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# FABRICATION, CHARACTERIZATION AND ANTICANCER ACTIVITIES OF GOLD & VIOLA (*VIOLA ODORATA*) BASED GREEN NANOPARTICLES

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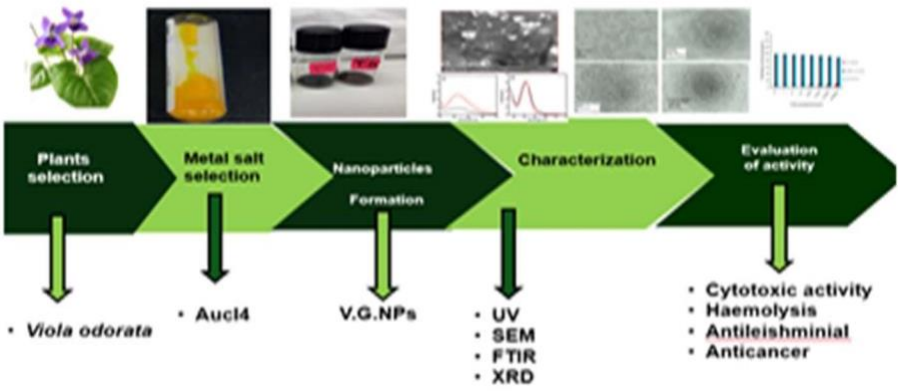
**Abstract**

Nano biotechnology has opened new ways of improved materials in biomedical integrations. Green synthesis with noble metals is an enormously emerging area of research in a nano biotechnology. It acknowledged great consideration from physicists, chemists, engineers and bio scientists who wish for the development of a new world nano medicines. The current contribution deals with one pot synthesis of green nanoparticles by gold via potent and traditional medicinal plant Viola odorata. The synthesized nanoparticles were characterized via different analytical procedures, included ultraviolet-visible spectroscopy(UV), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction(XRD) and scanning electron microscopy (SEM). The ultra violet-visible spectrum of nanoparticles e.g VGNPs peaks were recorded at 530nm. Optimization has gained at pH 8-9, 1mM conc., high heat and 48 hrs incubation period along with pH 9-10 and 3mM conc. enhanced the gold nanoparticles synthesis and small size. FTIR studies revealed some alcoholic and phenyl groups are involved in capping of *Viola* based GNPs. XRD and SEM confirmed the particles size and shape were found spherical and triangular for VGNPs size varied from 42-60nm. VGNPs proved best allaround for bioactivities like antileshminial, haemolysis studies, cytotoxicity testing and anticancer assey studies proved promising activity for VERO cells while also for MCF-7 breast cancer cell lines. The outcome of the recent reserch could make a way in the development of value-added products from *Viola odorata* with metallic salts of gold for biomedical, agriculture and nanotechnology-based productions.

**Key words:** Green nanoparticles, *Viola odorata*, VGNPs, MCF-7, FTIR, XRD, SEM

**Graphical abstract**



## 1. Introduction

Medicinal plants being potent are used by man long before the introduction of chemical medicines. Some favor the plants to be used for remedies due to their strong belief that plants have provided food, medical aid, and other benefits to mankind. According to WHO plants are always a way of easy and cost-effective treatment, both in the context of traditional preparations as well as in the form of active principle representation in their simplest form (Al-Snafi, 2013, Al-Snafi, 2015).

Pakistan for having a unique range of biodiversity and all climate zones. Approximately 6,000 plant species have been identified across the country. Approximately 600 to 700 species have therapeutic uses and roughly about 84 percent of the population relies on plant products Seventy percent of these medicinal plant species are located in certain parts of Pakistan, (Jan, H. A., et al. 2020).

### 1.1 Plant description (*Viola*)

Plants of the Violaceae family are commonly used in traditional Chinese medicine and therapies. The Violaceae family (sometimes known as Alsodeiace, Leoniaceae, or Retrosepalaceae) has approximately 800 species split into 20 genera. In Asia, *Viola odorata* is known as Gul-e-banafsha, as well as sweet violet, garden violet, wood violet, common violet, English violet, and florist's violet. Sweet violets are cultivated and self-generated on six continents, all of which are in the Mediterranean area and have a moderate climate. It has one genus (*Viola*) and 17 species and is found in Pakistan's Sawat, Hazara, and Dir regions. It was initially described in 1817 by English botanist James Edward Smith (Roqaiya., 2019), (Chandra and colleagues, 2015).

Table 2 1: Taxonomic position of *Viola odorata* and other species used in the study.

| **Viola Specie** | **Medicinal status** | **Plant part** | **References** |
|---|---|---|---|
| *Viola biflora* | Diaphoretic and intestinal pain | Flower | (Chandra, D.,et al 2015) |
| *Viola tricolor* | Anti-inflammatory, skin diseases, cystitis, cough, bronchitis rheumatoid arthritis | Aerial part | (Chandra, D.,et al 2015) |
| *Viola betonicifolia* | Bronchitis, kidney, cough,diuretic, sinusitis,anticancer,pneumonia, haryngitis | Leaves,Roots, flowers,fruits | (Muhammad, N.et al 2012) |
| *Viola hondoensis* | Cough, bronchitis, eczema, diuretic, skin eruption, anti-inflammatory rhmtoid hritis | Whole plant | (Asheesh, K., et al 2017). |
| *Viola pilosa/ Viola serpens* | Throat cancer, cough, fever, headache, diaphoretic, and skin diseases | Whole plant | (Muhammad,N.,et al 2012) |
| *Viola canescens* | Cold, cough, malaria, flu, anticancer drug | Whole plant | (Chandra, D., et al 2015) |
| *Viola arvensis* | Used in rheumatoid arthritis, used in edema nephrotic syndrome, cystitis, cough anti-inflammatory, bronchitis, | Aerial parts | (Asheesh, K., et al 2017). |
| *Viola falconeri* | Cold, cough, and jaundice | Roots & flowers | (Muhammad,N. et al2012) |
| *Viola cinerea* | Aphrodisiac | Whole plant | (Asheesh, K., et al 2017). |
| *Viola patrinii* | Cold, cough, constipation, purgative | Flower | (Chandra, D., et al 2015) |

Traditional Chinese medicine uses the dried entire plant (including the roots) to give toxic heat, puffiness, blisters, swellings, snake bites, carbuncles, bronchitis, hepatitis, acute nephritis, appendicitis, and enteritis. Extracts from *V. odorata* have been shown to suppress the human immunodeficiency virus (HIV), cancer, and definite microorganisms (Wang et al., 2007).

