Project entitled

Chemical Investigations on four Astavarga Plants

Jivaka, Kakoli, Mahameda and Vriddhi

UGC Reference No.F. No. – 43-230/2014 (SR) dated August 19, 2015

University Grants Commission

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

E-Mail: ugc.@bol.net.in

UGC Major Research Project (Under XIIth Plan)

By

Professor Braja Gopal Bag

Principal Investigator

Department of Chemistry and Chemical Technology
Vidyasagar University
Midnapore 721102
West Bengal, India
Email: braja@mail.vidyasagar.ac.in
Fax: 03222275297

Annexure -VIII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.

Final Report of the work done on the Major Research Project.
(Report to be submitted within 6 weeks after completion of each year)

1. Project report No. 1st/2nd/3rd/Final _____ Final

2. UGC Reference No.F. No. – 43-230/2014 (SR) dated August 19, 2015

3. Period of report: from July 01, 2015_____to June 30, 2018___

4. Title of research project

Chemical Investigations on four Astavarga Plants: *Jivaka, Kakoli, Mahameda and Vriddhi*

5. (a) Name of the Principal Investigator BRAJA GOPAL BAG

(b) Deptt. _ Chemistry and Chemical Technology

(c) University/College where work has progressed VIDYASAGAR UNIVERSITY

6. Effective date of starting of the project__ November 01, 2015

7. Grant approved and expenditure incurred during the period of the report:

a. Total amount approved Rs. 781600/-

b. Total expenditure Rs. 754877/-

c. Report of the work done: (Please attach a separate sheet)

Separate sheet attached.

i. Brief objective of the project

A. The four Astavarga plants *Jivaka, Kakoli, Mahameda and Vriddhi* will be collected from various altitudes of Himalaya,

B. The active parts of the plants will finely powdered and extracted with various solvents such as alcohol, ethyl acetate, petroleum ether, etc.,

C. The solvents extracts will be analyzed and purified by various chromatographic techniques such as GC, HPLC, TLC and column chromatography,

D. The purified compounds will be analyzed by various analytical techniques such as NMR, UV, FTIR, Mass spectroscopy,

E. Detailed morphology of the hydrogels obtained from some of the fractions will be carried out by polarized optical microscopy and SEM, TEM, AFM, XRD, CD, etc.

F. Antioxidant activity of the plant extracts will be estimated by various analytical techniques such as DPPH and FRAP assays,

G. Synthesis of metal nanoparticles will be carried out utilizing the plant extracts and the synthesized nanoparticles will be carried out by HRTEM, EDX, SAED, X-ray, etc.

ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication

Published before Date of Start of the Project

I. Study of Antioxidant Property of the Rhizome Extract of *Roscoea purpurea* Sm. (Kakoli) and its Use in Green Synthesis of Gold nanoparticles, Bag B.G., Dash S.S., Roy A., *Int J Res Chem. Environ* 4 174 (2014).

II. Study of Antioxidant Property of the Pseudobulb Extract of *Crepidium acuminatum* (*Jeevak*) and its use in the Green Synthesis of Gold nanoparticles, Bag Braja Gopal, Dash Shib Shankar and Patra Sumit Kumar, IJRCE, 2014, 4, 133-138.

Published after Date of Start of the Project

1. Study of Antioxidant Property of the Tuber Extract of *Habenaria edgeworthii* (*Vrddhi*) and its use in the green synthesis of Gold nanoparticles, Braja gopal Bag, Abir Chandan Barai, *Prayogik Rasayan*, 2016, 2, 20.
2. B. G.Bag, S.S. Dash, A, Mandal Study of Antioxidant Property of the Rhizome Extract of *Polygonatum cirrhifolium* (Mahameda) and its use in the green synthesis of Gold nanoparticles. *Prayog. Ras*. **2017**, 2(2), 53 -57.
3. B.G. Bag, R. Majumdar, Self–assembly of renewable nano–sized triterpenoids, *Chem. Rec.* **2017**, 17, 1, 1-34
4. B.G. Bag, R. Majumdar, Self–assembly of renewable nano–sized triterpenoids, *Chem. Rec.* **2017**, 17, 1, 1-34 R. Majumdar, B.G. Bag, Evolution of Vesicular Self-Assemblies of the Salts of a Natural Triterpenoid Arjunolic Acid into Superstructured Ambidextrous Gels and Study of Their Entrapment Properties, *ChemistrySelect,* **2018**, 3, 951 –957.

5. A. C. Barai, K. Paul, A. Dey, S. Manna, S Roy, B. G. Bag, C. Mukhopadhyay Green synthesis of *Nerium oleander*-conjugated gold nanoparticles and study of its in vitro anticancer activity on MCF-7 cell lines and catalytic activity *Nano Convergence*, **2018**. 5,10.
6. K. Chaudhuri, SK N. Hasan, A. C. Barai, S. Das, T. Seal and B. G. Bag, Green synthesis of gold nanoparticles using Wendlandia wallichii, a potent wild edible plant consumed by the tribal of north-eastern region in India, *The Pharma Innovation Journal,* **2018**, 7(6): 437-446.
7. B. G. Bag, A. C. Barai, Sk N. Hasan, S. Das, C. Garai, S. Ghorai, S. K. Panja, Terpenoids as Renewable Nano-Sized Building Blocks, *Prayog. Ras.,* **2018,** 2(1), 1-23.
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9. Sk N. Hasan, K. Paul, A. Dey, S. Manna, S. Roy, B.G. Bag, S. Mondal, One Step Biosynthesis of Gold Nanoparticles Using the Leaf Extract of Gymnema sylvestre and Study of Its In-vitro Anticancer Activity on MCF-7 Cell Lines, *IJRCE*, **2017**, 7 (2), 1-8
10. B. G. Bag, S. Das, Self-assembly study of ammonium oleanolate and generation of gel–gold nanoparticle hybrid material. *Prayog. Ras.* **2017**, 1, 43-47.
11. R. Majumdar, S. Tantayanon, B.G. Bag, A novel trihybrid material based on renewables: an efficient recyclable heterogeneous catalyst for C-C coupling and reduction reactions, *Chem. Asian J.*, **2016**, 11, 2406.

iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons

Yes, the progress is as per original research plan.

