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Calotropis gigantea extract incorporated nanofibers as a novel strategy for smart drug delivery systems

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ABSTRACT

Plants possess a wide range of biological and medicinal properties are highly safe, widely available, low cost, high efficiency, and demonstrate several properties that include antioxidant, antibacterial, anti-inflammatory, and even anticancer activity. Calotropis gigantea Stem Methanolic Extract (CGSME) was subjected to phytochemical, analysis, antimicrobial activity analysis, antioxidant analysis and synthesis of silver of nanoparticles analysis and all these studies demonstrated the scope of this extract to be ideal for application in nanofibers. Electrospinning has secured significant attention in several fields owing to its ability to produce continuous fibers from different polymers and composites in a simple manner. Electrospun nanofibers possess many merits such as diverse chemical composition, easily adjustable structure and diameter, high surface area and porosity, and good pore connectivity, which give them broad application prospects in the biomedical field. Nanoparticles were proved to have better antimicrobial action favouring Calotropis gigantea extract incorporated nano fibers to find immense scope as novel strategy in smart drug delivery such as controlled drug release, tissue repair, biological dressings and enzyme immobilization fields

Keywords: Calotropis gigantea, Nanofibers, Electrospinning, Drug delivery.

1.INTRODUCTION

1.1 Taxonomical Classification of *Calotropis gigantea*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Gentianales
Family	Asclepiadaceae
Subfamily	Asclepiadoideae
Genus	*Calotropis*
Species	*gigantea*

1.2 Ethanobotany of Plant:

A shrub found normally as small, erect and compact, growing wild across India in relatively drier and warmer areas, ranging up to an altitude 1200m. *Calotropis*

gigantea grows mainly on coarse, sandy and alkaline soils as they are good soil binders and recommended for deserts.*Calotropis gigantea* possesses a life span that varies from 15-20 years. Researchers are of the view that root-bark of older plants have significantly more percentage of acrid and bitter resinous matter than that of younger plants. In the supplement to the pharmacopoeia of India, there are reports that the older the plant, the more active is the bark in its effects.[5, 6]

1.3 Pharmacological Properties of *Calotropis gigantea*

Calotropis gigantea contains many biological active chemical groups including, cardenolides, steroids, tannins, glycosides, phenols, terpenoids, sugars, flavonoids, alkaloids and saponins.[1,2] Various pharmacological effects such as antimicrobial, anti-inflammatory, analgesic, antioxidant, anticonvulsant, anticancer, anthelmintic, antipyretic, anti-angiogenic, immunological, antidiabetic, cardiovascular, hypolipidemic, gastroprotective, hepatic protective, renal protective, antidiarrheal, enhancement of wound healing, antifertility and smooth muscle relaxant effects have been evidenced by earlier researchers[3-13]. Thus *Calotropis gigantea* is a plant with wide range of chemical constituents with many pharmacological effects. There is a great promise for the development of novel drugs from *Calotropis gigantea* to treat many human diseases as a result of the effectiveness of these phytoconstituents.

1.4 Electrospinning of nano fibres

Production of polymer and nanofiber composite materials could be achieved directly by electrospinning. The post-processing of electrospun fibers, forms other materials like ceramics and carbon nanotubes with a design that is versatile [15].

The simple and most common method applied for tissue framework production is electrospinning due to these advantages. The basis for the operation principle lies on filling the syringe with the aid of a polymer solution or melting in the high potential area and spraying the same from the syringe tip to the collector by application of a voltage to an electrode connected to the syringe tip. The solution that is being sprayed from the syringe is subjected to an electrical field and hence it elongates at the tip of the needle, and a conical appearance known as Taylor cone is obtained. An electrospinning process typical of its kind is subjected between a high voltage source with negative or positive polarity and a grounded surface such that the fibers can together clump. Spraying the solution present in the syringe commences when the potential difference exerted from the voltage source reaches the threshold value and equalizes with that of the electrostatic forces, and the process completed by spraying the same on the grounded surface. As the fibers collected on the surface are sprayed with a cosiderably high amount of pulling, they are found to be in a fine and regular structure [16-18].

Electrospun nanofibers possess many advantages as drug delivery systems, and have been researched for many years as oral, transdermal or injection dosage forms [14]. Polymer nanofibers obtained as a result of electrospinning method possess high surface area-volume ratio, have superior mechanical performance, are flexible in surface functions, and some electrospun fiber materials possess good biocompatibility and degradability, which could be applied as drug carriers. Electrospun nano fibers find immense scope for application in tissue repair, biological dressing, drug controlled release and enzyme immobilization [19].

