing agent and fine larvicidal activity. This plant not only rich in medical purposes but also can improve the bio-energy production where the leaves of *Eichhornia crassipes* can produce high-quality bio-fuels and bio-manures to enrich the world's wealth and health ⁸. Thus due to its high nutritional quality *Eichhornia crassipes* plant was utilized as cheap source for the extraction of lutein by solvent extraction technique. Presence of lutein in extract was confirmed using GC-MS and the lutein was further identified and purified by TLC and column chromatography techniques. Lutein micro-emulsion (O/W MEL) was synthesized and characterized for particle size distribution, zeta potential, antioxidant assay, *in-vitro* and *ex-vivo* drug release studies to enhance its effect on targeted drug delivery.

MATERIALS AND METHODS:

Materials: Standard lutein (Rankem), Methanol (AR), Hexane(AR), Sodium bicarbonate (NaHCO₃), Calcium Chloride (CaCl₂.2H₂O), Sodium Chloride (NaCl), Hydrochloric acid (HCl), column grade

silica gel (mesh 230-400µm) and pre-coated silica plates for thin layer chromatography (TLC) were purchased and used as received.

Simulated tear fluid (STF, pH 7.4) was prepared by dissolving 2.01g NaCl, 0.6g NaHCO₃ and 0.016g CaCl₂.2H₂O in 200ml distilled water and the resulting solution was adjusted with 0.2N HCl to a pH of 7.3 ± 0.1.

Methods:

Sample Collection: *Eichhornia crassipes* was collected from Kaveri River near Erode district, Tamil Nadu, India during the month of February 2016 and the plant material was identified by BSI (Botanical Survey of India, Coimbatore, Tamil Nadu, India). The voucher specimen *Eichhornia crassipes* was deposited in the Herbarium of Bannari Amman Institute of Technology, Sathyamangalam, Erode district, Tamil Nadu, India.

Sample Preparation: *Eichhornia crassipes* was collected from Kaveri River near Erode district, Tamil Nadu, India. Leaves of *Eichhornia crassipes* were washed with distilled water, air dried, and grinded and stored. The sample was mixed with suitable solvents for extraction. Three different solvents like acetone, methanol, and ethanol was mixed with grinded sample and stored at room temperature, overnight. The solution was filtered using whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator and stored at 4 °C after extraction ⁹.

Gas Chromatography – Mass Spectrometry: Gas chromatography (GC) analysis was carried out using Agilent 6890N gas chromatography equipped with photon multiplier tube as detector coupled to front injector type 1079. The chromatograph was fitted with HP 5 MS capillary column (30 nm × 0.25 mm ID., film thickness 0.25 µm). The injector temperature was set at 250 °C, and the oven temperature was initially at 70 °C hold for 4 min then programmed to 200 °C at the rate of 10 °C/min and finally held at 200 °C for 13 min. Helium was used as a carrier gas with the flow rate of 1.5 ml/min. A volume of 0.2µl of the sample (diluted with acetone 1:10) was injected in the splitless mode. The compounds were identified based on the comparison of their retention indices

(RI), retention time (RT), mass spectra of WILEY, NIST library data of the GC-MS system and literature data¹⁰.

Absorbance Measurement: The filtrate obtained was measured at 0 and 24th hour for its absorbance using UV Visible spectrophotometer at 450 nm. By plotting the concentration versus absorbance values, concentration of lutein was evaluated against the standard lutein concentration graph.

Carotenoid Test: The lutein content was confirmed primarily through carotenoid test. To the plant extract, 5% of sodium nitrite solution and 0.5M sulphuric acid were successively added. The presence of carotenoid was confirmed by the disappearance of dark colour. This test was performed in three extracts (methanol, ethanol, and acetone) of water hyacinth.

Thin Layer Chromatography (TLC): The pre-coated silica plates were used for detection of lutein. Hexane was used as the mobile phase for running the TLC and the TLC plates were treated using iodine. This was performed for both the standard and concentrated lutein sample.

Column Chromatography: The lutein was separated from the dried crude extract using column chromatography. The dried crude extract was dispersed in hexane. The column grade silica gel was used as the stationary phase and hexane was used as the mobile phase. The components in the extract was absorbed by the column and eluted based on its hydrophobicity. The fraction was collected and analyzed using UV visible spectrophotometer.

High Performance Liquid Chromatography (HPLC): The sample was prepared by dissolving 1mg of lutein in 1ml of acetonitrile. Both the standard and sample were quantified using HPLC (Agilent technologies). The mixture of water: acetonitrile in the volume ratio of 10:90 was used as mobile phase. About 20µl of sample was injected in the portal at the flow rate of 0.5ml/min and monitored at 450nm using UV Spectrophotometer¹¹.

Synthesis of O/W Micro-emulsion: An aqueous solution with 2% Tween 80 (HLB value = 15) was prepared by dissolving 2ml of Tween 80 in 98ml of

distilled water. Oil phase was prepared by fabricating coconut oil into medium chain fatty acid called caprylic / capric triglyceride. The fabrication was performed by hydrolysis of coconut oil with distilled water and medium chain fatty acid (oil phase) was separated from free glycerin (by product). The oil was then melted and cooled slowly to form the medium chain fatty acid. The known weight of lutein was dissolved in oil completely¹². The aqueous phase and oil phase were taken in the different ratios as mentioned in **Table 1**.

The oil phase with lutein was added drop by drop to the aqueous phase stirred continuously in the magnetic stirrer. To form the emulsion the solution was stirred continuously for 20 min. This facilitates the formation of lutein encapsulated emulsions. This solution was further sonicated using sonicator (20 KHz) for 2 cycle 30min to obtain the micro emulsion¹³. The reference micro-emulsion was prepared with the same ingredients but lutein. Then the micro-emulsion with lutein (MEL) and micro-emulsion without lutein (ME) with equal amount of ethanol was centrifuged at 10000 rpm for 30min¹⁴. The supernatant of ME was used as a blank (B) for the measurement of absorbance of lutein in MEL supernatant.

