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Ball Milling Synthesis of Herbal Nanopowders from Various Parts of Rauwolfia Serpentina, and their GC-MS Analysis and Evaluation Of Antimicrobial Activity.

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Abstract

In the field of nanotechnology Green synthesis of nanoparticles has received increasing attention because of its eco-friendliness, cost effective nature, safety and simplicity. The present study focuses on the production of herbal nanopowders from *Rauwolfia serpentina*, an important medicinal plant of Apocynaceae, employing ball milling technique which produces homogenous nanoparticles. These nanoparticles were then thoroughly characterized using UV–Visible spectroscopy, Scanning Electron Microscopy, X-Ray Diffraction. FTIR confirmed the presence of various functional groups in the herbal nanopowders. Then the bio-active constituents in root, stem and leaf nanopowders were evaluated using GC-MS. Different types of compounds were identified in root, stem and leaf nanopowders by comparison of GC-MS spectrum with Library searches. The Anti bacterial and anti fungal activity of methanolic root, stem and leaf nano powder extacts are tested against selected bacteria (*Klebsiella pneumoniae, Salmonella typhi, Vibrio cholerae ,Corynebacterium diphtheriae, Vibrio parahemolyticus*) and selected fungi (*Aspergillus fumigatus, Candida albicans, Mucor hiemalis*). The methanolic extract of synthesized herbal nanoparticles showed potent antimicrobial activity against all the test bacteria and fungi when compared to standard drugs.

Key words: *Rauwolfia serpentina,* Ball milling, Herbal nano particles, GC-MS.

Introduction

In recent years, green synthesis of nanoparticles has been an active area of research owing to their excellent properties and remarkable applications. Nanoparticles, the fundamental blocks of Nanotechnology possess some novel properties not seen with macro molecules. At Nano size (1 to 100 nm), atoms and molecules work differently and produce a variety of surprising and interesting results (1). Nanoparticles can be synthesized by physical, chemical and biological approaches. Biological approach for nanoparticle synthesis utilizing plants also called “Green Synthesis” is completely eco-friendly that connects Nanotechnology with plants. Synthesis of metal nanoarticles from plants has attracted the attention of researchers in recent years (2). Various types of metal nanoparticles like silver, gold, copper, zinc etc., have been widely used in different fields of science. Silver nanoparticles were widely used in nanomedicine as they have multi functional applications like anti-bacterial, anti-fungal, anti-viral, anti-parasite, anti-inflammatory, anti-angiogenic and anti-cancer properties (3). Although silver nanoparticles are most frequently used nanoparticles because

of their anti-bacterial activity, they produce harmful effects on human health. Cells upon exposure to silver nanoparticles generate reactive oxygen species (ROS) which are toxic. These ROS damage cell membrane, disrupt ATP production, DNA replication and alter gene expression (4, 5). Due to the hazardous effect of silver nanoparticles, there is need to develop eco-friendly, biocompatible nanoparticles and this can be achieved through ball milling which produces homogenous nanoparticles with enhanced biological activity (6). In the present study herbal nanoparticles were synthesized from different parts of medicinal plant *Rauwolfia serpentina* by top down method. In top down method bulk material is broken down into fine particles by size reduction employing various lithographic techniques (7). Ball milling is one of the top down approaches to produce homogeneous nanoparticles. Ball milling plant parts maintains the chemical composition of original material without adding any artificial ingredients or wasting any material (8). *Rauwolfia serpentina* (L). *Benth. ex Kurz.* is an endangered species belonging to the family Apocynaceae. It is an important medicinal plant of Indian sub-continent and South East Asian countries and is commonly known as Sarpagandha (9). A large number of secondary metabolites are localized in different plant parts of *R. serpentina* mainly roots and rhizomes (10). It is a medicinally famous herb in Ayurveda, Siddha, unani and western system of medicine (11). It is known for curing various disorders due to the presence of alkaloids, flavonoids, glycosides, steroids, tannins, resins, alcohols, sugars and fatty acids (12). Although different metal nanoparticles were synthesized from this plant till now, very little focus was exerted on the synthesis of herbal nanoparticles from *R. serpentina*. Present study focused on synthesis of herbal nanoparticles from different parts of *R. serpentina* and to evaluate their bioactive potentiality.

