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Antimicrobial study of *Arjuna Terminalia* loaded PLGA nanoparticle

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The bark of *Terminilia Arjuna* is known for its numerous health benefits in traditional system of medicine. Nanotechnology based delivery system have added advantages of action at targeted location and improved cellular uptake. In the present paper, polymeric nanoparticles of methanolic extract of *Terminilia Arjuna* (Arjun ki chhal) were synthesized by using solvent evaporation method. The synthesized polymeric nanoparticles were further characterized using SEM, TEM, XRD, FTIR followed by evaluation of antimicrobial activity. The SEM images showed spherical shaped nanoparticles. Further TEM images revealed that particles of size as small as 50-75 nm are formed. PLGA encapsulated nanoparticles shows entrapment efficiency as high as 96.8% and percentage yield comes out to be 45.3. Antimicrobial study using cup-plate method was carried out using two strains of gram (+) bacteria *S. aureus* and *B. pumilus* and two strains of gram (–) bacteria *E. Coli* and *Pseudomonas aeruginosa* which shows MIC at 2000 ppm for gram (+) bacteria (both *S. aureus and B. pumilus*) and 5000 ppm (*E. coli*) and 6000 ppm (*Pseudomonas aeruginosa)* for gram (–) bacteria. From the results obtained it is proposed that polymeric nanoparticles were successfully formed which will enhance the efficacy of active components in the bark of *Terminilia Arjuna* and also demonstrates promising use in various pharmaceutical formulations as they show considerable results in the inhibition of bacterial growth.

Keywords: Amorphous, Encapsulation efficiency, Herbal nanomedicine, Minimum inhibition concentration (MIC), Percentage yield

In recent years, several herbal plants have been tested for their ability to cure human diseases. In the present era, Nanotechnology is most commonly used and it has emerged as the new face of technology[1-3]. Nanoparticles are the structures that are having dimension between 1nm to 100 nm[4]. There are some herbs and plants which are rich in nutrition and have a high therapeutic value[5]. Selection of a particular herb to synthesis nanomaterial would depend on its applicability to the body and its bioactive potential. The compounds can be the extracts of leaf, root, whole body, flower, fruit or bark of an herbal plant[6-9]. In the present work we use to cure different diseases. The selected plant for nanoformulation, Arjuna tree is 60-80 M long, usually found in Himalayan region of India. It is an evergreen tree having conical shaped leaves and yellow flowers. The bark of Arjuna tree is of great utility as it contains many active components such as tannins, triterpenoids, saponins, flavanoids, magnesium salts, calcium salts and glucosides that have been used in traditional herbal ayurvedic medicine therapy[10-11]. Moreover, the Arjuna bark herbal therapy is quick to heal as it contains natural antioxidant and is very useful in heart diseases, fractures, urinary discharges, ulcers, asthma and tumours[12]. It also has an easy reach to a large number of populations. The medicines derived from Arjuna bark have very less side effects and also possess widespread popularity. The herbal medicine is the first and quite popular step of medicine in most of the developing countries[13-16]. The natural products obtained from Arjuna bark are further examined and experimented in laboratories to investigate their applications in modern medical science. The recent and nanoscience has proved to be a revelation for existing therapies and for future medicines[17-20]. Various plant extracts of *arjuna terminalia* is used for preparation of a variety of nanoparticles. Arjuna bark in natural form is shown in (Fig. 1). In present paper, research has been done to synthesise more effective *arjuna terminalia* nanoparticles to increase their efficacy towards different ailments. After which characterization of same is done using various techniques like SEM, TEM, XRD and FTIR. Antimicrobial study was done using two strains of gram (+) and two strains of gram (–) bacteria to ascertain the efficiency of nanoparticles formed.