TABLE 1: VARIOUS CONCENTRATION RATIOS OF AQUEOUS PHASE: OIL PHASE AND THEIR STABILITY

Emulsion Code	Aqueous Phase	Oil Phase	Stability
ME01L	8.0	2.0	Phase Separation
ME02L	8.2	1.8	Phase Separation
ME03L	8.4	1.6	Phase Separation
ME04L	8.6	1.4	Phase Separation
ME05L	8.8	1.2	Flocculation
ME06L	9.0	1.0	Stable
ME07L	9.2	0.8	Stable
ME08L	9.4	0.6	Stable

Particle Size and Zeta Potential: Zetasizer 6.32, Malvern instruments, was used to measure the Z-average particle size, poly dispersity index and zeta potential through dynamic light scattering. The surface charges either positive or negative charges present on the lutein micro-emulsion repel each other and prevent the aggregation¹⁵. This repulsion gives the stability of the micro-emulsion. From the published data it was found that the suspensions with zeta potential above ± 30 mV and above \pm

60mV 0 were physically stable and showed excellent stability respectively. Suspensions below ± 20 Mv and below ± 5 mV are of limited stability and undergo pronounced aggregation respectively. If the zeta potential is in the range of 40mV - 60mV the micro-particles will have very good stability¹⁶.

Entrapment Efficiency of Lutein in Micro-emulsion (MEL): Entrapment efficiency of lutein in micro-emulsion was measured spectrophotometrically. The synthesized MEL and ME with equal amount of ethanol were centrifuged at 10000 rpm for 30min and the supernatant of MEL was measured for its absorbance with blank (B) as reference at 450 nm. The concentration of lutein was calculated from the absorbance value, interpolated against standard graph of lutein. The loading efficiency of lutein in MEL was calculated using the formula¹⁷,

$$\text{Loading efficiency} = \left(\frac{\text{Amount of lutein lutein loaded (ml)}}{\text{Amount of lutein used in experiment (ml)}} \right) \times 100$$

The loading efficiency also depends on the amount of oil used so the weight percentage of lutein in MEL was calculated by,

$$\text{Wt \% of lutein in MEL} = \left(\frac{\text{Amount of lutein in MEL}}{\text{Total amount of MEL}} \right) \times 100$$

Antioxidant Assay: The antioxidant activity of the standard lutein, extracted lutein and MEL was done with DPPH radical scavenging assay. The MEL and free lutein were dispersed in equal amount of ethanol and centrifuged at 10000 rpm for 30 min respectively. The supernatants obtained were added with 2ml DPPH in ethanol. The solutions were

incubated at 37 °C for 30 min and the absorbance at 518nm was measured with UV- Vis spectroscopy.

In vitro Drug Release Profile: *In vitro* drug release studies was done using franz diffusion cell. The cell was filled with prepared Simulated Tear Fluid (STF) of pH 7.4 and the mouth of the cell was covered with trans-membrane layer¹⁸. Above the layer, the lutein micro-emulsion (MEL or EL) was added and the setup was stirred using magnetic stirrer. At known intervals of time a known amount of STF was taken by replacing them with same amount of fresh STF and the solution was measured for the absorbance at 450 nm using UV-Vis spectra¹⁹. The same experiment was done for lutein emulsion (EL) and the drug release was compared for both the formulations.

Ex vivo Drug Release Studies: *Ex vivo* drug release studies were carried out with the goat's eye cut with tissue purchased from the slaughter house. The eyes were washed with cold saline solution and maintained until it was taken to the laboratory. Eyes were then transferred to STF (pH 7.4). The drug release profile was obtained by placing the corneal layer of eye on the mouth of the franz diffusion cell which was filled with STF²⁰. Above the cornea of the eye, sample (MEL or EL) was placed and the STF was stirred using magnetic stirrer. At known intervals of time, the known amount of STF inside the apparatus was taken which was replaced by the same amount of fresh STF. The taken STF was measured for its absorbance at 450 nm using UV-Vis spectra. The profile of drug release studies of lutein micro-emulsion and lutein emulsion were compared.

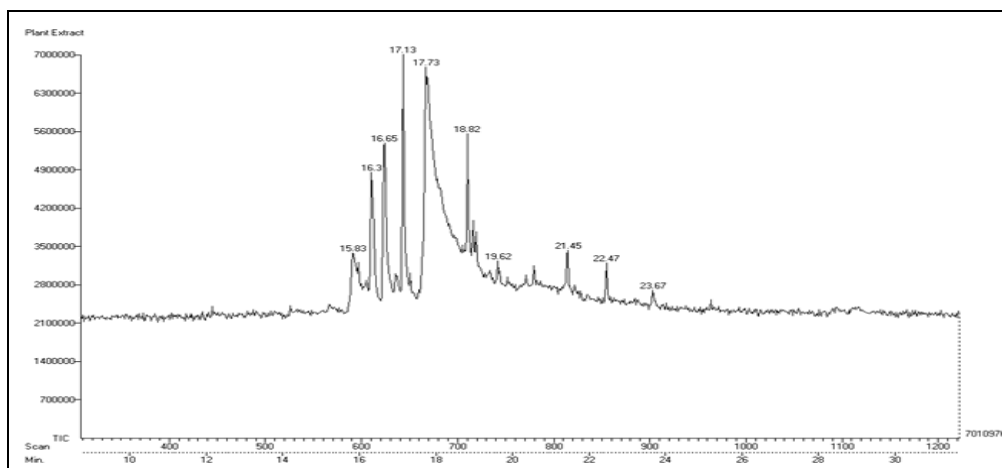


FIG. 1: GAS CHROMATOGRAPHY MASS SPECTROMETRY CHROMATOGRAM OF METHANOLIC EXTRACT OF *EICHHORNIA CRASSIPES*

RESULTS AND DISCUSSION:

Gas Chromatography - Mass Spectrophotometry: From GC-MS analyses the phytochemicals were identified. Currently 10 compounds were identified from the whole plant methanolic extract of *Eichhornia crassipes* that are deliberated in the **Table 1**. The spectrum profile of GC-MS **Fig. 1** confirmed the presence of 10 compounds Tetradecanoic acid, 2-Pentadecanone, 6, 10, 14-trimethyl, 1, 2-Benzene dicarboxylic acid, bis(2-methylpropyl)ester, 4,5,7-Trihydroxyisoflavone, Z,E-2 Methi-3,13-Octadecadien-1ol, Phytol, Z-13-Octadecen-1-yl acetate, 2-Cyclohexen-3,4-diol-1one, 2 tetradecanoyl, 2, 5- Cyclohexadiene-1,4-dione,2,5-dihydroxy-3,6-bis(4methoxyphenyl), Beta,beta-Carotene-3,3'-diol with retention time 15.83, 16.3, 16.65, 17.13, 17.73, 18.82, 19.62, 21.45, 22.47 and 23.67 respectively. The individual

fragmentation of the compounds was illustrated in **Fig. 2**.