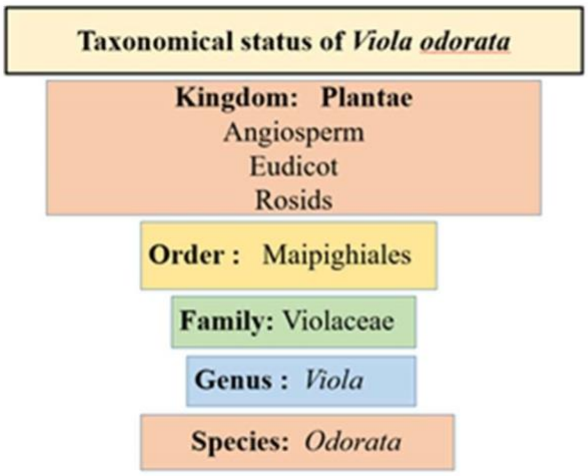



**Figure: 2-2:** Taxonomic position of *viola odorata* used in the study.

Roots and fruits are used to cure kidney disease, pneumonia, and bronchitis. The grasses are advantageous for treating boils (Orhan, I.E., et al., 2015).

### 2.1 Primary phytochemical status

An extract from the leaves of *Viola odorata* was found to have a high phenolic (35.4 mg/g) and flavonoid (22.8 mg/g) content (Ebrahimzadeh, et al., 2010). Whole plant extracts of *Viola tricolour* in aqueous, ethyl acetate, dichloromethane, and methanolic form have been studied and shown to be high in terpenoids, saponins, phenolic compounds, and flavonoids. HPLC, LC-MS, and NMR were used to investigate the flavonoid content of *Viola's* methanolic extract, and five minor flavonoids were identified.

### 2.2 Nutritional value

The fundamental compositions of *Viola odorata's* various individual parts (stem, leaves, petiole roots, branches, and flower) were examined for the detection of various elements such as carbon (C), oxygen (O), sodium (Na), calcium (Ca), magnesium (Mg), aluminium (Al), silicon (Si), chloride (Cl), and iron (Fe) (Fe). *Viola odorata* was discovered to be a potent and plentiful source of these nutrients (Bibi, S., et al., 2006).

### 2.3 Compounds isolated

Many pharmacologically dynamic mixtures have been identified from diverse classes of *Viola*, according to a study of the literature. Viola's natural compounds include cyclotide alkaloids, caffeic acid, flavonoids, derivatives, triterpenoids, and salicylic acid, among others (Ebrahimzadeh et al., 2010).

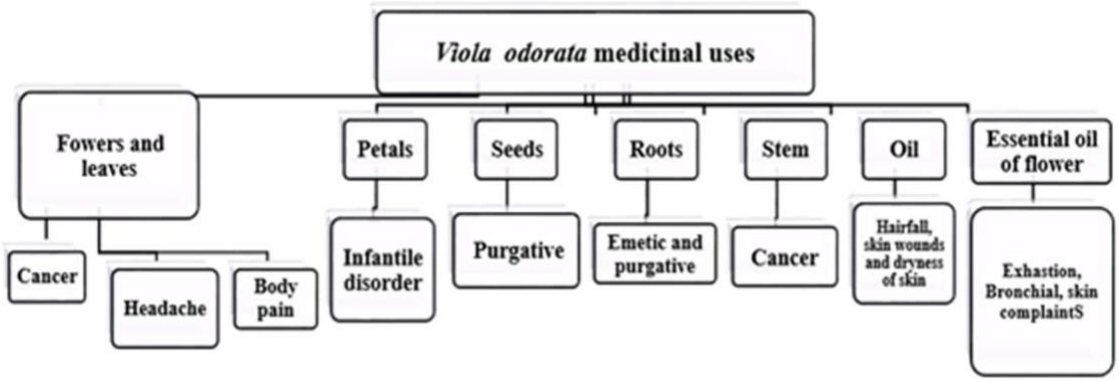



Figure:2 3: Showing medicinal uses of *Viola odorata* from different parts of the plant.

**2.4 Anticancer activity of *Viola odorata***

As stated by Singh and Dhariwal (2018), the entire aerial part of *V. odorata*, which includes the flowering stems and leafy stems, is utilised to cure cancer. The plant *Viola* sp has been characterised as a possible lead for anticancer medicines as well as a pharmacological method for treating cancer patients. cycloviolacin $O_2$, a cyclotide produced from the plant *V. odorata*, exhibits anticancer properties and is capable of inducing cell death by permeabilizing the cell membrane. The *Viola odorata* cyclotides are cytotoxic and have the potential to be used as chemosensitizing causes in the handling of drug-resistant breast cancer. It is possible to utilise the fresh leaves of *V. odorata* to cure cancers of the throat and mouth (Alipanah et al., 2018). *Odorata* was the subject of a few lab studies which have proved for its anti tumor activities. The different advantages of this medicinal plant have been studied in terms of patient satisfaction, favourable results, and the absence of negative side effects in patients with cancer (Parsley, N.C., et al., 2018).

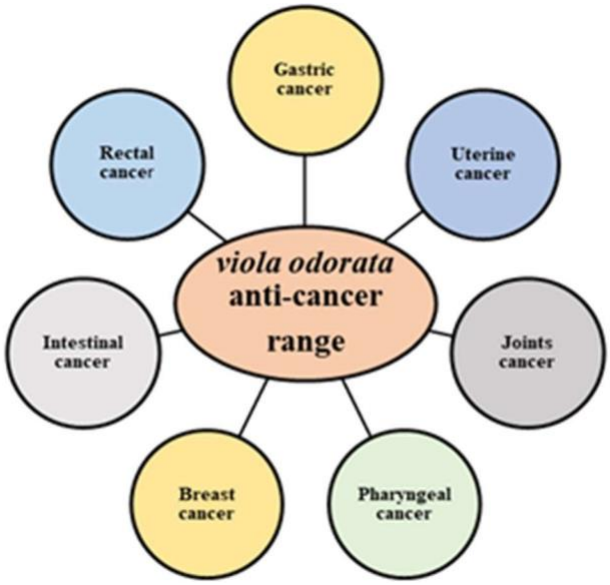



Figure: 2 3: *Voila odorata* is used for different types of cancer.

Recent study has revealed that *Viola Odorata* cyclotides have significant anticancer effects against uterine, stomach, intestinal, breast, joint, pharyngeal, and rectal cancers, the anticancer effects of

*Viola Odorata* and its cyclotides have been been investigated in vitro and in living organisms, including humans. Based on an in vitro study conducted in 2010, it was shown that *V. Odorata* had anticancer capabilities and induces cell death in a breast cancer cell line by way of membrane channels. (Jungwirth, et al., 2011).