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Fig. 1 — *Arjuna terminalia* Bark used for synthesis of nanoparticles

Materials and Methods

Preparation of *arjuna terminalia* loaded PLGA nanoparticles by solvent evaporation method

Arjuna terminalia was first ground and powdered and then sieved to attain a uniform size range. 8 g of powdered raw *arjuna terminalia* was dissolved in 100 mL methanol for 72 h to form its methanolic extract. This extract was filtered using a Whatman filter paper No.1. The obtained extract which was dried, was then kept and refrigerated in containers to be used further. The solvent evaporation method has been used to prepare *arjuna terminalia* nano-sized particles. For this, 50 mg of PLGA and 10mg of methanolic extract of *arjuna terminalia* were dissolved together in 10 mL of acetonitrile. Then this mixture was added drop wise to 25 mL aqueous phase which contained 0.1% PVA using a homogenizer at 50 watt. The solution containing nanoparticles was further stirred for 4 h using a magnetic stirrer to evaporate the organic solvent acetonitrile. Eventually, these suspended nanoparticles were centrifuged (REMI, INDIA) for 20 min at 15000 rpm, cleansed with deionized water, which was later dried and stored at 4°C for further use[19-20].

Particle Size and Morphology Characterization

Scanning electron microscopy

Zeiss EVOMA10 scanning electron microscope (SEM) has been used to observe the morphology of *arjuna terminalia*-loaded PLGA nanoparticles. Ion sputter was then used to sputter coat the samples with a palladium layer. After 30 nm palladium coating, observations were done at an accelerating voltage of 20 kV and 10 Pa.

Transmission electron microscopy

Using Transmission Electron Microscopy FEI Morgagni, nanoparticle size was determined and morphology was examined at IARI, New Delhi. To provide contrast under magnification, nanoparticles were suspended in water (1 mg/mL), placed on copper grids of 0.037 mm size, and then stained with a 2 g/100 mL uranyl acetate aqueous stain. Before viewing under 50000 to 120000 times magnification, surplus liquid on the Mesh was wiped off with filter paper, and the grid was allowed to air dry. Observations were performed at 80 kV[8].

XRD and FTIR Study

Using X-beam diffractometer, the XRD patterns of methanolic extract, PLGA, and nanoparticles of drug-loaded into PLGA are examined. The measurements were taken at 45 kV voltage and 40 mA anodic current. XRD patterns were acquired at diffraction edges (2) from 0° to 90° at examining study of 2° each moment. XRD patterns were done to find out the nature of the compound formed whether is crystalline or amorphous. Further, FTIR studies were also done on *arjuna terminalia* loaded PLGA nanoparticles to determine whether the polymer interacted with the drug during the nanoencapsulation.

Determination of entrapment efficiency and percentage yield

The UV analysis (Shimadzu, UV-1800) at 276 nm determined the amount of *arjuna terminalia* entrapment. The supernatant obtained after centrifugation and removal of 1st pellet was UV monitored at 276 nm wavelength and corresponding absorption maxima was recorded. A generic calibration curve was drawn using different concentrations of methanol extract (1-10 mg in 10 mL water) *vs* total supernatant absorption and, as indicated by UV monitoring, was used to analyze the samples indirect determination of encapsulation efficiency and percentage yield. The following equations were used to measure the efficiency and percentage of encapsulation.

$$\%\ \mathrm{EE} = \frac{(\text{Drug added} - \text{Free "unentrapped drug"})}{\text{Drug added}} \times 100$$

$$\text{Percentage Yield} = \frac{(\text{Weight of Nanoparticles})}{(\text{Weight of PLGA} + \text{Weight of methanolic Extract})} \times 100$$

Antimicrobial activity of arjuna terminalia loaded PLGA nanoparticles

Once tested against two grams (+) strains of bacteria-*Bacillus pumilus* and *Staphylococcus aureus*

and two grams (−) strains of bacteria- *Escherichia coli* and *Pseudomonas aeruginosa*, the activities and properties of *Arjuna terminalia*-loaded nanoparticle were studied. Using cup plate method, the MIC of these test compounds was ascertained. In this a solidified agar layer is taken in a petri-dish and the antimicrobial substance diffuses from the cup through this agar layer so as to entirely inhibit the growth of added micro - organism in a circular zone around the cavity having the solution of a known quantity of antimicrobial substance. Zone of inhibition is measured with a zone reader in millimetres to express the antimicrobial activity (The amount of nanoparticles which were in suspension form was used to study the antimicrobial activity). After a period of 24 h of incubation at the temperature of 37°C, the plates were then assessed to determine the MIC of the entire test compounds, and the lowest concentration which could inhibit the visible growth of different strains of bacteria was recorded[20-22].

