NANOTECHNOLOGY ASSISTED CONCOCTION OF BIO-NANOMATERIAL FOR THE INSTIGATION OF NANO MEDICINE AND THE BIOCHEMICAL REVELATION RELIABLE ANTI-BACTERIOLOGICAL ACTIVITY

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ABSTRACT

The stemming extensive manipulation in regard to native merchandise synthetic substances intergalactic lays directed to detect the unprecedented composites including pharmacological assets. Natural foodstuffs, moreover in their natural form or afterward crude abstraction of their energetic constituents and discovered as irreplaceable sources aimed at drug design. Medicinal plants shows a foremost protagonist in relation to the dispensation of medical management, predominantly amongst a-few rural populace concerning in India. Ethnomedicine prepared using plant extracts and their bioactive composites have been exploited for the dealing of numerous diseases. Our study was designed to nanotechnology based preparation of ethnomedicine and assessing the butchery activity including antibacterial commotion, of carefully chosen medicinal plant Catharanthus roseus used for the control of many diseases. The leaves of the selected plants were collected, shadow dried, crushed and further nano size powdered by mixer mill and then liquidity extracted through maceration process. The prepared nano leaf powder was nano characterized by using UV, XRD, SEM, and DLS as well as subjected to phytochemical tests, GC-MS analysis, butchery activity such as antibacterial schedules were evaluated. The nano material based ethnomedicine of Catharanthus roseus was most active in contradiction of tested bacterial and fungal strains. The ethnomedicine consists of alkaloids, saponins, phenols, flavonoids, and tannins. These phytochemicals in nano powder form of leaf material, used to prepare ethnomedicine is to empathetic their tackles of action.

KEY: antimicrobial, extract, ethnomedicine, mixer mill, nanoparticles, phytochemicals.

How to cite this article: Surekha K, Shamina S, Kavitha S, Suvitha A, Gunasekaran M, Banu Karthi G. Nanotechnology Assisted Concoction of Bio-nanomaterial for the Instigation of Nano Medicine and the Biochemical Revelation Reliable Anti-bacteriological Activity. Int J Drug Deliv Technol. 2026;16(56s): 971-979. DOI: 10.25258/ijddt.16.56s.103

Source of support: Nil.

Conflict of interest: None.

1.Introduction

Plants produce an enormously rich variety of specific secondary metabolites encompassing an massive number of energetic and complementary phyto compounds. Humans hold distinct pharmacological acquaintance of the healing properties of plants. The utilization of plant extracts, based ethnomedicine comprising hundreds of biochemicals as medicinal agents[1]. The conversion of traditional ethno-pharmaceuticals into nano medicine and its invention has shadowed a modern technical pathway, abetted through nano techniques for the isolation and characterization of medicinal particles [2-4].Some nanotechnology researchers recognized by means of incessant ethnomedicinal exploration, through the energetic phyto compounds arising subsequent extensive application of nanotechnology based analysis and testing [5-9]. In current years, actionable phytochemical substances were determined using various nano techniques (High energy ball-milling, XRD,TEM,SEM, Zeta potential, DLS etc.,[10-14]. Also, tracked by the proof of identity that the plants comprising the active components, recognized by present or reputed ethnopharmacological materials[15]. Nanotechnology has sustained to harvest extensive consideration completed its earlier limited periods owing through its widespread as well as beneficial claims in plant systems [16]. Nano techniques offers some dais in advance of effort at atomic and super molecular range referring to plant nanomaterial, in the range between 1 to100 nanometre [17]. This prospect facing brand new progressive plant based ethano products from

previously traditional ones, with improved functions, physical and biological characteristics[18]. Nanotechnology have formed the motivation enroute for the incessant attention to plant science research area , specifically in relation to of ethno-nanomedicine[19]. Therefore, nanonization of plant biomaterial products give the impression and could be an imminent nanomedicine. Intrinsically, one of the distinguished instances of nano biomaterials acknowledged significant consideration into current years and have to be extensively used is plant created nanocomposites and nanomedicines [20]. In our work, we have discussed the nanotechnical ideas and methods, from traditional ethnomedicine in to nano ethnomedicine as a novel drug discovery approach using *Catharanthus roseus* leaf extract an applicable to natural medicine products. Due to its biological actions, including antioxidant, anti-inflammatory, antimicrobial, and cancer control activities, it is considered as a hypothetical herbal medicine and also cast off as a traditional medicine.