**2.5 Gold nanoparticles green Synthesis**

For GNP synthesis, several biological and chemical techniques are suitable. Chemical methods include reducing tetrachloroauric ácid in the colloidal form (dimensions range 10–60 nm).A few years ago, researchers examined and evaluated the biological technique of synthesising gold NPs in different sizes and forms. (Li, X., et al., 2011).

Plant extracts from various plant sections may be utilised as a greener approach in the gold synthesis. One nice aspect here is that it is easy to understand the form and size of these manufactured green nanoparticles. A number of researchers recently reported synthesising gold NPs with various plant extracts including *Ficus religiosa, Brevibacterium casei, Citrus reticulata and Citrus sinensis (Piper pedicellatum), Murraya Königii, Cinnamomum Zeylanicum, Cochlospermum Terminalia chebula, Mangifera indica, Memecylon edule, Coriandrum sp and Ficus religiosa* (Rajeshkumar S., et al., 2016). The green production of gold nanoparticles, which includes spices and vegetables, was investigated using extracts from 25 medicinal plants (Panyala N. R., 2009).

Gold nanoparticles(AuNPs) is one of the nanomaterials most frequently utilized in commercial goods that have strong electrical conductivity, attractive optics, efficient catalytic and antibacterial activity and biocompatibility. In addition, AuNPs are the most well researched nanomaterials, anticancer, anticoagulants, cytototoxicity, and anti-oxidant immune modulator. Environmentally friendly NPAs have made them popular and suitable across broad areas. A clean, low cost, environmentally acceptable, non-toxic, dependable and safe method to AuNP's biogenic synthesis is available in a broad variety of applications. (Khan, A. U., et al., 2018).

**2.6 Toxicity of gold**

When pure metallic gold is used, it is non-toxic and non-irritating, and it is often used in the form of a gold leaf for food decoration. Metallic gold may also be found in the alcoholic drinks Goldschläger, Gold Strike, and Goldwasser. Metallic gold is approved as a food ingredient in the European Union (E175 in the Codex Alimentarius). Despite the fact that the gold ion is toxic, metallic gold is suitable as a food additive due to its chemical inertness and resistance to corroding or transformation into soluble salts (gold compounds) by every known chemical process encountered in the human body. Because elemental metallic gold is chemically inert, it interacts with the body (for example, ingested gold cannot be eliminated by stomach acid, thus it remains undigested and may cause ulcers, etc.) (Fratoddi, I., et al., 2015).

Gold salts are used alone to toxicate the soft tissues in the human body, because liver and kidney toxic soluble molecules like gold chloride have been discovered. Some of the other gold salts often employed in electroplating, such as gold cyanide and particularly potassium gold cyanide are hazardous, since both their cyanide and gold content react with biomolecules, although there are few instances of gold or potassium gold cyanide deaths. Chelation handling is a method for gold

to improve the gold toxicity of a dimercaprol agent. Gold metal was recognized by the American Contact Dermatitis Society as an allergin 2001. Most women have allergies to gold, although gold, as compared with other metals like nickel and others, are in reality a comparatively non-potent contact allergen (Fratoddi, I., et al., 2015).

**2.7 Anticancer activity of Au Nanoparticles**

Cancer is a disease in which cells grow uncontrollably owing to a variety of physical and chemical factors. It is one of the most dangerous illnesses, with a little possibility of survival. Pancreatic cancer is a kind of aggressive malignancy that is typically linked with a low or poor prognosis. Pancreatic cancer may be treated with a variety of chemotherapies and radiation treatments. However, the prognosis is uncertain and the survival rate is low. The MTT cytotoxicity test is a popular in vitro method for determining the viability of cancer cell lines. This method is often used to evaluate anticancer medication cytotoxicity in vitro. An MTT cytotoxicity assay was used in this research to evaluate the cytotoxicity of gold nanoparticles produced by *Scutellaria barbara*. Previous study has shown that depending on the samples and testing agents utilised, different types of cytotoxicity testing may provide different results. The gold nanoparticles produced have been found to exhibit substantial (p.001) cytotoxicity against dose-dependent human cancer cell types in recent research. The percentage of viability of cancer cell lines (PANC-1) was substantially decreased as the volume of gold nanoparticles produced increased (p.001). It shows that generated gold nanoparticles may cause cytotoxicity in human pancreatic cell lines (PANC-1) (Abbas., et al., 2019).

**2.8 Antileishmanial activity of Au nanoparticles**

Leishmania parasites proliferate in host macrophages. Therefore, to combat leishmanial parasite infestations, a particular supply of medicines is needed for macrophages. The usage of AuNPs for leishmanicidal medicines as vehicles for drug delivery have already been reported. Quercetin functionalized auNPs were manufactured to effectively combat selected drug-proof bacteria. The aqueous *Rhazya stricta* decne extract and *Maytenus royleanus* stem are other plant chemicals used as leishmanicidal agents for AuNPs (Katas et al. 2018).).

Leishmaniasis is one of the biggest health problems in the globe. It is currently widespread across the world in 98 nations. The overall annual prevalence of the disease is approximately 12 million and more than 350 million people at risk. Around 1.5 million new cases of skin leishmaniasis and 500,000 visceral leishmaniasis are anticipated to emerge worldwide every year. Moreover, due to an increase in the number of disease vectors induced by global warming, leishmaniasis develop. Pentavalent anti-monials, considered a gold standard in the therapy, are known to be highly harmful to humans among many medications used for leishmaniasis treatment.

**2.9 Gold toxicity**

Although gold is not required for plant growth, plants are exposed to significant quantities Studies have nevertheless revealed that plant species such as *Brassica juncea, B. peastris, Trifolium repens, Sorghum helense, Raphanus sativus, Kalanchoe serrate* and *Helianthus annuus* may accumulate to varyings in levels of absorption and dispersion in the plants, with species specific variations. (Katas H., et al., 2018).

In addition to the fast growth of nanotechnology, the negative impacts of these nanomaterials are not yet studied and must be taken into account. Such materials, which are based on environmental and biocompatible reagents, may at the same time reduce the toxicity of production materials and the environmental impact of the by-products. In order to achieve this goal, non-toxic plants are selected and evaluated for further investigation without affecting response medium and biological means (Katti K. V. 2018).

## 3. Methodology

### 3.1 *Theoretical Framework*

There are mainly two approaches suggested for nanoparticle synthesis, bottom up and top down. In present work second one has been adopted which involved the synthesis of nanoparticles from minimized atomic components through self-assembly. Which supported the nanoparticles formation through chemical and physical ways. It is cheap an approach; the following flow diagram is representing the whole methodology.