TABLE 2: CHEMICAL COMPOSITION OF METHANOLIC EXTRACT *EICHHORNIA CRASSIPES*

S. no	Retention Time (min)	Name of the compound
1.	15.83	Tetradecanoic acid
2.	16.3	2-Pentadecanone,6,10,14-trimethyl
3.	16.65	1,2-Benzene dicarboxylicacid,bis(2-methylpropyl)ester
4.	17.13	4,5,7-Trihydroxy isoflavone
5.	17.73	Z,E-2 Methi-3,13-Octadecadien-1ol
6.	18.82	Phytol
7.	19.62	Z-13-Octadecen-1-yl acetate
8.	21.45	2-Cyclohexen-3,4-diol-1one,2 tetradecanoyl
9.	22.47	2,5-Cyclohexadiene-1,4-dione,2,5-dihydroxy-3,6-bis(4methoxyphenyl)
10.	23.67	Beta,beta-Carotene-3,3'-diol

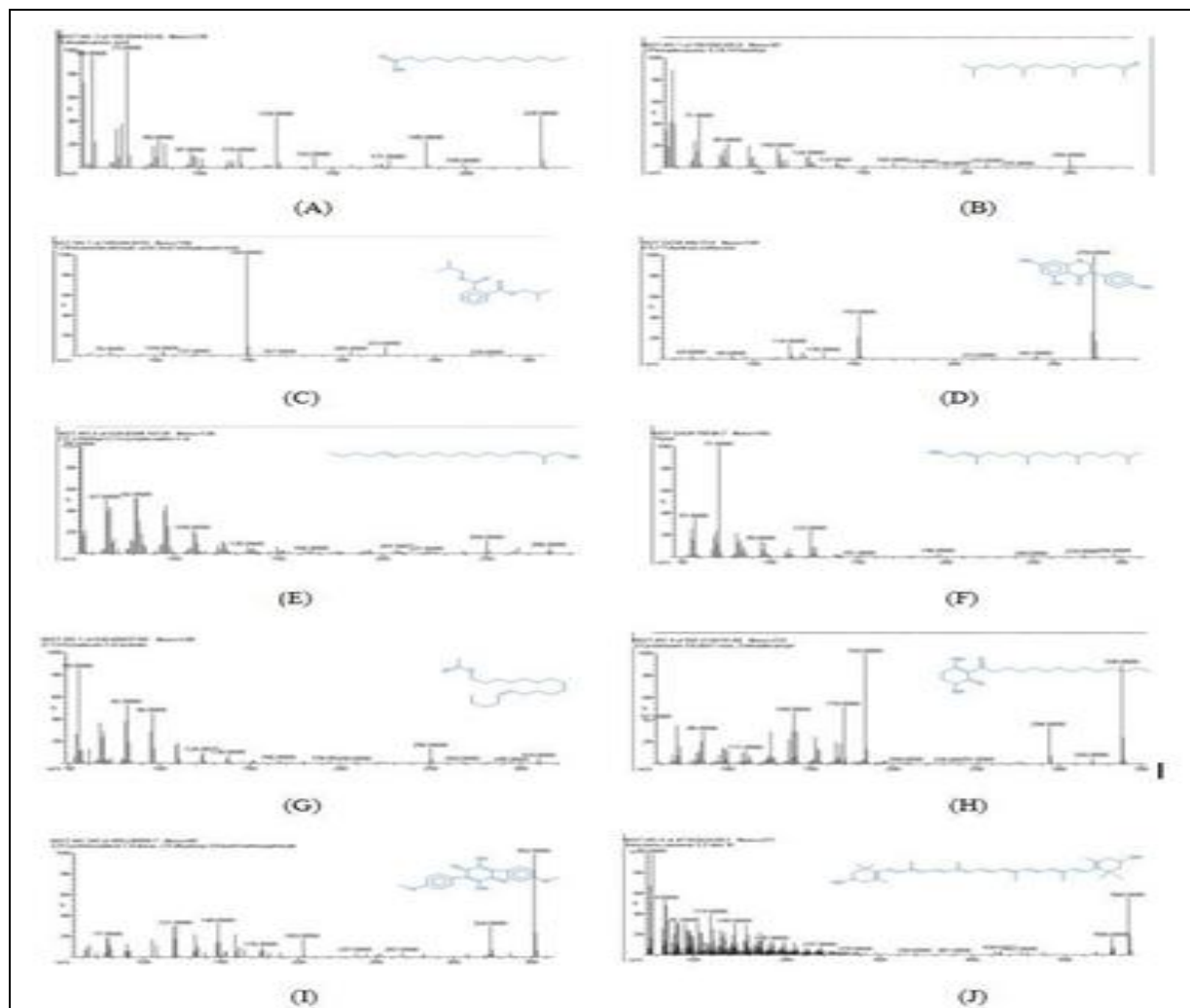


FIG. 2: (A) TETRADECANOIC ACID, (B) 2-PENTADECANONE,6,10,14-TRIMETHYL, (C) 1,2-BENZENE DICARBOXYLICACID,BIS(2-METHYLPROPYL)ESTER, (D) 4,5,7-TRIHYDROXY-ISOFLAVONE, (E) Z,E-2 METHI-3,13-OCTADECADIEN-1OL, (F) PHYTOL, (G) Z-13-OCTADECEN-1-YL ACETATE, (H) 2-CYCLOHEXEN-3,4-DIOL-1ONE,2 TETRADECANOYL, (I) 2,5-CYCLOHEXADIENE-1,4-DIONE,2,5-DIHYDROXY-3,6-BIS(4METHOXYPHENYL), (J) BETA, BETA-CAROTENE-3,3'-DIOL

Absorbance Measurement: The concentration of lutein in whole plant extract using three different solvents at 0 and 24th hour of elution was measured from the lutein standard graph shown in **Fig. 3** and their values are mentioned in **Table 2**.

TABLE 3: ABSORBANCE AND CONCENTRATION OF LUTEIN WITH THREE DIFFERENT SOLVENT EXTRACTS

Solvent		Standing time	
		0 hour	24 th hour
Acetone	Absorbance (AU)	0.42	1.83
	Concentration (mg/g)	0.08	0.39
Methanol	Absorbance (AU)	0.50	1.86
	Concentration (mg/g)	0.12	0.41
Ethanol	Absorbance (AU)	0.52	1.74
	Concentration (mg/g)	0.15	0.37

From the table, methanolic extract shows the maximum concentration of lutein with the standing time of 24 hours when compared to ethanolic and acetone extract. The concentration of lutein extracted from marigold in methanolic extract was found to be 0.00016mg/g; ethanolic extract contains 0.00106mg/g and acetone extract measures about 0.0053mg/g at 27 hour which was very low when compared to *Eichhornia crassipes* lutein concentration²¹. The concentration of lutein from marigold at 9th hour in butanol extract was found to be 0.0016mg/g, ethanolic extract had 0.0033mg/g and acetone extract measured about 0.0038mg/g which is still very low concentration of lutein when compared to lutein from *Eichhornia crassipes*²².

