A REVIEW ARTICLE: GREEN SYNTHESIS OF SOME MEDICINAL PLANTS - DERIVED NANOPARTICLES: A GREEN CHEMISTRY APPROACH WITH BIOMEDICAL APPLICATION INSIGHTS

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Introduction

Green synthesis is the organic synthesis of nanoparticles by utilizing plant extracts and the developed nanoparticles are called biogenic nanoparticles. Several findings have reported that a wide number of medicinal plants are used to synthesize the silver NPs such as Mulberry leaves , Alternanthera dentate , Ocimum sanctum , Azadirachta indica, Brassica rapa, Coccinia indica, Vitex negundo, Melia dubia are already been utilized to synthesize and stabilize metallic nanoparticles, more significantly biogenic silver (Ag) nanoparticles. Recently the development of green synthesis of silver nanoparticles has become more acceptable throughout the world for the treatment of several disorders due to having several advantages including less toxicity, effective mechanism of actions, improved bioavailability and targeting. This green synthesis is used for the treatment of antibiotic-resistant bacteria, cancer because of having effective characteristics in comparison with other chemical ways and drugs. To avoid the limitations of chemical methods, green synthesis methods (including bacteria, fungi, plant extracts, and small biomolecules like vitamins and amino acids) are mostly considerable which are simple, cost effective, dependable, and environmentally friendly options and high yield productions of AgNPs of defined size.

Sources of Green Synthesis

Ag-NP are synthesized at industrial stage using physio-chemical strategies including chemical reduction, gamma ray radiation, micro-emulsion, electrochemical strategies, laser ablation, autoclaving, electrochemical techniques, microwaving, photochemical reduction. Having toxic outcomes, excessive cost, requirement of excessive energy, now green synthesis gives a potential and novel method which involves usage of bacteria, fungi, yeast, algae, plant extract as reducing or stabilizing agents extensively used microorganisms are *Shewanella oneidensis*, *Trichoderma viride* (*T. viride*), *Bacillus sp.*, *Lactobacillus sp*., and a few vegetative parts of plants. Nanoparticles are frequently more catalytic, have electromagnetic capacity and are as a consequence more reactive.ROS technology ability makes them extra toxic than bulk counterparts. Distinctive plant metabolites which includes carbohydrates, alkaloids, terpenoids, tannins, phenolic acids play a prime function in fabrication of Ag-NP by way of inexperienced synthesis. The maximum essential plant parts liable for reducing Ag particles for the formation of Ag-NP are polysaccharides, aqueous solvent heterocyclic blends. In green synthesis of Ag-NP the use of plant components, the vital variables for Ag-NP preparations are plant source, herbal mixes in rough leaf elimination, concurrence of Ag particles, temperature, centralization of leaf concentrate shades and many others. Plant separation would possibly act as reducing in addition to stabilizing agent in Ag-NP formation. In a examine, A. R. Vilchis-Nestor et al. used extract of *Camellia sinensis* (in experienced tea) as reducing and stabilizing agents for the synthesis of gold silver nanoparticles in aqueous solution underneath ambient conditions. In another look at Kalishwaralal k. et al. mentioned synthesis of Ag-NP with the aid of lowering Ag+ ion in aqueous solution with supernatant of Bacillus licheniformis culture. Ag-Np synthesized by means of this method has high established order and this technique additionally has

an advantage over conventional strategies as microorganisms here used are nano pathogenic bacterium. Sarma et al. (2009) said several techniques for synthesizing Ag-NP under water the use of non-poisonous and value effective compounds. green synthetic approach confirmed more capability in synthesis. green synthetic methods encompass mixed-valence polyoxometalates, polysaccharide, Tollens, irradiation, and biological etc. The mixed-valence polyoxometalates were conducted in water solution of $AgNO_3$ that consists of glucose and starch in water produces starch protected silver nanoparticles. Dubey et al., (2010) pronounced a brand new and simpler technique for biosynthesis of gold and silver NPs. The method includes tansy fruit (*Tanacetum vulgare*) extract for synthesis of gold and silver nanoparticles of round and triangular shapes. The common diameter of silver and gold nanoparticle crystals had been decided as 16 nm and 11 nm with the aid of the Scherrer approach.

