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Synthesis of Silver Nanoparticles from *Swertia Chirata, Solanum Robustum* and its Biological Activity

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Abstract—*Green synthesis of metal Nano particles is gaining significance in the nanotechnology branch. Extensive use of these nano particles has paved way for its commercial demand. Nano-biotechnology is a developing field of applied biological science. In this paper, the environmentally benign, Green synthesis of silver nanoparticles using methanol leaf extract of* Swertia chirata *and Solanum robustum is studied. Investigation carried out for the synthesis of silver nanoparticles (SNPs) using the methanol leaf extract of* Swertia chirata *and Solanum robustum as a reducing agent from 1 mM silver nitrate ($AgNO_3$). UV–Vis, SEM and FTIR are used for characterizing silver nano particles thus resulted. Silver nanoparticles were synthesized within 24 hours of incubation period and synthesized SNPs showed an absorption peak at around 430 nm in the UV-visible spectrum. The morphological study of Silver nanoparticles using SEM suggests that the nanoparticles are spherical in shape. New active particles could be identified by the synthesis of silver nanoparticles using methanol leaf extract of* Swertia chirata *and Solanum robustum and the eco-friendly SNPs can be used effectively against drug resistant bacterial strains. This is rapid and simple method by avoiding hazardous chemicals as reducing or stabilizing agents and cost-effective in synthesizing SNPs.*

Keywords*:* Swertia chirata, *Solanum robustum, Green synthesis, Silver nanoparticles, UV-Vis, SEM, FTIR and Antibacterial activity.*

Introduction

Nanotechnology is an emerging field of life science that has various applications in medicine. The term Nano is derived from the Greek word which means dwarf *i.e* one billionth of meter or 10^{-9} m and also related to Spanish word Nino (Taylor, 2001). Green synthesis of nanomaterials is an ecofriendly, clean approach for nanoparticles preparation where chemicals used are non-toxic and reducing agents used are renewable (Moritz and Moritz, 2013). Nanoparticle synthesis using biological methods is called green synthesis. Researchers have attempted synthesis of nanoparticles of different sizes and shapes using a variety of biological materials (Saifuddin *et al*., 2009).

Medicinal plants have been in use from ancient times in treatment of wound healing and infectious diseases, etc. The phytochemicals in the plants is responsible for such activities. The phytochemicals includes terpenoids, steroids, saponin, alkaloids, flavonoids and carbohydrates (Akindele and Adeyemi, 2007). These phytochemicals also serves as a capping and reducing agents in synthesis of silver nanoparticles, whereas plant extract reduces the silver nitrate which forms Ag3+ ions to AgO ions. The development of resistance by micro-organisms to the existing synthetic drugs is the major problem now a days. There is also an increasing awareness among the peoples about the importance of medicinal plants. So there have been a shift from synthetic to nature (Thirumurguan *et al*., 2009).

In the present study, the phytochemical analysis of the medicinal plants *Swertia chirata* and *Solanum robustum* were done and silver nanoparticles were synthesized from them. Biosynthesized nanoparticles were evaluated for antimicrobial effect by disc diffusion method and comparative studies were performed with crude extracts.

The plant under investigation is *Swertia chirata* Ham which belongs to the family Gentianaceae. The Gentianaceae is a tropical family of small trees, herb and bitter tonic. It consists of 180 species. About 8-10 species exist in India (Hooker, 1885). This plant is indigenous to temperature Himalayas at altitudes above 4000 feet from Kashmir, Nepal and Bhutan (Chirangib Bownoshadhi of India). In this family all plants are used as medicine (Flora of Tamil Nadu, 1983).