2. METHODOLOGY

2.1 Preparation of Extract

2.1.1 Sample Preparation

Root, stem and leaves were collected separately and brought to the laboratory and thoroughly washed in running water to remove debris and dust and then rinsed using distilled water and finally dried by shade dry method, powdered in mixer grinder to obtain 200gms each of the respective sample powders.

2.1.2 Extract Preparation

Calotropis gigantea R.Br. (Linn) leaves and root powders were subjected to Soxhlet extraction. Methanol, ethanol, isopropanol and hexane solvents were used for phytochemical extraction in the order of polarity. The extraction was performed overnight to obtain concentrated extracts. Further the extracts were concentrated using a rotary evaporator and were stored at 4°C for further use.

2.2 Phytochemical Analysis

The four solvent extracts namely, methanol, ethanol, isopropanol and hexane respectively for each of leaf stem and root parts were subjected to qualitative phytochemical analysis for detecting the presence of

different phyto constituents using standard protocols as given below:

2.2.1 Test for Alkaloids

Mayer's test:

A fraction of extract was subjected to treatment with Mayer's test reagent (1.36 g of mercuric chloride and 5g of potassium iodide in 100 ml of water) and viewed for the formation of cream coloured precipitate.

Wagner's test:

A fraction of extract was subjected to treatment with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and viewed for the formation of reddish brown colour precipitate.

2.2.2 Test for flavanoids

NaOH test

A small fraction**2.1 Preparation of Extract**

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Wagner's test:

A fraction of extract was subjected to treatment with Wagner's reagent which is a mixture of 1.27 g of iodine and 2 g of potassium iodide in 100 ml water and viewed for the formation of reddish brown colour precipitate.

2.2.2 Test for flavanoids

NaOH test

A small amount of extract was subjected to treatment with a mixture of aqueous NaOH and HCl and viewed for the formation of yellow orange colour.

2.2.3 Test for sterols

Liebermann-Burchard test

1ml of extract was subjected to treatment with chloroform, acetic anhydride and drops of H_2SO_4 was added and viewed for the formation of dark pink or red colour.

2.2.4 Test for Terpenoids

Liebermann-Burchard test

1ml of extract was subjected to treatment with chloroform, acetic anhydride and following which drops of H_2SO_4 were added and viewed for the formation of dark green colour.

2.2.5 Test for Anthraquinones

Borntrager's test

About 50 mg of powdered extract was subjected to heating with 10% ferric chloride solution and 1ml concentrated HCl. This extract was then subjected to cooling and filtration and the resultant filtrate was shaken with diethyl ether. The ether extract was subjected to further with strong ammonia and viewed for the formation of pink or deep red colouration of aqueous layer.

2.2.6 Test for phenols

Ferric chloride test

A fraction of extract (1ml) was subjected to treatment with with 5% ferric chloride and observed for the formation of deep blue or black colour.

2.2.7 Test for Tannins

Acetic Acid Test

The extract (1ml) was subjected to treatment with acetic acid solution and viewed for the formation of red colour solution.

2.2.8 Test for Carbohydrates

Molisch's Test

In a test tube, 2 ml of the extract and 2 drops of α–naaphthol solution were added. The tube was inclined carefully and concentrated H_2SO_4 was poured drop

wise using a dropper along the sides of the tube. A violet colour was observed at the junction of the two liquids.

The extracts of stem of *Calotropis gigantea* R.Br. (Linn) in different solvents were screened for the presence of various bioactive phytochemicals. The phytochemical analysis was carried out in stem part for different extracts like methanol, ethanol, Isopropanol and Hexane.

Table 1: Phytochemical Analysis of *Calotropis gigantea* R.Br. (Linn) Stem Extract

Phytochemicals		Phytochemical Analysis of Calotropis gigantea R.Br. (Linn) Stem Extract			
		Methanol	Ethanol	Isopropanol	Hexane
Alkaloids	Mayer's Test	+	+	-	+
	Wagner's Test	+	-	+	-
Flavonoids		+	-	-	-
Steroids		-	-	+	-
Terpenoids		+	+	-	-
Anthraquinones		+	-	-	-
Phenols		+	-	+	-
Tannins		+	-	-	-
Carbohydrates		+	+	+	+

In methanol extract the presence of phytochemical constituents were more in number than the other solvents. Presence of organic solvents was found to be least in Hexane (Table 1).