Preparation of plant extract

Plants are a complex matrix that produces a large number of secondary metabolites with different functional groups and polarities. Although, many traditional protocols use water as an extractant. In modern extraction processes, organic solvents of different polarities are generally selected to take advantage of the different solubilities of plant components. Soxhlet extraction is often used for both initial and bulk extraction. Its main advantage is the continuous extraction of the material. The solvent saturated with the solubilized metabolite is discharged into the flask, and a new recondensation solvent extracts the substance in the thimble. This process consumes less time and solvent than bleaching and penetrating. 100 g of ground, weighed fine powder material was continuously extracted in a particular order using various solvents of hexane, chloroform and methanol based on the increased polarity. The Soxhlet heat extraction procedure for each of the above solvents was performed for approximately 6 hours until a colorless solvent was found in the siphon tube, indicating complete extraction. The solvent was removed by rotary evaporation under reduced pressure and controlled temperature. The extract was dried, stored in a clean glass bottle and stored at 46°C for further antibacterial screening at 4°C until further investigation. Aqueous plant leaf extracts have been used to produce silver nanoparticles based on cost-effectiveness, easy availability, and their medicinal properties. About 10 g of finely chopped leaves of each plant type were weighed separately, transferred to a 250 ml beaker containing 100 ml of distilled water and boiled for about 30 minutes. The extract was then filtered 3 times with Watman # 1 filter paper to remove particulate matter, a clear solution was obtained and cooled in a 250 ml Erlenmeyer flask (4°C) for further experimentation. At each step of the experiment, sterilization conditions were maintained for the effectiveness and accuracy of the clean results.

Maceration

4 kg of dried leaves were soaked in a 1 liter Erlenmeyer flask at room temperature for 24 hours using 8.6 liters of methanol. Next, the obtained extract was filtered using Whatman filter paper to obtain a methanol extract. The remaining residue was subjected to a second continuous extraction with methanol according to the procedure described above to obtain a second methanol extract. The TLC profiles of both extracts were then examined and mixed based on similar TLC patterns. The methanol extract thus obtained was concentrated on a rotary evaporator and dried in a vacuum oven to obtain a thick and viscous mass. Next, the yield was calculated, and the concentrated methanol extract was continuously extracted with hexane, chloroform, and methanol to obtain soluble hexane, chloroform, and methanol.

Phytochemical Tests

The phytochemical test of these extracts was performed using the method adopted by Harborne (1973) and Sofowora (1993).

1. **Test for Carbohydrates (Molisch's Test):** To 2 mℓ of plant extract, 1 mℓ of Molisch's reagent and a few drops of concentrated sulfuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.
2. **Test for Tannins (Ferric Chloride Test):** To 1 mℓ of plant extract, 2 mℓ of 5% Ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.
3. **Test for Saponins (Frothe's Test)**: To 2 mℓ of plant extract, 2 mℓ of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of a 1cm layer of foam indicates the presence of

saponins.

