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Antioxidant Activity of *Hemidesmus Indicus* (L.) R.Br. Encapsulated poly (lactide-co-glycolide) (PLGA) Nanoparticles

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Abstract: *Hemidesmus indicus (HI) belonging to the family Asclepediaceae has been used in Indian system of traditional medicine. The study was aimed to synthesize and characterize the aqueous root extract of HI encapsulated poly (lactide-co-glycolide) (PLGA) nanoparticles and further evaluate its antioxidant properties. The root extract was encapsulated on PLGA by solid-in-oil-water solvent evaporation method to improve the solubility, permeability and stability of the compounds in the extract. Physicochemical properties were characterized by Scanning Electron Microscope, X-ray diffraction and Fourier Transform Infrared techniques. The antioxidant properties of free and encapsulated root extract were determined by DPPH, Superoxide and Hydroxyl radical scavenging assays. The PLGA encapsulated nanoparticles showed reduction in their IC50 values in all the assays. The information obtained for this study facilitates the design and fabrication of polymeric nanoparticles as possible drug delivery system and therapeutic applications.*
Keywords: *DPPH, Hemidesmus indicus, PLGA, SEM, XRD*

I. Introduction

Numerous physiological processes in the human body may lead to the generation of oxygen-centered free radicals and other reactive oxygen species as byproducts [1]. These free radicals can cause oxidative damage to biomolecules (DNA, proteins, lipids etc.) leading to diseases like cancer, diabetes, aging and other degenerative diseases in humans [2]. They are degraded to their non-reactive forms by enzymatic [superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT)] and non-enzymatic [ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids] defense mechanisms. Medicinal plants possess large amounts of antioxidants, which can play an important role in neutralizing free radicals by quenching the singlet and triplet oxygen or by decomposing peroxides. Many of these phytochemicals has potent antioxidant capacities and can be used against several human diseases [3]. World Health organization estimated that approximately 80% of world's inhabitants rely mainly on traditional medicines for their primary healthcare and at least 119 chemical substances derived from 90 plant species can be considered as important drugs [4].

The traditional Indian medicine and the use of plant drugs as alternatives against various diseases receive considerable attention in recent days. *Hemidesmus indicus* (L) R.Br. (*H. indicus*) belonging to the Asclepiadaceae family, commonly known as Indian sarsaparilla, is a twining shrub that has been used as folk medicine and as an ingredient in Ayurvedic and Unani preparations against diseases of blood, inflammation, etc.[5]. Some important chemical constituents of the root include 2-hydroxy-4-methoxy-benzoic acid, β-sitosterol, α- and β-amyrins, lupeol, tetracyclic triterpene alcohols, resin acids, fatty acids, tannins, glycosides and a ketone [6]. The root has been used for treating blood diseases, diarrhea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation, rheumatism and in gastric ailments. The root extract also has potent chemoprotective anti-inflammatory, antipyretic and antioxidant properties [7].

There is a growing interest in use of phytochemical as nutraceutical agents in pharmaceutical and food formulations in the past few decades. Although, *H. indicus* has tremendous potential as a therapeutic compound, but its effectiveness and oral bioavailability is limited by poor solubility and poor formulation characteristics of high lipophilicity. In the recent past, research works are being focused on improving poor bioavailable drugs and phytocompounds by nanoencapsulation technique.

Many methods have been developed for preparing nanoparticles. Commonly used methods of preparing nanoparticles from biodegradable polymers include emulsion solvent evaporation [8], nanoprecipitation [9], salting out procedure [10], and a combined method [11]. Nano-encapsulation of drugs in biodegradable polymers like PLGA has received great attention as a possible drug carrier system due to its faster mobility, high drug loading capacity and the possibility of controlled drug release to the specific target site. Moreover, PLGA is approved by US Food and Drug Administration (FDA) and its final degradation products (lactic and glycolic acids) are compatible and safe, as they are either excreted by kidneys or enter the Krebs' cycle to be eventually eliminated as carbon dioxide and water [12].

Therefore, the aim of this study was to compare the antioxidant properties of methanolic (MeOH) and aqueous (Aq.) extracts of *H. indicus* and to encapsulate *H. indicus* extract within polymeric nanoparticles, using the biocompatible copolymer PLGA and characterize their physicochemical and antioxidant properties.

II. Materials And Methods

2.1 Chemicals and Reagents

Gallic acid, quercetin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid (TBA) and PLGA were obtained from Sigma (St. Louis, MO, USA.). All organic solvents were of spectral grade and general chemicals were of analytical grade and were purchased from local companies.

2.2 Preparation of *H. Indicus* Extract

Fresh samples of *H.indicus* roots were collected from Jnana bharathi campus, Bangalore University, Bangalore, Karnataka. Authentication of the plants was carried out in Department of Botany, Bangalore University Bangalore.

The roots were washed thoroughly under running tap water and shade dried at room temperature and finely powdered using a blender. The Aq. and MeOH extracts were prepared by soaking the powder in water and methanol respectively for about 24hours. The extracts were filtered through whatmann filter paper and concentrated using rotary evaporator at 40^0C. The extracts were further concentrated by lyophilization and stored at 4^0C until further use.