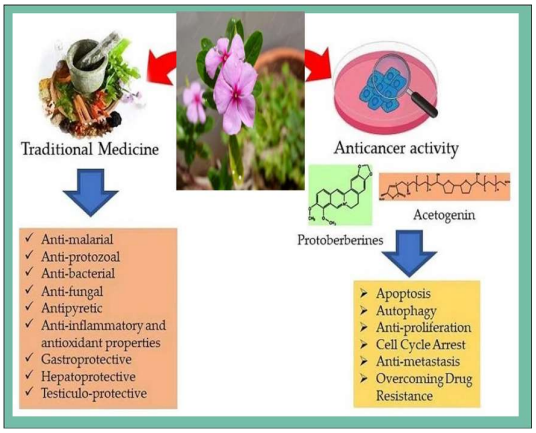

Figure 1. Almost valuable Medicinal activity of *Catharanthus roseus*

2.Materials and Methods

2.1.Plant material collection

The renewed *Catharanthus roseus* leaves were placid around Karaikudi near Kalanivasal area Sivagangai District , Tamilnadu ,India. The plant were identified by Dr. A. Arumugam Professor, Department of Botany Alagappa University, Karaikudi – 630 003, Tamil Nadu, INDIA.

Fig 2. Collected C*atharanthus roseus* plant leaves with flower

2.2. Nano Dispensation of leaf material

The picked *Catharanthus roseus* leaves subsequently rinsed through distilled water and dried in shadow and methodically homogenized as a crude powdery dirt and placed in a airtight bottles. The crude leaf powder consists of coarse micro sized particles. To get fine nano emulsion form of ethnomedicine 20 gram of crude leaf powder was taken and feed inside the mixer mill jars (Mixer mill MM -500 Nano), Retsch scientific Inc., Allee, Germany. The MM -500 Nano mixer mill is a compressed, multipurpose bench-on component. It has been technologically advanced and particularly for wet and dry cryogenic crushing 500 gm trial substantial within few seconds. Through an all-out 30-40 Hz frequency, it produces plentiful energy to yield particles in the nanoscale range.

2.3. Nanotechnological Characterization

The prepared nano powder extract of leaves were encompassed with various characterization techniques including UV-spectroscopic analysis, X-ray diffraction (XRD), SEM and DLS. It divulges and recognizes chemical conformations, crystal edifices, and photoelectric features of nano powder extract .All the characterization parameters related laboratory works were carried out in Central Instrumentation laboratory, Aalagappa University, Karaikudi – 630 003, Tamil Nadu, INDIA.

(a) Morphology of leaf Powder particles

The leaf particles surface morphology of powder extracts were detected by using SEM (Scanning Electron Microscope(Hitachi S-4000).The particle nano structure was resolute by electron dispersion at the particle surface .

(b) Particle size Determination by XRD method

The equipped powder extract of leaves were used to determine the particle size using ™ EQUINOX 100 X-ray Diffractometer(Thermo Scientific). Each obtained peak position were interpreted with Scherrer Equation:

$$Dp = (0.94\ X\ \lambda) / (\beta\ X\ Cos\theta)$$

Whereas, Dp (Average Crystallite size), β (Line broadening in radians), θ (Bragg angle), λ (X-Ray wavelength) and further the equation used in the determination of size of crystallites (grains)s in the form of powder.

(c) Ultraviolet visible spectroscopic analysis

This analysis was carried out by using a UV-Vis spectrometer (Lambda Model: 950,Perkin Elmer, USA) with a range girth of 2 nm, with a 10mm cuvette cell at 37°.