Materials and Methods

Collection of plant material

The plant *Rauwolfia serpentina* was collected from Chintapalli/Paderu area between 18^0 4^{l} 59.88^{ll} N and 82^0 40^{l} 1.20^{ll} E in Visakhapatnam District of Andhra Pradesh, India.

Synthesis of herbal nanoparticles

The roots, stems and leaves were separated from collected plants. All the parts were washed twice with tap water followed by double distilled water for multiple times to remove dust particles from the surface. The washed roots, stems and leaves were shade dried at room temperature for 3 weeks. Initially, a mixture grinder was used to grind shade dried roots, stems and leaves to obtain coarse powders. The obtained coarse powders were further subjected to ball milling (PM 200 RETSCH) with a steel vial of 125 ml volume. For ball milling 7 mm size zirconium balls were used. Root, stem and leaf powders were milled in the ball mill at 500 rpm for about 25 hours.

Characterization of herbal nanoparticles

UV–Visible spectroscopy (UV–VIS)

The herbal nanoparticles of root, stem and leaf were dissolved in carbinol placed in quartz cuvette for optical analysis at room temperature. The absorption spectra of all herbal nano particles were obtained using UV–Visible Spectrophotometer (UV–2400 PC series, Shimadzu) operated from wavelength range of 200-800 nm spectral regions at a resolution of 1.0 nm.

Scanning electron microscopy (SEM)

To identify the morphology and micro structure of nanoparticles, herbal nanopowders were subjected to SEM analysis. For SEM analysis, the sample was spread on carbon coated copper grid and thin film was formed under vacuum. SEM analysis was carried out using field emission scanning electron microscope (FE–SEM–JSM–7100 F/JEOL India Pvt., Ltd., Bangalore), with a resolution of 1.2

nm at 30 KV and 32 nm at 1 KV.

X-ray diffraction studies (XRD)

XRD is used to know the face centre cubic crystalline nature of the synthesized herbal nanoparticles. The dried powder was coated on XRD copper grid and analyzed for nanoparticles by using XPERT – PRO Diffractometer (PANALYTICAL) at a voltage of 45 KV and a current of 40 mA. The spectrum is recorded using CuK alpha radiation of 1.5406 angstorms in the range of 2 theta from 10^0 to 91^0. The crystallite domain size of nanoparticles is calculated from the width of the XRD peaks using the Debye – Scherrer formula,

$$D = 0.94\ \lambda\ /\ \beta\ \text{Cos}\ \theta$$

Where D is the average crystallite domain size perpendicular to the reflecting planes, **λ is the X ray wavelength i.e., 1.5406** angstorms, β is the full width at half maximum (FWHM) and **θ is the diffraction angle.**

Fourier transform infrared spectroscopy (FTIR)

The spectra of herbal nanoparticles were obtained using FTIR (SHIMADZU Spectrometer, IR Affinity 1S) in the frequency range from 400 to 4000 cm^{-1} at a resolution of 4 (1/cm) by an average of 10 scans per sample using KBr pellet. These pellets were used for FTIR analysis in transmittance mode. The pellet was obtained by mixing 200:1 of KBr, nanoparticles.