2.3 Preparation of Nano-Encapsulated Aqueous *H. Indicus* Extract (NEAE)

PLGA nanoparticles were prepared according to the solid-in-oil-in-water (s/o/w) emulsion technique [13]. Briefly, 50 mg of PLGA was dissolved in dichloromethane for 6 hours to obtain uniform PLGA solution. 10 mg Aq. extract was added and sonicated at 55w for 1min to produce s/o/w emulsion. The resulted nano-sized particles were stirred in the emulsion for 3 h for solvent evaporation. The final emulsion was centrifuged at 15,000 x g (Plasto crafts, India) for 15 min to remove the residual solvent. The nanoparticles obtained were washed thrice with deionized distilled water, and finally resuspended in deionized water and dried on a lyophilizer. The nanoparticles were stored at 4 °C till further use.

2.4 Characterization of PLGA Nanoparticles.

2.4.1. X -Ray Diffraction (XRD) Measurement

X-ray diffractograms of Aq., NEAE and PLGA nanoparticles were obtained with an x-ray diffractometer (Shimadzu XRD 7000 maxima, Japan) using Ni-filtered CuKa radiation (35 kV, 15 mA).

2.4.2. Surface Morphological Characterization of AHIE-PLGA Nanoparticles by Scanning Electron Microscope (SEM)

The NEAE and PLGA nanoparticles were characterized by SEM (TM 3000, Hitachi, Japan). The NEAE and PLGA nanoparticles were mounted on an aluminum stub using double sided carbon tape. The images were captured on SEM mode at desired magnification.

2.4.3 Spectroscopic Characterization

The Fourier transform infrared (FTIR) spectra of PLGA nanoparticles, Aq. extract and NEAE were recorded on KBr plates in the scanning range of 400–4000 cm^{-1} and at 1 cm^{-1} resolution [Perkin Elmer Spectrometer (Spectrum 1000)].

2.5. Determination of Phenol Content

The total phenol content was determined using the method of Singleton *et al*. [14]. Appropriate dilutions of the extracts were mixed with 2.5 mL of 10% Folin–Ciocalteu reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was

measured at 765 nm in a spectrophotometer (ELICO SL-210). The total phenol content was calculated using gallic acid as standard.

2.6. Determination of Flavonoid Content

The flavonoid content was determined using a modified method by Meda *et al.* [15]. Briefly, 0.5 mL of appropriately diluted sample was mixed with 0.5 ml methanol, 50 µl of 10% $AlCl_3$, 50 µl of 1M Potassium acetate and 1.4 ml water and allowed to incubate at room temperature for 30 minutes. Thereafter, the absorbance of the reaction mixture was measured at 415 nm. The total flavonoid content was calculated using quercetin as standard.

2.7. Antioxidant Assay (DPPH Assay)

DPPH assay was carried out according to the method of Shon *et al.* [16]. Briefly, 0.5 ml of the sample extracts and nanoencapsulated extract were added to 1 ml of DPPH solution (0.1 mM, in 95% ethanol) and incubated at room temperature for 20 min. The absorbance was read at 517 nm against blank.

$$\text{Scavenging effect (\%)} = \frac{\text{Abs}_{\text{Control(517)}} - \text{Abs}_{\text{Sample (517)}}}{\text{Abs}_{\text{Control (517)}}} \times 100$$

Where Abs control = absorbance of the control (reacting mixture without the test sample) and, Abs test sample = absorbance of reacting mixture with the test sample.

2.8. Degradation of Deoxyribose (Fenton's Reaction)

The ability of the extracts to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge [17]. Briefly, different concentrations of 20 µl of extracts and nanoencapsulated extract were added to a reaction mixture containing 120 µl 20 mM deoxyribose, 400 µl 0.1 M phosphate buffer, 40 µl 20 mM hydrogen peroxide and 40 µl 500 µM $FeSO_4$, and the volume was made upto 800 µl with distilled water. The reaction mixture was incubated at 37°C for 30 min, and the reaction was stopped by the addition of 0.5 ml of 2.8% TCA (trichloroacetic acid). This was followed by the addition of 0.4 ml of 0.6% TBA solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at measured at 532 nm in a spectrophotometer.

$$\text{Percentage OH radical scavenging ability (\%)} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{test sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

Where Abs control = absorbance of the control (reacting mixture without the test sample) and, Abs test sample = absorbance of reacting mixture with the test sample.

2.9. Superoxide Radical Scavenging Assay

Superoxide radical scavenging was measured according to the method of Nishikimi *et al*. [18]. 1ml of NBT was added to 1 ml of sample extracts and nanoencapsulated extract followed by 100µl of PMS in phosphate buffer. The reaction mixture was incubated at 25^0C for 5 min and the absorbance was measured at 560nm.

$$\text{Scavenging effect (\%)} = \frac{\text{Abs}_{\text{Control(517)}} - \text{Abs}_{\text{Sample (517)}}}{\text{Abs}_{\text{Control (517)}}} \times 100$$

Where Abs control = absorbance of the control (reacting mixture without the test sample) and, Abs test sample = absorbance of reacting mixture with the test sample.

2.10. Statistical Analysis

Values are expressed as mean ± SE. Statistical analysis was performed by one and two way analysis of variance followed by Tukey's test using SPSS (Armonk, NY: IBM Corp) 20 software package for Windows.The results were considered statistically significant for $p< 0.05$.