The *Catharanthus roseus* extract examined underneath the UV visible light source at the wavelength vacillating around 300 to 750 nm to confirm the formation of nanoparticles. The nano leaf powder extract was mixed with pure distilled water in 1:3 ratio and centrifugated at 6000 rpm enroute for 30 minutes and riddled over Whatman

No. 1 filter paper. Some sample filtrates were used for UV-VIS Spectroscopic analysis.

(d)Analysis of DLS - Dynamic light scattering

The nano sized particle dispersion in liquid form and polydispersity index leaf material were evaluated by DLS method (Malvern Nano system).Some limitations working for the analysis comprised a wavelength of 633 nm, RI(refractive index) liquid medium is 1.1.32, temperature 22 °C, viscosity of liquid medium 0.87873 and light scattering angle 173.

2.4. Phytochemical and GC-MS analysis

For qualitative phytochemical and GC-MS guesstimate of *Catharanthus roseus* leaf powder extracts were executed using typical procedures**,** for the finding of medicinally important flavonoids, tannins, amino acids, steroids, alkaloids, terpenoids, saponin, and phenols.

2.5. Preparation of Ethnomedicine

The 100 g nano powder form of *Catharanthus roseus* leaves underwent further corresponding extraction by means of maceration upto 24 hours further distillation about 30 minutes under chamber temperature around 30–32 °C and boiled at water bath at 100° C, categorized in favour of hot and cold concoction. The prepared extracts were mixed in 90% ethanol and the resultant ethnomedicine kept near to 4 °C in refrigerator until further laboratory work.

2.6. Antibacterial activity against human pathogen

The antibacterial activity of nanotech assisted ethnomedicine was tested against some human bacterial pathogens together with *Salmonalla Typhi, Staphylococcus aureus, Shigella* and *Klebsiella Pneumonia,* along with the standard allopathic antibiotics were assessed using Kirby-Bauer well diffusion assay. For the facilitation of diffusion by prepared ethnomedicine in inoculated medium of the plates with 6mm diameter wells were encumbered with 1mg/ml ethnomedicine sideways with positive and negative controls by means of a micro pipette. The positive rheostat delimited 1mg/ml tetracycline while the negative control pure distilled water. All the plates were categorized and allowed nurturing for 24 hrs at 37 °C. At the end of incubation, the zones of inhibitions were compared to matching the butchery activity against tested bacterial pathogens were measured in mm.

2.7. Activity against plant pathogen

The prepared solution form of ethnomedicine application on plant pathogen was conceded out on home garden rose plants they were 6 months old. The *Catharanthus roseus* nano extract based ethnomedicine was diluted by means of pure fresh water at a ratio of 100 ml of ethnomedicine in 1 liter. The prepared ethnomedicine spraying was carried out using a Hand-Sack sprayer on the black spot infected rose plants. It is most prevalent and severe disease, instigated through a fungal species *Diplocarpon Rosae,* and it is easily induced black spot on leaves. The preliminary signs leads to minor black colored spots on upper and lower parts of the leaves, finally the leaves fall off. In our experiment, spraying was carried out on experimental rose plants infected with black spot disease, not carried out for control plant. Application was repeated on experimental plants every 2 days up to 10 trails. The results were observed.

2.8. Statistical analysis

The research outcome data attained from our work has been articulated by mean (± SD error). Data comprises graphs, plotting all are confirmed by means of Version 5.0, Graph Pad Prism Software (San Diego, CA, USA).

3.Results

The mixer milled leaf powder particles were categorized to fix their morphology in addition to size through Scanning Electron Microscope (SEM). Each resultant SEM images shape were gotten which are shown in Fig. 3 and the shape of the leaf particles from place to place look like a spherical or spheroid shape. Likewise, the predictable typical particles size were roughly 21 to 32 nm.

