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DESIGN, SYNTHESIS AND CHARACTERISATION OF SILVER NANOPARTICLES (*BRYOPHYLLUM PINNATUM*)

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Fig. 1: Bryophyllum Pinnatum.

ABSTRACT

The production of silver nanoparticle(AgNPs) using an environmentally benign process from the ***BRYOPHYLLUM PINNATUM*** plant (family; **Crassulaceae**). Plant based nanoparticle offer sustainable, Eco-friendly alternatives to conventional methods. UV and other analytical techniques are used to analyze the biosynthesis of silver nanoparticles. ***Bryophyllum pinnatum*** is an indigenous and exotic plant used widely by the traditional Practitioner for treating various ailments like renal calculi, hypertension, asthma, cold, bleeding disorders. Prepared silver nanoparticles were examined for in vitro antioxidant activity using DPPH and ascorbic acid [VITAMIN-C] as standard. Additionally, protein denaturation assay was used to test for in vitro anti-inflammatory activity using diclofenac sodium as standard. The production of silver nanoparticles during biosynthesis is confirmed by color shifts from from colourless to reddish brown, with maximal absorption occurring between 400 and 450 nm. Comparing AgNPs to standard under identical settings, they exhibit good anti-inflammatory and

considerable antioxidant activity. These in vitro data are suitable for in vivo animal studie**s.** ***Bryophyllum pinnatum*** is the good source for the medicinal drug and have the properties of antidiabetic, anti-pyretic etc. ***Bryophyllum pinnatum*** were used for fresh utilisation by washing, weighing and crushing their leaves, then mixing them with distilled water and heating at 50℃and 60℃ for 30 mins.

INTRODUCTION

Bryophyllum pinnatum *is* plant is an environmental weed from the family **Crassulaceae**, but commonly used traditionally as a medicine in different regions of India mainly to treat urinary stones, as well as in other part of world. The traditional practitioners in various parts of world use this plant in numerous conditions like hypertension, skin disorders, asthma, cold, insect stings, abscesses etc. A large number of people are suffering from urinary stones and it is prevalent in approximately 12% of the world population with a recurrence rate of 70%–80% in males and 47%–60% in females.

- syn. *Kalanchoe pinnata* (Lam.), and *B. calycinum* (Salisb.) is a widely distributed perennial medicinal herb. It is native to **Madagascar, but has been naturalized in several other regions, including the temperate regions of Asia, Australia, and New Zealand**
- *Bryophyllum pinnatum*. linn plant commonly known as **love plant1, miracle leaf, life plant…etc**. In Nigeria this plant is locally called as **"Never Die"** plant and one of the popular plants in folklore medicine. In Ayurvedic science it is commonly known as *Parnabeeja4*. These are also used in bleeding disorder, ulcer, and diarrhoea5. The main chemical constituents of this plant include alkaloids, flavonoids, glycosides, steroids, bufadienolides, and organic acid.6. It is a perennial herb which is about 1m tall. Stem is fleshy and cylindrical and youngest stem are reddish in colour. It is growing primarily in rain forests and distributed worldwide. It is astringent and sour in taste2. The nutritive value of fresh and dried leaves of *Bryophyllum pinnatum* shows that the carbohydrate values were the highest and ash had the least value. Calcium and potassium levels are high and lead and zinc levels are law in both fresh and dried samples.

Presence of large amount of various chemical constituents in the plant *Bryophyllum pinnatum* shows various pharmacological actions. It has been used for the treatment of a variety of conditions in tropical America, India, China, Australia and Africa. These Herbs can warm the body, speed metabolism, cleanse the blood, improve surface circulation, improve waste

disposal, reduce inflammation, and relax and soothe irritation. They can be used topically or taken internally as syrups, infusions, or capsules.