III. Results

3.1. Total Phenol and Total Flavanoid

Table 1 represents the total phenol and total flavonoid content in MeOH and Aq. extracts. Insignificant changes were noticed in the flavonoid content between the extracts. Whereas, Aq. extract showed significantly higher phenolic content (33 %) over MeOH extract.

Table 1. Polyphenols content in *H. indicus* extracts

Sample	Total phenols(mg GAE/g extract)	Total flavonoids(mg QE/g extract)
Aqueous extract	12.25 ± 2.25 a	0.028 ± 0.002 a
Methanolic extract	4 ± 0.5 b	0.019 ± 0.001 a

Values are represented as mean ± S.E (n=3). Significance between the extracts is represented in lower case (p<0.05). Those not sharing the same letters are significantly different.

3.2. Characterization of Nanoparticles

The XRD patterns for Aq. extract, NEAE and free PLGA nanoparticles are represented in **Fig. 1**. For PLGA and *H. indicus* loaded PLGA nano particle, the XRD pattern showed no characteristic peak. Aq. extract exhibited characteristic signals of their crystalline structures. The SEM micrographs of Aq. extract and NEAE were recorded. The Aq. extract shows larger size triangular crystals while PLGA encapsulated nanoparticle extract appeared as spherical shape with smooth surface and the size of the particles was found to be < 200 nm **(Fig.2).**

The intermolecular interaction between Free PLGA, Aq. extract and NEAE was determined by FTIR spectroscopy are shown in **Fig. 3.** The characteristic spectra of the PLGA polymer showed the –CH, $–CH_2$, $–CH_3$ stretching at 2850–3000 cm^{-1}, C–O stretching at 1050–1250 cm^{-1}, carbonyl –C=O stretching at 1700–1800 cm^{-1} and –OH stretching at 3200–3600 cm^{-1}. The plant extract spectra showed characteristic bands corresponding to the functional groups. For *H. indicus* loaded PLGA nanoparticles, the spectra showed that the OH stretching band (3200–3600 cm^{-1}) is slightly shifted to a lower wave length and some peaks were not observed in the free PLGA and *H. indicus* Aq. extract.