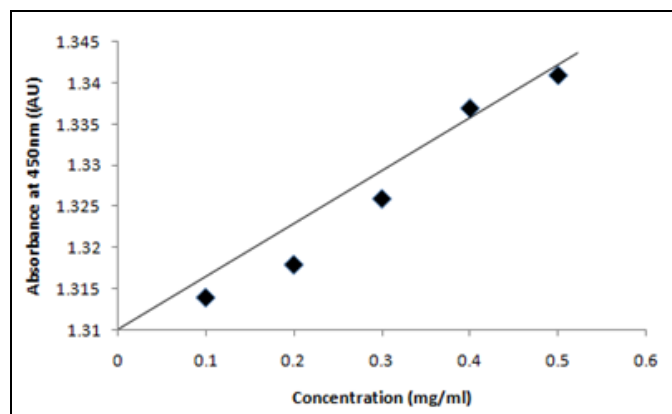


FIG. 3: STANDARD GRAPH OF LUTEIN

Carotenoid Test: The whole plant extract of *Eichhornia crassipes* with three different solvents (acetone, ethanol, methanol), when reacts with sodium nitrite and sulphuric acid, the carotenoids present in the plant extract turns dark green colour

(**Fig. 4**) to spoiled green colour (**Fig. 5**). This disappearance of colour indicates the presence of lutein which is a carotenoid²³.



FIG. 4: PLANT EXTRACT OF DIFFERENT SOLVENTS OF DARK GREEN COLOUR



FIG. 5: PLANT EXTRACT OF DIFFERENT SOLVENTS FORMING SPOILED GREEN COLOUR

Thin Layer Chromatography: The TLC performed for the plant extract and the standard lutein (**Fig. 6**).

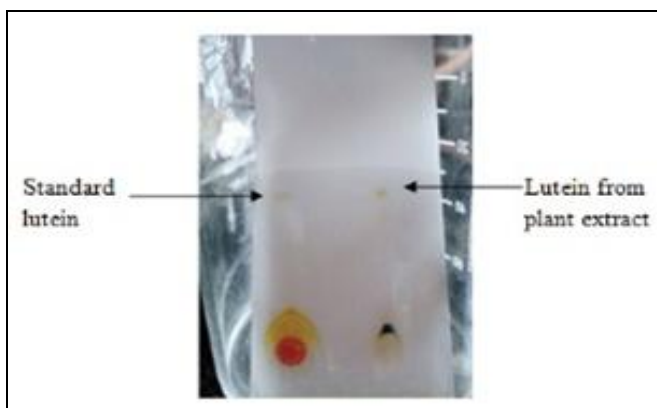


FIG. 6: TLC PLATE OF STANDARD LUTEIN AND PLANT EXTRACT CONTAINING LUTEIN

The plant extract band was in resonance with the standard lutein band migration. Hexane was used as the mobile phase. Since lutein was the most hydrophobic of all carotenoids, hexane drives lutein first and elutes it which was shown as light yellow band. This discloses that hexane can be

used as a mobile phase for the column chromatography by which lutein elutes can be obtained. Suggested that hexane: ethyl acetate mixture at 70:30 ratio was found to be the most suitable mobile phase for the separation of free lutein¹¹. This was further confirmed by another author, who stated the best separation and the quantification of lutein was obtained by the solvent mixture ethyl acetate: acetone 5:4 (v/v)²⁴.

The R_f values of lutein standard and plant extract were calculated and found to be 0.66 and 0.68 respectively. This partially confirmed the presence of lutein in the plant extract.

Column Chromatography: The plant extract concentrated in rotary evaporator was then separated using column chromatography. Column grade silica gel (60 – 120 mesh) was used as stationary phase and hexane, a non polar solvent was used as the mobile phase. Since lutein is hydrophobic it gets eluted by the hexane first. The collected lutein sample was yellowish in colour. The preparative column was carried with mobile phase (Hexane: ethyl acetate (70:30) v/v) at flow rate of 10min/ml can yield 97.1% pure free lutein¹¹. Repeated column chromatography was carried

out with petroleum ether (PE), ethyl acetate (EA) and methanol as a mobile phase. Orange colour fraction was eluted using PE: EA (9:1)²⁵.

High Performance Liquid Chromatography (HPLC): Lutein present in the plant extract and column fraction was quantified by HPLC with the standard lutein shown in **Fig. 7, 8, 9**. The plant extract has three peaks at 450 nm at 2.2, 2.9 and 5.15 minutes as retention time, which indicates the presence of carotenoids. The quantified area percentage for three peaks of plant extract was 17.64% for peak 1, 28.23% for peak 2 and 54.11% for peak 3. The column fraction of lutein forms a single sharp peak at 450 nm in 5.152 minutes of retention time which has the 100% of peak area. The standard lutein forms a single sharp peak at 450nm in 5.09 minutes of retention time with 100% of peak area. The similar results were obtained in HPLC chromatogram of standard lutein and free lutein showing peak at 6.7minutes of retention time and 90.7% lutein⁶. HPLC analysis of lutein indicated the absence of chlorophyll at 660nm and the presence of lutein showed the peak at 18 minutes of retention time for both the standard lutein and lutein extract of *Commelina benghalensis*²⁶.