- The Palikur mix the leaf juice with coconut oil or andiroba oil and then rub it on the forehead for migraines and headaches.
- In Peru, indigenous tribes mix the leaf with aguardiente (sugar cane rum) and apply the mixture to the temples for headaches; they soak the leaves and stems overnight in cold water and then drink it for heartburn, urethritis, fevers and for all sorts of respiratory conditions. The root infusion is also used in Epilepsy.
- In the Amazon squeeze the juice from fresh leaves and mix it with mother's milk for earaches.
- In Mexico and Nicaragua it is also used to promote menstruation and assist in childbirth
- Externally, the pulp of the leaves or the juice is applied on traumatic injuries to arrest the bleeding as it contract the minute arterioles and promote the healing of wounds. It is also used for headaches, toothaches, earaches, eye infections, wounds, ulcers, boils, burns and insect bites.
- On traumatic wounds, the heated leaves are crushed and applied.
- It reduces the edema and promotes the wound healing without leaving a scar. Internally, the leaves juice and cumin seeds are given along with the double amount of ghee in dysentery. The herb is highly recommended in bleeding disorders, piles and menorrhagia.
- High levels of calcium in diet, insufficient drinking water required for body metabolism, gout, hyperparathyroidism, obesity, and continuous calcium supplement intake are some of the high risk factors responsible for urinary calculi.
- The plant are used by the tribals of Kerala for treating cancer symptoms. Also plant induce the typical symptoms of cardiac poisoning, but repeated small doses also cause cotyledonis, an intoxication affecting the nervous and muscular systems of small animals, particularly sheep, in the Karoo area of South Africa.
- However, currently using therapies for prevention as well as for cure is not effective in all cases and is highly expensive with common reoccurrence and potential side effects, Hence, more engrossment is given towards medicinal plants.

- **ORIGIN**

Native to Madagascar and South Africa.

MORPHOLOGY

Bryophyllum pinnatum is a succulent herb 0.3 1.2m high. Stems obtusely four angled, older one pale coloured and younger ones are reddish with white. Leaves are usually simple/compound, upper ones are 3-5/7 foliolate with long petioled.(12) The bell-shaped (i.e. tubular), drooping (i.e. pendulous), flowers (up to 7cmlong) are arranged in branched clusters at the terminal of the stems (i.e. in terminal inflorescences). Each flower is present on a stalk (i.e. pedicel) 10-25mm Long. That Are Partially Connected To The Tube (i.e. Calyx) And Streaked with pink or reddish colored blotches.(13) The yellowish green to dark red coloured petals (3-6cm long) are also partially fused into a tube (i.e. a corolla tube) that differentiate into four petal lobes (i.e. corolla lobes) near the tip. Flowers are produced mainly during winter and spring.

COMMON NAME

- Air plant
- Good luck leaf
- Green mother of millions
- Leaf of life
- Live leaf
- Mexican love plant
- Sprouting leaf.