All the four Astavarga plants namely *Jivaka, Kakoli, Mahameda and Vriddhi* have been collected from different regions of Himalaya and chemical analysis of various parts of all the four plants have been carried out. DPPH assay has indicated the presence of large quantities of antioxidants. Detailed GCMS and NMR analysis have indicated the presence of terpenoids, polyphoenols, steroids, fatty acids including erucic acid and stigmasterol.

Evidence for the presence of polyphoenolic compounds encouraged us to utilize the extracts of the plants for the successful green synthesis of gold nanoparticles under very mild reaction condition in aqueous medium at room temperature.

iv. Please indicate the difficulties, if any, experienced in implementing the project___

The second instalment of the project was not received though we submitted UC and participated in the Mid-Term evaluation. Proper justification for the utilization of 80% utilization of the budget (except the fellow) was given. Such an information of 80% utilization during release of the 1st instalment should have been given. This could have eased the implementation. I met the the UGC officers twice in UGC New Delhi to explain this. But still then, the second instalment of the project was not released.

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet.

We have completed the proposed work in the project. The indication of the presence of new plant secondary metabolites such as erucic acid, stigmasterol, low molecular weight terpenoids and polyphenols have encouraged us to study the properties and utilization of those compounds (as an extension work of the completed project).

vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission.

Summary of the findings is enclosed

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

(a) Sri Abir Chandan Barai joined as a Project Fellow (JRF) in this project and took fellowship for about two months. Then he got his own UGC fellowship. Even though, he continued investigations on this UGC MRP research problem, he did not take any fellowship after that. At the end of the project Mr. Subhajit Das joined as SRF and worked for two months as SRF.

(b) Both Abir Chandan Barai and Subhajit Das are Registered Research Scholar of Vidyasagar University. They are yet to be awarded PhD degree.

(c) In addition to four publications on Astavarga plants, additional investigations have been carried out on six more plants and additional nine publications have come out in leading journals of RSC, Wiley, Springer, etc.

Reprints of all the thirteen publications have been enclosed.

(d) other impact, if any

The results have also been presented in National Symposia which have drawn the attention of a large number of audiences. They have highly appreciated admired.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

REGISTRAR/PRINCIPAL

(Seal)

SIGNATURE OF THE CO-INVESTIGATOR

FINAL REPORT OF WORK DONE ON THE UGC MRP 43-230/2014 (SR)

dated August 19, 2015

Entitled

Chemical Investigations on four Astavarga Plants Jivaka, Kakoli, Mahameda and Vriddhi

Thousands of years ago, an old and feeble sage (Risi) Cyavan wasrejuvinated and his youth and strength was restored on treatment with a special formulation containing a set of eight medicinal plants called Astavarga. Since then, this special formulation of plants is known as Cyavanaprasa and Astavarga plants are known to be the active 'Vitality strengthening' ingredients of Cyavanaprasa .

However, because of the lack of proper scientific documentation and traditional knowledge, proper identification of the eight plants could not be done until recently. In the beginning of 21st century, a group of scientists and sages made extensive trips and expeditions to the inaccessible places of the higher ranges of Himalaya and identified those plants.[1,2,3,4,5] We have recently been able to collect four of those plants namely Jivaka, Kakoli, Mahameda and Vriddhi (Figure 1) and initiated investigations on the chemical constituents and their activities. Initial investigations in our laboratory have shown that all those four plants contain large quantities of antioxidants including flavanoids, steroids, alkaloids, etc. Antioxidants inhibit the cell damage and cell death by quenching the free radicals generated by uncontrolled oxidation in the physiology. Moreover, the antioxidants prevent age related diseases, promote proper functioning of the immune system, fight and prevent against cancer, etc. This has prompted us to quantify the amount of antioxidants present in the Astavarga plants by DPPH assay (Figure 2). Additionally, green synthesis of gold nanoparticles was carried out utilizing the plant extracts under very mild conditions (Figure 3). The aim of this project is to isolate the chemical constituents of the four Astavarga plants in larger quantities, characterize them by various analytical techniques such as HPLC, GCMS, UV-visible spectroscopy, FTIR, NMR, etc. and study their antioxidant activities and metal ion reducing properties. Attempts will also be made to address the structure-property relationships.

Study of Antioxidant Property of the Rhizome Extract of *Roscoea purpurea* Sm. *(Kakoli)* and its use in the green synthesis of Gold nanoparticles

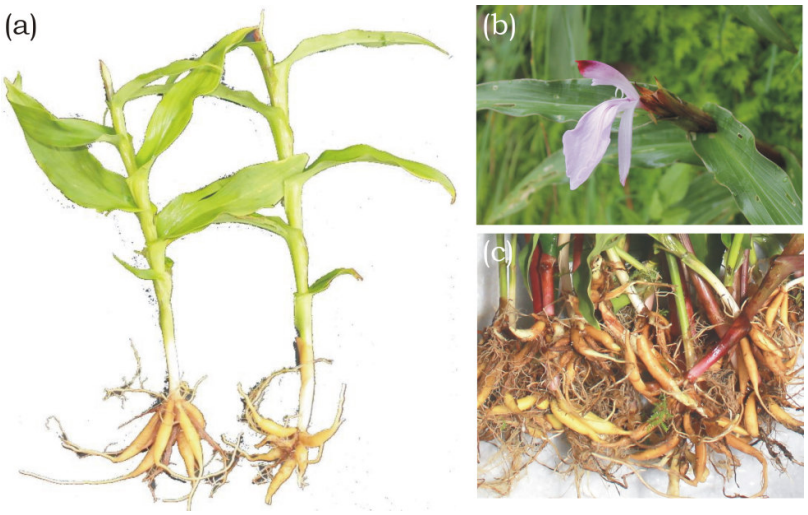
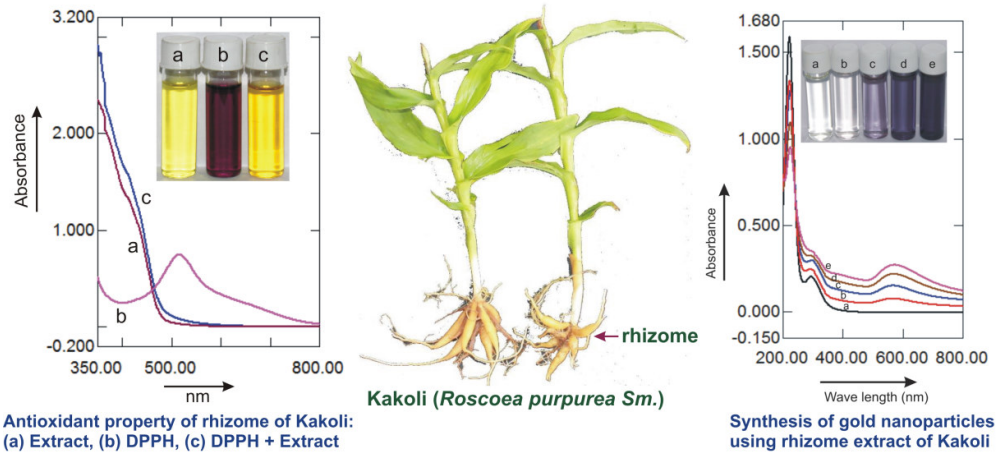