Results and Discussion

Formulation, optimization, and characterization of nanoparticles

The methanolic extract of *arjuna terminalia* was obtained as sticky and earthy brown in colour. The nanoparticles were formulated using acetone and acetonitrile solvents with Pluronic F68 and Poly (vinyl alcohol) as surfactants were optimized for further studies based on the particle size and encapsulation efficiency. The encapsulations efficiency and percentage yield so obtained are discussed in (Table 1).

Observing the results of (Table 1), the conclusion was drawn that the percentage yield of formulations F2 and F3 was found to be moderate, however, the drug encapsulation efficiency resulted to be high in the formulation F2. So, different parameters such as Morphological analysis, size, and *in vitro* drug release and antimicrobial studies were carried out using F-2.

Table 1 — Characteristics of drug- loaded PLGA nanoparticles

Formulation	Stabilizer	Solvent	Drug-Polymer ratio	Process Yield (%)	Encapsulation Efficiency (%)
Formulation 1	Pluronic F-68	Acetonitrile	1:5	12.33	93.75
Formulation 2	PVA	Acetonitrile	1:5	45.3	96.8
Formulation 3	Pluronic F68	Acetone	1:5	37.86	83.8
Formulation 4	PVA	Acetone	1:5	24.66	64.4

Particle Size and Morphology Characterization

Scanning electron microscopy

To study the particle size nanoparticles were observed after removing the organic phase and SEM analysis of the nanoparticles obtained shows they are spherically in shape shown in (Fig. 2).

Transmission electron microscopy

Further TEM images show that spherically shaped nanoparticles of *arjuna terminalia* PLGA nanoparticles were formed having a size as small as 50-75 nm. Figure 3 represents the *arjuna terminalia* loaded PLGA nanoparticles using PVA as a stabilizer. Nanoparticles so obtained by the solvent evaporation method came out to be highly uniform and mono dispersed (0.115 PDI, Fig. 4). In the solvent evaporation method, an excessive amount of energy was released during the

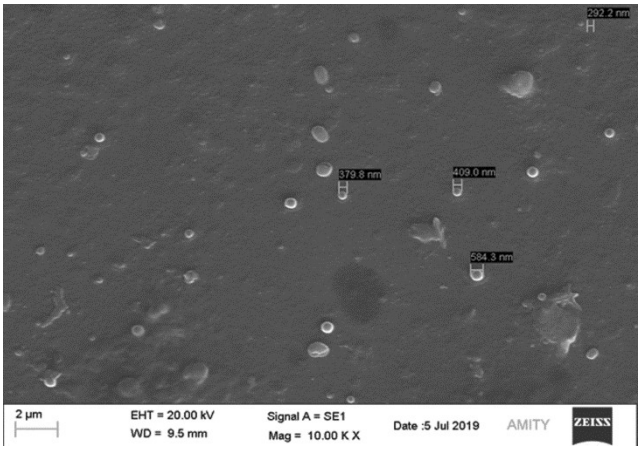

Fig. 2 — SEM images showing spherically shaped PLGA encapsulated nanoparticles of *arjuna terminalia*