### 3.2 Plant extraction

The utilized items were thoroughly sterilized, all the chemicals were of analytical grades bought from the Merck Int. and the whole experiment was conducted using fresh DD water.Viola (*Viola odorata*) was chosen for experimentation. Plant extract was used for the synthesis of green gold nanoparticles as capping agents. Powdered plants of *Viola* were bought from neighboring Islamabad's standard pharmaceutical agency (Pakistan). Weighted 1 g of powder and washed twice in DD water. The powder has been soaked and was mixed at interval of 24- 48 hours. After complete soaking period the extracts were portrayed in jars and placed on a hot plate at 60Co. Mixtures were made ready for the experimentation using magnetic stirrers for the homogenious mixing. The extracts were subsequently filterd into whatman filter paper (90 mm porous size) and centrifuged for 10 minutes at 10,000 rpm to induce free redicals. The filtrate was collected in a 250 mm Erlenmeyer flasks, and the remaining were stored at 4 Co to help in the preparation for a week (Sagar R.et al, 2015 and Vidya Shilpa, 2013).


Figure: 3 2: Showing prepared extracts of *Viola* plant.

### 3.3 Phytochemical tests of plant extract

The formed plant extracts of *Viola* was examined for the presence and confirmation of specific chemical components by phytochemical and qualitative screening. The literature review standard approach was used for these tests (Hedge et.al 2010).

**1.Saponins test:**

This test is carried out by placing 10 ml of aquous extract of each of two types in a test tube with 5 ml of water. The tubes were closed and the samples are shaked constaly. The formation of a foam layer, that lasts at least 30 minutes, indicating that saponins may be present. The results were tabulated and compared for each extract type between the various plant sections.

**2.Alkaloids test:**

The 3ml of the extract is taken in clean glass test tubes and were added to the mix, along with 1 mL HCl, gently heated for 20 minutes, cooled, and subjected to following tests.

a) **The wagner test:** Wagner's reagent was used to treat 1 ml of the extract, and the construction of a brownish-reddish precipitate indicates the presence of alkaloids.

b) **Test dragen droff's test:** In 1 ml of extract, 2 drops of the Dragen droff's reagent were added. Alkaloids were fonfirmed by the creamy ppt production.

c) **The hager's test:** The Hager's reagent was used to treat 1 ml of extract and alkaloids were confirmed by instant yellow ring in test tube.

**3. Steroid test:**

1 ml extract was subjected in 10ml chloroform, and the same quantity of resolute H2SO4 acid was added from the tube side. A red layer at top and yellow or green by H2SO4 was confirmation for steroids.

**4.Tannin test:** 4ml extract has been preserved with 4ml FeCl3 green color development shows condensed tannin attendance.

**5.Test of anthocyanin:** To Adding 2ml of aqueous extract to 2ml of 2N, HCl & $NH_3$; the color change by pink, red or violet sighns for anthocyanin.

**6.Test of coumarin:** 3ml of 10% NaOH was added to 2ml of extract formation of yellow color predicts for coumarins.

**7.Test proteins and: Xanthoprotein test:** Extract has been treated with few droplets of $HNO_3$ production of intense yellow color indicates for protein.

**8.Test amino acids:** Test of Ninhydrin: 2ml of ninhydrin mixture to extract was added and boiled for a few minutes, blue color formation shows the presence of amino acid.

**9.Flavonoid test:**

**Alkaline reagent test:** 10 percent NaOH abstract solution was treated with intensive yellow color production indicating Flavonoid presence.

a. **NH4OH test:** 3ml of extract with 10% NH4OH solution by development of yellow fluorescence indicates positive for flavonoids.

b. **Mg turning test:** Extracts have been treated, HCl with 5 ml of 95 % ethanol a scarlet of red color indicates for flavonoids.

c. **Test Zn:** 2ml of Zn dust and conc. extract have been mixed with some HCl, red color development suggests for flavonoid content.

**10. Diterpenes test:**

Extracts were dissolved in water and treated with 10 drops of copper acetate solution for a copper acetate test. Diterpenes are present when a bright green color forms.

**11. Salkowski's test:**

The chloroform extract of plants has been treated and filtered for the Salkowski test. The filtrate was simply treated with a few drops of concentrated H2SO4 and shaken, the golden-red color is suitable for the test.

**12.Test of phenol:** Ferric Chloride Test: The extract was treated with 4 drops of alcoholic FeCl3 solution. The presence of phenol is indicated by a bluish-black color expansion.

**13. Carbohydrate test:** Extracts were individually dissolved in 5ml of distilled water. and the filtrate was utilized for followindg tests.

a. **Molish test**: Filtrate was treated with 2 drops of alcoholic naphthalene solution; violet color at junction confirms carbohydrate content.

b. **Barford's test:** 1ml of Barfoed's reagent was added in a test tube and maintained in boiling water bath, brick red ppt at the bottom, indicates for the carbohydrate.

c. **Test Iodine:** 2ml extract was treated with 5 drops of Iodine solution, blue color indicates for positive test.

d. **Missing test:** 2ml extract has been diluted with HCl hydrolyzed, alkaline neutralized and heated with the Fehling solution, the red ppt development indicates existence of reducing sugars.

e. **Test for Benedict:** The extract has been treated with filtrate (multi-functional, copper sulfate, sodium citrate and sodium carbonate in combination) orange-red ppt shows the presence of reducing sugars.

**14.Phenolic substances test:** The extract was added with 3ml, 10% lead acetate solution. The presence of thick white precipitate indicates for phenols.

**15.Carboxylic acid test:** Sodium carbonate hydrogen test. Carboxylic acid interacts with sodium carbonate and hydrogen, which may be observed in the form of a carbohydrate effervescence.

**16.Test of acetate anhydride:** The plant extract was treated with a few drops of acetic anhydride solution, followed by an ml of conc. HCl black color indicates for positivity.

### 3.4 Nanoparticles synthesis

Zinc sulfate ($ZnSO_4$) and gold chloride ($AuCl_4.2H_2O$) were bought from Johnson Matthey Plc. The UK along with other required chemicals such as NaCl, HCl (used for stabilizing pH and particle size management). For nanoparticles production, various quantities of freshly produced extract were treated with varying concentrations of salt solution. The nanoparticles formation was indicated by the gradual color change and were confirmed by a UV spectrophotometer. Further the produced nanoparticles were optimized by different factors like time, pH, temperature, and different concentrations. In the instance of the gold solution, it was avoided to light exposure for its specific light responses, so, the initial suspension of the AuNP was kept in dark and were remained covered with aluminum foil. The color of the solutions, owing to the gold and zinc ions of the precursor, changed from yellow to dark brown for zinc and ruby red or cherry red for gold, which is a feature of successful gold and zinc nanoparticle production.