Gas chromatography-mass spectroscopy (GC-MS)

GC-MS analysis of the sample was performed using a GCMS (QP 2010 SHIMADZU). Chromatography was performed on a 30 m X 0.25 mm ID fitted with DB – 5 methyl phenyl siloxane column. Injector temperature was set at 260 ^{0}C, ion source temperature at 200 ^{0}C. Helium was used as carrier gas with flow rate of 1 ml/m and the sample injected was 1 micro litre. The oven temperature was programmed from 80 ^{0}C for 2 minutes to 260 ^{0}C for 10 minutes. Total GC running time was 28 minutes. Compounds of Methanolic extracts of different parts of *R. serpentina* nanopowders were identified by comparison of their mass spetra and retention indices with those published in literature and contained in The National Institute Of Standard And Technology (NIST) library data base**.** The name, molecular weight, molecular formula and structure of the components of the test materials were ascertained.

Antibacterial activity

The antibacterial activity of methanolic extract of *R. serpentina* root, stem, leaf nano powders was assessed against human pathogenic gram positive bacteria i.e. *Corynebacterium diphtheriae* and gram negative bacteria i.e. *Klebsiella pneumoniae, Salmonella typhi, Vibrio cholerae and Vibrio parahaemolyticus*. The antibacterial activity was determined by agar well diffusion method using 4 various concentrations of herbal nano powders (50 **µ**g/ml, 100 **µ**g/ml, 150 **µ**g/ml, 200 **µ**g/ml). Antibiotic chloramphenicol (30 **µg/ml) was used as positive control and DMSO** (30 **µl/ml) was used as negative control.** Antibacterial activity recorded when ZOI was greater than 8mm and studies performed in triplicate and mean values calculated.

Antifungal activity

The anti fungal activity of methanolic extract of *Rauwolfia serpentina R*oot, Stem, Leaf nano powders was tested against human pathogenic fungi viz *.Aspergillus fumigatus, Candida albicans, Mucor hiemalis* by agar well diffusion method. The fungal cultures grown overnight at 28^0c were spread on to the surface of potato dextrose agar plates with sterile glass spreader and 4 wells were made at equal distance with sterile cork borer. Antifungal activity recorded when ZOI was greater than 8mm and studies performed in triplicate and mean values calculated. Nystatin was used as positive control and anti fungal activity was determined using 4 various concentrations of

herbal nanopowders (50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml).

MIC determination

The cultures were then incubated and subsequently, serially diluted to reach the density of 2 × 10^4 cells per ml. Cell counting was done using hemocytometer. Two milliliters of broth were dispensed in tubes, and 100 µL of cell culture was inoculated in it. Then, 50 - 200 µg/ml of different concentration of extract was added to each tube. Each experiment was carried out in a triplicate set. All the experimental tubes were incubated in 24-48 h. After completion of incubation period, the optical density was measured at 600 nm using spectrophotometer. The MIC was defined as the minimum concentration of extract that caused 20% inhibition in growth of test microorganism (13, 14). The percentage of bacterial inhibition by each extract was computed using the following equation.

$$\text{PERCENTAGE INHIBITION} = \frac{\text{O.D IN CONTROL} - \text{O.D IN TEST SET}}{\text{O.D IN CONTROL}} \times 100$$

Results

Herbal nanoparticles characterization studies

UV-Visible spectral analysis

UV-Visible spectrophotometry was used to confirm the presence of nanoparticles. The surface Plasmon resonance of root nanoparticles produced peak centered near 360.50 nm, 307.50 nm, 291.50 nm, 224.50 nm, 205.50 nm. Stem nanoparticles produced a single narrow peak at 204.50 nm and leaf nanoparticles produced peaks near 664.50 nm, 409 nm , broad peak at 356 nm, 266 nm and narrow peak at 202 nm (Figures 1). The UV absorption spectra of herbal nanoparticles shows correlation with UV absorption spectra of metal nanoparticles reported in this species [15, 16].

