Synthesis of Thernostic Herbal Biomaterial for Human Infections

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***Abstract*—The main aim of this paper is to analyze the wound healing properties of Chrysopogon zizanioides and Acalypha indica as nanoparticles mixed in the ratio of 1:1. Infectious diseases caused by bacteria remains a large global issue and in recent days have led to the production of many disease-resistant microbes. In this project, and Chrysopogon zizanioides Acalypha indica are selected by virtue of their wide availability and presence of wound healing properties, evidenced from folklore. The herbal nanoparticles were prepared from shade dried and Chrysopogon zizanioides Acalypha indica plant leaves employing ball milling technique. The obtained nanoparticles were comprehensively characterized using Particle size analyzer, Fourier transform infrared spectroscopy, UV–visible spectroscopy, Atomic Fluorescence Microscopy, and Antimicrobial analysis techniques. Even though the particle size changed due to milling technique, the crystalline of the nanoparticles was retained. The antimicrobial activities of the prepared herbal nanoparticles mixed in a ratio of a 1:1 against Staphylococcus aureus, Escherichia coli, Pseudomonas aeringinosa, and Proteus vulgaris were explored to understand the influence of mixed herbal particles on antimicrobial, anticoagulation, anti-inflammatory, antioxidative activities and their wound healing properties. The process of mixing these two medicinal plants in 1:1 ratio, resulted in better wound healing properties when coated on cotton fabric than when coated individually. The observed knowledge on the influence of a mixture of medicinal herbal nanoparticles at equal ratios will help to optimize the production of potential herbal nanoparticles for different biomedical applications.**

Keywords*— Chrysopogon zizanioides, *Acalypha indica, antioxidative, anti microbial, anti- inflammatory anticoagulation

I. INTRODUCTION

Nature serves as a source of medicinal agents with a remarkable number of modern drugs being isolated from natural resources. Traditional medicine has provided potentially useful new compounds to develop chemotherapeutic agents. The pathogenic microorganisms that exist and offer resistance to major classes of antibiotics have made a tremendous improvement in recent years due to indiscriminate usage of synthetic antimicrobial drugs. Antimicrobial agents of plant origin have enormous therapeutic potential proved effective to treat infectious diseases while simultaneously mitigating the side effects which are often allied with synthetic antibiotics. Diversified Investigations of extracts prepared from various medicinal plants by using miscellaneous polar and non-polar solvents are utilized as an optimistic source of antimicrobial agents pertaining to the various formulations of the drug**. [1]**

Chrysopogon zizanioides **(C. zizanioides)** is flowering plant species procurable throughout India and is employed as indigenous medicine for a variety of ailments. It is substantially applied in the Ayurvedic system of medicine and is conferred as "Bhringraj" remarkably employed to treat liver disorder. It has been contrived to possess notable medicinal properties against blood pressure, malaria, dysentery, diarrhea, stomach ache, headache, wound healing, it also prevents hair fall and stops hemorrhage from cuts and bruises. Its leaves and flowers retain antiseptic, insecticidal and parasiticidal properties. It harnesses various pharmacological activities like Immuno-modulatory, Anti-diabetic, Anti hepatotoxicity & Anti-oxidant, Anti-inflammatory, Analgesic, and marked depressant action on respiration.

Acalypha indica (A. Indica) plant is commodiously used in the conventional medicinal system of India and many other countries. It has been proclaimed to possess anti-cancer, anti-diabetic, anti-oxidant, anti-bacterial, antifungal hepatoprotective, anti-inflammatory and also used to check anti-ulcers and wounds healing. It possesses a splendid source of glycosides, flavonoids and tannins. The medicinal properties and therapeutic utilities of Acalypha indica and its secondary metabolites investigations substantiate its significance as a valuable medicinal plant.