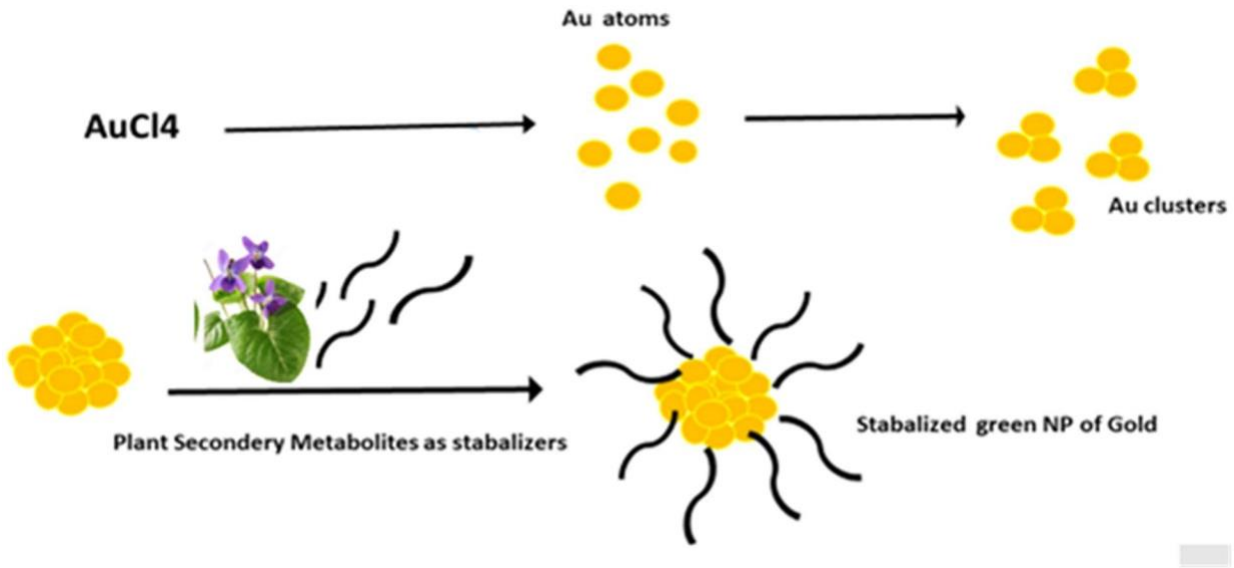



Figure: Showing green synthesis of *Viola* gold nanoparticles formation.

The following formula was used for the molar solution preparation of metal salts,

$$\text{Salt quantity g/litre} = \frac{\text{Mol required} \times \text{Molecular wt. of salt calculated} \times \text{Volume required}}{1000 \times 1000 \text{(per liter)}}$$

### 3.5 Characterization

The produced zinc and gold nanoparticles of all four types were subjected to physical analysis by several methods such as UV (ultra violet vis-spectrophotometry), XRD (X- ray diffraction), FTIR (fourier transform infra-red), SEM (scanning electron microscope). Which has confirmed the particle size, shape and fuctional groups of secondary metabolites from plant extracts involved in capping of metallic salts.

#### A. UV-visible spectrophotometry

All samples comprising plant extracts and nanoparticles samples were diluted 2X with deionized water (DI) for UV-vision spectrophotometric analysis. Each sample has been examined in surface plasmon resonances using a UV-visible spectrophotometer (T80+T70/T80 series v spectrometer), which assesses light absorption at a typical sample speed of 400–1000 nm at wavelengths. In each absorbent band, the highest peak was considered to represent the surface plasm resonance of all samples. For UV spectroscopy Inc, the basic line was fixed at 400-700nm for gold and its nanoparticles. For band analysis, the data is recovered and stored.

#### B. FTIR (Fourier transform infrared)

The Perkin-Elmer 1425X FTIR spectrometer using the KBr pellet method for the identification of surface functional groups was conducted in the 400-4000cm-1 spectral range units. Moreover, the samples were well dried for proper analysis, and 5 mg of every sample were collected and properly mixed with 245 mg potassium bromide (KBr) and treated accordingly for pellet formation used

for further analysis. The data was coded for peak creation and utilized for functional group identification based on the following table peaks.

Table 3 1. Showing functional groups identification for band intensity in FTIR analysis.

Fluctuation Group wave numbers

| **Fluctuation Group wave numbers** | | |
|---|---|---|
| **Bound** | **Wave#** | **Intensity** |
| Alcohol (O - H) | (3650-3200.) | Strong, bound. |
| Alkane (C - H) | (3300-2700. ) | Medium. |
| Amine (N - H) | (3500-3300. ) | MEDD, BOUIAM. |
| Nitrile (C = N) | (2260-2220. ) | Medium, Bound. |
| Alkyne (C =C) | (2260-2100. ) | Week, Mediam. |
| Alkene (C = C) | (1680-1600. ) | Medium |
| Amine (C = N) | (1650-1550. ) | Medium. |
| Ketone (C = Q) | (1780-1650. ) | Strong. |
| Acyl (C - O) | (1250-1050. ) | Strong. |

**C. XRD (x-ray Diffraction)**

This analysis was performed at Physiscs department of Allama Iqbal Open University Islamabad Pakistan. A small amount of sample colloidal solution was evaporated by the X-ray diffraction, samples were washed, dried, and a fine film of sample was left on a glass slide subjected to XRD analysis. The raw data were transformed into excel sheets using Powell convertin software and graphs were drawn and compared appropriately.

**D. SEM (scanning electron microscope)**

SEM imaging for size, shape and morphology of all samples were performed at NUST (National University of Science and Technology) Department of chemistry school of natural studies Islamabad Pakistan, Model No. JEM 3145LV, JOEL, Germany and at by SEM (Hitachi S-4800, 30 kv) at department of engineering IIUM. All the samples were produced by dissolving 2mg samples in 100µl of tetrahydrofuran thoroughly blended with vortex for 100 minutes and then sounded at room temperature for 30 minutes following a tiny piece of glass slatting on the rotating

**3.6 Leishmanial culture maintenance**

For the bioactivity analysis (antileishminial and anticancer activities) of synthesized nanoparticles, *lashminia Tropica (KWH23)* were collected from available sources. In the M199 medium (pH 7.2), *L. tropica KWH23* was incubated at 23Co with sodium bicarbonate, 10% fetal bovine (FBS), and 25mM HEPES (4-(2)-1-piper-azo-neethanesulfonic acid). For all studies, 1X107 cells/mL were concentrated in cells. The 22oC-25oC culture was maintained for promastigote development and proliferation. *Lashminia* was cultivated following the aforementioned method. During the experiment, a serial dilution technique was employed.