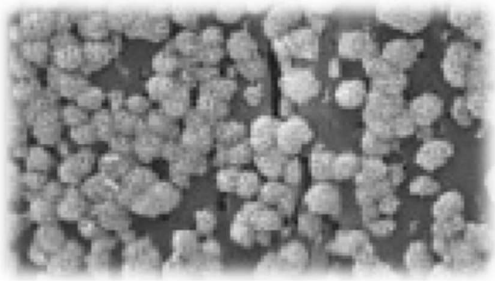

Fig 3. SEM image display the trajectories of leaf powder nano particles

The crystallite of the nanosized leaf powder particles using *Catharanthus roseus* leaf extract was inspected over X rays diffractions (Fig. 4). Determined nanoparticles size of the leaf powder was premeditated by Scherrer equation: ($D = k\lambda/\beta\cos\theta$). The angle of diffraction regarding the (111) lattice planes respectively. The approximate size of the crystallites (grains) were flinch about 30 nanometre range. The lattice limitations for the leaf powder nanoparticles were stubborn to be c = 0.4086 nm.

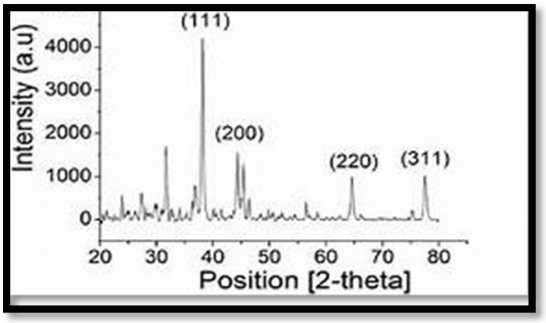

Fig 4.XRD pattern of nanoparticles present in leaf powder display peaks such as 111, 200, 220, and 311are confirmative to crystallite points

UV–Vis spectrophotometric study was approved for the prime examination of leaf nanoparticles . The absorbance of green coloured plant extract solution was investigated for one week. The absorption spectrum of the nano meter at room temperature showed in Fig. 5 and the spectral range was established from 200 nm to 800 nm. The determined peak wavelength was detected at 376 nm, it confirms that the solution form of leaf extract consists of polydisperse nano particles.

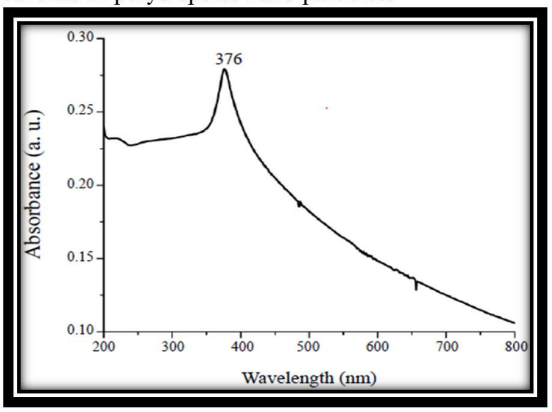

Fig 5. UV-Vis absorption spectra of leaf nanoparticles in aqueous medium

By using DLS- Dynamic light scattering technique the size of dispersal silhouette of nano particles present in any solution or suspension can be determined. By the way of our study the mean nanoparticles size dispersal in suspension(ethnomedicine), exposed that size referring to ball milled leaf based liquid form nanoparticles assumed the size range around 39 to 44 nm. The fig. 6, clearly shows, that the leaf nanoparticles suspension had diverse sizes of nanoparticles.