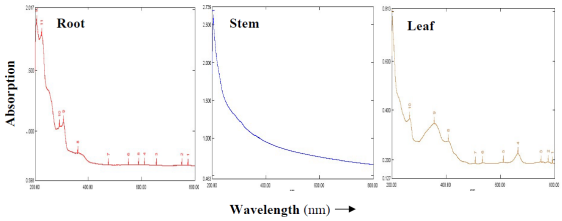

Figure 1. UV-Vis analysis of *R. serpentina* 3.1.2 SEM analysis

Herbal nanoparticles are clearly seen in the micro-graph. Root and stem nanoparticles shows spherical structure whereas leaf nanoparticles showed sponge like structure. The size of root, stem and leaf nanoparticles were 45-65 nm; 56-71 nm and 55-72 nm respectively (Figure 2).

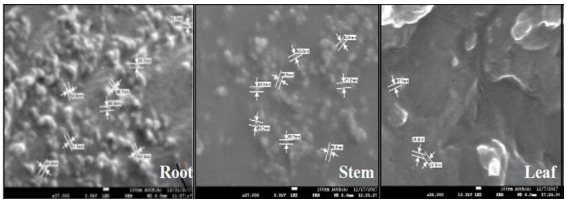

Figure 2. SEM analysis of *R. serpentina*

XRD analysis

The X-Ray diffraction profile of root, stem and leaf nanoparticles was analyzed (Figure 3). The XRD pattern confirms the crystalline nature of nanoparticles. The average crystallite size was calculated from Scherrer formula. Smaller nanoparticles were obtained from leaf nano powders (17.60 nm). The size of the nanoparticles formed was calculated and presented in Table 1.

Table 1. XRD data of studied herbal nanoparticles of *R. serpentina*

Plant Part	Peak Position (2θ)	Average Crystalline Size (nm)
Root	27.064330	26.16 nm
Stem	38.603450	26.29 nm
Leaf	31.223130	17.60 nm

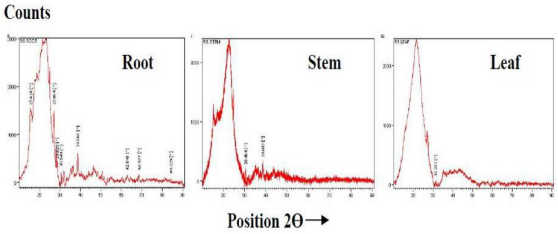

Figure 3.XRD Analysis of *R.serpentina.*

FTIR analysis

FTIR analysis confirms the presence of functional groups in the nanopowders. The FTIR spectroscopical data and functional group identification of root, stem and leaf nanopowders were presented in Tables 2 and Figure 4.

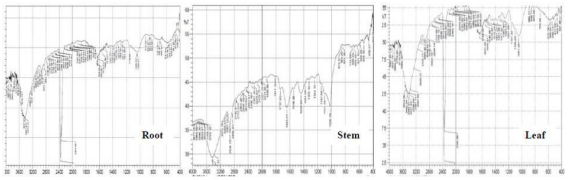

Figure 4. FTIR analysis of *R. serpentina*

GC-MS analysis

The GC-MS analysis of root, stem and leaf shows various chemical compounds. The methanolic mixture of root nano powder showed the chemical compounds such as isopropyl alcohol, Methane Sulfinyl Bis, argon, 2,4dichloro-2,4-Diflouro-1,3-Dithiethane-1,1-Dioxide, 1-(Pent-4-Ynyl)Pyrano{3,4-B}

Table 2. FTIR spectroscopical data of *R. serpentina*

Wave Number (cm^{-1})	Functional Groups
Root	
2343.59	CO_2
2243.29	C ≡ N stretch
2926.11(weak peak)	Alkane C-H stretch
3527.92, 3421.83, 3414.12 (strong broad peaks)	Intermolecular Alcohol O-H stretch
3857.76, 3844.26(weak sharp peaks)	N-H stretch
Stem	
2341.66, 2359.02	CO_2
3524.06, 3462.34, 3446.91, 3421.83(strong broad peaks)	Intermolecular Alcohol O-H stretch
2924.18(weak peak)	Asymmetric C-H stretch
1641.48, 1610.61(weak broad peaks)	N-H bend of Amine
Leaf	
3423.76, 3439.19(strong broad peaks)	Intermolecular Alcohol O-H stretch
2924.18(strong sharp peak)	Alkane C-H stretch
1643.41, 1438.94(strong broad peaks)	C-H bending mode
1020.38(strong sharp peak)	Flouro Alkanes C-X bend