II. REVIEW OF LITERATURE

Chrysopogon zizanioides leaf extract containing carbohydrates is a major component which acts as a reducing agent. By reduction of ferric chloride solution with Chrysopogon zizanioides leaf extract containing carbohydrates, the iron oxide nanoparticles were synthesized. The results indicated as water-soluble carbohydrates containing aldehyde groups may cause the formation of iron oxide nanoparticles. The purification process does not require any expensive methods for obtaining the final product, since solid product is obtained from the reaction in the liquid phase. The investigation for Gram-negative bacteria by using iron oxide nanoparticles against Pseudomonas aeruginosa was done for antibacterial effect. The Potato Dextrose Agar (PDA) medium on solid agar plates intended for bacteriological tests in liquid systems augmented with various concentrations of nanosized iron oxide particles. The effective bactericides were shown on these particles. The resulting iron oxide nanoparticles

were characterized by X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), UV-VIS absorption and Fourier Transform Infrared (FTIR) spectroscopy. Chrysopogon zizanioides was also reported for its anti-inflammatory and antioxidant activity [2].

In Murraya koenigii leaf extract, biological synthesis of silver nanoparticles and broth concentration effect in reduction mechanism and particle size is obtained.

By using UV-visible spectrophotometry, the rapid reduction of silver (Ag+) ions was monitored and showed the formation of silver nanoparticles within 15 minutes. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) analysis indicates that the synthesized silver nanoparticles are varied from 10-25 nm with spherical shape. Investigations suggest the increasing broth concentration increases the rate of reduction and decreases the particle size [3,4].

A. Indica extracts showed that the antibacterial activities against Staphylococcus aureus and Bacillus cereus, whereas no significant antibacterial activity was found against Escherichia coli and Salmonella typhimurium [5].

III. MATERIALS AND METHODS

A. *Preparation of Herbal Nanoparticles*

The leaves of C. zizanioides and *A. Indica* collected from areas of Coimbatore district and washed with the distilled water to remove dust particles stuck on the leaves. The leaves were dried under land shade for 10 days and then grinded into coarse particles with normal mixer grinder before subjected to ball milling technique. Syntheses of herbal nanoparticles are shown in the figure 1.

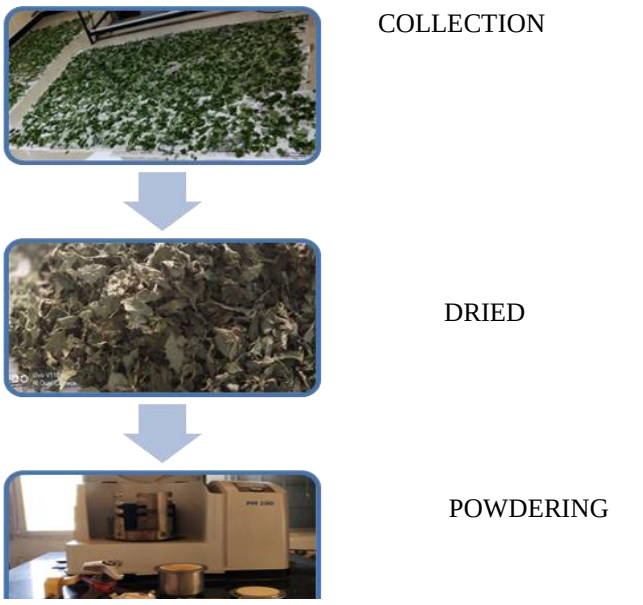


Fig.1. Flowchart for obtaining herb nano particles.

The obtained fine nanoparticles were comprehensively characterized and used for further studies dealing with wound healing properties.

1) Particle size analysis: Particle size analyzer [6] is used to measure and visualize a particle size distribution for a given sample and plays a vital role to achieve good final product quality. It works on the principle that when a beam of laser light is scattered by a group of particles, the angle of light scattering is inversely proportional to particle size. Particle size distributions are reported as 2D graphs with particle size on the x-axis and quantity of material on the y-axis.

2) UV test: The different phytoconstituents, confer specific characteristics and properties, present in plants include flavonoids, alkaloids, saponins, coumarins, phenol carboxylic acids and terpenes.. The analysis was conducted on the plant extracts of *C. zizanioides and A.indica* using a UV Visible Spectrophotometer [7](Agilent Technologies), with a slit width of 2nm, using a 10mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 300 to 800nm for proximate analysis, and helps in determining various biological activities of plants. The sample was diluted with ethanol as solvent in the ratio of 1:10.