4. **Test for Flavonoids (Shinoda Test)**: To 2 mℓ of plant extract, 1 mℓ of 2 N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.
5. **Test for Alkaloids (Mayer's Test)**: To 2 mℓ of plant extract, 2 mℓ of concentrated hydrochloric acid was added. Then a few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids.
6. **Test for Quinones**: To 1 mℓ of extract, 1 mℓ of concentrated sulfuric acid was added. Formation of red color indicates presence of Quinones.
7. **Test for Glycosides (Molisch's Test):** To 2 mℓ of plant extract, 3 mℓ of chloroforms and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.
8. **Test for Cardiac Glycosides (Keller–Kiliani Test)**: To 0.5 mℓ of extract, 2 mℓ of glacial acetic acid and a few drops of 5% ferric chloride were added. This was under layered with 1 mℓ of concentrated sulfuric acid. The formation of brown ring at the interface indicates presence of cardiac glycosides.
9. **Test for Terpenoids (Salkowski Test):** To 0.5 mℓ of extract, 2 mℓ of chloroform was added and concentrated sulfuric acid is added carefully. Formation of red brown color at the interface indicates presence of terpenoids.
10. **Test for Phenols (Ferric Chloride Test)**: To 1 mℓ of the extract, 2 mℓ of distilled water followed by a few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.
11. **Test for Proteins:** To the 0.5 mℓ of extract, 0.5 mℓ of extract, 2 mℓ of 40% sodium hydroxide and 1 mℓ 1% CuSO4 were added, formation of pink colour indicates presence of proteins.

DPPH Radical Scavenging Assay

Free radical scavenging is one of the mechanisms involved in antioxidant action, a good antioxidant (AH) able to scavenge the DPPH (1,1 Di phenyl 2-picryl hydrazyl) radical and retain its own stability due to its reduction ability as shown in the equation below.

DPPH* + AHDPPH H + A* $\longrightarrow$

Procedure

Plant extracts were tested for blocking effect by the DPPH radical method and 2 ml of extracts from different solvents (hexane, chloroform, and methanol) were collected at different concentrations (5, 50, 100, and 400 µg). A 0.4 mM / ℓ methanolic DPPH solution was added. A solution containing 2 ml of methanol and 2 ml of DPPH solution was used as the negative control and the synthetic antioxidant ascorbic acid was used as the positive control. Different concentrations were stored in the dark at room temperaturefor 30 minutes. The removal activity of the DPPH was determined by measuring the absorbance at 517 nm until the reaction reached steady state using a spectrophotometer. All the determination was performed five times.

The DPPH radical scavenging activity was calculated using the following equation.

$$\% \, inhibition = \frac{1 - A1}{A0} \times 100$$

A1 and A0 are the absorbance of the tested sample and control respectively.

Ferric reducing power assay (FRAP)

Ferric reducing/antioxidant power (FRAP) Zhao, H. et al 2008 described it previously Briefly, 100 µl of each concentration (100-600 µg / ml) of the extract was mixed with 2.5 ml of 200 mM phosphate buffer (pH 6.6). And 2.5 ml of 1% potassium ferricyanide was incubated at 50°C for 20 minutes. Next, 2.5 ml of 10% trichloroacetic acid was added and the tubes were centrifuged at 10,000 rpm for 10 minutes. 5 ml of the top layer of the solution was mixed with 5.0 ml of distilled water and 1 ml of 0.1 µl chloride and the absorbance of the reaction mixture was measured at 700 nm. The final result was expressed as mg ascorbic acid equivalent / g dry weight.

$$(Controlabsorbance - Sampleabsorbance)$$

$$\% \ inhibition = Control absorbance \times 100$$

Ferric reducing antioxidant power

The antioxidant capacity of medicinal plants was estimated spectrophotometrically according to the method of Benzie and Strain. This method is based on reducing the Fe3+ TPTZ complex (colorless complex) to Fe2 + tripyridyltriazine (blue complex). It is formed by the action of electron-donating antioxidants at low pH values. This reaction is monitored by measuring the change in absorbance at 593 nm. Ferric Reducing Antioxidant (FRAP) Reagent is prepared by mixing 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl, and 20 mM FeCl3,6H2O at 37 ° C in a 10: 1: 1 ratio. Did. The freshly prepared FRAP working reagent was pipetted with a 15 ml variable micropipette (3.995 ml) and mixed with 5 µl of the corresponding diluted plant sample for complete mixing.