Figure: Photo of Roscoea purpurea Sm. (Kakoli): (a) the whole plant, (b) flower of Kakoli, (c) rhizome of Kakoli



The size distribution, shape and morphology of the AuNPs were studied by high resolution transmission electron microscopy (HRTEM) (Figure 6). Spherical shaped AuNPs of nearly 6 nm size was observed along with *flower like assembly* of AuNPs of nearly 200 nm sizes was obtained. The average size of the AuNPs formed at 400 mg/L concentration of rhizome extract was calculated to be almost 6 nm (calculated from 60 spherical particles).

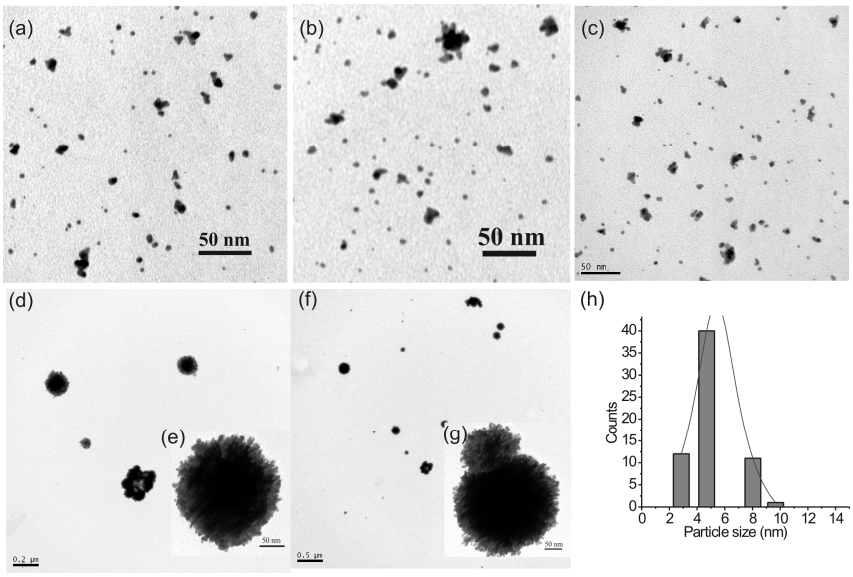
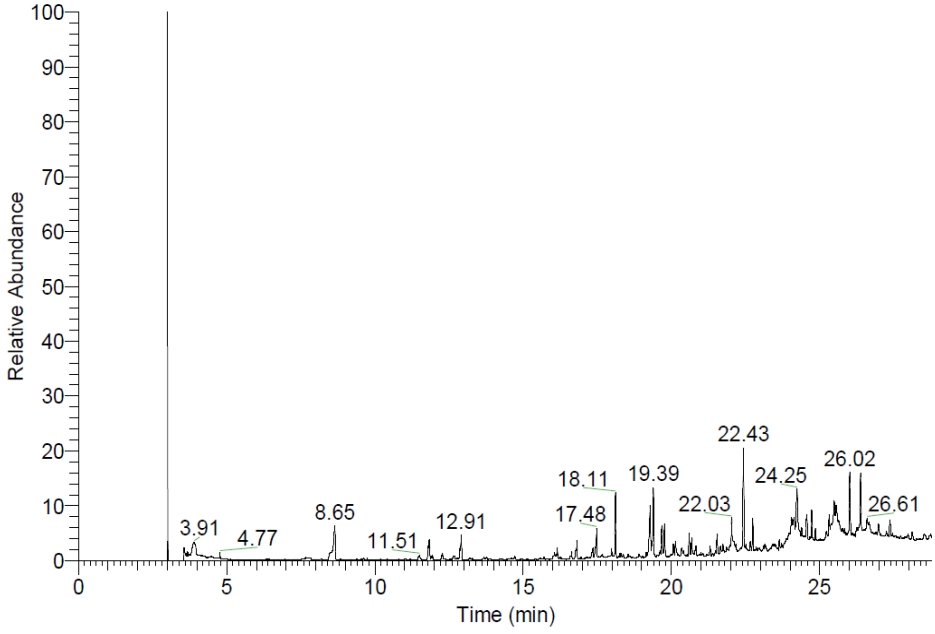