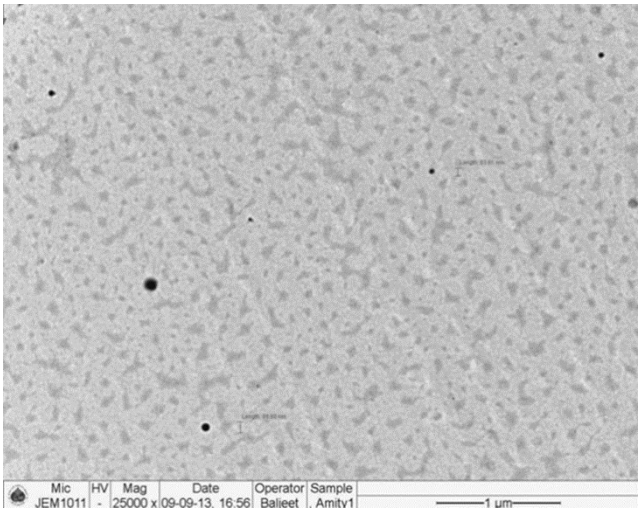

Fig. 3 — TEM images of *arjuna terminalia* loaded PLGA nanoparticles

homenization process, and sonication led to a quick dispersion of polymeric organic phase in the form of small size nano-droplets. Using acetonitrile as an organic solvent led to a smaller size because it is water-miscible and the size obtained is below 100 nm even. So, when the organic phase having acetonitrile was added to aqueous phase, nanoparticles got diffused in water leading to a smaller size.

The results obtained through SEM indicate that nanoparticles are spherical in shape. TEM images from the F2 formulation showed spherical nanoparticles (prepared with acetonitrile and PVA) ranging from 50-75 nm.

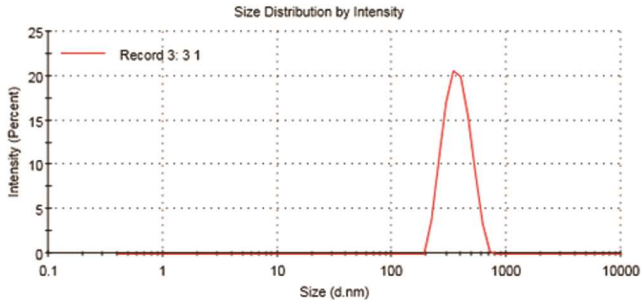

Fig. 4 — Particle size distribution of *arjuna terminalia* loaded PLGA nanoparticles

XRD and FTIR Studies

Sample Characterization

The FT–IR spectra of the sample were recorded using Fourier transformed infrared (FT–IR) spectrometer (Perkin Elmer spectrum 65, FT–IR spectrometer; Perkin Elmer) in the range 4000–400 cm–1 to study the molecular structure of the sample preparation. For scanning through IR radiation, the powdered nanoparticle sample was diluted with KBr (materials: KBr =1:100) to form pellets. 100 interfero grams with a spectral resolution of ± 4 cm^{-1} were averaged to improve the signal to noise ratio for each spectrum. From the sample spectra, background spectra collected under identical conditions were subtracted. Therefore, the intensities of the absorption bands can be directly related to the concentration of the corresponding functional groups in the present study. Using FTIR spectroscopy technique, the structural characteristics of the methanolic extract, nanoparticles of the drug- loaded PLGA and PLGA were studied. The studied FTIR spectra of the nanoparticles of PLGA, methanolic extract of *arjuna terminalia*, and the drug- loaded PLGA nanoparticles are shown in (Fig. 5A-C), respectively. FTIR studies on *arjuna terminalia* loaded PLGA nanoparticles