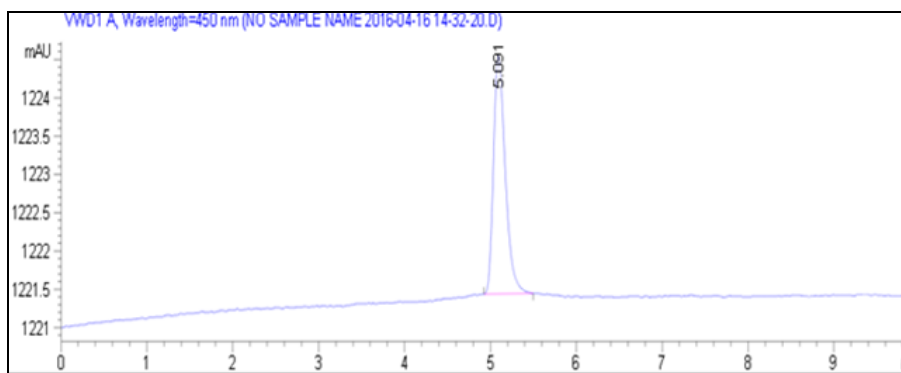


FIG. 7: HPLC OF STANDARD LUTEIN FORMING PEAK AT 5.09 MINUTES OF RETENTION TIME AT 450nm

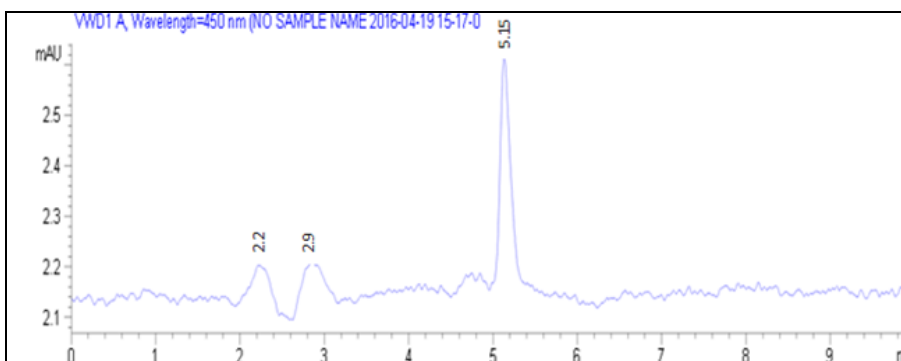


FIG. 8: HPLC OF PLANT EXTRACT FORMING PEAK AT 2.2 (PEAK 1), 2.9 (PEAK 2) AND 5.15 (PEAK 3) MINUTES OF RETENTION TIME AT 450nm

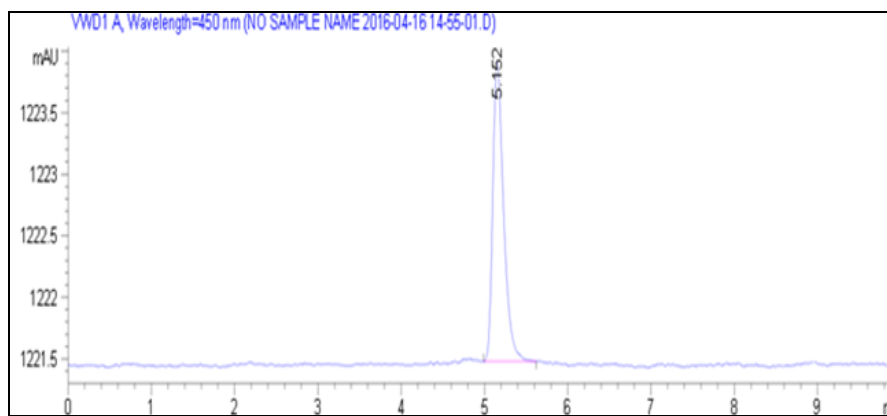


FIG. 9: HPLC OF LUTEIN SEPARATED FROM COLUMN FRACTION FORMING PEAK AT 5.152 MINUTES OF RETENTION TIME AT 450nm

The plant extract having one peak at 5.15 minutes and column fraction forming single peak at 5.15 minutes quantifies the amount of lutein, which is similar to the peak formed for the standard lutein at 5.09 minutes. The peak of standard lutein similar to the peaks at plant extract and column fractionated compounds confirmed and quantified the presence and percentage area of lutein respectively. The supercritical fluid method of extraction showed 90.7% purity at 6.7min⁶. Different solvents used to remove the chlorophyll and increase the concentration of lutein from different combination of solvent for extraction of lutein diethyl ether and methanol in 2:1 ratio, absence of chlorophyll was confirmed by HPLC profile²⁶. In this study, methanol extract of column fraction showed single clear peak of lutein in accordance with standard lutein.

Synthesis of Micro-emulsion: The synthesized micro-emulsion forms the monophasic solution after sonication. In the first cycle of 30 minutes solution forms the milky white solution and during second cycle of 30 minutes forms the turbid isotropic micro-emulsion. The similar results have been obtained by researchers earlier¹²⁻¹⁴.

Characterization of Lutein Micro-emulsion:

Particle Size and Zeta Potential: Particle size distributions of MEL at stable ratios of aqueous phase and oil phase as 9:1 (ME01L), 9.2:0.8 (ME02L) and 9.4:0.6 (ME03L) were shown in **Fig. 10, 12** and **14**. The particle size and poly dispersity index of ME01L, ME02L, ME03L were found to be (0.41µm, 0.507), (1.37µm, 0.516) and (0.813µm, 0.296) respectively. The zeta potentials of the lutein micro-emulsion of different ratios ME01L, ME02L, ME03L were shown in the **Fig. 11, 13, 15**, which indicates the stability of droplets in micro-emulsion. This was found to be -9.95 mV, -0.902mV, -7.15mV which shows the limited stability, micro-emulsion undergo pronounced aggregation and limited stability respectively. This shows that at very low particle size the stability of the suspension increases in the 9:1 ratio of aqueous phase and oil phase respectively. Lipid lutein nanocarrier (O/W) showed good short term stability at room temperature for one month and the zeta potential was in the range of -40 to -63mV in a claimed²⁷. Zeta potential of the nano emulsion deliberated improved stability in the range between -30 to 60 Mv²⁸.