3) Fourier Transform Infrared spectroscopy (FTIR) test: ATR-FTIR [7] test was done to identify the characteristic functional groups in the extract. The samples placed in the path of an infrared beam will absorb and transmit light and then the light signal will penetrate the sample to the detector. The detector measures the intensity of the radiation moving into a sample and the intensity of the radiation transmitted through a sample. It provides information about the structure of the molecule that could frequently be obtained from the absorption spectrum. The herbal nanoparticles of *C. zizanioides and A.indica* in the ratio 50:50 was coated in the cotton fabric by spin coating method and let to dry.

4) Atomic force microscopy (AFM) test: The herbal nanoparticles of *C. zizanioides* and *A.indica* in the ratio 50:50 were coated in the cotton fabric by spin coating method and let dry. This coated cotton fabric was subjected to scanning the sample line by line and using a calibration file for each mode of operation and cantilever type a topography image of the surface is reconstructed by the software that drives the scanning process [8].

5) Anticoagulation Test: The plant extract of *C. zizanioides and A.Indica* in the solvent ethanol was added to two test tubes, containing blood samples added with 2 drops of EDTA solution, at 0.2gm/ml and 0.4gm/ml. Control group is obtained by adding saline (0.4gms) to blood plasma. Calcium chloride (0.3gms) was added to all the three test tubes. Control group is obtained by adding saline (0.4gms) to blood plasma. All the three test tubes were placed in a water bath of 37°Celsius and the time taken for plasma in each test tube for clotting is measured. The obtained results proved that the thrombolytic activity of the herbal extract was increased with increase in the amount of herbal extract used [9].

6) Antimicrobial test: Pathogenic strains of Staphylococcus aureus,Pseudomonas aeringinosa, Escherichia coli and Proteus vulgaris were utilised in this work. The qualitative antibacterial assessment was

consummated using Agar well diffusion method. The Pathogenic strains were swabbed on each Nutrient agar plate. Wells of 5 mm diameter were embossed into the agar plates supported by sterilized cork borer (5 mm). Employing a micropipette, 20µl, 40µl and 60µl of the plant extracts (DMSO solvent) were aggregated to the wells made in the plate. The plates were incubated aerobically in an upright position at 37 °C for 24-48 hrs. Antimicrobial activity was evaluated by formulating the zone of inhibition (mm) in contempt of the strains. The test was accomplished in triplicates with tetracycline cephalosporin (30µg) as reference or positive control [10].

7) Antioxidative Test: The ethanolic extract of herbal nanoparticles of *C. zizanioides* and *A. Indica* mixed in the ratio of 1:1 was taken in five test tubes along with 2,2-diphenyl-1-picrylhydrazyl of 5µl was added. The sample and the standard were incubated in dark for about 30mins. The percentage inhibitory concentration values of standard and samples are obtained by optical density values at 517nm in the UV Spectroscopy indicates the antioxidative property[11,12].

8) Anti-inflammatory test: The anti-inflammatory property of the two herbal nanoparticles (*C. zizanioides and A. Indica*) was checked by Protein denaturation method with Dichlofenac Sodium as standard. Bovine serum albumin was added with the herbal nanoparticles at different concentrations of 200µg, 400µg, 600µg, 800µg, 1000 µg. The sample and standard were incubated at the dark room for 30 mins at 30° celsius. The absorbance of samples and standard were obtained at 660nm and the percentage inhibitory concentration was calculated [11,12].

IV. RESULTS AND DISCUSSION

The results of various analyses performed on the wound healing property of herbal nanoparticles from the plants C. zizanioides and *A. Indica* in the ratio of 50:50 proved the increased healing property than when used individually.