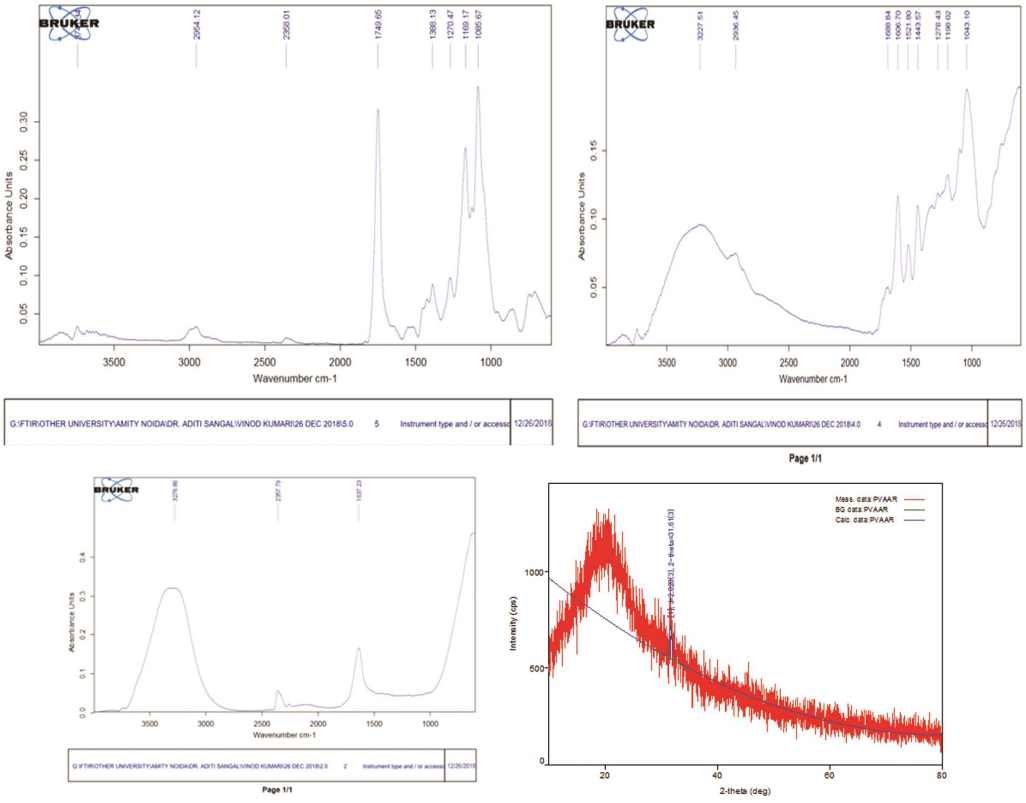

Fig. 5 — (A) FTIR of PLGA; (B) FTIR of Methanolic Extract of *arjuna terminalia*; (C) FTIR of drug (*Arjuna terminalia*) loaded PLGA nanoparticles; and (D) The XRD spectra for the *arjuna terminalia*-loaded PLGA nanoparticles

Table 2 — Various zone of inhibition obtained against different concentrations for different bacterial stains

S. No	Bacteria	Mic(In Ppm)	Zone of Inhibition (Np)	Bacteria stain
1	*Staphylococcus Aureus*	10000	19.75	gram (+) bacteria
2		8000	17.24	gram-positive bacteria
3		4000	13.11	gram (+) bacteria
4		2000	8.59	gram (+) bacteria
5		1000	No Zone of Inhibition	gram (+) bacteria
6	*Bacillus pumilus*	10000	18.11	gram (+) bacteria
7		8000	16.44	gram (+) bacteria
8		40000	12.57	gram (+) bacteria
9		2000	7.15	gram (+) bacteria
10		1000	No Zone of Inhibition	gram (+) bacteria
11	*Escherichia coli*	10000	19.12	gram (–) bacteria
12		8000	17.20	gram (–) bacteria
13		6000	14.13	gram (–) bacteria
14		5000	11.25	gram (–) bacteria
15		3000	No Zone of Inhibition	gram (–) bacteria
16	*Pseudomonas Aeruginosa*	12000	21.15	gram (–) bacteria
17		10000	18.70	gram (–) bacteria
18		80000	15.13	gram (–) bacteria
19		6000	10.45	gram (–) bacteria
20		2000	No Zone of Inhibition	gram (–) bacteria

further revealed that the polymer does not interact with the drug during the nanoencapsulation. The XRD spectra obtained in (Fig. 5D) for the drug-charged polymeric nanoparticles suggested that no characteristic peaks for the drug trapped in PLGA were observed, possibly because the drug was amorphous.

Entrapment efficiency

Entrapment efficiency related to *arjuna terminalia* loaded PLGA nanoparticle for active compound *arjuna terminalia* was found to be 96.8%. For nanoformulation F2, using PVA as stabilizer and acetonitrile as a solvent, percentage yield comes out to be 45.3. The selection of the method used in the production of nanoparticles strongly depends on the drugs that were about to be encapsulated.