When the iron (III) tripylidyltriazine (Fe3 + TPTZ) complex is reduced to iron (II) form and disappears at 593 nm with respect to the reagent blank (3.995 ml FRAP reagent + 5 µl distilled water), it is a deep blue complex. Was formed. After incubating at 37 ° C for 30 minutes. All decisions were made three times. The calibration curve was established by plotting the absorbance at 593 nm for various concentrations of FeSO4. The concentration of FeSO4 was plotted again against the concentration of the standard antioxidant Trolox. The FRAP value is obtained by comparing the change in absorbance of the test mixture with that obtained by increasing the concentration of Fe3 + and is expressed as the Trolox equivalent mg per gram of sample.

***In vitro* antimicrobial assays:**

The development of a simple in vitro pre-screening may provide a first impression of the biological activity of the plant extract and its compounds. Microbial cultures and bioassay studies generally require two media, solid (agar) and liquid (broth). This solid matrix is ideal for presenting microorganisms on the surface of the medium as it is required for the separation of pure colonies to prevent significant mixing of colonies in the culture. Liquid media, on the other hand, are more mobile media and are most useful for producing concentrated inoculations of microorganisms [Kanika Sharma, 2005; Kanika Sharma, 2005, Handbook of microbiology.,Tools and Techniques, 133-183]. Antibacterial activity was performed using two methods: agar disk diffusion of essential oil extracts and agar groove or well diffusion of solvent extracts (Perez et al., 1990; Murray et al., 1995). Of these two methods, the agar well diffusion assay was used to screen for antibacterial activity of extracts from various plant species. This is the most common type of antibacterial activity identification that utilizes the diffusion of antibacterial compounds through agar media to demonstrate inhibition of bacteria and fungi.

Antibacterial activity:

Composition of nutrient agar medium:

Peptone	: 5g
Sodium chloride	: 5g
Meat extract	: 10g
Agar agar	: 15g
Distilled water	: 1000 mℓ
pH adjusted to	: 7.2 to 7.4

Agar cup plate method/ Agar well diffusion method:

In the agar diffusion method, a small amount of peptone (0.5 g), meat extract (1.0 g), sodium chloride (0.5 g), and agar (1.5 g) in distilled water are dissolved in a water bath and heated, and the volume is increased with purified water. I made it 100 ml. The pH of the nutrient broth was adjusted to 7.2 using 5M sodium hydroxide and sterilized in an autoclave maintained at 121 ° C, (15 lbs) for 20 minutes. After sterilization, the medium was inoculated with 3 µl culture aliquots containing approximately 105 CFU / ml of each of the tested organisms, tilted for 24 hours under sterile conditions, transferred to sterile Petri dishes and allowed to solidify at room temperature for approximately 10 minutes. Store in the refrigerator for 30 minutes for a few minutes. After setting, beaker bits # 3 (6 mm) in diameter were properly flame sterilized and used to create 4-5 uniform beakers / wells in each Petri dish.

A drop of molten nutrient agar medium was used to seal the bottom of each beaker. The cups/wells were filled with 50µl of the different extracts of 100mg/ml, 250mg/ml, and 500mg/ml so final drug concentration will be 5mg/well, 15mg/well, and 25mg/well respectively and allow diffusing of plant extract into the medium for about 45 minutes. The standard drug ciprofloxacin (10 µg / ml), control (0.1% DMSO), was transferred to a beaker on each agar plate using a sterile pipette under a laminar flow unit. The plates thus produced were left in the refrigerator for 2 hours for diffusion and then stored in an incubator at 37 degrees Celsius. After 24 hours, the inhibition zone on the agar plate was examined and the zone was measured in millimeters. Table No. 7-10 shows the observed data on the activity of the methanol extract. Inhibition zones were measured in mm on the antibiotic zone scale and the experiment was performed 3 times.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC value of the extract was determined based on the microbroth dilution method on 96 multi-well microtiter plates with minor modifications. In the susceptibility test, 1 g of plant material powder was first dissolved in 1000 µl of dimethyl sulfoxide (DMSO) to achieve a final concentration of 1 g / ml. 100 µl of this suspension was added to the first test well of each microtiter line as a sterile control, then 50 µl of scalar diluent was transferred to the 2nd to 10th wells. The eleventh well was considered growth control because no extract was added. 50 µl broth was distributed to the 2nd to 12th wells. Next, 50 µl of bacterial suspension was added to each well. The plates were incubated at 37 ° C for 24 hours. Ciprofloxacin (10 µg / m²) was prepared and used as a standard drug for positive controls. As an indicator of bacterial growth, 40 µl of pyodonitrotetrazolium (INT) violet dissolved in water was added to the wells and incubated at 37 ° C. for 30 minutes (Buwa and van Staden, 2006). The lowest extract concentration that did not show growth was defined as the minimum inhibitory concentration (MIC) and was confirmed by plating 5 µl samples from clear wells on agar medium. The tetrazolium salt acts as an electron acceptor and is reduced to a red formazan product by bioactive organisms (Eloff, 1998). When bacterial growth was suppressed, the solution in the well remained clear after incubation with INT.