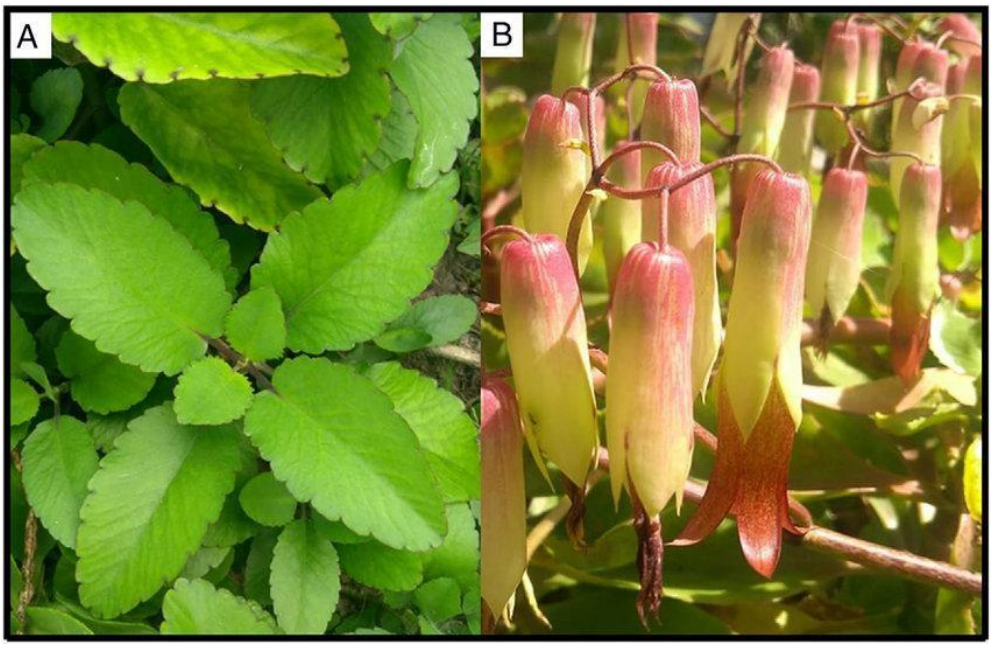


Fig 2: A:- BRYOPHYLLUM PINNATUM PLANT.

B:-BRYOPHYLLUM PINNATUM FLOWER

TAXONOMICAL CLASSIFICATION

- **Kingdom-**Plantae.
- **Divison-** spermatophyta
- **Class**- Magnoliopsida
- **Order**- Rosales
- **Family**- Crassulaceae
- **Genus**- Bryophyllum
- **Species** - pinnatum

MAIN PHARMACOLOGICAL ACTIVITIES

BRYOPHYLLUM PINNATUM plant, leaves are have several pharmacological Activity like:

- **Anti-leishmanial activity**
- **Anti-cancer activity**
- **Anti-hypertensive activity**
- **Anti-ulcer activity**
- **Anti-diabetic activity**
- **Anti-inflammatory activity**
- **Anti microbial activity**
- **Anti-diabetic activity**
- **Anti-hypertension activity**
- **Neuroprotective activity.**

Manily we are focused 2 activities which are **ANTI-INFLMATORY AND ANTIOXIDANT ACTIVITY.**

- **T**hese plant leaves are also treated for ***menstrual disorder, migraines, wounds and kidney & urinary bladder stone.***
- In unani the bark have some poisonious effect.
- In ayurvedha system the leaves have poisonious to insects.

ETHANOPHARMACOLOGY

The Bryophyllum pinnatum leaves and bark are bitter tonic, astringent, analgesic and carminative, so ethanopharmacologically it is used for the treatment of diarrhoea, vomiting,

ear ache, burns, abscesses, gastric ulcers, insect bites and lithiasis. The fresh leaf juice is used for the treatment of smallpox, otitis, cough, asthma, palpitation and general debility". The leaf powder is used for wound dressing. The juice of the leaves is used to treat dysentery with ghee. Two tea spoon leaf juice is used in the treatment of renal calculi. In Nagpur the steamed leaf juice along with ghee or garlic is used for the treatment of cough. The leaves with palm oil are also used for the treatment of sore eyes externally.

AYURVEDIC PROPERTIES

- ✧ Rasa:Kashya, Amla
- ✧ Guna:Laghu
- ✧ Virya:Sheeta
- ✧ Vipaka:Madhura
- ✧ Doshaghnata:Vatakaphahara
- ✧ Karma:Ashmarighna, Shonita

Dosage

Leaf powder 2.5 to 5gm.

Reproduction

Seed and plantlets.