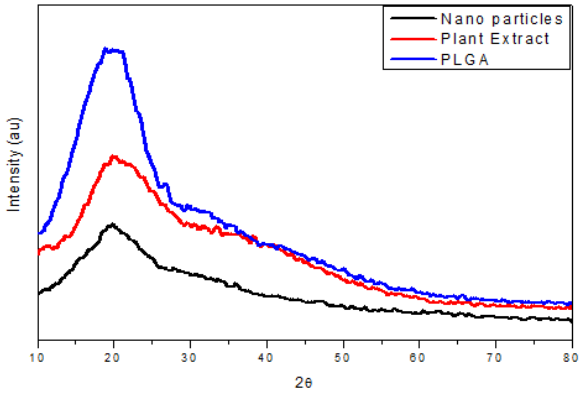

Fig 1. XRD spectra of aqueous extract, free PLGA and NEAE

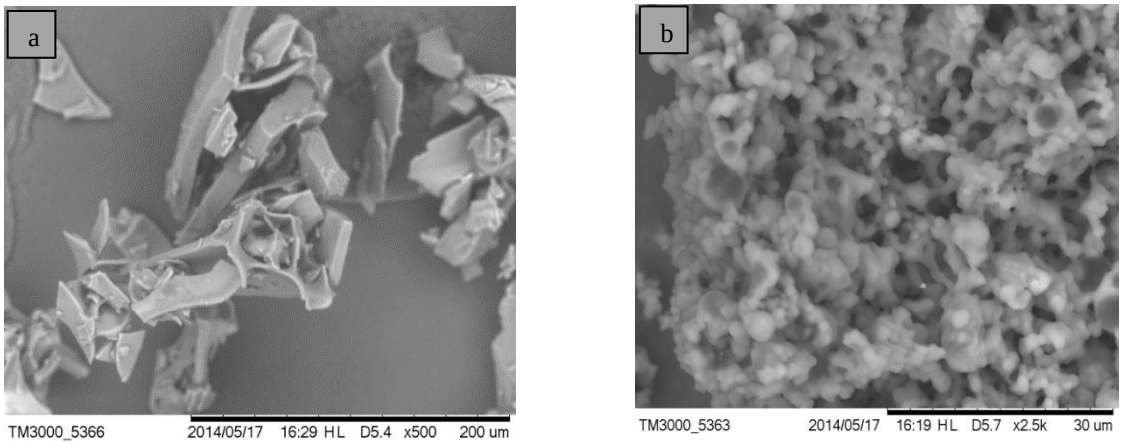

Fig.2: SEM micrographs of (a) Aqueous extract (b) NEAE

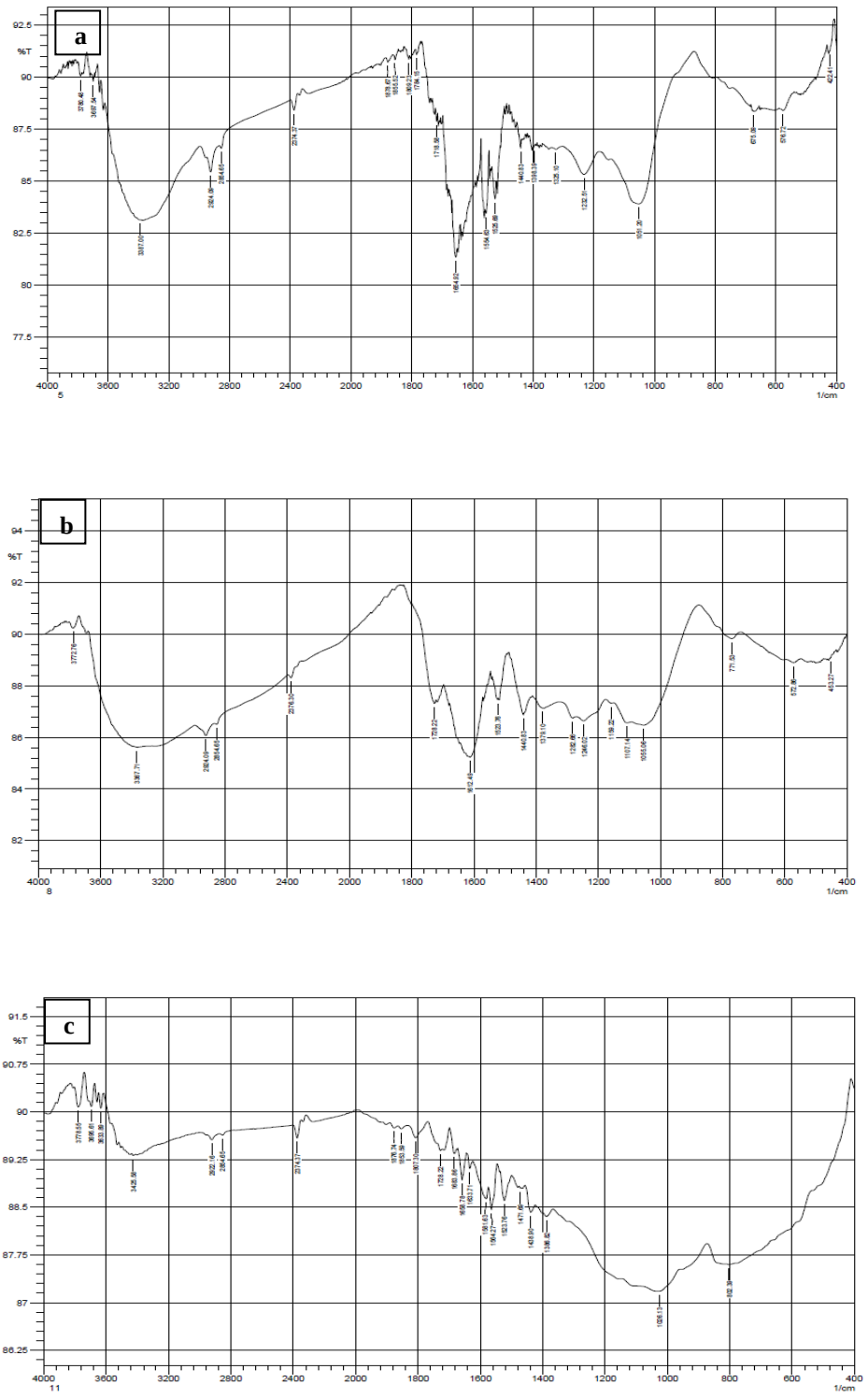

Fig 3. FTIR spectra of (a) Aqueous extract (b) Free PLGA and (c) NEAE

3.3. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the extracts is represented in **Fig. 4a.** The MeOH and Aq. extracts of *H. indicus* exhibited concentration dependent antiradical activity. The Aq. extract was significantly ($p< 0.05$) more effective in scavenging the free radicals than BHT with an IC_{50} value of 90µg/ml.

Fig. 4b represents the scavenging activity of the extracts and NEAE. The NEAE showed similar trend as that of the extracts and exhibited significant ($p< 0.05$) percent of inhibition (17%) at the same concentration compared to extracts with an IC_{50} value of 80µg/ml.