Indol-3-O, 1,2-Dodecanediol2-0Benzoylester, trideuteroacetonitrile, 1,1'-Bibicyclo(2.2.2) Octyl-4-Carboxylic acid, trideuteroacetonitrile, 1,2-Propanediol, isocyanic acid, 2-Nitropropane, trideuteroacetonitrile and 1,1'-Bibicyclo(2.2.2) Octyl-4-Carboxylic Acid. The methanolic herbal nanopowders of stem contains following chemical compounds isopropyl alcohol, beta ionone epoxide, isobutyl ester of nitrous acid, 1,2-Dodecanediol2-0 benzoyl ester, trideuteroacetonitrile, 1,1'-Bibicyclo(2.2.2) Octyl-4-Carboxylic Acid, 1-(Pent-4-Ynyl) Pyrano{3,4-B}Indol-3-O, argon. The leaf nanoparticles extracted with methanol recorded the chemical constituents isopropyl alcohol, 5-Hexenal Oxime, N,N'-Bis(2-Methyl-

2-Nitrosopentan-4-One, 1,1'-Bibicyclo(2.2.2) Octyl-4-Carboxylic acid, argon, 5-Hexenal Oxime.

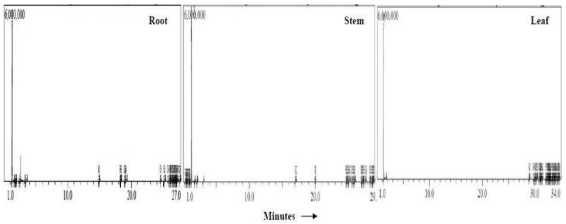

Figure 5. GC-MS chromatogram of herbal nanoparticles

Evaluation of antibacterial and antifungal activity

The herbal nanoparticles obtained from different parts of *R. serpentina* showed significant antibacterial and antifungal activity with degrees of variation. The methanolic herbal nanopowder extract of root showed ZOI between 14-16 mm against all test bacteria, more than positive control chloramphenicol with maximum ZOI 16 mm against *Klebsiella pneumoniae.* Similarly stem and leaf nano powder extracts showed more ZOI than positive control against all test bacteria, the maximum ZOI of stem nano powder being 18 mm against *Klebsiella pneumoniae* and leaf nanopowder being 16 mm against *Vibrio parahemolyticus.*

Table 3. Compounds identified through GC-MS analysis

Sr. No	Retention Time	Area%	Matching	Name of the Compound
Root				
1	1.26	87.47	97	Isopropyl Alcohol
2	2.59	11.82	98	Methane Sulfinyl Bis
3	26.875	0.05	100	Argon
4	3.449	0.04	94	2,4dichloro-2,4-Difflouro-1,3-Dithiethane-1,1-Dioxide.
5	26.798	0.04	99	1-(Pent-4-Ynyl) Pyrano{3,4-B} Indol-3-O
6	26.475	0.02	100	1,2-Dodecanediol2-0 Benzoyl Ester
7	18.325	0.02	100	Trideuteroacetonitrile
8	24.555	0.02	97	1,1'-Bibicyclo(2.2.2) Octyl-4-Carboxylic Acid
9	14.892	0.02	100	Trideuteroacetonitrile
10	2.442	0.02	99	1,2-Propanediol
11	1.875	0.02	98	Isocyanic Acid
12	1.641	0.02	95	2-Nitropropane
13	25.767	0.02	100	Trideuteroacetonitrile
14	26.097	0.02	96	1,1'-Bibicyclo(2.2.2) Octyl-4-Carboxylic Acid
Stem				
1	1.243	99.34	99	Isopropyl Alcohol
2	0.483	0.06	97	Beta Ionone Epoxide
3	2.138	0.06	93	Isobutyyl Ester Of Nitrous Acid
4	28.73	0.04	100	1,2-Dodecanediol2-0 Benzoyl Ester
5	0.725	0.03	100	Trideuteroacetonitrile
6	25.825	0.03	98	1,1'-Bibicyclo(2.2.2) Octyl-4-Carboxylic Acid
7	28.576	0.03	86	1-(Pent-4-Ynyl) Pyrano{3,4-B} Indol-3-O
8	25.933	0.02	100	Argon
Leaf				
1	1.245	96.75	99	Isopropyl Alcohol
2	32.947	0.17	83	5-Hexenal Oxime
3	33.433	0.17	88	N,N'-Bis(2-Methyl-2-Nitrosopentan-4-One
4	34.575	0.17	82	1,1'-Bibicyclo(2.2.2) Octyl-4-Carboxylic Acid
5	34.426	0.12	93	Argon
6	32.875	0.11	83	5-Hexenal Oxime