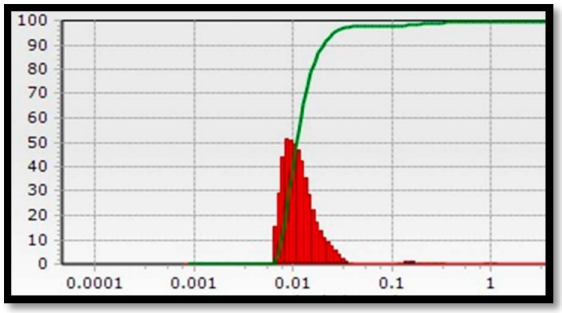

Fig 6. Dynamic light scattering ((DLS) shows leaf nano particle size in solution

In solution or suspension form particle under going Brownian moment are shown on the X- axis (red), characteristic examples of a well-fit autocorrelation curve (green).The intensity of light scattering is taken as a consideration in Y-axis. The nano technology pragmatic preparation of ethnomedicine shows potent therapeutic effects. It is due to the presence of valuable phytochemicals in *Catharanthus roseus* . In phytochemical determination alkaloid (reddish brown), Tannins (bluish black),flavonoids(yellowish), saponins(foam or froth) and phenol derivatives(white precipitate) were identified. The identified phytochemicals exhibited in Table 1 and Fig .7 and GC-MS analysis of biochemical constituents in figure 8; Table 2 respectively.

(A) (B) (C) (D) (E)

Fig.7- Phytochemical determination **(A)** Alkaloids**(B)**Tannins **(C)** Flavonoids **(D)** Saponins and **(E)** Phenolics

Table-1

Phytochemicals	Methods	Observation	Results
Alkaloids	Wagner's test	Reddish brown	Presence of alkaloids
Tannins	Ferric chloride test	Blackish blue	Presence of tannins
Flavonoids	Ammonia test	Yellowish	Presence of flavonoids
Saponins	Foam test	Foam formation	Presence of saponins
Phenolics	Gelatin test	White precipitate	Presence of phenolics

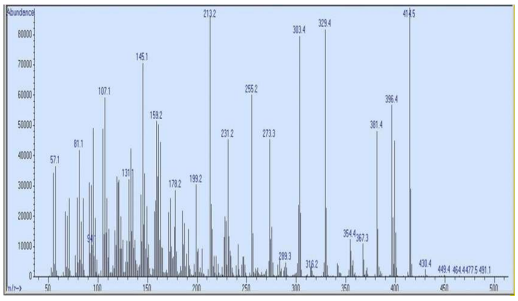

Fig.8.GC–MS analysis of Biochemical constituents from *Catharanthus roseus.*

Table-2

GC-MS evidence of all typical Biochemical compounds involved in Antimicrobial Activity

R. T.	Compound Name	M. W.	Formulae	AREA %	Co. Nature	Activity
31.30	cis-13-Octadecenoic acid	282	$C_{18}H_{34}O_2$	1.58	Fatty acid	Antibacterial
20.14	Phenol, 2,4-bis (1,1-imethylethyl)-	206.32	$C_{14}H_{22}O$	7.45	organic compound	Antifungal and Antimicrobial
23.74	Hexacosane	226.45	$C_{26}H_{54}$	0.4	alkane	Antimicrobial
25.21	Tetradecanoic acid	228.38	$C_{14}H_{28}O_2$	1.7	Fatty acid	Cancer preventive
25.51	5-Octadecene, (E)-	252	$C_{18}H_{32}$	0.47	long-chain fatty acid	Stronger sexual characters
28.81	Pentadecanoic acid	242	$C_{15}H_{30}O_2$	12.52	Fatty acid	Antimicrobial
28.81	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$	12.52	Fattt acid	Cytotoxic
29.53	Heptadecanoic acid	270	$C_{17}H_{34}O_2$	0.26	Fatty acid	Antimicrobial
31.40	cis-Vaccenic acid	282.46	$C_{18}H_{34}O_2$	1.65	Fatty acide	Antimicrobial
31.66	Octadecanoic acid	284	$C_{18}H_{36}O_2$	1.00	Fatty acid	Antimicrobial
30.26	Palmitoleic acid	254.41	$C_{16}H_{30}O_2$	9.31	Fatty acid	Antibiotic and Pesticide
34.79	1-Nonadecene	266	$C_{19}H_{38}$	0.242	long-chain fatty acid	Anti-fungal activity
32.96	9-Hexadecenoic	298.54	$C_{20}H_{42}O$	2.85	Organic cid	antibiotic
36.62	Bis(2-ethylhexyl) phthalate	390.56	$C_{24}H_{38}O_4$	2.94	organic compound	intitoxicity and intioxide.
36.62	Diisooctyl phthalate	390.5	$C_{24}H_{38}O_4$	2.94	organic compound	Antimicrobial and Antifouling
39.86	Decanedioic acid, bis(2-ethylhexyl) ester	426.67	$C_{26}H_{50}O_4$	1.7648	organic compound	Antimicrobial and antioxidant
40.02	Squalene	410	$C_{30}H_{50}$	1.05	organic compound	Antibacterial and antioxidant