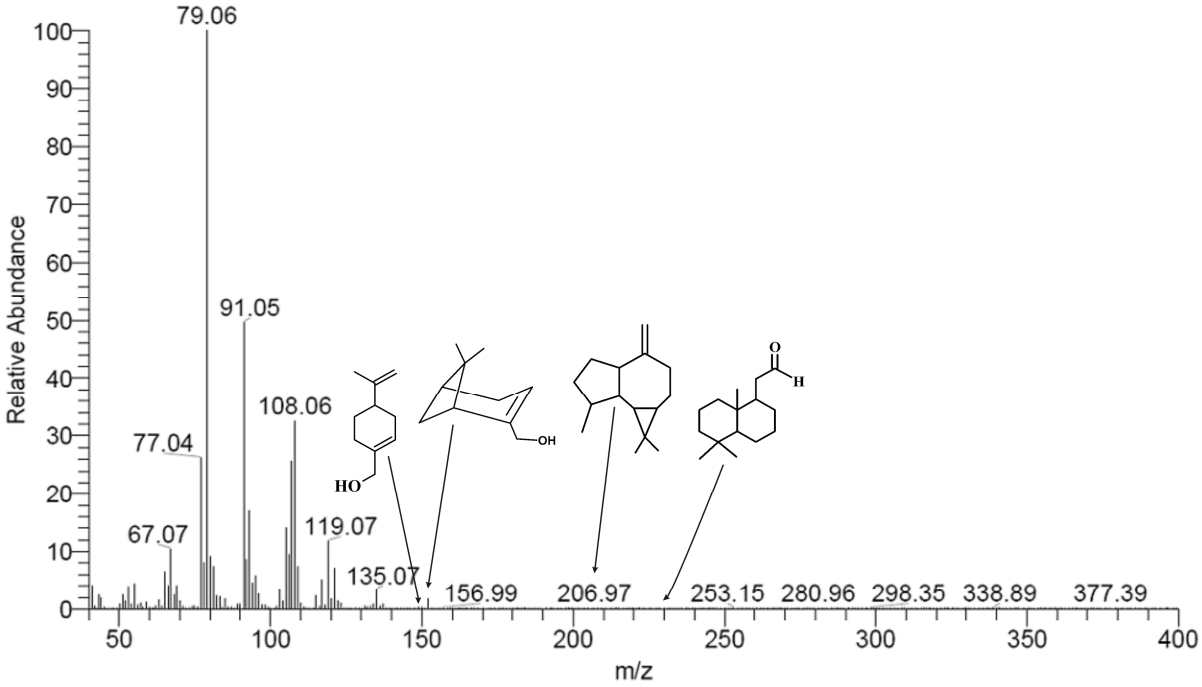
Figure : (a-f) HRTEM Images Kakoli rhizome stabilized AuNPs at 400 mgL^{-1} concentration of rhizome extract.(d) Histogram

Chemicals present in leaves of *Roscoea purpurea* (Kakoli)

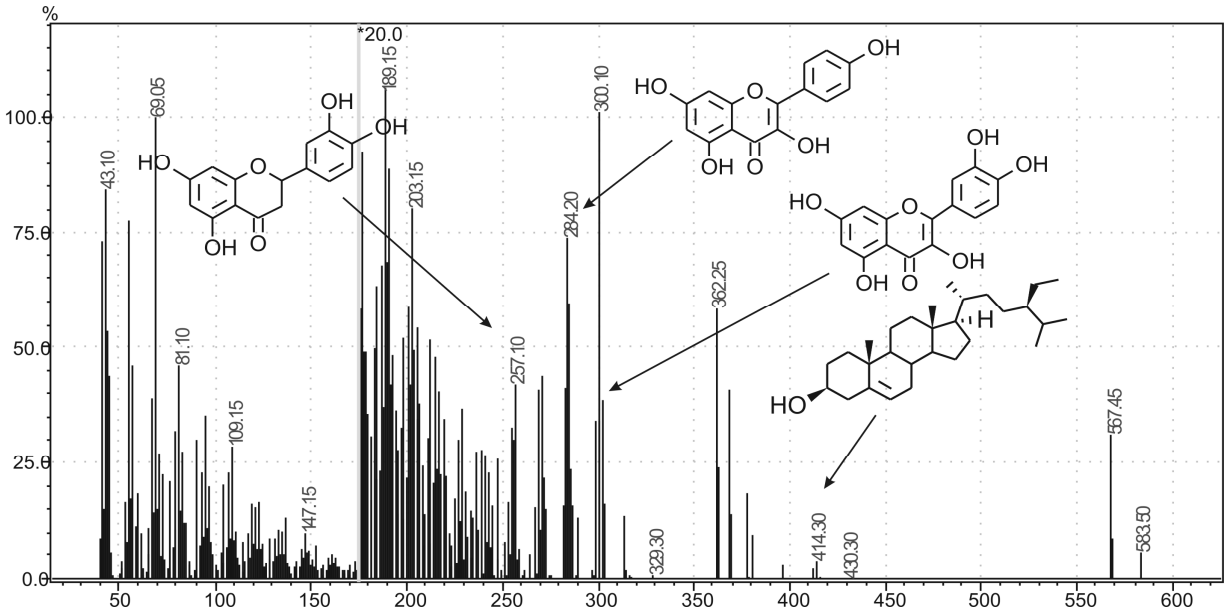
Laves of Kakoli was collected and dried in air. Then it was finely powered using a grinder. Finely dried powdered of leaves (10 g) was suspended in ethanol (100 mL) and refluxed with continuous magnetic stirring for 5 h, cooled at room temperature and then filtered using a sintered glass funnel.Volatiles of the greenish filtrate were removed under reduced pressure to afford a greenish yellow sticky material. GCMS studies of the leaves extract revealed that the presence of several chemicalcompouns compounds mainly the mono-terpenoids.

GCMS study:





Molecular structure of the possible compounds present in the ethanolic extract of leaves of *kakoli*

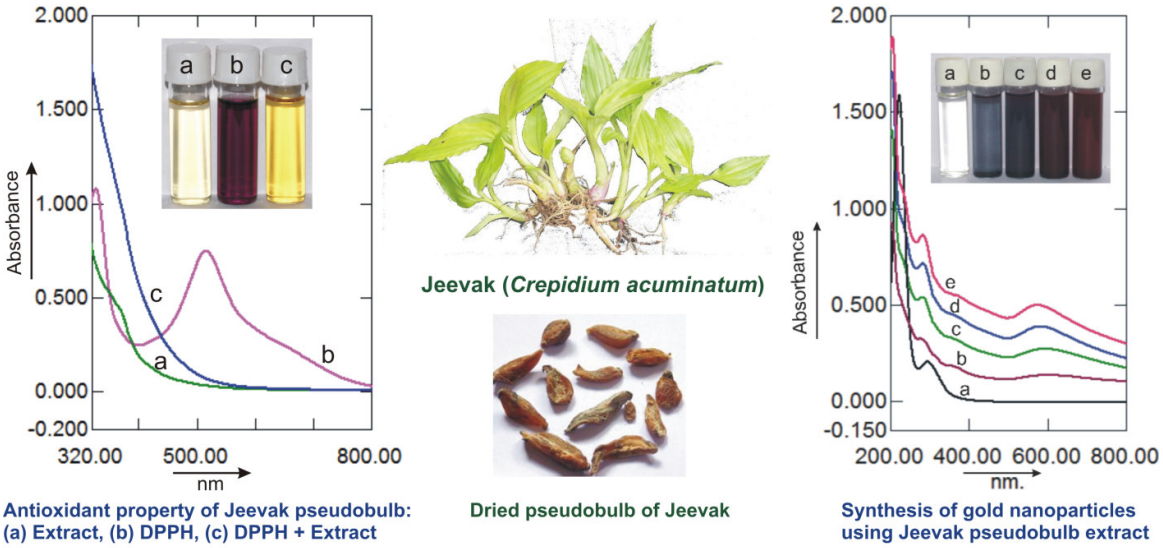


Molecular structure of the possible compounds present in the ethanolic extract of leaves of *kakoli*

Isolation of sigmasterol from the leaves of *Roscoea purpurea* (Kakoli):

Finely powdered leaves of *Roscoea purpurea* (Kakoli) (16 g) were extracted with ethyl acetate (250 mL) by using an extraction apparatus (capacity 500 mL) during 24 h at room temperature. The volatiles were removed under reduced pressure to afford a brownish solid material (0.636 g). The crude extract was purified by successive column chromatography (thrice, Si-gel, 100–200 mesh) using 10-20 % ethyl acetate/ petroleum ether as the eluant.