Table 4. Zone of inhibition observed on different bacteria

Plant Part (µl)	Zone of Inhibition (mm)																			
	Klebsiella pneumoniae				*Salmonella typhi*				*Vibrio cholerae*				*Vibrio parahemolyticus*				*Corynebacterium diphtheriae*			
	50	100	150	200	50	100	150	200	50	100	150	200	50	100	150	200	50	100	150	200
Root	12±0.2	13±0.1	14±0.1	16±0.2	10±0.1	11±0.1	13±0.1	15±0.2	10±0.1	12±0.1	13±0.1	15±0.2	10±0.1	10±0.1	12±0.1	14±0.1	11±0.1	13±0.2	15±0.2	15±0.1
Stem	12±0.0	14±0.1	16±0.1	18±0.2	-	10±0.1	10±0.1	12±0.0	-	12±0.1	14±0.1	16±0.2	10±0.1	12±0.0	12±0.1	16±0.1	10±0.0	12±0.0	12±0.1	14±0.2
Leaf	10±0.0	12±0.1	12±0.0	14±0.2	10±0.0	10±0.1	12±0.1	14±0.1	10±0.0	12±0.0	12±0.0	14±0.2	10±0.1	12±0.1	12±0.1	16±0.1	10±0.1	12±0.1	12±0.2	14±0.1

Plate1. Antibacterial activity of nanoparticles on *test bacteria*

MIC

The MIC 50 refers to concentration of plant extract required to prevent the growth of 50% of organisms tested. MIC of methanolic root nano powder extract was observed at 150 µg/ml for *Klebsiella pneumoniae*, *Corynebaccterium diphtheriae* and 200 µg/ml for *Salmonella typhi, Vibrio parahemolyticus*. The MIC 50 of *Rauwolfia serpentina* stem nano powder is 100 µg/ml for *Klebsiella pneumoniae*, 150 µg/ml for *Vibrio cholerae*, *Vibrio parahemolyticus* and 200 µg/ml for *Corynebacterium diphtheriae*. The MIC 50 of *R. serpentina* leaf nano powder is 200 µg/ml against *Klebsiella pneumoniae*, *Salmonella typhi*, *Vibrio cholerae*, *V. parahemolyticus*.

Rauwolfia_serpentina root nanopowders showed good antifungal activity against all tested fungi with ZOI between 17-20 mm more than control Nystatin, and leaf nanopowders showed ZOI between 15-16 mm.