The antibacterial activity of prepared ethnomedicine exhibit constructive result, while equated to tetracycline assayed as standard or control antibiotics .

The Doubled distilled water, used as a negative control did not show the inhibition zone. The inhibition zone of standard antibiotics tetracycline shows 12 mm diameter(Table 3; Fig 8).The butchery activities of prepared ethnomedicine *Catharanthus roseus* counter to bacterial pathogenic strains and resulting inhibition zones were paralleled with antibiotic Tetracycline. The ethno-nanomedicine shown more active antimicrobial proficiency and butchery activity against, *Staphylococcus aureus, Klebsiella Pneumonia, Shigella* and *Salmonalla Typhi* when comparatively analysed to the standard antibiotics.

Table-3

Antibacterial activity against Human pathogens

Test pathogens	Negative control (solvent Only)	Positive Control (tetracycline)	Ethnomedicine
S.aureus.	Nil	18 mm	16mm
K.pnumoniae	Nil	14mm	12mm
S.typhi	Nil	16mm	14mm
Shigella	Nil	21mm	18mm

Statistical study: Mean frequency of 3 changed evaluates

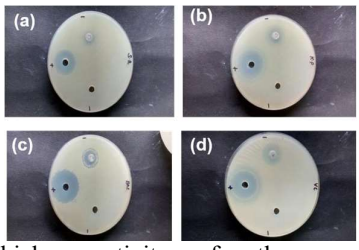

Fig 8. Antimicrobial activity of ethno-nanomedicine against selected human pathogens. The culture plates confirms susceptible inhibitory zones. (a) S.aureus, (b) K.pnumoniae , (c) S.typhi and (d) Shigella

Ethno-nanomedicine spray treatment on black Sopot leaves had a significant impact on the degeneration of black spots on leaves indicates P value = 0.03. Almost, 99 % leaf black spots disappeared and normal condition was reimbursed in all infected leaves (Fig 10), when equated to the normal uninfected plants. The results showing that the ethnomedicine were found to be significant ($P<0.05$) in clampdown of black spot development at advanced level.

Fig 9.Liqufied Ethnomedicine

Fig 10(a). Rosa indica leaves infected by black spot disease (before treatment),

Fig 10 (b). Step by step recovery of black spot disease infected leaves into normal condition after 14 days treatment of ethnomedicine spray

4.Discussion

Nanotechnology partakes noteworthy progress over the usage of assets built on nanomaterials. Plants have the capability to convert as a nanomaterials, which canister to changes the composition and structure [21]. Nanoparticle amalgamation by means of many plant portions for example leaf, flower, roots, and fruits [22]. The conversion of plant materials into nano material and nano ethnomedicine has develop challenging research extent owing to its immense application in numerous fields including medicine and disease control. The preparation of ethnomedicine using nanotechnology synthesis to avoiding poisonous chemicals, eco-friendly, and cost efficiency engrossed countless researchers on the way to it [23]. Usage of plant portions and its biotic activities increases the efficacy of nanoparticles. Such plant based nanoparticles advantageous in medicinal ground due its butchery activity against cancer cells, bacterial cells and also have antiviral activities. This chattels correspondingly imperative in drug delivery, diagnosis of disease and as a biomarkers [24].These properties were revealed by the ethnomedicine prepared by us using nanotechnology. Apart from the methods above-mentioned, we have used another approach employed to advance the solubility, bioavailability of unwell water-soluble plant material based drugs is mixer milling. This milling technique useful to reduce size reduction of leaf material mad about nanonized particles.