**3.7 Anti-promastigote assay:**

Biogenically produced *Voila* nanoparticles stock solution was made by suspending nanoparticles 1mg/1ml dist.$H_2O$. The nanoparticles were mixed for 1-2 minutes by vortex and the solution was

sonicated for 10 minutes for homogeniousity of aggregates. The M199 medium containing crop promastigotes was suspended to produce a 96 well platform of 1106 cells/ml each. Subsequently, drug nanoparticles were put into each well. In all studies, the final volume was 1000 l with M199 medium for each 96-well microplates. Cells were exposed to LED light at 25Co for 10 minutes and then incubated in dark at 25Co -27Co overnight. The leishmanial colonies in control were not exposed to nanoparticle solution. The vitality of Prastigotes was evaluated under the optical microscope using the Neuba Chamber (MARIENFELD, Germany) after 24, 48, and 72 hours of incubation in (micros, AUSTRIA, MC700) incubator. All responses were repeated three times and in between mid-November and the end of December. The growth of *Leishmania T.* is favoured when the external temperature is amongst 23 and 26 degrees Celsius.

**3.8 Cytotoxicity studies**

The hemolysis test was performed to evaluate the cytotoxicity of nanoparticles for human blood. Blood samples from healthy individuals (groups O, A+, and B+) had drawn using technical methods. For erythrocytes separate the the blood was centrifuged for 3 minutes at 3000 rpm and rinsed 2-3 times with phosphate buffer. Phosphate buffer solution was used to dilute 10:90. The erythrocyte solution is a mixture of erythrocytes and a (PBS). Each eppendorf has received 100 liters of water and has been treated with all sample solutions. Negative controlI was maintained by the phosphate buffer and red blood cells suspended without nanoparticles while positive control was with red blood cells lysed in 0.1 percent triton X-100.The reaction was conducted 10 minutes in the LED photo-meter and samples were incubated for 3hours in dark at 37Co. Later on samples were centrifuged to separate the hemoglobin at 6000rpm for 10 minutes. The absorption of hemoglobin by UV-visible spectrophotometry was finally determined at 576nm. All the tests were conducted in three replicates. Following formula was used to determine the percentage of hemolysis.

$$\%Hemolysis = \frac{(OD\ at\ 576nm\ inthe\ nanoparticle\ solution - OD\ at\ 576nm\ inPBS)}{(OD\ at\ 576nm\ in\ 0.1\%\ Triton\ X - 100 - OD\ at\ 576nm\ in\ PBS)} \times 100$$

All the values were obtained as the mean of three replicates and the results were shown by mean standard deviation (SD). For assessing significant differences, the student t-test was used using SPSSS software, and P-values of 0.05 were considered statistically significant.

**3.9 Anticancer assay**

***a) Chemicals and reagents***

All the chemical, cell culture media (dulbecco eagle medium), DMEM (high glucose and Lglutamine) in powder form, FBS(Fetal Bovine Serum) and PBS (phosphate-buffered saline Sulforhodamine) B powder was obtained from Sigma-Aldrich Chemical USA, and Gibco ® manufacturers Germany. And others like 70% ethyl alcohol, ethanol, taxol (Paclitaxel), the commercial medicine came from Chemolab Supplies, Malaysia.

***b) Cells lines***

MCF-7 breast cancer cell (ATCC® HTB-22TM) was derived from US Type Culture Collection and African Green Monkey VERO cell (ATCC® CCL-81TM) (ATCC).

***c) Preparation of the sample***

During experimentation freshly produced green nanoparticles were utilized. In the dimethylsulfoxide (DMSO) 0.005 mg of each kind of nanoparticle was dissolved in 1 ml of 100% dimethyl sulfoxide and kept at a -20 °C freezer for future usage. Each sample of four nanoparticles were subjected for cytotoxic/anticancer testing. For each test run, three separate experiments were performed and all the procedures were followed as by (Abbas et al., 2018).

***d) Assay of cell attachment (CAA)***

The previously stored cells in liquid nitrogen were maintained and re-grown into three additional subcultures, P1, P2, P3, and P4, the experimental MCF-7 (cell lines of breast cancer) were stored for experimentaion. The cell attachment test was conducted using Abbas et al. 2018 techniques. Nanoparticle samples were added to the culture medium with cell concentration of 1 x 105 cell/ml in a T-25 cm2 culture bottle while adjusting flask contents. The control treatment was given a 10% (v/v) dose of DMSO with cell lines. The cultures were incubated in PBS, 37 °C and in 5%CO2, after 24 hours of incubation the cells were rinsed with phosphate-buffered saline. Accurate trypsinization was used to trypsinize cell cultures, which were then counted using the trypan blue dye removal method (Kaewroek, K., 2009).

***e) Assay of cell viability (CVA)***

The cell attachment test was performed according to the protocol. Healthy culture cells were maintained in fresh T-25 $cm_2$ tissue culture bottles with 5 ml of culture media by a concentration of 1 x 105 cells/ml. These flasks were afterward incubated for 24 hours at 37°C/5% $CO_2$. The consumed media were discarded and each flask was introduced to new media that contained samples at modified concentrations. 10 percent (v/v) DMSO was added to the control flask. During 24 hours, cells were exposed to final incubation at 37°C/5%$CO_2$. Finally the cells were rinsed with phosphate-buffered saline (PBP), trypsinized, followed by a cell counting process using the trypan blue dye exclusion method (Abbas, et al, 2018). f) Assay for sulforhodamine B (SRB)

The cytotoxicity test was carried out as stated (Abbas, et al, 2018). In 10 percent (v/v) DMSO, all samples were dissolved. Plates 1 x 105 cells/ml in 190 l of cultivated medium were incubated for 24 hours at a temperature of 37°C/5 percent of $CO_2$. After that, 10 l of every sample was put to each well with dilution and incubated for 72 hours. Cold trichloroacetic acid (TCA) was mixed with SRB solution and washed with acetic acid before the staining process. To each well containing the protein-bound dye for solubilization, a 10mM tris base solution was also added. Plates were then put in a rotary shaker for 10 minutes and readings of the optical density (OD) at 510 nm were taken.

$$Controlled\ cell\ growth\ \ \% \ = \frac{(\text{Mean } ODsample - \text{Mean } ODblank)}{(\text{Mean } OD)\ negative\ control - \text{Mean } ODblank) \times 100}$$