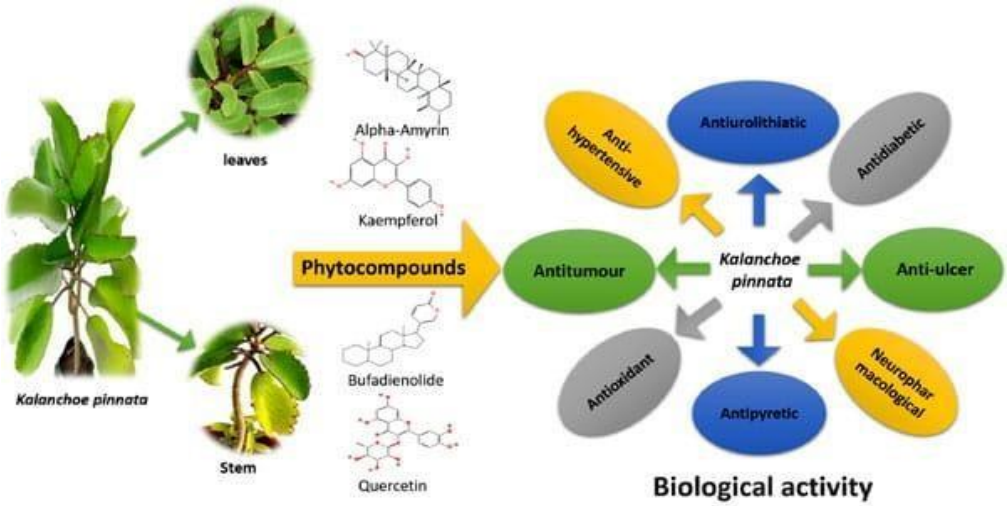
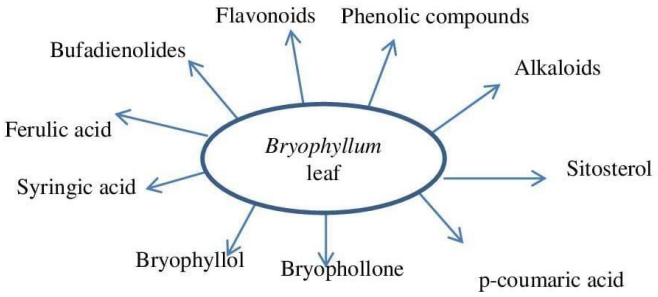


Fig 3: Pharmacological Activity.

PHYTOCHEMICAL CONSTITUENTS



Some Common methods used to synthesizing silver nano particles

- ✓ Laser ablation: A method that uses intense laser pulses to focus on a silver target immersed in a solvent. This method is efficient and doesn't require chemical reagents.
- ✓ Green synthesis: An eco-friendly method that uses biological agents like bacteria, fungi, algae, and plant extracts.
- ✓ Evaporation-condensation: A physical method for synthesizing silver nanoparticles.
- ✓ Electrical irradiation: A physical method for synthesizing silver nanoparticles.
- ✓ Gamma irradiation: A physical method for synthesizing silver nanoparticles.
- ✓ Lithography: A physical method for synthesizing silver nanoparticles.

Some methods for characterizing silver nanoparticles

- ✓ Scanning electron microscopy
- ✓ Used to confirm the shape, size, and crystalline nature of silver nanoparticles.
- ✓ UV–Vis spectroscopy
- ✓ Used to measure the characteristic surface plasmon band of silver nanoparticles.
- ✓ Silver nanoparticle spectroscopy for mercury detection
- ✓ Used to quantify the mercury content in a sample by observing the color change that occurs when silver nanoparticles react with mercury.

MATERIAL AND METHODS

COLLECTION OF SAMPLES

Bryophyllum Pinnatum leaves were collected around the KG Herbal garden, for this investigation.

METHOD OF PREPARATION OF SAMPLES

1 or 2 drops of leaf extract add to silver nitrate solution. Bio Reduction of AgNO3 by chemical constituents was observed by color change from colorless to reddish brown in color.