Antifungal activity:

Composition of Potato dextrose agar medium:

Potatoes (peeled): 200.0g Dextrose: 20.0g
Agar-Agar: 15.0g
Distilled water: 1000 mℓ

Procedure:

Peeled potatoes (20 grams) were chopped and boiled in 100 ml of water for 30 minutes. Crush during cooking, cool and filter with a muslin cloth to remove pulp. After adding dextrose (2 grams) and agar (1.5 grams) to a volume of 100 ml, the medium was sterilized in an autoclave at 121 degrees Celsius (15 lbs) for 30 minutes. Aftersterilization, inoculate the medium using a 4-day culture of the test organism, adjust the turbidity to 0.5 McFarland units (equivalent to 1.5 x 105 or 106 CFU / m²) under sterile conditions, and transfer to a sterile Petri dish. Allow to solidify for 10 minutes at room temperature. For each agar plate, 4-5 beakers with a diameter of 6 mm were prepared on the medium at regular intervals using a sterile drill. With methanol extracted at various concentrations (100 mg / m², 250 mg / ml and 500 mg / ml), the final active ingredient concentration is 5 mg / well, 15 mg / well, or 25 mg / well, Control (DMSO). And standard (fluconazole), 10 µg / ml) were transferred to the beaker of each agar plate. The plates thus prepared were diffused for 2 hours and then incubated at 28 ° C. at room temperature. After 36 hours, the inhibition zone on the agar plate was examined and the zone was measured in millimeters. Table 11-13 shows the observed data on the activity of the methanol extract. Inhibition zones were measured in mm on the antibiotic zone scale and the experiment was performed 3 times.

Minimum fungicidal concentration (MFC)

The minimum fungicidal concentration (MFC) of the various extracts was determined by a microdilution method using a serially diluted (2-fold) plant extract according to the 2000 Clinical and Laboratory Standards Institute (NCCLS). Equal amounts of each extract and broth were mixed in a test tube. In particular, 0.1 ml of standardized

inoculum material (12 x 10^5 CFU / ml) was added to each tube. The tubes were aerobically incubated at 37 ° C for 24-48 hours. Two control tubes were stored for each test lot. These include antifungal controls (tubes containing growth medium without extracts and inoculum) and biological controls (tubes containing growth medium, saline, inoculum). The lowest concentration (maximum dilution) of the extract that did not result in visible fungal growth (no turbidity) when compared to the control tube was considered to be MFC. However, MFC was determined by subculturing the test diluent in PDA medium for an additional 72 hours (Rubio et al., 2005). The highest dilution that did not result in fungal colonies on the solid medium was found to be MFC.

Preparation of 1 mM AgNO3 solutions

One millimolar solution of AgNO3 (0.017 gms) was prepared by dissolving in 100 mℓ deionized water (DIW) and stored in amber coloured bottle in cool and dry place.