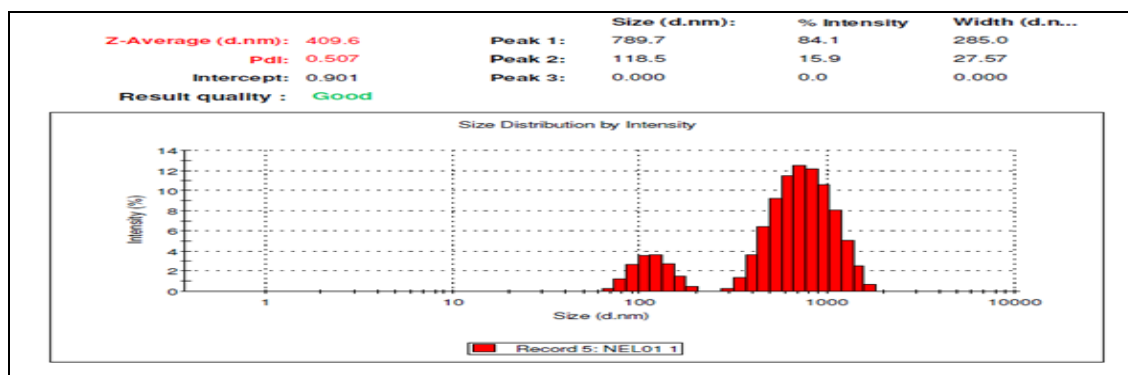


FIG. 10: PARTICLE SIZE DISTRIBUTION OF ME01L

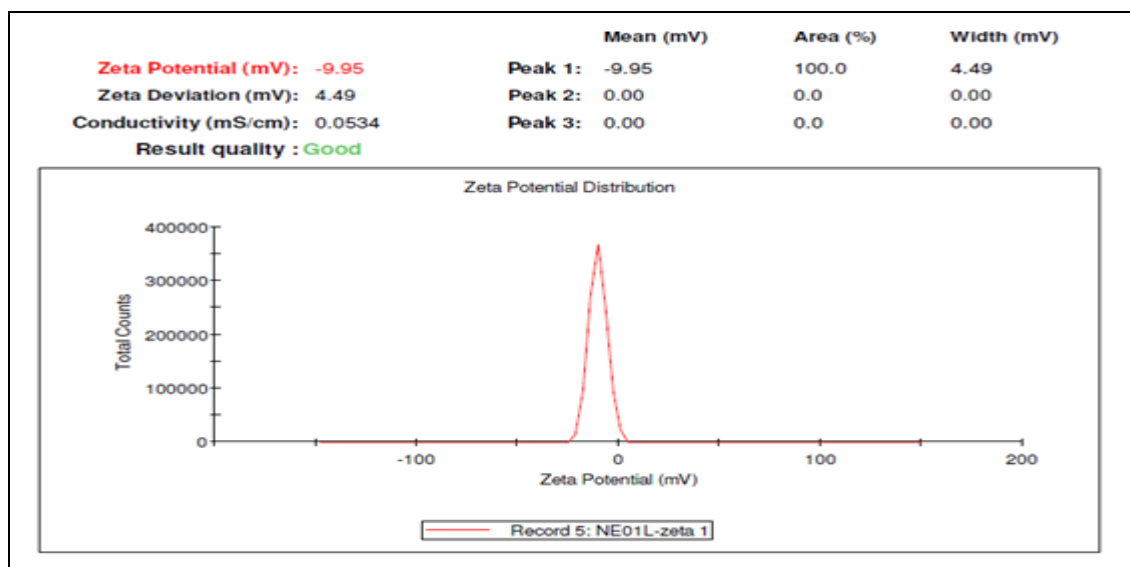


FIG. 11: ZETA POTENTIAL OF ME01L

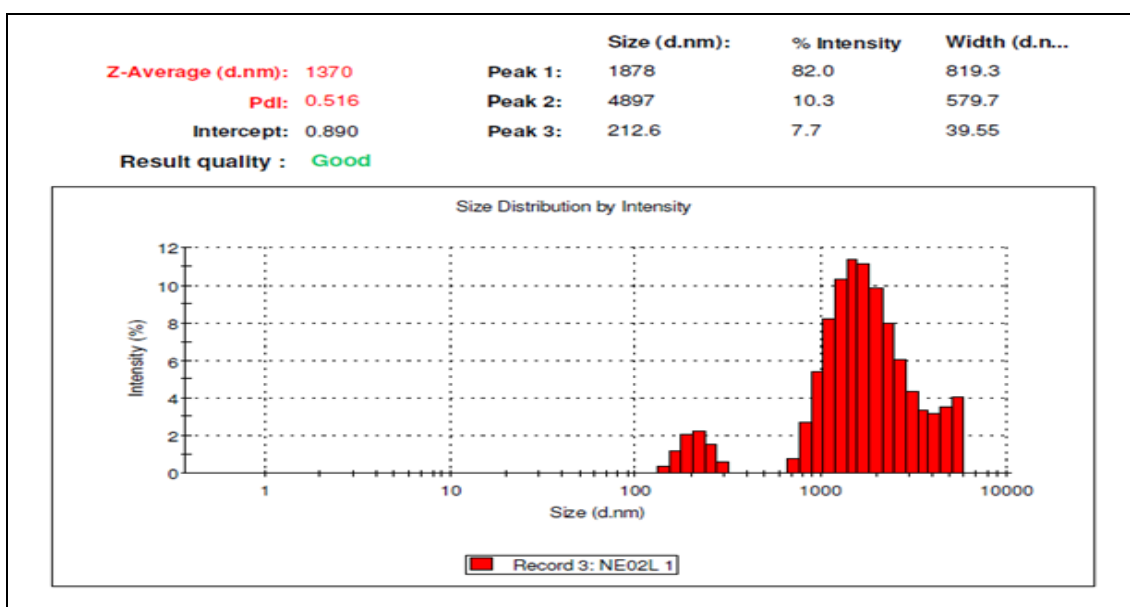


FIG. 12: PARTICLE SIZE DISTRIBUTION OF ME02L

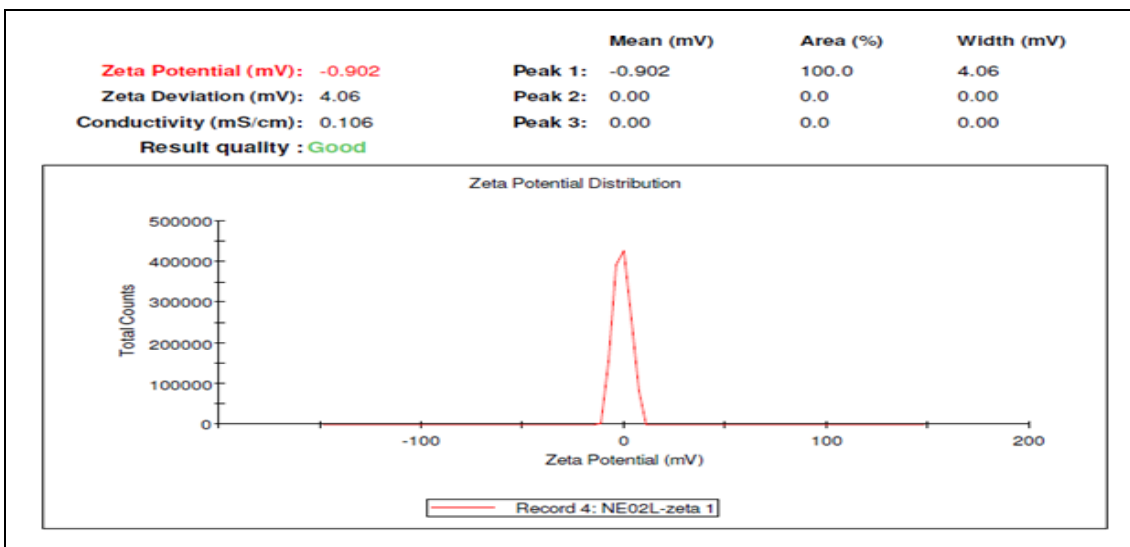


FIG. 13: ZETA POTENTIAL OF ME02L

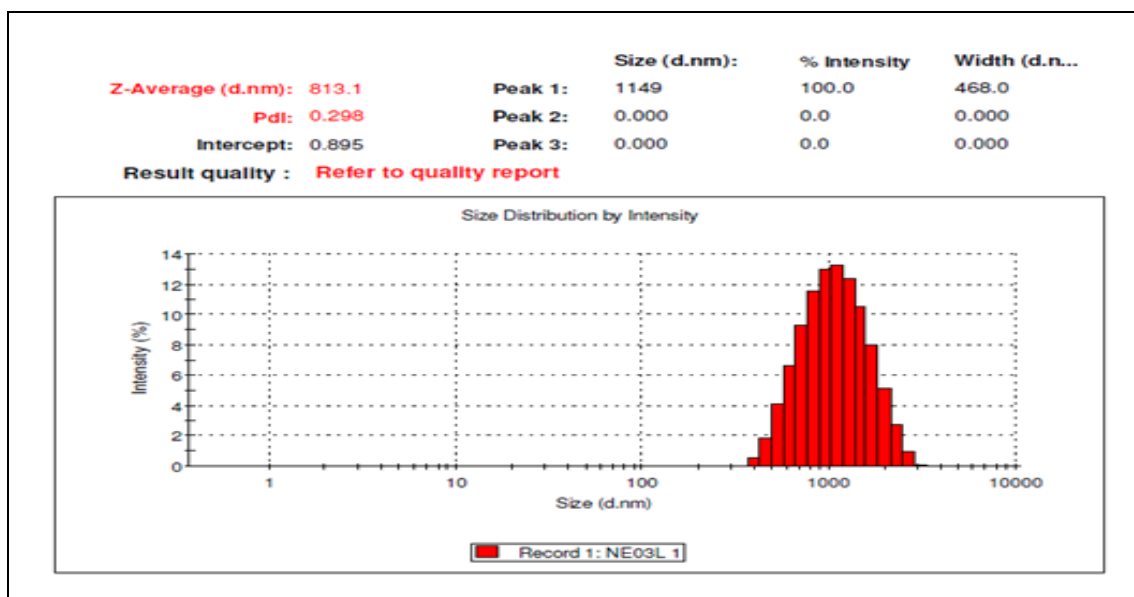


FIG. 14: PARTICLE SIZE DISTRIBUTION OF ME03L

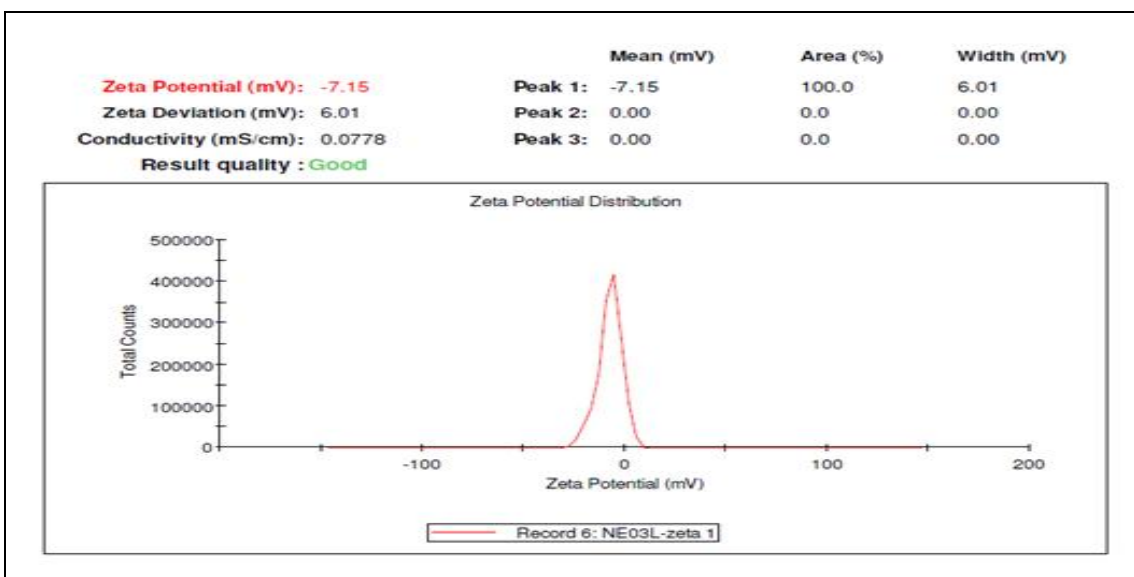


FIG. 15: ZETA POTENTIAL DISTRIBUTION OF ME03L

Entrapment Efficiency of Lutein in Microemulsion (MEL): The lutein entrapped in the micro-emulsion was calculated using the absorbance measured which is interpolated with the standard lutein graph (Fig. 4.1). The concentration of lutein entrapped was measured as 0.11mg/ml from the standard graph. The loading efficiency was calculated from the formula as 99.75% and the weight percentage of lutein in MEL was calculated from the formula as 48.78%. Similar findings were posted by,²⁹ which explain encapsulation efficiency of curcumin in nanoparticle was found to be 88.72% and its weight percentage was found to be 30.72%. In yet another study by,¹⁷ the encapsulation efficiency of curcumin in lipid nanoparticle was found to be 93.8% - 100%.

Antioxidant Assay: The antioxidant activity of standard lutein (SL), extracted lutein (CL) and lutein micro-emulsion (MEL) was calculated for the DPPH radical scavenging activity. The percentage inhibitions of free radical scavenging activity of SL, CL and MEL were measured by interpolating the absorbance measured at 518nm in the standard ascorbic acid graph (Fig. 16). The inhibition percentages of SL, CL and MEL were calculated to be 32.9%, 28% and 44.5% respectively (Fig. 17). MEL exhibited the highest inhibition of free radicals with percentage inhibition of 44.5% than standard and extracted lutein. This increase in anti-oxidant activity in entrapped lutein than readily available lutein can be attributed to the property of uniform dispersive

ability of micro emulsion droplets in the solution and sustained release of lutein into the solution. The comparable result was given ²⁰.