A. Particle Size Analysis(PSA) of C. zizanioides

x10= 15.72 nm x50=23.11 nm x90= 33.90 nm
x16= 17.02 nm x84=31.61 nm x99 = 47.78 nm

The result was obtained under a temperature of about 25.00 °C, laser power of 100%, and duration of about 75.61seconds.The result of PSA on shows that the maximum density distribution of particles is in the range between 31.61 to 47.78nm and is shown in figure 1.

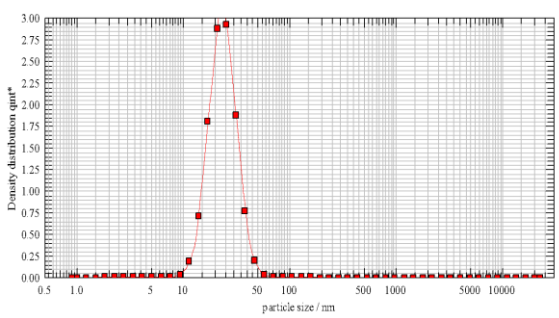


Fig.2. PSA of C. zizanioides

B. PSA of A. INDICA

x10= 14.38 nm x50=21.17 nm x90= 31.30
x16= 15.76 nm x84=28.04 nm x99= 41.63 nm

The result was obtained under a temperature of about 24.99 °C, laser power of 100%, and duration of about 75.61seconds. The result of PSA on *A. Indica* shows that the maximum density distribution of particles is in the range between 28.04 to 41.63nm and is shown in figure 3.

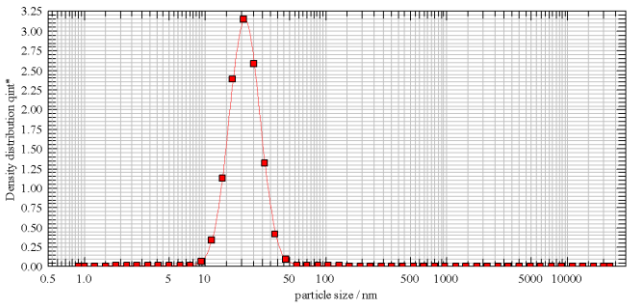


Fig.3. PSA of *A. Indica*

C. Ultra Violet Visible spectroscopy (UV) result of *C. zizanioides* and *A. Indica*

The UV –VIS analysis performed for the identification of phytoconstituents present in the ethanolic extract of Chrysopogon zizanioides and Acalypha indica. The UV-VIS spectra were performed to identify the compounds containing π bonds, φ bond, and lone pair of electrons, chromophores, and aromatic rings. The qualitative UV-VIS profile of ethanolic extracts of *A. Indica* was taken at the wavelength of 200 to 700 nm due to the sharpness of the peaks and the proper baseline. The profile showed the peaks at 438,469,490,591, 678nm with the absorption at 0.682, 0.64, 0.62, 0.489, 0.48 respectively shown in figure 4.

The ethanolic extracts of, the profile showed peaks at 201,206,282,471,490nm with the absorbance of 3.84,3.37,1.27,0.81,0.79 respectively shown in figure 5. In the UV-VIS spectra, the appearance of peaks in the region of 200 to 400nm is a clear indication of the presence of unsaturated groups and heteroatoms such as S, N, O.