Study of Antioxidant Property of the Pseudobulb Extract of *Crepidium acuminatum* (Jeevak) and its use in the green synthesis of Gold nanoparticles



Morphology of the AuNPs formed at different concentration of the pseudobulb extract of Jeevak were studied by high resolution transmission electron microscopy (HRTEM). Mostly spherical shaped AuNPs were observed along with some triangular, and pentagonal shaped AuNPs. The average size of the AuNPs formed at 1200 mgL-1 concentration of the plant extract was 10.7 nm (calculated from 75 particles, Figure 4f). The colloidal AuNPs samples were coated over a glass plate, the volatiles were removed and X-ray diffraction analysis of the AuNPs was carried out. The reflections of the planes (111), (200), (220) and (311) at 2θ = 38.24°, 44.54°, 64.82° and 77.66° respectively resembled the characteristic reflections of crystalline metallic face centered cubic Au (JCPDS file no. 04-0784). The

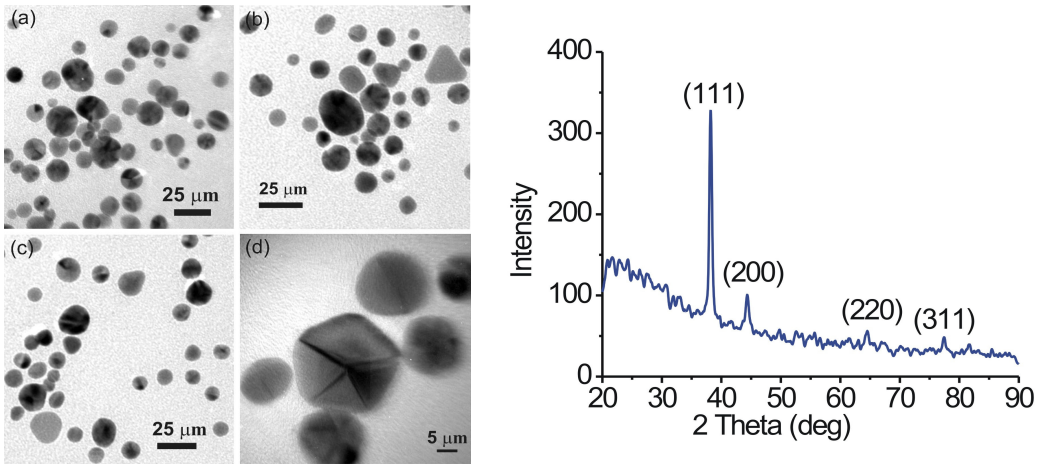
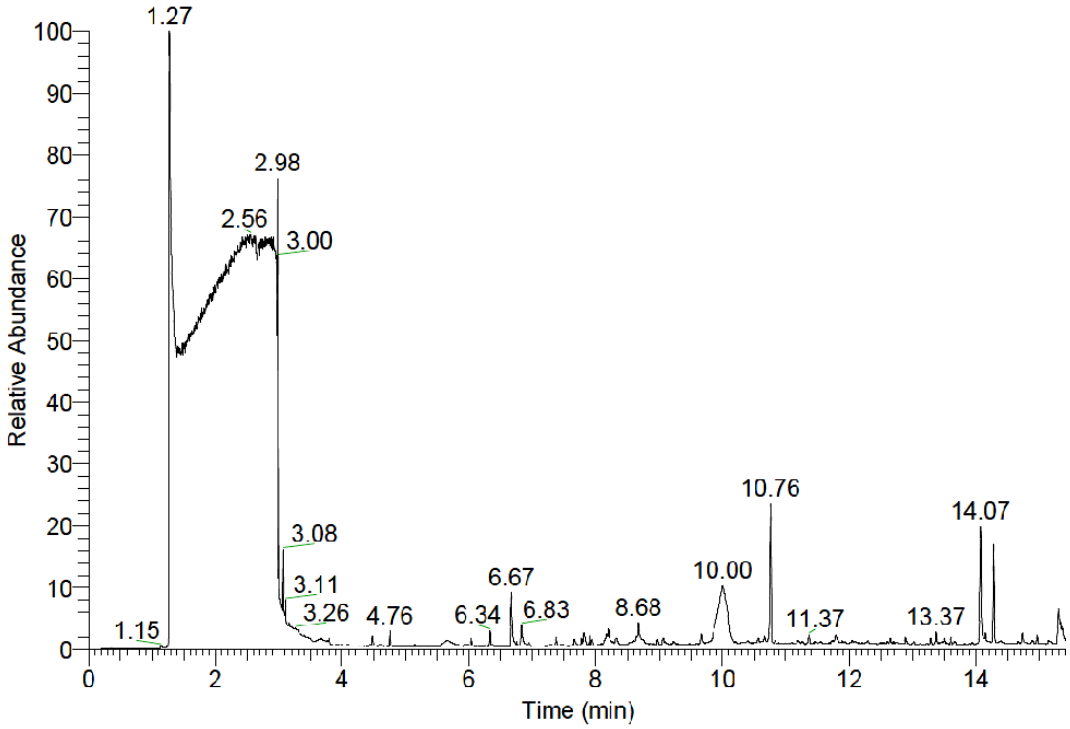


Figure : (a-d) HRTEM Images *Crepidium acuminatum*pseudobulb stabilized AuNPs at 400 mgL^{-1} concentration of rhizome extract. And XRD

comparatively greater peak intensity of the (111) plane indicated the predominant orientation of the (111) plane.