Solanum robustum belongs to the family solanaceae. It is a thorny perennial shrub, origin of northeastern South America of the genus *Solanum.* Hence related to the potato and tomato plants. It is a shrub of medium type, the plant grows up to 4 to 8 feet (1.2 - 2.4 m.) with velvety leaves and stems due to dense stellate trichomes present on all faces of the plant *S. robustum* blooms between late spring and mid fall with small clusters of white to yellow-white star shaped inflorescence

followed by white or yellowish marble sized berries. In the present study *Swertia chirata* Ham and *Solanum robustum* were selected for phytochemical and biological study (Natural Resources Conservation Service PLANTS Database, 2015). Qualitative analysis of phytochemical constituents by using methanol leaf extracts of *Swertia chirata* and *Solanum robustum*; and biological synthesis of SNPs and screening of SNPs for microbial efficacy are dealt in the present paper.

Materials and Methods

Collection and Preparation of Extracts

The fresh samples of *Swertia chirata* and *Solanum robustum* were collected from Yercaurd, Tamil Nadu. The air dried samples were homogenized to fine powder and kept in refrigerator in airtight bottles for storage. By Soxhlet extraction method, extract of crude sample was prepared. With 250ml of methanol extract, about 20gm of powdered sample material was consistently packed into a thimble and extracted. Until the solvent in siphon tube of extract become colourless, the process of extraction was continued. The extract was taken in a beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for future use.

Phytochemical Screening

Preliminary phytochemical analysis was carried out for the extracts of *Swertia chirata* and *Solanum robustum* as per standard methods described by Brain and Turner, 1975; Evans 1996.

Detection of alkaloids

The presence of alkaloids was tested from the filtrate of extracts that were dissolved individually in dilute hydrochloric acid and filtered. Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Detection of Flavonoids

H_2SO_4 test: Formation of orange colour, when the extracts treated with few drops of H_2SO_4 indicates the presence of flavonoids.

Detection of Steroids

Colour change from violet to blue or green when 2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4 indicate the presence of steroids.

Detection of Terpenoids

Salkowski's test: A reddish brown coloration of the inner face when 0.2g of the extract of the whole plant sample mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) indicates the presence of terpenoids.

Detection of Anthroquinones

Borntrager's test: The filtered extract which was extracted when about 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath was allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Pink colour formed when few drops of 10% NH_3 were added to the mixture and heated, indicates the presence anthroquinones.

Detection of Phenols

Ferric chloride test: Formation of bluish black color when the extracts were treated with few drops of 5% ferric chloride solution, indicates the presence of phenol.

Detection of Saponins

Froth test: About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

Detection of Tannins

Ferric chloride test: A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green color formation indicates the presence of tannins.

Detection of Carbohydrates

Fehling's test: 0.2gm filtrate is boiled on water bath with 0.2ml of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

Detection of Oils and Resins

Spot test: Test solution was applied on filter paper which develops a transparent appearance, it indicates the presence of oils and resins.

SYNTHESIS OF SILVER NANO-PARTICLES

1 Mm silver nitrate solution was prepared in 100 ml flask. 1 ml of plant extract was mixed with 9 ml of 1 mM of silver nitrate. The leaf methanol extracts of the *Swertia chirata* and *Solanum robustum* and silver nitrate solution were used as a control throughout the experiment (Smetana *et al*., 2005). The final solutions were centrifuged at 18,000 rpm for 25 mins. The collected pellets were stored at -4^0c.The supernatant was heated at 50^0c to 95^0c .A change in the colour of solution was observed during the heating process.

Antimicrobial susceptibility test

To screen the antimicrobial activity, the disc diffusion method (Bauer *et al.*, 1966) was used. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Hi-media (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1%

inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The concentration of extracts (40 mg/disc) were loaded on 6 mm sterile disc. The loaded discs were placed on the surface of medium and the extracts were allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^{o}C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

CHARACTERIZATION OF SILVER NANOPARTICLES:

UV-Vis Analysis:

The optical property of Ag-NPs was determined by (UV-Vis Spectrophoto meter Perklin- Elmer, Lamda 35, Germany). Spectra's were taken at 24 hrs interval between 350 nm to 500 nm, after the addition of $AgNO_3$ to the plant extract.