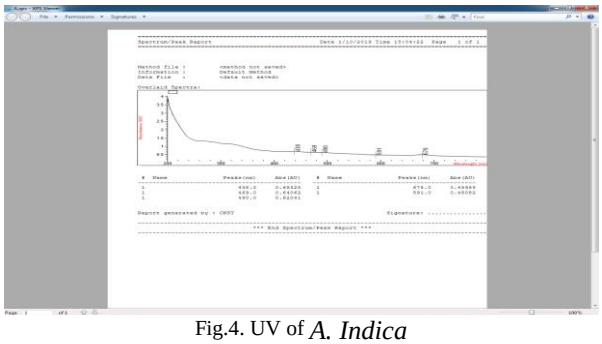


Fig.4. UV of *A. Indica*

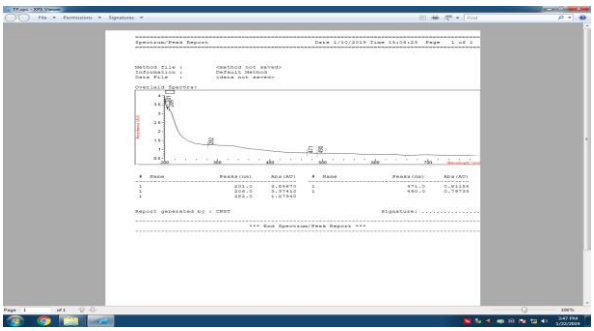


Fig.5. UV of C. zizanioides

D. Attenuated Total Reflectance-FourierInfrared Spectroscopy (FTIR) result of C. zizanioides and *A. Indica*

The ATR-FTIR spectrum of the herbal nanoparticles of C. zizanioides and *A. Indica* mixed in the ratio 50:50 coated on cotton fabric is shown in figure 6 and the table I lists the absorption range and their corresponding compound class present in the herbal nanoparticles.

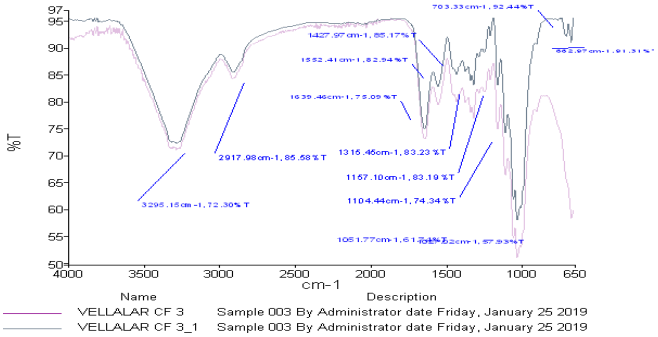


Fig.6. FTIR of C. zizanioides and *A. Indica* in ratio 50:50

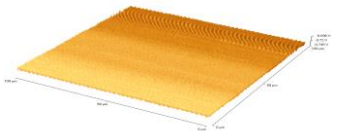
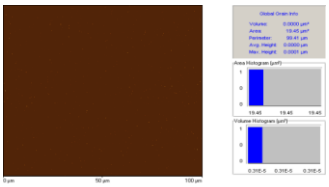
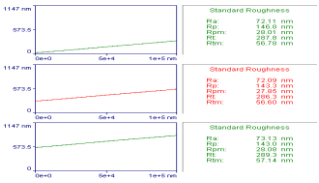
TABLE I. FTIR ANALYSIS OF C. zizanioides AND *A. INDICA* COATED IN THE RATIO 50:50

Absorption	Appearance	Group	Compound Class
1250-1020	Medium	C-N Stretching	Amine
1205-1124	Strong	C-O Stretching	Tertiary alcohol
1310-1250	Strong	C-O Stretching	Aromatic ester
1440-1395	Medium	O-H Bending	Carboxylic acid
1550-1500	Strong	N-O Stretching	Nitro compound
1648-1638	Strong	C=C Stretching	Alkene
3300-2500	Strong	O-H Stretching	Carboxylic acid
3000-2800	Strong, Broad	N-H Stretching	Amine salt

E. Atomic Fluorescence Microscopy (AFM)

The AFM result of coated fabric with the herbal nanoparticles proves that peak value of amplitude was obtained at 5.631 to 10.029V and that of deflection was obtained at 0.696 to 0.745V. The roughness of the coated fabric was obtained to be 72.11nm in all three different places of the fabric that were checked and is shown in table II.