Chemicals present in pseudobulb of *Crepidium acuminatum* (Jeevak)

The plant Jeevak was collected from Dhanolti region of Himalaya, identified by a group of scientists and Baidyas from Patanjali Yogpeeth, Haridwar and deposited in their herbarium. Air dried pseudobulb of Jeevak was finely powdered using a grinder. Finely powdered pseudobulb of Jeevak (6.07 g) was suspended in methanol (50 mL) and refluxed with magnetic stirring for 7 h, cooled at room temperature and then filtered (sintered glass funnel). Volatiles of the filtrate were removed under reduced pressure to afford a foamy solid (1.05 g).



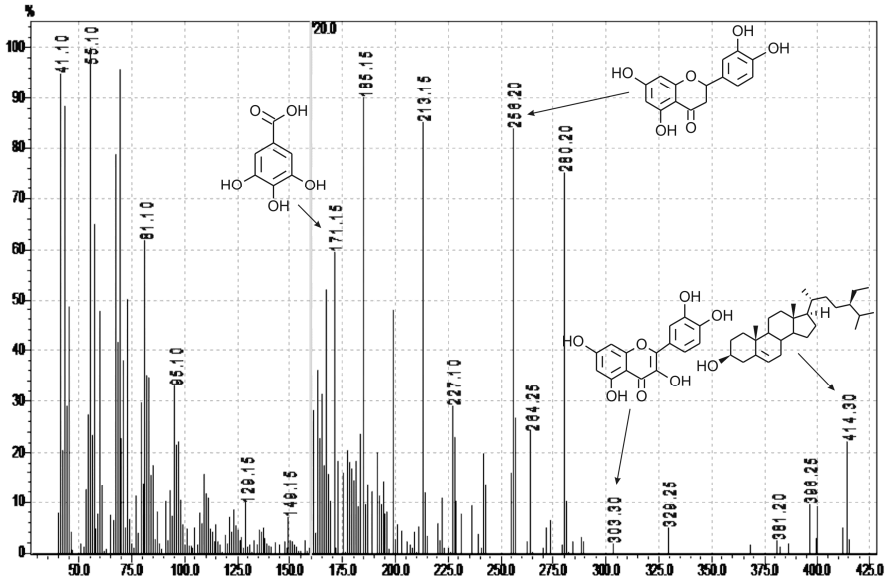
GCMS profile of the pseudo-bulb extract of *Crepidium acuminatum* (Jeevak)

Isolation of Erucic acid from the pseudobulb of *Crepidium acuminatum* (Jeevak):

Finely powdered pseudobulb of *Crepidium acuminatum* (Jeevak) (10 g) was extracted with ethyl acetate (100 mL) by using an extraction apparatus (capacity 500 mL) during 24 h at room temperature. The volatiles were removed under reduced pressure to afford a yellowish solid material (0.636 g). The crude extract was purified by successive column chromatography (thrice, Si-gel, 100–200 mesh) using 10-20 % ethyl acetate/petroleum ether as the eluant.

Study of Antioxidant Property of the Rhizome Extract of *Polygonatum cirrhifolium* (*Mahameda*) and its use in the green synthesis of Gold nanoparticles

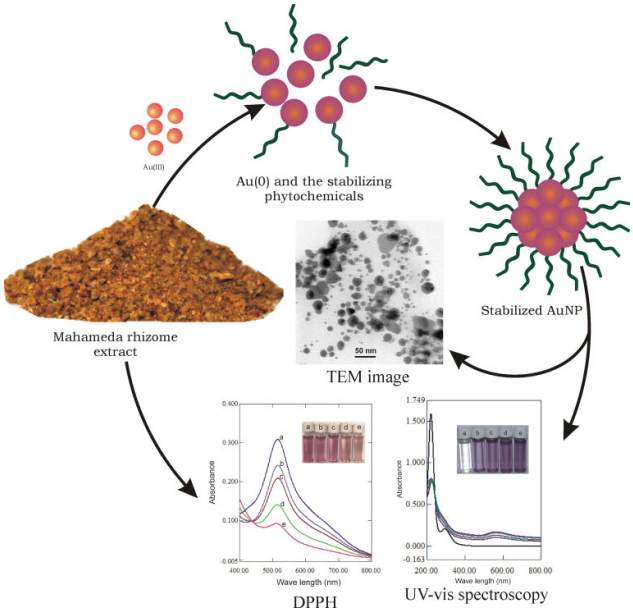
Ethanolic extract of *Mahameda* rhizome was utilized for the synthesis of AuNPs at room temperature. The UV-visible spectrum of stabilized AuNPs at various concentration of rhizome extract is given in Figure 3. The charge transfer interaction between the metal and



Molecular structure of the possible compounds present in the methanolic extract ofpseudobulb of Jevaka

chloro ligands leads to the formation of two peaks at 220 and 290 nm in the UV-visible spectrum of $HAuCl_4$ solution. But interestingly, on addition of stabilized AuNPs the intensities of these two peaks reduced and concomitantly a new peak appeared in the region of 550 nm due to surface plasmon resonance (SPR) phenomenon of AuNPs. With increasing the concentration of the plant extract a blue shift of the SPR band was observed due to the formation of small sized AuNPs. Mostly spherical shaped AuNPs were observed along with some tri angular, and pentagonal shaped AuNPs. The reflections of the planes (111), (200), (220) and (311) at 2θ = 38.24°, 44.54°, 64.82° and 77.66° respectively resembled the

characteristic reflections of crystalline metallic face centered cubic Au (JCPDS file no. 04-0784). The comparatively greater peak intensity of the (111) plane indicated the predominant orientation of the (111) plane.