Table 5. Zone of inhibition observed on different fungi

Plant part (µl)	Zone of Inhibition (mm)											
	Aspergillus fumigatus				Candida albicans				Mucor hiemalis			
	50	100	150	200	50	100	150	200	50	100	150	200
Root	13±	15±	17±	20±	12±	13±	15±	17±	12±	13±	14±	18±
Stem	12±	14±	15±	17±	13±	14±	15±	17±	12	14±	15±	18±
Leaf	13±	14±	17±	18±	12±	13±	14±	15±	12±	13±	14±	17±

Ball milling synthesis of herbal nanopowders

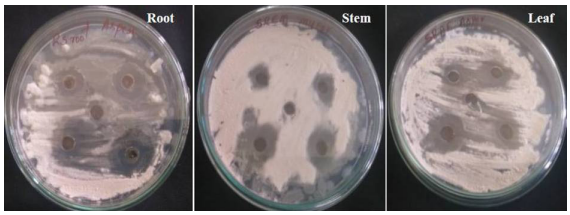

Plate 2. Antifungal activity of nanoparticles against *A. fumigatus and M. hiemalis*

MIC

The MIC of methanolic root nanopowder extract was observed at 100 µg/ml for *Aspergillus fumigatus*, 150 µg/ml for *Candida albicans* and 200 µg/ml for *Mucor hiemalis*. The MIC 50 of stem nanopowders was 100 µg/ml for *Candida albicans, Mucor hiemalis,* 150 µg/ml for *Aspergillus fumigatus*. The MIC of leaf nanopowders was 150 µg/ml for *Aspergillus fumigatus,* 200 µg/ml for *Candida albicans, Mucor hiemalis*.

Discussion

In the present study a novel pathway was presented in the synthesis of herbal nanoparticles from different parts of *Rauwolfia serpentina* by ball milling method without applying heat treatment or any chemical reagent. Although sufficient volume of published literature is available on the synthesis of silver nanoparticles through green route in this plant species , in this paper it is aimed to produce nanoparticles by one of the top down approaches namely ball milling technique. These nanoparticles are further analyzed under SEM and their morphology appeared spherical and sponge like shape and the size ranges between 46-72 nm. The XRD pattern in the present study confirms the crystalline nature of nanoparticles. The obtained nano particle size of root, stem and leaf in XRD pattern is 26.16 nm, 26.29 nm and 17.60 nm respectively and tally with the results of sliver nanoparticles derived through bio reduction in *R. serpentina* [15, 16]. The UV Visible spectral analysis showed that the surface plasma resonance absorbance bands of root, stem and leaf nanoparticles produced peaks at 360 nm 307 nm, 291 nm, 224 nm, 205 nm, 204 nm and 664 nm, 409 nm, 356 nm, 266 nm, 202 nm respectively which confirms the presence of nanoparticles, as per other studies reported in case of metallic nanoparticles in this plant [16,17]. It is also in agreement with results obtained in case of herbal nanoparticles synthesized by ball milling from *Tridax procumbens* leaf [18]. The UV Visible spectrum indicates the presence of flavanoids as the flavonoid spectra typically consists of two absorption maxima in the range of 230-290 nm and 300-350 nm [19]. The results of FTIR spectroscopic analysis revealed the presence of certain functional groups present in the methanolic extract of root, stem and leaf nanopowders. The absorbance peaks of root nanoparticles observed at 2343.59, 2243.29 cm^{-1} confirms the presence of CO_2 and C ≡N stretch, 2926.11 cm^{-1} confirms alkane C-H stretch, 3527.92, 3421,83, 3414.12 cm^{-1} confirms intermolecular bonded alcohol O–H stretch of alcohols, phenols [20] and 3857.76, 3844.26 cm^{-1} confirms N-H stretch. The absorbance peaks of stem nanoparticles observed at 2341.66, 2359.02 cm^{-1} confirms CO_2 peaks at 3524.06, 3462.34, 3446.91, 3421.83 cm^{-1} confirms intermolecular bonded alcohol O-H stretch of phenols, alcohols, hydrogen bonded flavanoids, tannins [20, 21], peak at 2924.18 cm^{-1} confirms asymmetric C–H stretch and peaks at 1641.48, 1610.61 cm^{-1} confirms N-H bend of amine of proteins [18, 21]. The absorbance peak of leaf nanoparticles at 3423.76, 3439.19 cm^{-1} confirms the presence of intermolecular bonded alcohol O–H stretch. Peak at 2924.18 cm^{-1} confirms the presence of C–H bending mode and peak at 1020.38 cm^{-1} confirms the presence of Floro alkanes C–X bend [16]. The peaks observed between 800-1700 cm^{-1} in the herbal nano particles studied indicate the presence of flavanoids [22].