2.3 ANTIMICROBIAL ANALYSIS

The solvent extracts viz. methanolic, ethanolic, isopropanol and hexane were screened against the four microbial species viz., Escherichia coli, Staphylococcus aureus, Klebsiela pneumonia and Pseudomonas aeruginosa by disk diffusion method for anlysis of Antimicrobial activity.

2.3.1 Preparation of MHA Test discs

Mueller-Hinton agar (MHA) plates of 90 mm size were selected for the above analysis.

2.3.2 Innoculation of Test Organisms

6 mm wells were made using 6mm diameter cork borer. With help of sterilepipettes, varying concentrations of the extracts i.e. 62.5, 125, 250, 500mg/ml were introduced into each of the wells. The plates were allowed to stand for a pre-diffusion time of 15 minutes after which they are incubated at 37°C for 24 hours.

2.3.3 Measurement of Zone of Inhibition

After incubation, zones of inhibition were measured. Antibacterial activity was expressed as mean diameter of the zone of inhibition. It was compared with commercial standard antibiotics namely, Ampicillin, Tetracycline and Vancomycin (10µg/disc)

The Zone of Inhibition determination was performed for each of the four solvent extracts i.e., Methanol, Ethanol, Isopropanol and Hexane individually for stem, root and leaf parts respectively of *Calotropis gigantea* R.Br. (Linn) R.Br. (Linn) by disk diffusion method using Mueller Hinton Agar (MHA). Extracts of 62.5, 125, 250 and 500 µg/ml concentration respectively were prepared and inoculated into the tubes. The inoculated tubes were incubated at 37ºC for 18 hrs. The Zone of Inhibition values for all test organisms were read by naked eye for each of the strains performing the experiment twice or thrice if necessary and their corresponding MIC average values determined and tabulated.

Calotropis gigantea R.Br. (Linn) Stem Methanolic Extract (CGSME) when analyzed for antimicrobial activity revealed the most significant activity when compared to other solvent extracts of other plant parts, which were studied. The MIC value of CGSME was 27 mm almost the highest inhibition concentration among all the strains studied and displayed increasing activity with increasing concentrations of the extract. The MIC value of Hexane extracts was the least significant in the above studies.

2.4 ANTIOXIDANT ANALYSIS

There are increasing evidences that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants that are constituent in spices, medicinal plants and herbs.

Polyphenols are a class of large and diverse compounds, of which many are found to be preaent naturally in a wide variety of food and plants. The largest and best studied group among polyphenols are flavanoids. A fair variety of plant polyphenols is either being actively developed or already sold as dietary supplements or as herbal and derived medicines. Although these compounds perform an unknown role in nutrition (non-nutrients), many of them possess properties which include antioxidant, anti-mutagenic, anti-carcinogenic and anti-inflammatory effects that might be beneficial

potentially in preventing disease and protecting the stability of genome. Antioxidant quality stands good as a measure of the effectiveness of the antioxidant(s) present as a pure compound or a mixture. The percentage scavenging and IC50 values were therefore calculated.

2.4.1 DPPH Assay of CGSME

To 1 ml of 100.0 µM DPPH solution in methanol, equal volume of the plant sample in methanol of varying concentration was added and incubated in dark for 30 minutes. The change in coloration was studied in terms of absorbance utilizing a spectrophotometer at 514 nm. To the control tube was added 1 ml of methanol instead of the test sample. Different concentrations of ascorbic acid were utilized as reference compounds. Calculation of percentage of inhibition was.done using the equation - [(Control's Absorbance - Test's Absorbance)/ Control's Absorbance)] × 100

IC50 value was calculated using Graph pad prism 5.0.

The reactivity of extract of *Calotropis gigantea* R.Br. (Linn) extract was subjected to analysis with DPPH, a stable free radical.

Concentration	OD Value	% IC50	IC50
50	0.189	40.00	196.38
100	0.192	42.20	
250	0.217	60.74	
750	0.219	62.22	
1000	0.225	66.66	

Table 2 DPH Scavenging Activity of CGSME

As DPPH picks up one electron in the presence of a free radical scavenger, there is decrease in absorption and the resultant discoloration is stochiometrically related to the gain in the number of electrons. The DPPH radical scavenging (%) activity of *Calotropis gigantea* R.Br. (Linn) Stem Methanolic Extract for different concentration was carried out such as 50 - 1000 µg/ml. The inhibition concentration 50 percentage was determined to be 196.38 µg/ml.