The rhizome extract of *Mahameda* is rich in various plant phytochemicals such as polyphenols including flavanoids, steroids, etc. DPPH study and positive ferric chloride test also support the presence of polyphenolic compounds in the rhizome part of this plant (supporting information). Mass spectral analysis of the rhizome extract carried out by us supported the presence of the several polyphenolic compounds (supporting information Figure S1) such as gallic acid (m/z= 171), quercetin (m/z= 302), β-sitosterols (m/z= 414) or their analogues. A schematic representation of the possible mechanism for the formation of AuNPs and their stabilization by the phytochemicals present in the extract is shown in Figure 5. Polyphenolic compounds along with other easily oxidizable phytochemicals present in the rhizome extract can reduce Au (III) to Au (0) with concomitant oxidation of the phytochemicals to a higher oxidation state. Collision of the neighboring Au (0) atoms with each other leads to the formation of the AuNPs. The AuNPs can be stabilized by the polyphenolic compounds, quinones as well as the other coordinating phytochemicals present in the rhizome extract of *Mahameda.*

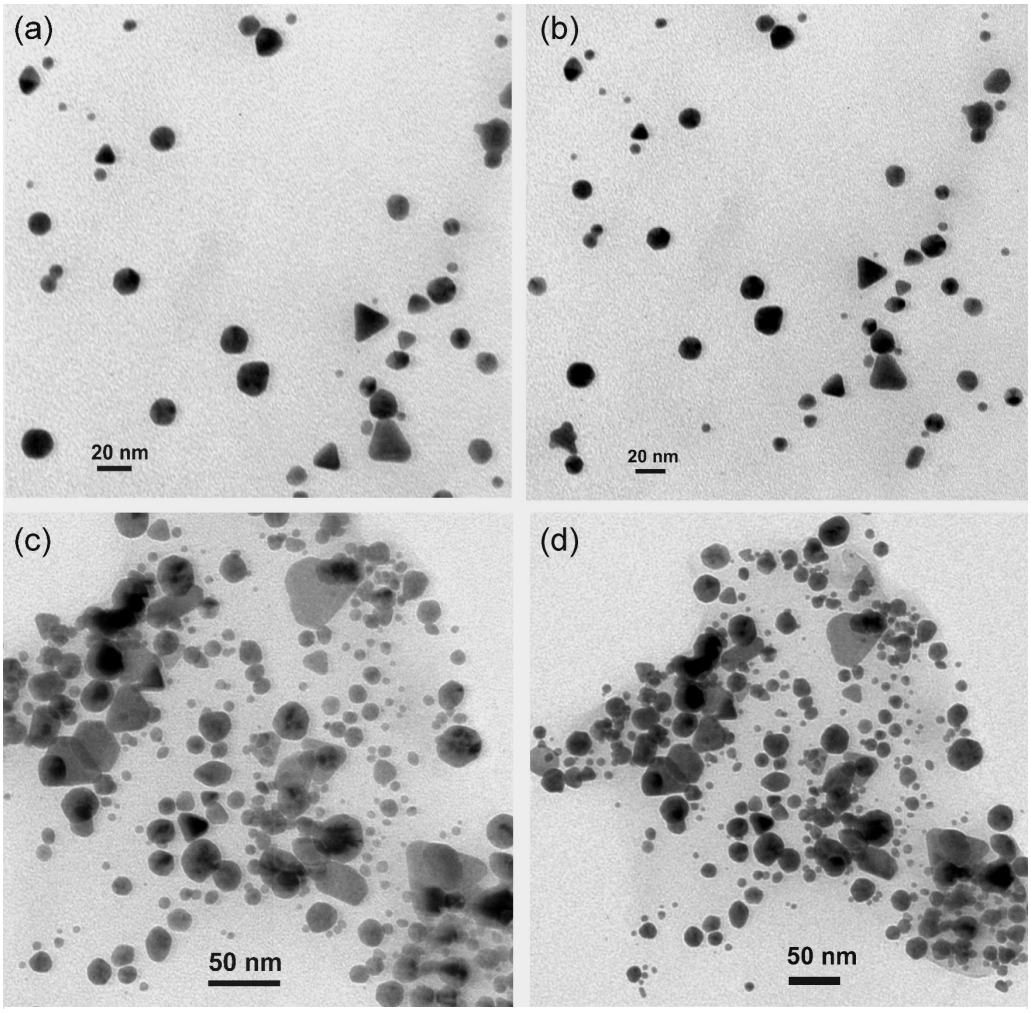
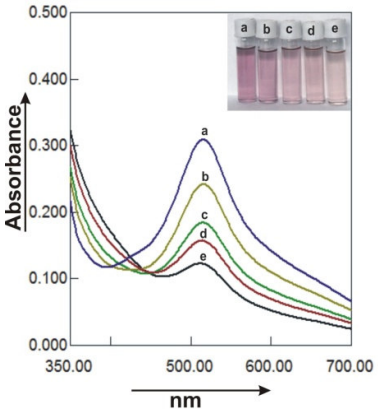


Figure : HRTEM images of rhizome extract stabilized AuNPs.

Study of Antioxidant Property of the Tuber Extract of *Habenaria edgeworthi* (Vrddhi) and its use in the green synthesis of Gold nanoparticles

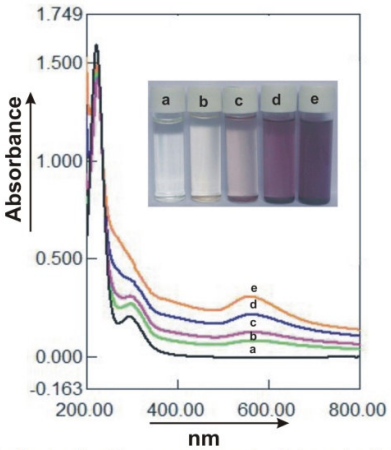
Vriddhi, a terrestrial tuberous orchid, growing upto 75 cm in height, is found across the Himalayas at an altitude 2500 – 3000 m above sea level (Figure 1). Tuberous roots are small, ellipsoid, usually 1-2.5 cm long, 0.5 - 1 cm in diameter, white inside and fleshy (Figure 1b). It is used for the treatment of asthma, fever, skin diseases, leprosy, blood disorders, burning sensation, general debility, etc. Mass spectral studies of the methanol extract of the tubers carried out in our laboratory showed the presence of several polyphenolic compounds including flavanoids along with steroids and other plant secondary metabolites (supporting information Figure S1) (Figure 1c). Evidence for the presence of phenolic compounds was also obtained from a positive ferric chloride test. As the phenolic compounds have antioxidant properties, we tested the antioxidant activity of the ethanol extract of the dried tubers against a long lived 2,2-diphenylpicrylhydrazyl (DPPH) radical at room temperature