Antimicrobial activities study

The Minimum Inhibitory Concentration of *arjuna terminalia*-loaded nanoparticles against *Bacillus pumilus and Staphylococcus aureus* the gram-positive bacteria show lower MIC than *Escherichia coli*, *Pseudomonas aeruginosa* gram-negative bacteria. The efficiency related to nanoparticle during inhibiting growth in bacteria was because of the insertion of nanoparticles in bacterial cells leading to comparatively rapid delivery of the *arjuna terminalia* at the site. The MIC with different gram (+) and gram (–) bacteria and the Zone of Inhibition obtained is listed in (Tables 2 & 3), and the zones obtained are depicted in (Fig. 6)

Table 3 — Summarization zone of inhibition obtained against different concentrations for different bacterial stains

S. No	Bacteria	MIC	Zone of inhibition (NP)
1	*Escherichia coli*	5000 ppm	11.25mm
2	*Pseudomonas aeruginosa*	6000 ppm	10.45 mm
3	*Staphylococcus aureus*	2000 ppm	8.59 mm
4	*Bacillus pumilus*	2000 ppm	7.15 mm

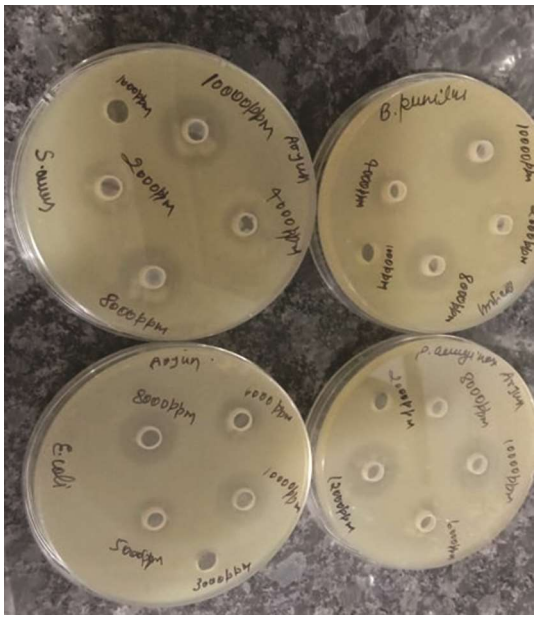

Fig 6 — Zone of inhibition against two gram (+) bacteria *B. pumilus* and *Staphylococcus aureus* and two gram (–) bacteria *E. coli* and *Pseudomonas aeruginosa*

Conclusion

In the present work a successful attempt has been shown for the first time to formulate spherical PLGA nanoparticles loaded with methanolic extract of *Arjuna terminalia* by solvent evaporation method. The results reveal reduced size of nanoparticles (73 nm to 100 nm) having entrapment efficiency as high as 96.8% and percentage yield of 45.3. The antimicrobial studies of the nanoparticles showed significant activity against various gram (+) bacteria (*S. aureus and B. pumilus*) and various gram (–) bacteria (*E. Coli and P. aeruginosa*). The present formulation can be used for safe antimicrobial formulations.

Conflict of interest

All authors declare no conflict of interest.

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References

1 Rashmi JB, Naveen G, Phytochemical analysis and antibacterial activity of different leaf extracts of *Murraya koenigii*. *Int J Biochem Biomol*, 2 (2016) 1.

2 Ajayi AO, Babalola SB & Osuntuyi R, Antimicrobial Activities of Natural Honey, Caesalpinia pulcherrima and acalypha wilkesiana against some pathogens. *Int J Biote Bioch*, 101 (2018) 10.

3 Aladji MM, Tchiagam JB, Yanou NN & Bell JM, Genetics of seed phenolic content and antioxidant activity in diallel crosses of sesame, *Sesamum Indicum* L. *J Biotechnol Biomater*, 2018 (2018) 1.

4 Kumari V & Sangal A, Study of Antimicrobial activity of Star Anise loaded poly (DL-lactide-co-glycolide) nanoparticles. *Res J Pharm Technol*, 12 (2019) 499.