PHARMACOLOGICAL EVALUATION

Preparation of Silver Nanoparticle

The fresh leaves of B. pinnatum were washed thoroughly with tap water to remove dust particles and then washed with double distilled water. Twenty-five grams chopped leaves were added to 100 ml of distilled water and boiled at 80°C till it turn slight green The extract was filtered twice with 0.2 µm size of vacuum filter. From 100 ml, 10 ml of pure plant extract was diluted in 90 ml of distilled water in a conical flask. To this, 100 ml of diluted leaf extract and equal quantity of 1 mM silver nitrate (AgNO3) solution was added and mixed thoroughly on a magnetic stirrer for 2–5 min. Later, the mixture was placed in bright sunlight tillthe colour changes from transparent to dark brown which indicated formation of AgNPs. The purification of nanoparticles was done by repeated centrifugation at 10,000 rpm for 10 min and the subsequent displacement of the supernatant with sterile distilled water was performed, so that the unreacted plant metabolites, nitrate and silver ions could be removed and nanoparticles were made to settle at the bottom forming the Precipitate. Furthermore, the precipitate was collected and oven dried, and the powdered AgNPs obtained were stored at room temperature and used for further study.

Characterisation of AgNPs UV-Vis spectra analysis

The AgNPs powder was dissolved in sterile water and subjected to UV-Visible analysis. Preliminary confirmation of synthesised AgNPs was done with the help of UV-Visible double beam spectrophotometer, operated at a resolution of 1 nm by scanning the absorbance spectra from 200 to 800 nm of the colloidal sample.

S.no	nm	Maximum absorbance (n=3)
1.	430	0.42
2.	500	0.39
3.	520	0.38
4.	620	0.36
5.	680	0.34
		MAX 400-450nm

Fig. 4: UV ANALYSIS.

UV- ANALYSIS vitro BPET AgNps Antioxidant activity

Leaf (BPET AgNps)aqueous extract investigated for in vitro antioxidant activity by DPPH, ABTS, FRAP and NO for the estimation of antioxidant potential ***BRYOPHYLLUMPINNATUM (*BPET AgNps)**aqueous extract. ***Bryophyllum pinnatum* (BPETAgNO3)** me also tested by DPPH assay.

BPET AgNPs				
S.no	COD	SOD	%inhibiton	Average
1.	1.30	0.82	67	61.9%
2.	1.39	1.15	56.3	
3.	1.30	0.83	62.3	
STANDARD VIT C ASCORBIC ACID				
1.	0.34	0.04	88.24	87.67%
2.	0.34	0.04	88.24	
3.	0.34	0.05	85.29	

Fig. 5: Invitro Antioxidant Activity.

Determination of DPPH radical scavenging activity

- Antioxidant activity in the sample ***BRYOPHYLLUM PINNATUM*** were estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (BrandWilliams et al., 1995). 100µL of SC extract was taken in the microtiter plate. 100µL of 0.1% methanolic DPPH was added to the samples and incubated for 30 minutes in dark condition.

- The samples were then observed for discoloration; from colorless to reddish brown were considered as strong and weak positive respectively. Read the plate on Elisa plate reader at 490nm. Standard ascorbic acid was used as reference. All the analysis was performed in triplicates and the average values were taken.

Radical scavenging activity was calculated by the following equation

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100.

In-vitro CGET AgNps Anti-inflammatory activity - Inhibition of albumin denaturation

The reaction mixture was prepared separately by mixing 0.5ml aqueous extract of BPET AgNps and its compounds A, B, and C (1mg/ml) with 0.45 ml aqueous solution of bovine albumin fraction (5%). The pH (6.3) of the solution was adjusted using a small amount of 0.1N HCl at 37 ˚C for 20 min, then heat to 57 ˚C for 30 min. Cool the solution and transfer it to the 96 well plates and measure the absorbance at 660nm. Standard was used as Diclofenac sodium (1000µg/ml) and the control contain 0.05ml distilled water. The percentage of inhibition of albumin denaturation was calculated by the following formula, Percentage of inhibition (%) = [(A control – A sample) / A control] x 100 Where A control – Absorbance of reaction mixture except drug. A sample – absorbance of the reaction mixture with the Sample.