FTIR analysis:

The chemical composition of the synthesized silver nanoparticles were studied by using FTIR spectrometer (perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 75^{o} C and the dried powders were characterized in the range 4000–400 cm $^{-1}$ using KBr pellet method.

SEM Analysis:

The morphological features of synthesized silver nanoparticles from *Swertia chirata* and *Solanum robustum* plant extracts were studied by Scanning Electron Microscope (JSM-6480 LV). After addition of $AgNO_3$ the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV.

Result and Discussion

The phytochemical investigation of *Swertia chirata* and *Solanum robustum* showed that the leaves are rich in alkaloids, flavonoids, steroids, terpenoids, anthroquinones, phenols, Saponin, tannin, carbohydrates, oil and resin and it is lacking in anthroquinones of *Swertia chirata* (Table 1). The secondary metabolites like anthroquinones, flavonoids, emodins and phenolic compounds are medicinally used as anti-stomatic, anti-diarrhoea, anti-inflammatory, anti-cancer and anti-oxidative (Jeong *et al.*, 2011; Kubo *et al*., 2011, Chukwujekwu *et al*., 2011; Venkatesh *et al*., 2011)

Table 1: Phytochemical analysis for *Swertia chirata* and *Solanum robustum*

Phytochemicals	Observations	Methanol Extracts	
		Swertia chirata	Solanum robustum
Alkaloids Mayer's test Wagner's test	Cream color Reddish brown solution/ precipitate	+ +	+ +
Flavonoids Lead acetate test H2SO4 test	Yellow orange Reddish brown / Orange color precipitate	++ ++	++ ++
Steroids Liebermann-Burchard test	Violet to blue or Green color formation	++	+
Terpenoids Salkowski test	Reddish brown precipitate	++	++
Arthroquinone Borntrager's test	Pink color	-	+
Phenols Ferric chloride test Lead acetate test	Deep blue to Black color formation White precipitate	+ +	+ +
Saponin	Stable persistant	++	++
Tannin	Brownish green / Blue black	+	+
Carbohydrates	Yellow / brownish / blue / green color	+	+
Oils & Resins	Filter paper method	-	+

Core of nano-biomaterial is formed by nanoparticle. It can be used as a convenient surface for molecular assembly, and may be composed of inorganic or polymeric materials. It can also be in the form of nano-vesicle surrounded by a membrane or a layer (Jitendra *et al*., 2011). Nanoparticles can offer significant advantages over the traditional delivery mechanisms in terms of high stability, high specificity, high drug carrying capacity, ability for controlled releases, possibility to use in different types of drug administration and the capability to transport both hydrophilic and hydrophobic molecules (Nilesh *et al*., 2011).

The green synthesis of silver nanoparticles through plant extracts *Swertia chirata* and *Solanum robustum* were carried out. It is well known that silver nanoparticles exhibit yellowish - brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Thirumurgan *et al.*, 2010). The appearances of yellowish-brown colour in the reaction vessels suggest the formation of silver nanoparticles (SNPs) (Shankar *et al.*, 2004).