TABLE II. AFM OF TP AND AI COATED IN THE RATIO 50:50

Amplitude	
Deflection	
Grain	
Roughness	

F. Anticoagulation And Antimicrobial Test

Extracts of the herbal nanoparticles from C. zizanioides and *A. Indica* in the ratio of 50:50 were used to check the anticoagulation and antimicrobial property. The obtained result (Table III) proves that the clotting time of the blood sample increases when the quantity of plant extract used was increased, i.e. the clotting time was higher for 4mgs of ethanol extract than 2mgs of extract. The antimicrobial activity of the plant extracts of C. zizanioides and *A. Indica* with DMSO solvent against various clinical pathogens Candida albicans, Proteus Vulgaris, and Klebsiella pneumonia were evaluated (shown in Table IV) in which Acalypha indica, the maximum zone of inhibition was obtained at 40 microliters and in the case of the maximum zone of inhibition was observed in C. zizanioides 20, 40 and 60 microliters against Candida albicans and Klebsiella pneumonia.

TABLE III. ANTICOAGULATION TEST

Experiment	Amount of Extract	Amount of Plasma	Amount of CaCl2	Time of Coagulation
Control group	Saline	0.2ml	0.3ml	4.2 mins
C. zizanioides **and *A.indica* in 50:50 (0.2gm/ml)**	0.1ml	0.2ml	0.3ml	12.7 mins
C. zizanioides **and *A.indica* in 50:50(0.4gm/ml)**	0.1ml	0.2ml	0.3ml	16.4 mins

TABLE IV. ANTIMICROBIAL TEST

Test Organism	Zone of Inhibition in diameter (cm)						
	AP			TP			Chloramphenicol (C10)
	20	40	60	20	40	60	
Candida Albicans	-	0.9	-	0.9	1.2	1.6	2.1
Proteus Vulgaris	-	-	-	-	1.2	1.3	1.9
Klebsiella Pneumoniae	-	0.9	0.9	-	0.9	0.9	2.1
Staphylococcus Aureus	-	-	-	-	-	-	2.4

G. Antioxidative Activity and Anti-Inflammatory Activity

The antioxidative and anti-inflammatory property of the two herbal nanoparticles (C. zizanioides and *A. Indica*) in the ratio of 50:50 was checked by DPPH method of free radical scavenging activity against the standard L.ascorbic acid (Shown in Table V) and Protein denaturation method against the standard Dichlofenac sodium (Shown in Table VI) respectively.

TABLE V ANTIOXIDATIVE TEST

COMPOUND	VALU	DUPLICATE VALUE	MEAN	% INHIBITORY	Ic50 VALUE
BLANK	0.619	0.846	0.732	-	-
	0.144	0.156	0.15	79.5	-
S2	0.132	0.122	0.127	82.65	-
S3	0.113	0.136	0.125	82.92	-
S4	0.125	0.114	0.120	83.60	-
S5	0.123	0.118	0.121	83.46	-
T1	0.064	0.214	0.371	49.31	60.60
T2	0.101	0.679	0.390	46.72	60.62
T3	0.046	0.339	0.192	73.77	60.4
T4	0.057	0.475	0.209	71.45	60.55
T5	0.025	0.986	1.071	24.63	60.5

COMPOUND	VALUE	DUPLICATE VALUE	MEAN	% INHIBITOR
BLAN	1.698	1.623	1.66	-
S	0.916	0.923	0.919	44.63
S	0.908	0.894	0.901	45.72
S	0.625	0.664	0.644	61.2
S	0.394	0.398	0.396	76.14
S	0.203	0.209	0.206	87.59
T	1.165	1.095	1.13	31.92
T	1.101	1.112	1.106	33.37
T	0.825	0.813	0.819	50.66
T	0.752	0.691	0.721	56.56
T	0.502	0.523	0.512	69.15

TABLE VI ANTI-INFLAMMATORY TEST

V. CONCLUSION

Plant extract of Chrysopogon zizanioides and Acalypha indica shows the best antibacterial activity against the bacteria like Candida albicans, Proteus Vulgaris, Staphylococcus aureus, and Klebsiella pneumonia while comparing with a standard antibiotic like chloramphenicol these extracts give the best results. The results of this project showed that the leaf and extracts of C. zizanioides and Acalypha indica mixed in the ratio 50:50 may have great potential as antibacterial, anti-inflammatory, and antioxidative agents in the treatment of wounds caused by infectious organisms.

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