Silver nanoparticle (Ag NP) synthesis

Green synthesis refers to the supplementation of biomaterials using plant extracts. The development of biocompatible, non-toxic and environmentally friendly methods for synthesizing AgNPs is a topic of green chemistry. Silver nanoparticles (Ag NPs) were synthesized using the redox method. Silver nanoparticles were created using plant extracts as a reducing agent. In this study, the synthesis is started with a 1 mM aqueous solution of silver nitrate (AgNO3) and is used for the synthesis of silver nanoparticles. Add 2 ml of plant extract to 8 ml of 1 mM silver nitrate aqueous solution to reduce Ag + ions and store at room temperature. To minimize the photoactivation of silver nitrate, the reaction was carried out overnight at room temperature in the dark until the color changed from colorless to yellow. By visually observing the color change from pale yellow to dark brown in the reaction mixture, rapid synthesis of AgNP was observed after 5 hours of incubation. The fully reduced solution was cooled to room temperature and then centrifuged at 10,000 rpm for 30 minutes in a high speed centrifuge. The pellet was dispersed in water and this procedure was repeated to obtain a colored supernatant solution. The sample was then stored at 4°C for further use. The supernatant was then decanted and the precipitate washed with millipore water and then ethanol and then dried in a hot air oven. The paste was then collected in a ceramic crucible and heated in an air-heated oven at 400 °C for 2 hours. A pale yellow powder was obtained, which was carefully collected and packaged for characterization. This was repeated 3 times to better separate AgNP and used for characterization studies.

Characterization Methods

Several characterization methods have been devised to investigate size, distribution, shape, surface charge and porosity of nanoparticles in different environments. Here, we discuss the main techniques for the characterization of these key parameters both in the dry state and in solution. We also introduce some specialized techniques for nanoparticle characterization, which enable to expand the accessible range of information to gain deeper insights into specific nanoparticle properties. Given the large number of existing approaches and techniques, including the combination of different methods in “hyphenated” techniques, different variations of the same techniques, and different approaches to data analysis for a same technique, this article cannot provide an exhaustive list of all available methods for nanoparticle characterization. We rather provide a selection of methods that in our opinion are best suited to characterize a broad range of nanomaterials, which are commonly used and well established.

The AgNPs were phyto-synthesized by adding 100 ml of 1 mM silver nitrate with 5 ml of extract and incubated at room temperature for 30 min. After 45 min of reaction, the sediment was washed three times with distilled water by centrifugation at 13,000 rpm for 20 min, and the product was kept at 60°C for 2 hr 17 . The absorption spectrum of prepared AgNPs was measured using a UV-vis spectrophotometer in the wavelength range of 200-700 nm. The dried powder of AgNPs was further analyzed by XRD (X-ray diffraction) and Fourier Transform Infrared spectrophotometer (FTIR). Size, shape, and morphology of the AgNPs were determined by Scanning Electron Microscopy (SEM) (XL30 electron microscope, Phillips, Japan) and Transmission Electron Microscopy (TEM) (Leo 906, TEM model 100 KV, Zeiss, Germany). The Energy-Dispersive X-ray Spectrometer (EDX) analysis was performed using the EDS X Sight Oxford instrument.

Characterization in Dry State

1. Transmission Electron Microscopy (TEM)

Transmission electron microscopy is undoubtedly one of the most important nanoparticle characterization techniques. TEM employs a focused electron beam on a thin (typically less than 200 nm) sample to produce micrographs of nanoscale materials with high lateral spatial resolution. Current electron microscopes can achieve resolutions down to 0.05–0.1 nm by reducing image distortion by aberration correctors, hence providing high-resolution images with atomic resolution. TEM also enables studying the crystalline structure of selected microscopic regions of crystalline materials by spatially confining and focusing the impinging beam and detecting the resulting electron diffraction pattern. Thanks to this high spatial resolution and selectivity, TEM enables the investigation of size, shape, and crystal structure at the single-particle level. Once a representative group of images of the nanoparticle sample is acquired, the individual size of ≈1000 randomly selected nanoparticles should be measured to obtain meaningful statistics for size distribution determination. This procedure can be done either manually (inherently affected by human errors, bias, and subjectivity) or using automated particle analysis methods.