High - energy ball milling including our technique so called mixer milling is a component process, wherever mechanical energy is functional to materially break down rough particles to more atomic level nano particles. Henceforth, is observed as a “top–down” method in the production of fine nano particle [25]. As effectively every one drug can be mashed to fine particles irrespective of its soluble properties in liquid based or non-liquid based solvents[26,27] The acceptable size nano medicinal particles are created after their liquified molecular status and appropriate solvents/non-solvents of the drug necessity to be prepared [28]. Leaf powder nanoparticles appear green in colour in liquefied medium as a outcome of surface plasmon

ambiences[29]. Similar vicissitudes in colour in the same way been detected in previous studies [30]. This was signified by expansion of the peak which showed the development of poly dispersed leaf nanoparticles due to slow reduction rates carried out by the phytochemical constituents.

The SEM, XRD, and DLS analysis of leaf nanoparticles were seen that particles of dwarf size and spherical shapes. Similar results were attained in circumstance of different leaf extracts[31].The phytochemicals such as flavonoid, tannin being act as reducing and capping agents[32]. Neem, Banana, and Hibiscus extracts formed almost spherical nanoparticles. In this study, the butchery activity in relation to antimicrobial property shown inhibition zones with all-out antibacterial activity of the prepared test ethnomedicine sample. Results attained in preceding studies[33], also sustenance the antibacterial potential of ethnomedicine. The butchery activity (killing activity) against the fungus of *Rosa indica* plants obtained wide-ranging meaningfully ($p < 0.05$) with each treatment.

The prepared ethnomedicine were causing carnage activity associated with antibiotic resistance. The ethnomedicine efficiently inhibited the expansion of all tested bacterial and fungal strains. The ethno-nanomedicine caused the cell membrane deformation. The membrane devastation is ascribed to the nano size and the spherical shape of leaf nanoparticles, which existing a larger surface zone[34]. The nanoparticles have the properties to facilitating better superficial reactions contrary to target organisms including bacteria and fungi [35].

In the stretch that crude ethnomedicine have coarse big size medicinal particles they cannot penetrate the pathogens cell matrix, the nano particles cross the bacterial and fungal cell matrix and express the noticeable butchery activity by destruction of the cell membrane [36]. The same results were confirmed in the present study, due to the preparation of nanosized ethnomedicine.

5.Conclusion

By using *Catharanthus roseus* plan*t* ethno-nanomedicine was successfully obtained from bio reduction of leaf biomaterial . The leaf powder nanoparticles have been properly characterized by means of UV-vis spectroscopy, SEM, XRD and DLS analysis. Results represented the ethnomedicine unchanging nature and microbial killing property, these ethno-nanomedicine may be well exploited in industrial and therapeutic purposes. However, plant based ethnomedicine approval and utilization necessitate more comprehensive research. This study acquaint with nanotechnology based preparation of ethnomedicine as antibacterial and antifungal agents. The medicinal features of the ethnomedicine with nano sized bio particles can be used as a potential forthcoming antibacterial and antifungal therapeutic. Therefore, it is suggested that upcoming studies proceeding on such ethnomedicine should be protracted to embrace in vivo mockups and medicine wellbeing researches.

Ethical Approval: Our **s**tudy procedures have no any ethical issues.

Acknowledgements: We are thankful to all of our Institutional staff members for their constant encouragement throughout the study.

Funding: Funding for the research, authorship, and/or publication of this article was not received from any organizations.

Conflict of Interest: All Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Authors' Contribution:

1 and 2: Experimentation on antibacterial and antifungal activity, Phytochemical identification, analysis and graphical presentation.
3-6: Ethno-nanomedicine formulation, nano technological characterization and applications.
All authors jointly drafted and approved the article in revised version to be published.

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