There are several other mechanisms by which antioxidants could act. One such method is by the scavenging of reactive oxygen and nitrogen free radicals. There are several different experimental methods by which the free radical scavenging activity could be estimated. One such method, by which total free radical scavenging can be evaluated, is by determining their efficiency to scavenge DPPH radicals. This method finds its basis on the reduction of DPPH, a stable free radical and any molecule that could donate an electron or hydrogen to DPPH which could react with it and thereby bleach the DPPH absorption. Because of its odd electron, DPPH gives a strong absorption maximum at 514nm by visible spectroscopy (purple colour). When the odd electron of the radical becomes paired off in the presenceof a hydrogen donor, that is, a free radical scavenging antioxidant, suchthat there is decrease in absorption strength and the resultant decolorization is stoichiometric with respect to the number of electrons that were captured. Therefore the methanolic extract of stem of *Calotropis gigantea* R.Br. (Linn) methanol extract when tested for the DPPH free radical scavenging ability, it showed strong radical scavenging activity with inhibition percentage of 196.38 µg/ml. Whereas the reducing power assay demonstrated the inhibition percentage as 505.14 µg/ml (Table 2).

2.4.2 Reducing power Assay

The sample together with the Ascorbic acid solutions was spiked with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was kept in a 50°C water-bath for 20 min. The resulting solution was cooled rapidly, spiked with 2.5ml of 10% trichloroacetic acid, and centrifuged at 3000rpm for 10 min. The supernatant (5ml) was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride and incubated for 10min. The absorbance was detected at 700nm on spectrophotometer. The extract concentration providing the absorbance was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid is used as standard. The higher absorbance indicated higher reducing power activity.

The antioxidant could donate one electron to the free radicals, which in turn leads to the radical neutralization.. Measurement of reducing power is by direct electron donation in the reduction of $Fe^{3+}(CN^{-})_6$–$Fe^{2+}(CN^{-})_6$. Visualization of the product was by forming the intense Prussian blue color complex and then measured at λ 700nm. A higher absorbance value aids to indicate a stronger reducing power of the samples.

Table 3: Reducing power Assay of CGSME

Concentration	OD Value	% IC50	IC50
50	0.193	32.19	505.14
100	0.198	35.61	
250	0.218	49.31	
750	0.225	59.10	
1000	0.237	62.32	

Calotropis gigantea R.Br. (Linn) stem methanolic extract showed concentration-dependent reducing power. However, its reducing power was found to be weaker than that of BHT, which demonstrated ed the strongest reducing power. The reducing power activity of 50% of *Calotropis gigantea* R.Br. (Linn) Stem Methanolic Extract 50 and 1000 µg were significantly higher. Sodium metabisulphate antioxidant compounds are able to donate electrons to reactive radicals, reducing them into a species that is more stable and unreactive..

2.5 Silver nanoparticles green synthesis

The silver nitrate solution 1 mM solution was prepared in 100 ml flask. 1 ml of CGSME was mixed with 9 ml of 1 mM of silver nitrate. The leaf extracts of the CGSME and silver nitrate solution were used as a control throughout the experiment. The final solution of 200 ml was prepared and centrifuged at 18,000 rpm for 25 minutes. The collected pellets were stored at -40C. The supernatant was was subjected to heating temperatures varying from 500C to 950C. A color change of the solution was viewed during the heating process.

Plant materials were collected and plant leaf extracts were prepared both by conventional and homogenization methods. Biosynthesis of silver nano particles by the filtrate of *Calotropis gigantea* R.Br. (Linn) was confirmed by change in the colour of the filtrate to brown after addition of silver nitrate and it was optimized under various conditions. The obtained nano particles were recovered and stored. This resulted due to excitation of surface plasmon vibrations in the silver nanoparticles.

2.6 *Calotropis gigantea* extract incorporated nano fibers

Extensive research have been conducted by researchers of biomedicine on the process of electrospinning and its applications. In the recent past, as a result of advancements in nanotechnology, electrospinning technology has considerately become a hotspot of research.

Since the diameter of electrospun nanofibers is smaller than that of cells, it aids favorably for mimicking that of the structure and biological functions of natural extracellular matrices. Most of the human organs and tissues are found to be structurally similar to nanofibers, which therefore benefits for the electrospun nanofibers in the repair the human tissues and organs. Electrospun nanofibers possess significant specific surface area and porosity, and some electrospun fiber materials have good biocompatibility and degradability, which could be employed for drug carriers.

Several types of nanomaterials have been synthesized following green approach using *Calotropis gigantea* extracts; evaluated for different pharmacological activities and potential studied for nano drug delivery applications. Nanomaterials tried to get synthesized include nanoparticles and nanophophors. Importantly, there is an upsurge in the green synthesis of nanoparticles as new nanobiotechnology in the production of eco-friendly and cost-effective synthetic processes for highly stable nanoparticles which could emerge as an alternative that is safer than conventional methods.