2. Scanning Electron Microscopy (SEM)

Scanning electron microscope enables imaging the sample surface by detecting secondary electrons emitted from the sample upon interaction with the impinging electron beam. In SEM, lower beam energies are utilized for sample imaging as compared to TEM characterization, which results in a limited penetration depth of the beam and, hence, in being sensitive solely to the specimen surface. However, this superficial interaction also implies that SEM characterization can be used for the analysis of the morphology of "thick" (>100 nm) samples, which is not possible with TEM. The moderate electron energies employed for SEM analysis limit the resolution to typically >2–3 nm, however at the same time drastically decrease the possibility of beam-induced sample damage compared to TEM. In addition, SEM is by far more user-friendly and enables faster measurements, and features lower acquisition and maintenance costs than TEM.

3. X-Ray Diffraction (XRD)

X-Ray diffraction is a versatile technique used to investigate a wide range of structural aspects in crystalline samples. The attainable information ranges from microscopic features, such as the arrangement of the crystal components, to macroscopic information, such as the mean shape and size of crystals. This information can be obtained by analyzing the full width at halfmaximum (FWHM) of the Bragg reflections. Since every Bragg peak is associated with a unique crystallographic direction, the FWHM is influenced by the number of atoms contributing to the scattering events and this number is directly connected to the size of the crystal planes generating the specific reflection. Different sizes for different crystallographic directions are associated to a specific shape (i.e., morphology) of the crystallites, which can therefore be refined against the XRD pattern.

4. GC/MS analysis of plant extract

The chemical composition of plant extract was studied using a Gas Chromatography-Mass Spectrometer (GC/MS) system. GC/MS analysis was done on Schimadzu 15A gas chromatograph equipped with a split/spitless injector (250°C) and a flame ionization detector (250°C). Nitrogen was used as carrier gas (1 ml/min) and the capillary column used was a DB-5 (50 m 0.2 mm, film thickness 0.32 μm). The column temperature was kept at 60°C for 3 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. Relative percentage amounts were calculated from the peak area using a Shimadzu C-R4A Chromatopac, without the use of correction factors.

Conclusion

India has a rich biodiversity of medicinal plants that has not yet been fully investigated. Of the estimated 250,000-500,000 plant species, only a few have been investigated in detail and only a few have undergone biological or pharmacological screening. Medicinal plants provide basic raw materials for various industries such as pharmaceuticals, cosmetics, perfumes and foods. Medicinal plants are used by about 80% of the world's population as herbal ingredients for health care. Medicinal plants are the most exclusive source of life-saving medicine for the majority of the world's population. They are still important therapeutic tools for alleviating human suffering. The

whole plant or individual parts thereof can be examined for their therapeutic, medicinal, aromatic or spicy properties. It also plays an important role as an antibacterial agent. Plants are an important source of structures that help develop new chemotherapeutic agents. The first step towards this goal is an invitro assay for antibacterial activity. There are many reports on the antiviral, antibacterial, antifungal, anthelmintic, antisoft and anti- inflammatory properties of plants. Some of these observations have helped identify the active ingredients responsible for such activities and develop drugs for therapeutic use in humans. However, there are not many reports on the development of commercial formulations of antibacterial chemotherapeutic agents that utilize the antifungal or antibacterial properties of plants. Resistance to antibacterial agents such as antibiotics is found in a wide variety of organisms around the world, and multiresistant organisms pose a serious threat to the treatment of infectious diseases. For this reason, plant antibacterial agents have received considerable attention and need today. Many years of new antibacterial active ingredients from plants have received a great deal of attention in the current research world due to the costs and higher side effects associated with chemically produced active ingredients.

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