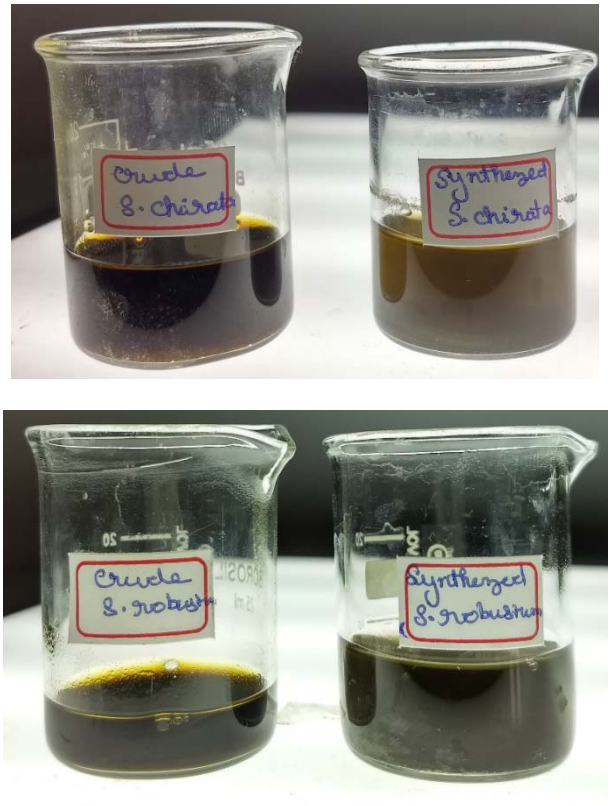


Figure 1: Synthesis of silver nanoparticles for *Swertia chirata* and *Solanum robustum*

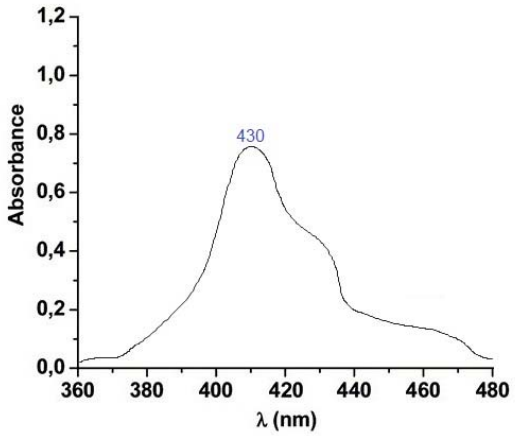
The SNPs from the leaves of *Swertia chirata* and *Solanum robustum* showed highest percentage of bacterial inhibition against *B.cereus* followed by *S.aureus, E.coli, E.faecalis, K.pneumoniae* (Table.2 and Fig.2). The maximum toxicity was observed in plant extract treated cells than SNPs. The smaller size of the particles which leads to increased membrane permeability and cell destruction might be the reason. Similar results were found in different plant extract (Savithramma *et al*., 2011). The antimicrobial effect of green synthesized SNPs is attributed that the micro-organisms having of peptidoglycan, which is a complex structure and often contains teichoic acids or lipoteichoic acids and these are having strong negative charge. This charge may contribute to the sequestration of free silver ions. The findings of Sereemaspun *et al*., 2008 suggested that the inhibition of oxidation based biological process by penetration of metallic nano sized particles across the microsomal membrane. SNPs have an ability to interfere with metabolic pathways and bacterial growth signaling pathway by modulating tyrosine phosphorylation of putative peptide substrates critical for cell viability and division (Shrivastava *et al*., 2007).

The SNPs synthesized from leaves of *Swertia chirata* and *Solanum robustum* are toxic to multi-drug resistant microorganisms. It shows that they have great potential in biomedical applications. As silver has distinctive properties such as good conductivity, catalytic and chemical stability, silver nitrate is used as reducing agent. Formation of silver hydrosol was formed when the aqueous silver ions when exposed to herbal extracts (*Swertia chirata* and *Solanum robustum*) were reduced in solution. The time period of modification in colour differs from plant to plant. Synthesize of silver nanoparticles in *Swertia chirata* took 10 min while it was 15 minutes for *Solanum robustum.*

Table 2: Antimicrobial activity for crude and synthesized samples of *Swertia chirata* and *Solanum robustum*

S.No	Organisms	Control	Crude sample		Synthesized sample	
			S. chirata	S.robustum	S. chirata	S.robustum
1	B.cereus	23	38	10	11	16
2	E.faecalis	24	36	15	18	19
3	S.pneumonia	26	31	12	19	13
4	K.pneumonia	26	35	20	15	15
5	B.substilus	22	33	12	12	15
6	S.aureus	25	37	17	16	18
7	E.coli	23	36	11	19	18
8	P.vulgaris	20	26	10	22	18
9	V.prahaemolyticus	21	28	16	11	15
10	P.aeroginosa	23	26	15	15	18

Measuring the UV-V spectrum of the reaction media confirms the synthesis of SNPs. Absorbance peaks were noted at 430 nm and 420 nm respectively for *Swertia chirata* and *Solanum robustum* in the UV-Vis spectrum of colloidal solutions of SNPs synthesized; and the broadening of peak indicated that the particles are poly-dispersed (Fig-2).