Antioxident property of *Vrddhi*

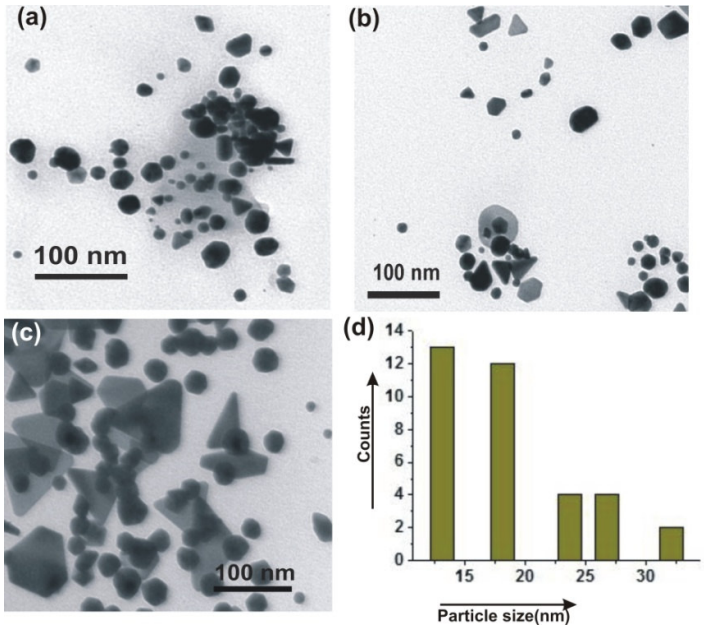
Vrddhi (*Habenaria edgeworthii*)

Dried tuber of *Vrddhi*



Synthesis of gold nano particle using tuber extract of *Vrddhi*

The size distribution, shape and morphology of the AuNPs formed at different concentration of the ethanol extractof tuber of *Vriddhi* were studied by high resolution transmission electron microscopy (HRTEM) (Figure 4). Trigonal, tetragonal, pentagonal, hexagonal along with spherical shaped AuNPs were observed. The average size of the AuNPs formed at 1200 mgL^{-1} concentration of the plant extract was 15.2 nm (calculated from 75 particles, Figure 4d). The AuNPs were held inside the organic matrix derived from the tuber extract of *Vriddhi*.



To test the presence of polyphenolic compounds in the tuber extract of *Vrddhi*, $FeCl_3$ test was performed. A freshly prepared aqueous solution of $FeCl_3$ was added to the aqueous tuber extract of *Vrddhi*. The mixture was shaken at room temperature. Instant appearance of greenish color indicated the presence of phenolic compounds in the tuber of *Vriddhi*.

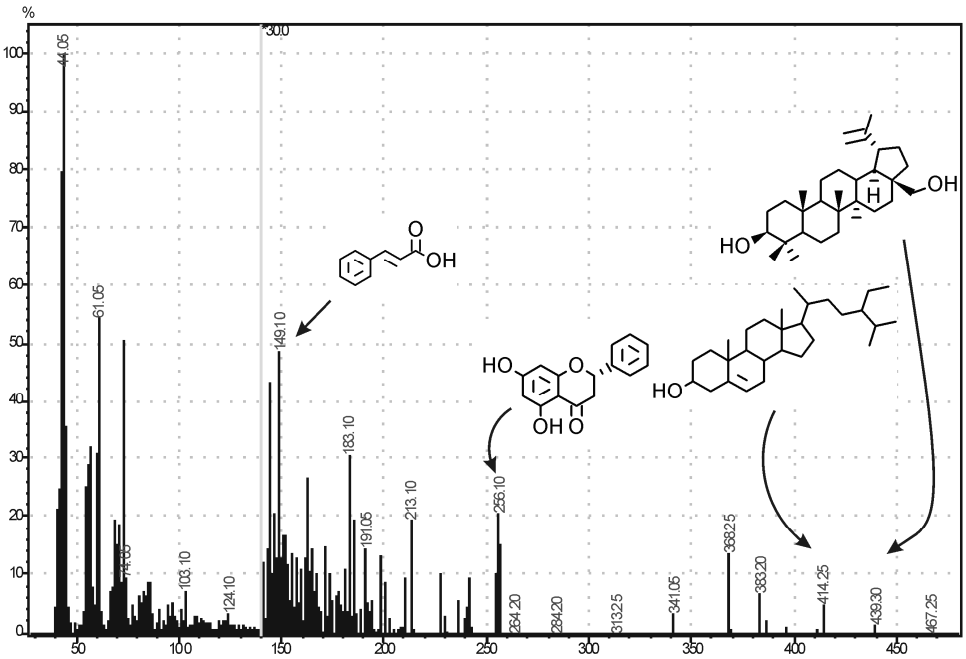


Figure : Molecular structure of the possible compounds present in the methanolic extract of tuber of *Habenaria edgeworthii* (*Vriddhi*).

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A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet.

The four Astavarga plants namely *Habenaria edgeworthii (Vrddhi), Polygonatum cirrhifolium (Mahameda), Crepidium acuminatum* (Jeevak), *Roscoea purpurea* (Kakoli) were collected from the Dhanaouti region of Himalaya in larger quantities. The different parts such as pseudo bulb, roots, stems, leaves, flowers and fruits were separately dried in air, powdered and kept in the refrigetator at 4 ºC until use.

Evidence for the presence of antioxidants including polyphenols has been obtained in the extracts of *Habenaria edgeworthii (Vrddhi), Polygonatum cirrhifolium (Mahameda), Crepidium acuminatum* (Jeevak), *Roscoea purpurea* (Kakoli). The antioxidant activity of the ethanol extracts of all the four plants has been studied against the long lived 2,2-diphenylpicrylhydrazyl (DPPH) radical at room temperature. The phytochemicals present in the tuber extract have been utilized for the synthesis of gold nanoparticles at room temperature under very mild conditions without any additional stabilizing or capping agents. Mechanism for the synthesis of the gold nanoparticles from Au(III) has also been proposed. According to our knowledge, this is the first report of the study of antioxidant property of the extracts of *Habenaria edgeworthii (Vrddhi), Polygonatum cirrhifolium (Mahameda), Crepidium acuminatum* (Jeevak), *Roscoea purpurea* (Kakoli) and its utilization in the green synthesis of gold nanoparticles. Additionally, evidences for the presence of phytosterols and fatty acids such as stigmasterol and erucic acids have also been obtained. As the plant extracts have tremendous medicinal significance, the results obtained in this project will be useful in biomedical applications as well as nanoscience and nanobiotechnology.

Professor Braja Gopal Bag
Dept. of Chemistry & Chem.Tech.
Vidyasagar University
Midnapore- 721102, W.B.

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