These nanoparticles were proved to have better antimicrobial action favouring *Calotropis gigantea* extract incorporated nano fibers to find immense scope in:

a. Drug controlled release

The drug loaded nano fibers exhibit excellent photothermal effects and controlled near-infrared irradiation (NIR)-triggered release of drug, enabling to chemo-photothermal therapy of melanoma concerning skin.

b. Biological dressings

The high surface area of the electrospun nanofibers facilitates efficient loading of hemostatic agents, drug molecules and hence results in speeding up wound healing. Therefore, electrospun nanofibers possess several advantages and potential in the development of wound dressings.

c. Tissue repair

Electrospinning facilitates continuous ultrafine fibers being prepared with diameters similar to Natural Extracellular Matrices (ECMs). Nanofiber scaffolds that are electrospun can therefore mimic the ECM structure in humans to a great extent. Electrospun fiber membranes or mats having characteristics such as high porosity, good pore connectivity, a large specific

surface area and biocompatibility could grant a good microenvironment for cell survival to aid cells in adherence, differentiation, and proliferation. The thickness, the three-dimensional structure and mechanical characteristics of the nanofiber scaffolds that are electro spun could be regulated by adjusting the electrospinning parameters. Moreover, bioactive molecules which include growth factors, cell regulators, and even living cells, and inorganic molecules which include hydroxyapatite could be added into to nano-scaffolds in electrospinning, which grant electrospun fiber scaffolds varied functions. Nanofiber materials that are electrospun find wide application in tissue repair such as trachea, nerves, skin, cartilage, bones, tendons, ligaments and blood vessels.particles.

d. Anti-inflammatory pain killers

Electrospun PCL nanofibers have been researched as a novel nano-delivery system. They have been adopted for oromucosally administering poorly water soluble drugs such as ibuprofen or carvedilol. The release of the drug from the PCL matrix was found to have been affected by the drug solubility and molecular weight. The PCL nanofibers loaded with drugs aided to release almost 100% of incorporated ibuprofen in four hours whereas 77% only of the incorporated carvedilol was released during the same time period.

e. Antimicrobials

Utilization of electrospinning in loading a variety of antibacterial drugs in a reasonable manner could demonstrate a role for drugs that is synergistic. Using electrospinning two hydrophilic drugs, such as metronidazole and ciprofloxacin hydrochloride, were loaded into the PCL matrix by Zupancic, individually and in combination. The result showed that the combination of drugs of the two antibacterial loaded fibers had beneficial effects by inhibiting the growth of pathogenic bacterials in periodontal diseases.

6. Other uses like enzyme mobilization

The porosity and high specific surface area of the fibers electrospun could alleviate effectively the diffusion resistance of the matrix and improve greatly the catalytic ability of the enzyme that is loaded. The method of enzyme immobilization in electrospun fibers is the surface loading method which include the chemical crosslinking surface modification and the active functional group loading method. Currently, the enzyme immobilization in electrospun fibers is mostly by surface loading method. Various loading modes provide different binding capacities between enzymes and enable to explore enzyme applications.

3. CONCLUSION

This study intends to highlight the potential of *Calotropis gigantea* extract incorporated nanofibers. By demonstrating the antimicrobial and antioxidant efficacy of the plant's stem extracts, this study underscores the importance of exploring the advantages of phyto extracts incorporated nanofibers as an alternative to synthetic nanofibers. This emphasis on natural compounds not only aligns with the global interest in phyto extract incorporated nano therapeutic applications but also holds promise for mitigating the adverse impacts of synthetic nano fibers.

The phytochemical, antimicrobial, antioxidant and silver nanoparticles analysis results provide valuable insights into the therapeutic potential of *Calotropis gigantea* Stem Methanolic Extract (CGSME). This study not only contributes to the scientific understanding of phytochemicals but also paves the way for the development of natural extract incorporated nano fibers as novel strategy for smart drug delivery systems. This study strengthens the credibility of CGSME as botanical extracts which provide helpful with antimicrobial, antiinflammatory, cytotoxicity, analgesic and wound healing activity. Moreover, this paper adeptly highlights the viability of CGSME extract incorporated nanofibers in drug controlled release, biological dressings, tissue repair, anti-inflammatory pain killers and antibacterials. This advocacy is crucial in promoting *Calotropis gigantea* extract incorporated nanofibers as a novel strategy for smart drug delivery systems and in addressing the growing concerns surrounding synthetic nanofibers and their health implications.

Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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