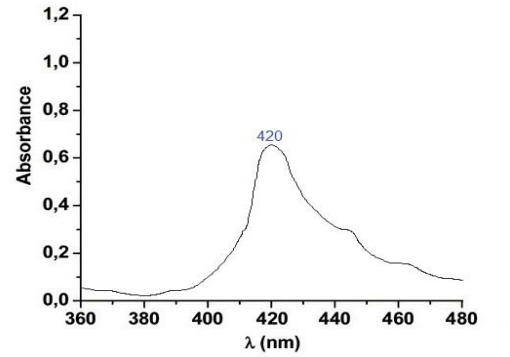
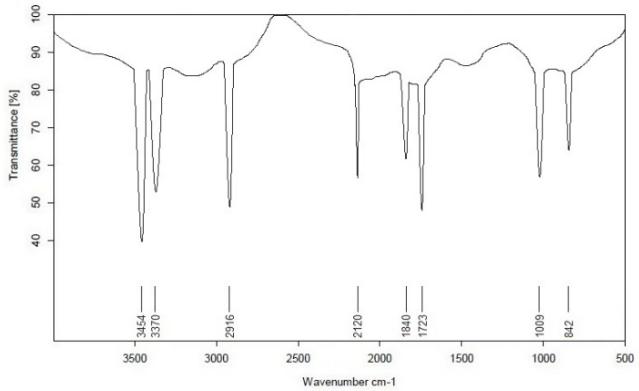


Figure 2: UV Analysis of Silver Nanoparticles from *Swertia chirata* and *Solanum robustum*

To identify the possible biomolecules responsible for capping and efficient stabilization of AgNPs synthesized in *Swertia chirata* and *Solanum robustum* extract, FTIR measurement were carried out. The spectrum as shown in (Fig.3) lot of absorption bands indicates the presence of active functional groups in the synthesized AgNPs. FTIR spectroscopic study confirm the carbonyl group of amino acid residues and peptides of proteins has a stronger ability to bind metal, so that the proteins might probably form a capping of metal nanoparticles, to prevent the agglomeration of the particles, and thus, the nanoparticles are stabilized in the medium. The FTIR spectrum of synthesized AgNPs using *Swertia chirata* extract shows a strong band at 3450 cm^{-1}, 3370 cm^{-1} indicates the presence of primary amines (weak to medium), 2916 cm^{-1} presence of alkanes (strong), 2120 cm^{-1} presence of terminal alkyne (weak), 1840 cm^{-1} Vinyl terminal (medium), 1723 cm^{-1} Aromatic esters (very strong), 1009 cm^{-1} and 842 cm^{-1} indicates Cycloalkanes (medium).



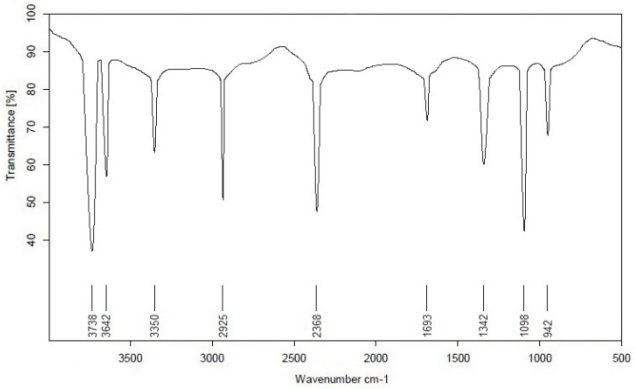


Figure 3: FTIR Analysis of Silver Nanoparticles from *Swertia chirata* and *Solanum robustum*