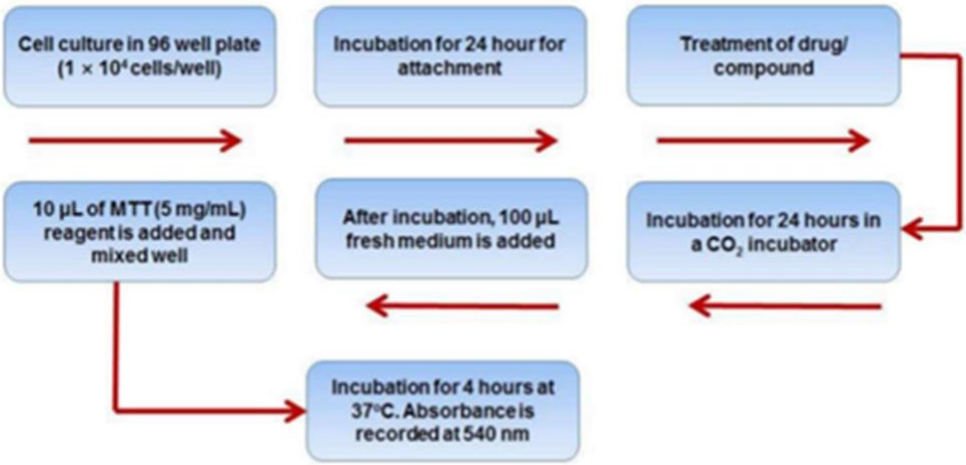



Figure: 3 4: Showing schematic presentation of MTS assay for both types of cells used in the anticancer activity of synthesized nanoparticles (Ashutosh, B. et al., 2017).

***g) Statistical analysis***

The results have been shown as (SEM) standard mean errors. In anti-cancer and antileishminial activity IC50 (the 50 percent inhibitory concentration) was calculated utilizing a linear regression equation. Comparisons and p-value calculations were performed using the single-way ANOVA by using statistical package statistics 8.1and by SPSS. The mean values were compared and assigned different letters by using the least significance test (LSD).

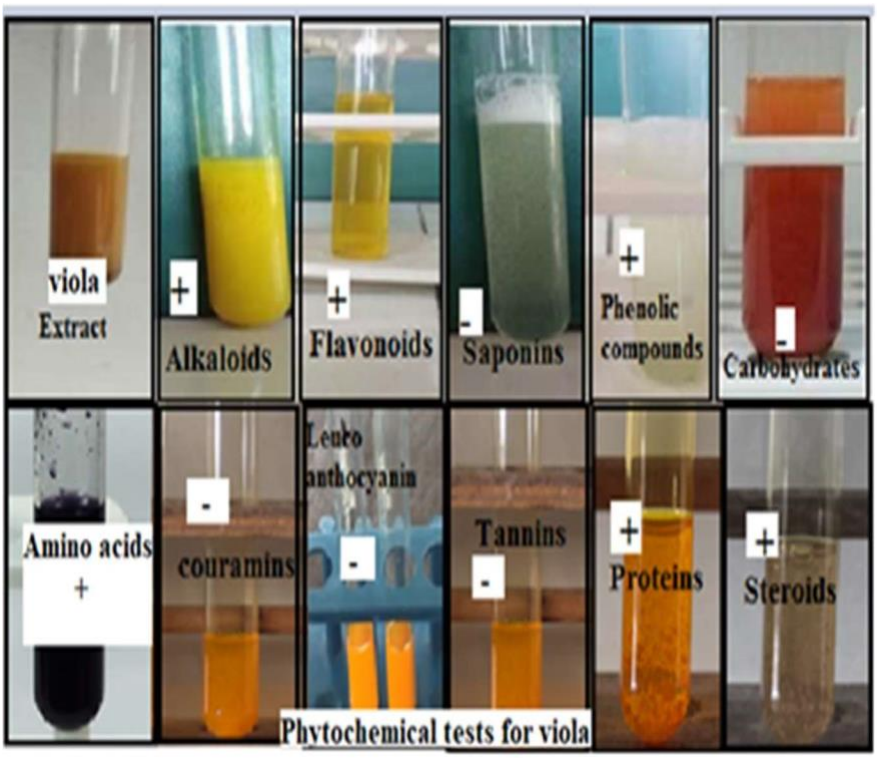
## 4. Result and Discussion

Green nanoparticle applications have demonstrated promising results in the area of medicine. In any event, owing to a paucity of data on the physical properties of AuNPs, only a few gold green nanoparticles have gone through clinical studies and been proven to have useful uses. This takes into account the following integration of their in vitro investigations of these nanoparticles, their absorption by living systems, whether plants or animals, as well as their exams as a necessary evaluation.

The current research shows *Viola odorata* aqueous extracts one-step production of green nanoparticles from gold metallic salts. The herb utilised in this research have been used to treat fevers, stomachaches, and cancer and have stong bioactivities against vast range of pathogens in the past (Kelly K.2009). Its anticancer, antioxidant, and antibacterial effects have also been widely documented. These plants include an infinite amount of phytochemicals with various medicinal actions (Asheesh et al. 2017), which may serve as a reducing agent for decreasing precursor Au ions. The antileishminial, anticancer cytotoxic cell studies and haemolysis of these new nanoparticles were examined after they were synthesised.

### 4.1 Plant phytochemical analysis

The plant extracts were subjected to different phytochemical tests which have confirmed many secondary metabolites in these plants the list of which is mentioned below.