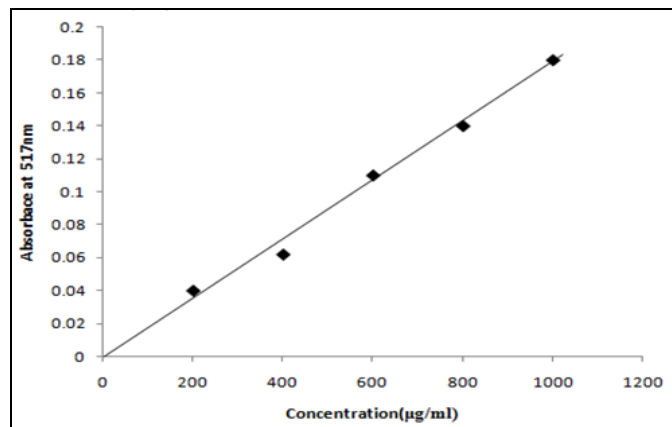


FIG. 16: STANDARD GRAPH FOR ASCORBIC ACID

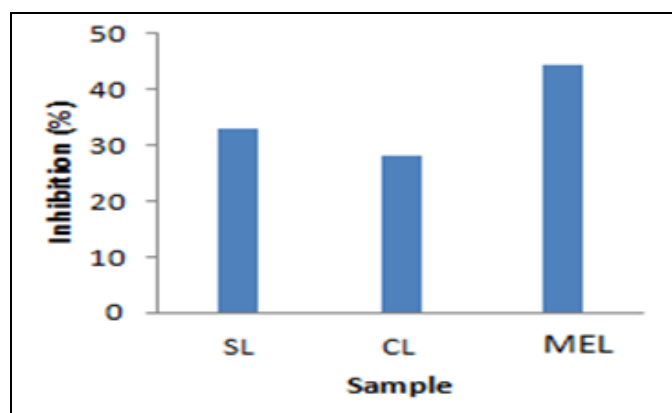


FIG. 17: PERCENTAGE INHIBITION OF SL, CL AND MEL

In vitro Drug Release Profile: The drug release profile (Fig. 18) was constructed for MEL. Based on the absorbance measured at different time intervals, the drug release percentage was calculated. The percentage drug release of MEL was calculated to be 31.8% and that of EL was 8.7% during 150th min. This illustrates the sustained release (increased significantly) of drug over time. Released drug concentration was measured at different time intervals, where MEL showed better percentage release than EL. This result indicates the polished up release of lutein by controlling the particle size of the emulsion to micro-scale level. This hypothesis was already stated ³⁰. The percentage drug release of Glipizide from Glipizide nanoemulsion of F9 and F29 was calculated ¹⁹ and found to be 25% during 180 min. The percentage drug release of dorzolamide hydrochloride nano emulsion was found to be 90% at 150 min ³¹.

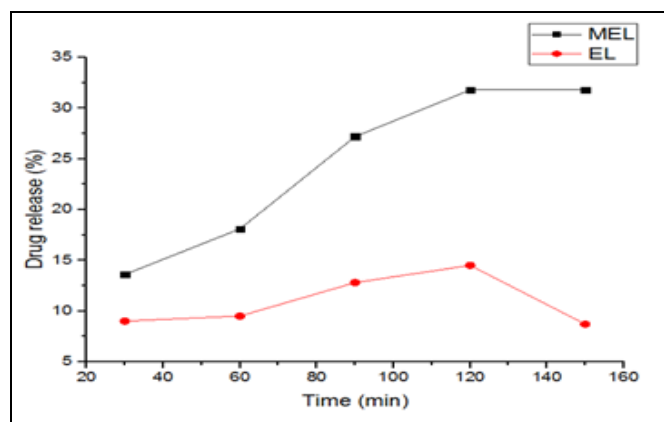


FIG. 18: IN-VITRO DRUG RELEASE PROFILE OF MEL

Ex vivo Drug Release Profile: The *ex vivo* drug release profile (Fig. 19) was constructed for MEL and lutein loaded emulsion (EL). The absorbance was measured for both MEL and EL which releases the drug through cornea of goat's eye over different periods of time intervals. The percentage drug release was measured from the absorbance obtained at different time intervals. This shows the 5.5% release of drug at 150 minutes for EL which was very sluggish, gradual and low amount of release when compared to MEL showing percentage drug release of 10.5% at the same time interval. Thus the MEL forms the fine-tuned release of lutein with reduced size of droplets in emulsions to microscale. This comparison was studied ³² in which the drug release was speculated to be affected by the increase of aqueous content in the nanoemulsion formulation and was justified by the amount of drug permeation per unit area.

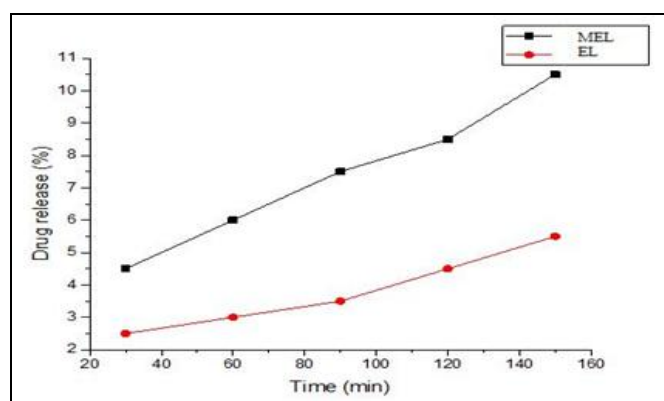


FIG. 19: EX- VITRO DRUG RELEASE PROFILE OF MEL AND EL

CONCLUSION: The *Eichhornia crassipes* isolated lutein entrapped low cost coconut oil: water micro emulsion was prepared which is thermodynamically stable. Entrapment and release

of lutein was better than the kinetically stable nano emulsion. This property may be due to the composition of mid chain fatty acid in the outer surface of the droplets, which will control the transport of carotenoids in and out of the droplet. Drug release from inside of the drop to the solution can be due to the pressure gradient. The pressure from inside of the droplet may be more than the ocular pressure and hence the lutein entrapped inside the droplets is forced to release out of the droplets. Thus this method of lutein delivery to eye sight can effectively target the age related macular degeneration and other eye related diseases by filtering the unfavourable ultraviolet rays.

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