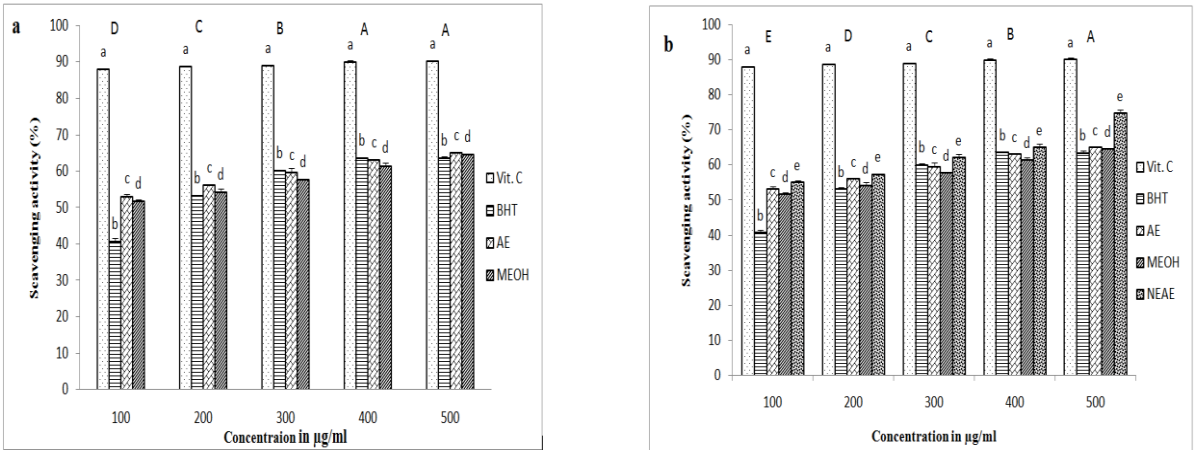

Fig 4. DPPH radical scavenging activities of *H.indicus* (a) aqueous and methanolic extracts (b) NEAE.
Values are represented as mean ± S.E (n=3). Significance between the extracts is represented in lower case ($p<0.05$) and between the concentrations is represented in upper case. Those not sharing the same letters are significantly different. BHT-Butylated hydroxytoluene; AE- Aqueous extract; MeOH-Methanolic extract; NEAE- Nanoencapsulated aqueous extract

3.4 Fenton's Reaction

The ability of the MeOH and Aq. extracts of *H. indicus* to inhibit Fe (II)/H_2O_2- induced decomposition of deoxyribose as an index for hydroxyl radical scavenging ability is represented in **Fig. 5a.** The extracts significantly scavenged the hydroxyl radicals in a dose dependent manner compared to the BHT. The Aq. extract significantly showed better inhibition (62%) with an IC_{50} value of 34 µg/ml at concentration of 80 µg/ml.

Another remarkable feature is that Nano extracts significantly showed a better scavenging activity than vitamin C. The NEAE exhibited maximum inhibition (63%) compared to Aq. extract with an IC_{50} value of 13.5µg/ml (**Fig. 5b**).

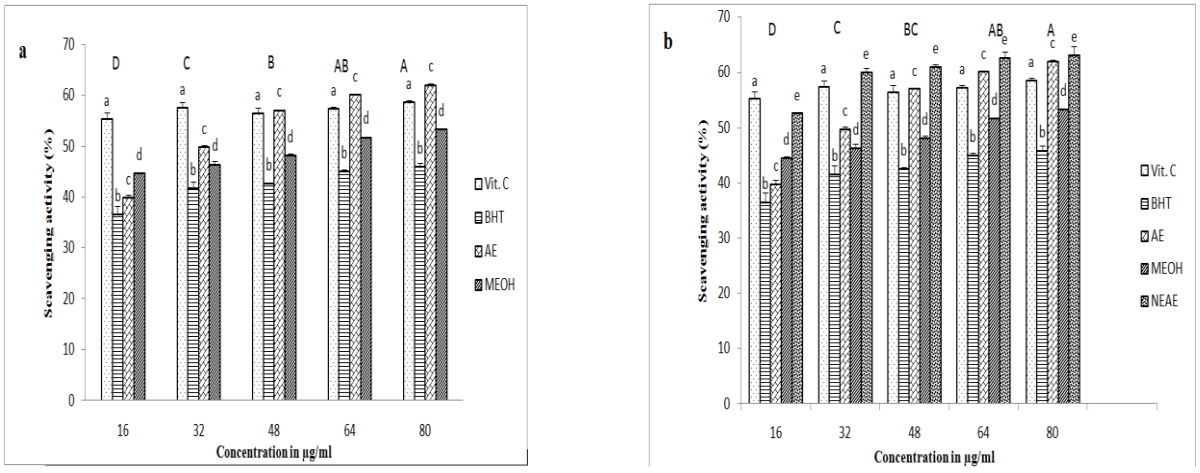

Fig 5. Hydroxyl radical scavenging activities of *H.indicus* (a) aqueous and methanolic extracts (b) NEAE.
Values are represented as mean ± S.E (n=3). Significance between the extracts is represented in lower case ($p<0.05$) and between the concentration is represented in upper case. Those not sharing the same letters are significantly different. BHT-Butylated hydroxytoluene; AE-Aqueous extract; MeOH-Methanolic extract; NEAE-Nanoencapsulated aqueous extract

3.5 Superoxide Radical Scavenging Activity

The superoxide radical scavenging activities of the extracts and NEAE showed similar trend as that of hydroxyl radical scavenging activity. **Fig. 6** indicates that NEAE rendered higher inhibition (74%) with an IC_{50} value of 145µg/ml compared to Aq. extract with an IC_{50} value of 265µg/ml. The scavenging activity was significantly better than BHT.