The FTIR spectrum of synthesized AgNPs using *Solanum robustum* extract indicates a strong band at 3738 cm^{-1}, 3738 cm^{-1} which is primary alcohol (variable), 3350 cm^{-1} indicates intermolecular hydrogen bonded OH (Strong), 2925 cm^{-1} presence of Acids (Medium), 2368 cm^{-1} indicates Aromatic methane (weak), 1693 cm^{-1} Aromatic acids (very strong), 1342 cm^{-1} secondary amines (strong), 1098 cm^{-1} indicates cycloalkanes (medium) and 942 cm^{-1} cycloalkanes (strong). The presence of active functional groups in *Swertia chirata* and *Solanum robustum* extract results in the swift reduction of silver ions to silver nanoparticle. For identification and characterization of a substance, FTIR is a significant technique. To detect the possible biomolecules and cell metal ions interface in the plant extract responsible for the steadiness of the newly synthesized silver nanoparticles, FTIR spectroscopic analysis was carried out (Ankamwar *et al*., 2010; Govindaraju, 2010).

In fig. 4 are the SEM images of nano particles of different sizes ranging which are dispersed in the form of different aggregates and particles. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by capping agents. The silver nanoparticles synthesized from *Swertia chirata* and *Solanum robustum* characterized by Scanning Electron Microscopy (SEM). SEM is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan patterns. SEM technique was employed to visualize the size and shape of silver nanoparticles.

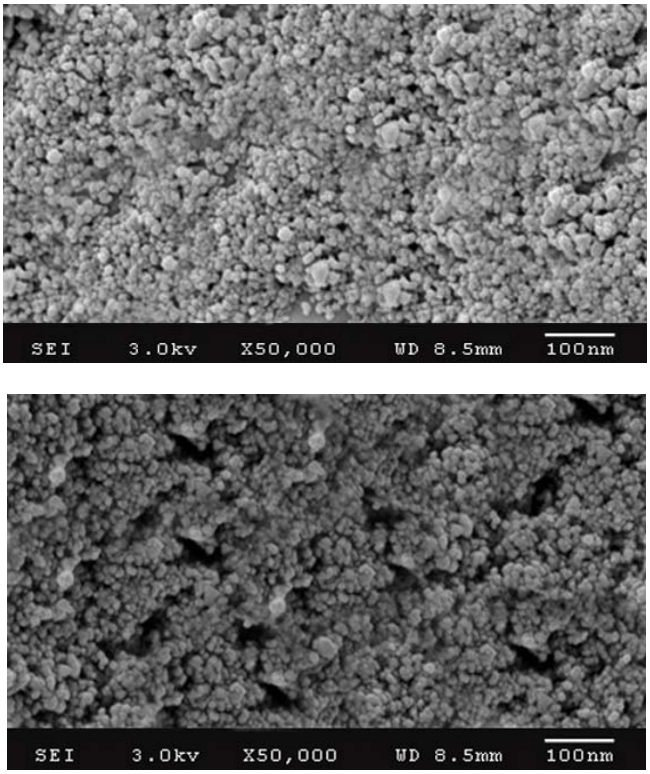


Figure 4: SEM Analysis of Zinc Nanoparticles from *Swertia chirata* and *Solanum robustum*

Conclusion

The present study covered the bio-reduction of silver ions through medicinal plants extracts of *Swertia chirata* and *Solanum robustum* and testing for their antibacterial activity. The synthesis of silver nanoparticles were confirmed by the change of colour of plant extracts of *Swertia chirata* and *Solanum robustum*. These eco-friendly silver nanoparticles were established further by using UV-Vis spectroscopy, FTIR and SEM. The results proved the good antibacterial activity against different microorganisms of silver nano partcilces. Further it was proved that the silver nanoparticles are capable of rendering high antibacterial effectiveness and hence has a great prospective in the formulation of drugs used against bacterial diseases. The phytochemical screening indicates that the plant *Swertia chirata* and *Solanum robustum* is a good source for bio active principle for pharmaceutical industry.

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