BPET AgNPs				
S.NO	COD	SOD	%inhibition	Average
1.	0.36	0.27	25	24.5%
2.	0.39	0.30	23.1	
3.	0.38	0.28	26.3	
STANDARD DICLOFENAC				
1.	0.36	0.04	88.89	87.96%
2.	0.36	0.04	88.89	
3.	0.36	0.05	86.11	

Fig. 6: Invitro Anti-Inflammatory Activity By Protein Denaturation.

NANOPARTICLE SIZE ANALYSIS

The average size of the synthesised nanoparticles was characterised by the nanoparticle tracking analysis (NTA) system NTA is a laser-based light-scattering technique, in which particles suspended in the liquid medium were injected into the LM viewing unit and

visualised under the optical element. At controlled conditions, the sample preparations and measurements were carried out and analysed by NanoSight LM 20 using a beta version of NTA 2.3 software.

PARTICLE SIZE DISTRIBUTION

Zeta potential measurement

Zeta potential of AgNPs was measured by using a Zetasizer with a zeta dip cell. For the sample analysis 10 µl of AgNPs colloidal solution was diluted in 1000 µl of distilled water. The surface charge of nanoparticles was measured using Zetasizer. A potential distribution around the particles was obtained as a result of surface charges. Zeta potential usually falls in the range of –70 mV to þ70 mV and is expressed in millivolts (mV).

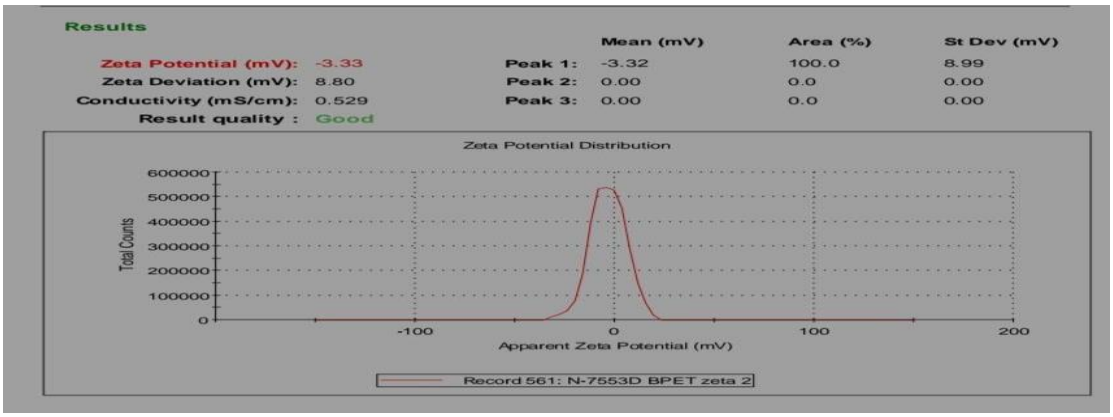


Fig 7: ZETA POTENTIAL

RESULT AND CONCLUSION

Ethanol extract of ***Bryophyllum pinnatum*** leaf has shown anti-inflammatory, antioxidant properties. biosynthesis of silver nanoparticles. ***Bryophyllum pinnatum*** synthesized AgNPs were initially confirmed by UV-visible spectrophotometer absorbance. Preliminary

confirmation of synthesised AgNPs was done with the help of UV-Visible double beam spectrophotometer. ***Bryophyllum pinnatum*** 4-5 leaf extract has shown in-vitro antioxidant activity by DPPH assay in the current investigation. The silver nanoparticle was prepared from ethanolic extract of

BRYOPHYLLUM PINNATUM and it was verified and confirmed by UV analysis and colour observation. The biosynthesis of BPET AgNPs was characterized by FTIR and SEM analysis. The ethanolic extract BPET prepared silver nanoparticle had significant anti oxidant activity by DPPH assay and mild anti inflammatory activity.

In-vitro CGET AgNps Anti-inflammatory activity

- Inhibition of albumin denaturation is done. Invitro anti-inflammatory activity by protein denaturation.

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