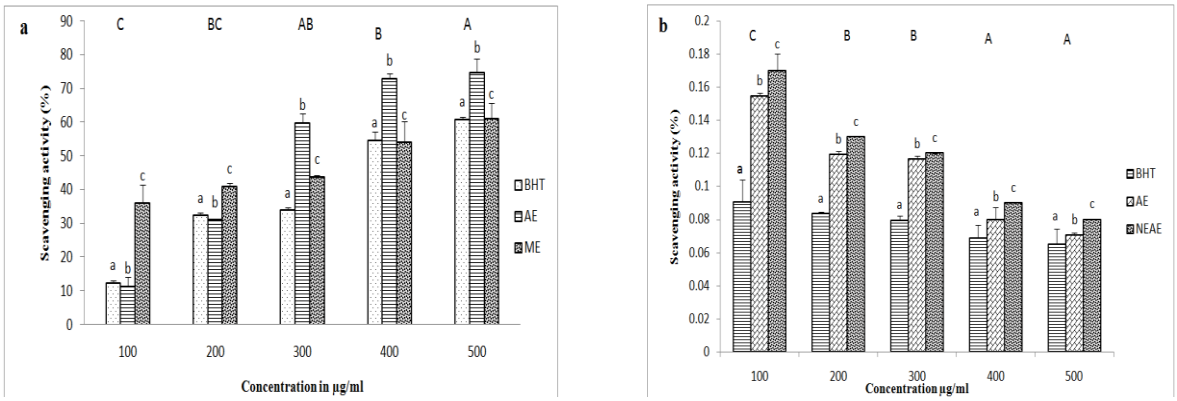

Fig 6. Superoxide radical scavenging activities of *H.indicus* (a) aqueous and methanolic extract (b) NEAE
Values are represented as mean± S.E (n=3). Significance between the extracts is represented in lower case (p<0.05) and between the concentrations is represented in upper case. Those not sharing the same letters are significantly different. BHT-Butylated hydroxytoluene; AE-Aqueous extract; NEAE-Nanoencapsulated aqueous extract

IV. Discussion

Phenolic compounds and flavonoids are the major constituents in most plants reported to possess antioxidant and free radical scavenging activity [19]. Phenolic compounds are efficient free radical scavengers exhibiting antioxidant activity by inactivating lipid free radicals, or by preventing the decomposition of hydroperoxides into free radicals. Flavonoids, the largest among the natural phenolics, possess antioxidant properties acting as effective scavenger of deleterious free radicals and reactive oxygen species [20]. Their antioxidant activity depends on the number and location of hydroxyl groups in the aromatic ring system [21].

In the present study, high phenolic and flavonoid content was observed in the Aq extract of *H. indicus* whichmay contribute to its potential antioxidant property and curative ability by adsorbing and neutralizing free radicals. The results are in agreement with the studies of Kumar et al. [22] where in higher phenolic and flavonoid content was obtained in Aq. extracts of *H. indicus*.

Encapsulated bioactive compounds into nanometric delivery systems are being increasingly tested in food system with the intention to improve the bioavailability of the hydrophobic phytocompounds. Selection of right kind of polymer becomes essential to minimize the impact on the quality attributes of the final product. At present, PLGA is extensively used in drug delivery systems. There are several reports published in the recent years on the use of PLGA polymer as an ideal carrier system for the encapsulation of substances like curcumin [23], ferulic acid [24], quercetin [25], vitamin E [26], saponin [27], and plant extracts [4,28] etc. PLGA has the property to cross the blood-brain barrier and therefore it can be used as a suitable polymer for compounds used in treating neurological and psychological disorders as well.

Information on the structural organization of the bioactive molecules and polymeric matrix within the nanoparticles was determined by XRD. In the present study, XRD pattern of free PLGA showed no characteristic peak, a fact that indicated that the polymer is amorphous in nature [25]. The Aq. extract exhibited characteristic signals of their crystalline structures. The absence of characteristic peaks in the NEAE indicate that Aq. extract was dispersed in a noncrystalline state within the PLGA polymeric matrix.

The structure of the nanoparticles plays an important role in determining their adhesion, interaction and absorption with the body cells. NEAE displays a spherical shape with a smooth surface. These results are in agreement with the previous studies of Pool *et al*. [25] and Nallamuthu *et al*. [29].

FTIR measurement was carried out to detect the functional groups of compounds. It is based on the fact that bonds and groups vibrate at characteristic frequencies. A molecule that is exposed to infrared rays absorbs

infrared energy at frequencies which are characteristic to that molecule. The spectra obtained for free PLGA nanoparticles showed characteristic bands that were consistent with the studies of Pool *et al*. [25] and Nallamuthu *et al*. [29]. The slight shift in the spectra of OH-stretching band (3200–3600 cm^{-1}) for *H.* indicus loaded PLGA nanoparticle may be due to increase in terms of energy absorption. These observations suggest that *H.indicus* Aq. extract is associated with the PLGA polymer by hydrogen bonds. Also, the band corresponding for C=O stretching (1700–1800 cm^{-1}) was broader, indicating that the extract is associated with the PLGA polymer by interactions between the carbonyl and the carboxyl groups of the flavonoids in the extract and the polymer.

The antioxidant capacity was determined by the $DPPH^{\bullet}$ radical scavenging activity. $DPPH^{\bullet}$ is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The methodology involves the reaction of a specific compound or extract with DPPH in methanol solution. In the presence of hydrogen donors, DPPH is reduced and a free radical is generated from the scavenger. The reaction of DPPH is monitored by measuring the decrease of the absorbance of its radical at 517 nm. Upon reduction of this radical by an antioxidant, the absorbance at 517 nm disappears [30].

The results revealed that extracts showed higher potency in scavenging the DPPH free radicals than BHT due to its high content of phenolic and flavonoid compounds. The NEAE showed higher inhibition compared to the extracts. These results indicate that the NEAE maintained its full antioxidant activity. Previous reports by Das *et al*. [4] and Bhattacharyya *et al*. [31] also revealed enhanced scavenging activity in PLGA encapsulated plant extracts.