5 Daniel M, Medicinal plants: chemistry and properties (CRC press) 2016, 1.

6 Badyal A, Sharma V, Kour N & Singh SK, Anticancer efficacy of methanolic extracts of some medicinal plants from Jammu region. Jammu & Kashmir, India. *Indian J Biochem Biophys*, 53 (2016) 51.

7 Sinha R, Pal AK & Singh AK, Physiological, biochemical and molecular responses of lentil (*Lens culinaris Medik*.) genotypes under drought stress. *Indian J Plant Physiol*, 4 (2018) 772.

8 Ikumawoyi VO, Agbaje EO, Awodele O & Akinyede AA, Biochemical, hematological, and hormonal profile of rats orally administered methanol stem bark extract of Napoleona vogelii Hook and Planch (Lecythidaceae). *Drug Chem Toxicol*, 6 (2019) 592.

9 Farooq S, Mehmood Z, Qais FA, Khan MS & Ahmad I, Nanoparticles in Ayurvedic Medicine: Potential and Prospects. *In new look to phytomedicine* (Academic Press) 2019, 581.

10 Kaur C & Kapoor HC, Anti-oxidant activity and total phenolic content of some Asian vegetables. *Int J Food Sci Tech*, 37 (2002) 153.

11 Biswas M, Kar B, Bhattacharya S, Kumar RBS & Ghosh AK, Antihyperglycemic activity and antioxidant role of Terminalia arjuna leaf in streptozotocin-induced diabetic rats. *Pharmaceutical Bio*, 49 (2011) 335.

12 Mukherjee PK, Rai S, Kumar V, Mukherjee K & Hylands PJ, Plants of Indian origin in drug discovery. *Expert Opin Drug Discov*, 2 (2007) 633.

13 Matteucci F, Sprocati A, Alisi C, Chiavarini S & Ercole C, Microbial degradation of chlorinated solvents: a microcosm study and a microbial genetic analysis to remediate a contaminated area in Central Italy. *Int J Bioremed Biodeg*, 110 (2018) 10.

14 Ashok A, Rejeesh CR & Renjith R, Biodegradable polymers for sustainable packaging applications: a review. *Int J Bionic Biomater*, 1 (2016) 11.

15 Sarkar K, Banerjee R & Chatterjee S, Characterization of a Staphylococcus haemolyticus Strain Isolated from Agroindustrial Waste Effluent and Susceptibility of its Biofilm on Polyethylene to Antibiotics. *Int J Biochem Biomol*, 1 (2015) 27.

16 Bhardwaj S, Antimicrobial and antioxidants screening through phytochemical evaluation and nutrient analysis of different medicinal trees. *Int J Biochem Biomol*, 2 (2016) 1.

17 Halder J, Sanwal SK, Rai AK, Rai AB, Singh B & Singh BK, Role of physico-morphic and biochemical characters of different okra genotypes in relation to population of okra shoot and fruit borer, *Earias vittella* (Noctuidae: Lepidoptera). *Indian J Agric Sci*, 2 (2015) 278.

18 Homaee MB & Ehsanpour AA, Physiological and biochemical responses of potato (*Solanum tuberosum*) to silver nanoparticles and silver nitrate treatments under *in vitro* conditions. *Indian J Plant Physiol*, 20 (2015) 353.

19 Kumari V & Sangal A, Synthesis, characterization, antimicrobial activity and release study of cinnamon loaded poly (DL-lactide-co-glycolide) nanoparticles. *Res J Pharm Technol*, 2 (2019) 499.

20 Kumari V & Sangal A, Preparation, Characterization and Optimization of Cinnamon-Loaded PLGA Nanoparticles. *In Green Chemistry in Environmental Sustainability and Chemical Education* (Springer, Singapore) 2018, 219.

21 Tavakoli SA, Mirzaei S, Rahimi M & Tavassolian I, Assessment of peppermint, clove, cumin essential oils and silver nano particles on biochemical and shelf life of *Citrus limon* (L.). *Indian J Biochem Biophys*, 56 2019) 269.

22 Tazehkand MN & Valipour E, Cytotoxic, antimicrobial and DNA breaking activity of Salgam. *Indian J Biochem Biophys*, 56 (2019) 169.