The GC-MS chromatogram of methanolic extract of root nanopowder showed 23 peaks indicating the presence of 12 different

compounds, besides a number of peaks with very narrow retention time. The GC-MS chromatogram of methanolic extract of stem nanopowder showed 19 peaks indicating the presence of 9 different compounds, besides a number of peaks with very narrow retention time. The GC-MS chromatogram of methanolic extract of leaf nanopowder showed 12 peaks indicating the presence of 11 different compounds, besides a number of peaks with very narrow retention time. In accordance with the previous findings, most of the identified compounds from the GC-MS were not reported elsewhere in this species and other species [23, 24].

The chemical compounds identified in the methanolic extract of root, stem and leaf nano powders are presented in Table 3. In accordance with the previous findings, some of the identified compounds from this study have also been reported elsewhere in other species. According to Nowrangi *et al.* [25], argon identified in root, stem and leaf nanopowders possess neuro protective ability. According to Ansel *et al.* [26] methane sulfinyl bis (DMSO) identified in root nano powder possess antibacterial activity. According to Nalawade *et al.* [27] 1, 2 propane diol (propylene glycol) identified in root nanopowder possess antibacterial activity.

The methanolic extracts of root, stem and leaf nanopowders showed good antibacterial and antifungal activity against all tested bacteria and fungi than standard antibiotic. The *R. serpentina* stem and root nanopowders showed highest ZOI 18 mm, 16 mm respectively against *Klebsiella pneumoniae*. Leaf nanopowders showed maximum ZOI 16 mm against *Vibrio parahemolyticus* although all the nanoparticles showed greater ZOI than standard against all tested bacteria. Root nanoparticles showed maximum ZOI against *Aspergillus fumigates* (20 mm) compared to ZOI of antibiotic Nystatin which is only 12 mm. Stem nanoparticles showed maximum ZOI 18 mm against *Mucor hiemalis*. The leaf nanopowders maximum ZOI is 18 mm against *Aspergillus fumigatus*.

In MIC assay for both bacteria and fungi, as the concentration of nanopowder extract was increased, inhibition percentage of bacterial and fungal growth also increased. The root nano powder extract showed MIC 50 at a conc. of 150 µg/ml against *Corynebacterium diphtheriae, Klebsiella pneumoniae*. Stem nanopower extract showed MIC 50 at a conc. of 100 µg/ml against *Klebsiella pneumonia* where as leaf nanopowder extract showed MIC 50 at a conc. of 200 µg/ml against *Vibrio parahemolyticus.* The root nanopowder extract showed MIC 50 at a conc. of 100 µg/ml against *Aspergillus fumigates*, stem nanopowder extract at a conc. of 100 µg/ml against *Mucor hiemalis* and leaf nanopowder extract at a conc. of 200 µg/ml against *Aspergillus fumigatus*.

Conclusion

In this work, a novel method was developed for the synthesis of herbal nanoparticles from different parts of *Rauwolfia serpentina* by high energy ball milling and these nanoparticles proved to have excellent antibacterial and antifungal properties. The present study gives new insight on the potential of herbal nanoparticles prepared from this plant when compared to metal nanoparticles as good antibacterial and antifungal agents. These herbal nanoparticles can be produced on large scale with less cost when compared to silver nanoparticles production which is costly and cannot be produced in bulk amounts. This observation helps in the use of herbal nano particles in biological applications. Thus the simple and rapid method of biosynthesis of herbal nanoparticles by ball milling can further be explored for application in various bio medical and bio technological fields.

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