The hydroxyl radical ($^{\cdot}OH$) is the most reactive radical known to initiate lipid peroxidation and damage of biomolecules [2]. The effect of the extracts on $^{\cdot}OH$ generated by Fe3+ ions was measured by determining the degree of deoxyribose degradation. The NEAE scavenged $^{\cdot}OH$ radical more effectively compared to the extracts. The antioxidants in the plant extracts competed with deoxyribose against the $^{\cdot}OH$ radical generated from the Fe^{3+}-dependent system. The antioxidants in these plant extracts could be acting as chelators of the Fe^{3+} ions in the system, thereby preventing them from complexing with the deoxyribose, or simply donating hydrogen atoms and accelerating the conversion of H_2O_2 to H_2O [32].

Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive species. Superoxide anion, derived from dissolved oxygen by PMS–NADH coupling reaction, reduces NBT. The decrease in absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion. Our results showed that encapsulated Aq. extract produced greater inhibition of NBT reduction. These findings suggest that Aq. extract dispersed in the PLGA nanoparticles are protected from molecular oxygen that could be produced during incubation process. Therefore, after incubation, encapsulated extracts were released and scavenged the $O_2^{\bullet}$ radicals, as opposed to the case of free extracts, which are degraded after incubation, therefore decreasing their scavenging activity.

V. Conclusion

In conclusion, the present study demonstrated the antioxidant activities of *H. indicus* extracts, encapsulation and characterization of PLGA loaded *H. indicus* Aq. extract. Aq. extract exhibited significantly higher ($p<0.05$) ability to scavenge the free radicals which may be attributed to its higher content of phytochemicals. PLGA loaded *H. indicus* nanoparticles showed better therapeutic efficacy compared to Aq. extract as seen by the reduction in IC_{50} values. The study suggests that PLGA nanoencapsulation can be applied to other herbal drugs with poor solubility thereby increasing its bioaccessibility and bioavailability.

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References

[1]. Y. Cai, Q. Luo, M. Sun, and H. Corke, Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer, Life science, 74(17), 2004, 2157-2184.

[2]. B. Halliwell, Free radicals, antioxidants, and human disease: curiosity, cause, or consequence?, Lancet, 344(8924), 1994, 721-724.

[3]. A. Djeridane, M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker, N. Vidal, Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds, Food Chemistry, 97, 2006, 654-660.

[4]. J. Das, S. Das, A. Samadder, K. Bhadra, and A.R. Khuda-Bukhsh, Poly (lactide-co glycolide) encapsulated extract of Phytolacca decandra demonstrates better intervention against induced lung adenocarcinoma in mice and on A549 cells, European Journal of Pharmaceutical Sciences. 47, 2012, 313-324.

[5]. S. Das and S.S. Bisht, The Bioactive and Therapeutic Potential of Hemidesmus indicus R.Br. (Indian Sarsaparilla) Root, Phytotherapy Research, 27, 2013, 791-801.

[6]. P.C. Das, O.C. Joshi, S. Mandal, A. Das, A. Chatterjee, New coumarinolignoids from Hemidesmus indicus R. Br, Indian journal of chemistry, 31B, 1992, 342-345.

[7]. S. Jayaram and S.M. Dharmesh, Assessment of antioxidant potential of free and bound phenolics of Hemidesmus indicus (L) R. Br against oxidative damage, Pharmacognosy research, 3(4), 2011, 225-231.

[8]. S.K. Sahoo, J. Panyam, S. Prabha, and V. Labhasetwar, Residual polyvinyl alcohol associated with poly (D, L-lactide-coglycolide) nanoparticles affects their physical properties and cellular uptake, Journal of Controlled Release. 82, 2002, 105-114.

[9]. E. Leo, B. Brina, F. Forni, and M.A. Vandelli,In vitro evaluation of PLA nanoparticles containing a lipophilic drug in water-soluble or insoluble form, International journal of pharmaceutics. 278(1), 2004, 133-141.

[10]. Y.N. Konan, R. Gurney, and E. Allemann,Preparation and characterization of sterile and freeze-dried sub-200 nm nanoparticles, International journal of pharmaceutics, 233(1-2), 2002, 239-252.

[11]. P.A. McCarron, R.F. Donnelly, and W. Marouf, Celecoxib-loaded poly(D,L-lactide-co-glycolide) nanoparticles prepared using a novel and controllable combination of diffusion and emulsification steps as part of the salting-out procedure, Journal of Microencapsulation: Micro and Nano Carriers, 23(5), 2006, 480-498.

[12]. A. Paul, J. Das, S. Das, A. Samadder, and A.R. Khuda-Bukhsh,Poly (lactide-co-glycolide) nano-encapsulation of chelidonine, an active bioingredient of greater celandine (Chelidonium majus), enhances its ameliorative potential against cadmium induced oxidative stress and hepatic injury in mice, Environmental Toxicology and Pharmacology, 36, 2013,937-947.

[13]. P. Sankar, A.G. Telang, S. Suresh, M. Kesavan, K. Kannan, R. Kalaivanah, and S.N. Sarkar, Immunomodulatory effects of nanocurcumin in arsenic-exposed rats, International Immunopharmacology, 17(1), 2013, 65-70.

[14]. V.L. Singleton, R. Orhofer, and R.M. Lamuella-Raventos, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin -Ciocalteau reagent, Methods in enzymology, 299, 1999, 152-178.

[15]. [15] A. Meda, C.E. Lamien, M. Romito, J. Millogo, and O.G. Nacoulma, Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity, Food Chemostry,91(3), 2005, 571–577.

[16]. M.Y. Shon, T.H. Kim, and N.J. Sung,Antioxidants and free radical scavenging activity of Phellinus baumii (Phellinus of Hymenochaetaceae) extracts, Food Chemistry,82(4), 2003, 593-597.

[17]. Halliwell, B and Gutteridge, J.M.C, Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. FEBS Letter, 128, 1981, 139-205.

[18]. M. Nishikimi, N. AppajiRao, and K. Yagi, The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen, Biochemical and Biophysical Research Communications.46(2), 1972, 849-854.

[19]. R.J. Williams, J.P. Spencer, and C. Rice-Evans, Flavonoids: antioxidants or signalling molecules?,Free Radical Biology & Medicine,36(7), 2004, 838-849.

[20]. [20] O.O. Olajuyigbe and A.J. Afolayan, Phenolic content and antioxidant property of the bark extracts of Ziziphus mucronata Willd. subsp. mucronata Willd, BMC Complementary & Alternative Medicine, 11, 2011, 1-8.

[21]. G. Cao, E. Sofic, and R.L. Prior, Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships, Free Radical Biology & Medicine, 22(5), 1997, 749-760.

[22]. S. Kumar, M. Pooja, K. Harika, E. Haswitha, G. Nagabhushanamma, and N. Vidyavathi. In-vitro antioxidant activities, total phenolics and flavonoid contents of whole plant of Hemidesmus indicus (Linn.), Asian Journal of Pharmaceutical and Clinical Research, 6(2), 2013, 249-251.

[23]. P. Sankar, A.G. Telang, S. Suresh, M. Kesavan, K. Kannan, R. Kalaivanah, and S.N. Sarkar, Immunomodulatory effects of nanocurcumin in arsenic-exposed rats, International Immunopharmacology,17(1), 2013, 65-70.

[24]. J.P.J. Merlin, N.R. Prasad, S.M.A. Shibil, M. Sebeela, Ferulic acid loaded Poly-D,L-lactide-co-glycolide nanoparticles: Systematic study of particle size, drug encapsulation efficiency and anticancer effect in non-small cell lung carcinoma cell line in vitro, Biomedicine & Preventive Nutrition, 2(1), 2012, 69-76.

[25]. H. Pool, D. Quintanar, J.D. Figueroa, C.M. Mano, J.E.H. Bechara, L.A. Godinez, and S. Mendoza, Antioxidant Effects of Quercetin and Catechin Encapsulated into PLGA Nanoparticles,Journal of Nanomaterials, 2012, 2012, 1-12.

[26]. P. Chaiyasat, A. Chaiyasat, S. Teeka, and S. Noppalit, Preparation of Poly(l-Lactic Acid) Microencapsulated Vitamin E, Energy Procedia, 34, 2013, 656-663.

[27]. H.V.D Ven, M. Vermeersch, A. Matheeussen, J. Vandervoort, W. Weyenberg, S. Apers, P.L. Cos, and M.A.L. Ludwig, PLGA nanoparticles loaded with the anti leishmanial saponin β-aescin: Factor influence study and in vitro efficacy evaluation., International Journal of Pharmaceutics, 420 (1), 2011, 122-132.

[28]. A. Samadder, S. Das, J. Das, A. Paul, and A.R. Khuda-Bukhsh, Ameliorative Effects of Syzygium jambolanum extract and its Poly (lactic-co-glycolic) Acid Nano-encapsulated Form on Arsenic-induced Hyperglycemic Stress: A Multi-parametric Evaluation. Journal of Acupuncture and Meridian Studies, 5, 2012, 310-318.

[29]. I. Nallamuthu, A. Parthasarathi, and F. Khanum, Thymoquinone-loaded PLGA nanoparticles: antioxidant and anti-microbial properties, International Current Pharmaceutical Journal. 2(12), 2013, 202-207.

[30]. N. Roy, R.A. Laskar, S.K. Ismail, D. Kumari, T. Ghosh, and N.A. Begum, A detailed study on the antioxidant activity of the stem bark of Dalbergia sissoo Roxb., an Indian medicinal plant,Food Chemistry, 126, 2011, 1115–1121.

[31]. S.S. Bhattacharyya, S. Paul, and A.R. Khuda-Bukhsh, Encapsulated plant extract (Gelsemium sempervirens) poly (lactide-co-glycolide) nanoparticles enhance cellular uptake and increase bioactivity in vitro, Experimental Biology and Medicine, 235, 2010, 678-688.

[32]. F.M. Awah, P.N. Uzoegwu, P. Ifeonu, J.O. Oyugi, J. Rutherford, X. Yao, F. Fehrmann, K.R. Fowke, and M.O. Eze, Free radical scavenging activity, phenolic contents and cytotoxicity of selected Nigerian medicinal plants, Food Chemistry. 131, 2012, 1279–1286.