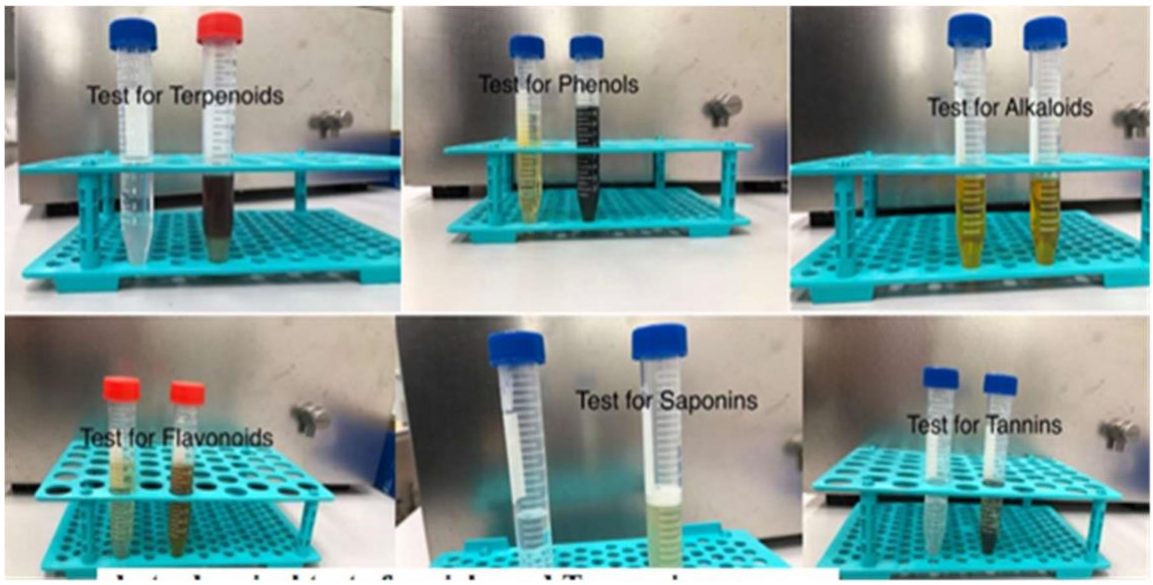




Figure: 4.1 Showing phytochemical test results for *Viola Odorata.*

Table 4.2: Showing phytochemical tests for *Viola odorata.*

| | Basic plant contents% | Minimum | Maximum | (Mean) ± Std. Deviaton |
|---|---|---|---|---|
| Proximatevalue | Moisture % | 5.150 | 5.20 | 5.183± 0.035 |
| | Protein % | 15.75 | 3.31 | 7.45± 8.8 |
| | Fat % | 10.53 | 12.0 | 11.51± 1.04 |
| | Ash % | 9.78 | 9.80 | 9.79±0.014 |
| | Crude fiber % | 38.97 | 39.02 | 39.00± 0.03 |
| | Carbohydrate % | 21.42 | 22.0 | 21.80± 0.41 |
| | Metabolization.energy (Kcal.Kg) | 2898.5 | 2901.7 | 2900.6±2.26 |
| Mine | Fe** (mg/100 g) | 1.19 | 1.186 | 1.87± 0.002 |
| | Zn** (mg/100 g) | 1.23 | 1.4 | 1.34 ± 0.12 |
| | Mg** (mg/100 g) | 0.2 | 0.43 | 0.35± 0.16 |
| | Ca** (mg/100 g) | 194.23 | 194.91 | 194.68± 0.48 |
| | K** (mg/100 g) | 98.10 | 98.75 | 98.53± 0.45 |
| | Na** (mg/100 g) | 10.46 | 10.57 | 10.53± 0.07 |
| | Pb (mg/100 g) | 0.50 | 0.50 | 0.5± 0 |
| | Cu (mg/100 g) | 46.89 | 47.25 | 47.13± 0.25 |
| | Cr (mg/100 g) | 33.95 | 34.50 | 34.31± 0.38 |
| | Mn (mg/100 g) | 24.87 | 25.0 | 24.95± 0.09 |
| | Ni (mg/100 g) | 1.48 | 1.45 | 1.46± 0.02 |
| Vitamins | Ascorbic acid (mg/100 g) | 150.1 | 152.4 | 151.63± 1.62 |
| | Couramines (mg/100 g) | 40.0 | 41.20 | 40.8± 0.84 |
| | Salicylates (mg/100 g) | 91.83 | 92.21 | 92.083± 0.26 |
| | Flavoniods (mg/100 g) | 201.0 | 230.678 | 220.78± 20.98 |
| Phytochemicals | Alkaloids (mg/100 g) | 2.55 | 3.76 | 3.36± 0.85 |
| | Tocopherols (mg/100 g) | 30.0 | 29.89 | 29.3± 0.07 |
| | Tannins (mg/100 g) | 2.4 | 4.5 | 3.8± 1.48 |
| | Saponins (mg/100 g) | 0.65 | 1.13 | 0.97± 0.33 |
| | Esters(mg/100 g) | 23 | 21 | 21.67± 1.42 |
| | Cyanogenic glycosides mg/100 g) | 57 | 57.67 | 57.44± 0.47 |
| | Phenols | 29.9 | 30.4 | 30.23± 0.35 |

Table 4 3: Showing phytochemical tests for *Viola odorata.*

| S.No. | Phytochemical | Test | V.Ext. |
|---|---|---|---|
| 1. | Alkaloids | Dragondroff's testWagner,s reagent | + |
| 2. | Saponins | Foam test | - |
| 3. | Tanins | Ferric chloride test | - |
| 4. | Terpenoids | Copper acetate test | - |
| 5. | Amino acids | Ninhydrin | + |
| 6. | Reducing sugars | Benedict test | + |
| 7. | Card. Glycosides | Legal's test | - |
| 8. | Flavonoids | HCl-Mg reaction | + |
| 9. | Carbohydrates | Molisch'stest,Benedict test Barfored's test | _ |
| 10. | Steroids | Salkovaski method | + |
| 11. | Proteins | Xanthoproteic test | + |
| 12. | Carboxalic acid | Sodium hydrogen carbonate test | + |
| 13. | Resins | Acetic anhydride test | + |
| 14. | Coumarins | NaOH test | - |
| 15. | Leucoanthocyanin | Isoamyl alcohol test | - |
| 16. | Phenolic compounds | Lead acetate test | + |
| 17. | Anthocyanins | HCl & NH3 test | - |

Note: + = Present and - = Absent

Thus the phytochemical tests preliminary showed the presence and percentage of various secondary metabolites found in plant tand provides an evidence for protective and disease defensive nature of the plant.

The identification of various elements such as carbon, magnesium, sodium, oxygen, calcium, chloride, aluminium, silicon, and iron has been shown in phytochemical investigations for *Viola odorata* and prior literature. *Viola odorata* entire plant powder, crude methanolic extract, and later solvent fraction have all been reported for different macro and micro nutrients (lead, cadmium, copper, chromium, zinc, nickel, magnesium, calcium, sodium and potassium) (Bibi., et al., 2006). The different phytochemical tests performed for *Viola* extract proved many secondary metabolites like, it contained alkaloid, amino acids, flavonoids, steroids, proteins, carboxalic acid, and phenols

while it gave negative tests for anthocyanins, carbohydrates, terpenoids and saponins (Table. 4-2). So, it stood proved that the complete plant of *Viola odorata* is a good source of various nutrients and secondary metabolites these results are also in line to that of (Muhammad et al., 2012), (Mittal P. et al., 2015).

The extracts of *Viola odorat* reported that the main plant extract /100g had maximum amount of crude fiber as 39.00± 0.03 mg/100g while in case of phytochemicals a large number of flavonoids 220.78±20. 98 mg/100g were counted along with phenols 30.23 ± 0.35mg/100g (Table 4-2, 4-3). The study results are more or less in range to that of (Bibi et al., 2006), (Orhan I. E. et al., 2015).

## 4.2 Characterization and properties of *Viola* green nanoparticles

### A. *UV Spectrophotometer*

The green nanoparticles synthesiszed were confirmed by the color of the *Viola* extract transformed in case of GNPs the colour of suspensions changed gradually from yellow to pinkish yellow and stood at dark purple. After effective synthesis of nanoparticles, the neo particles were optimized for best values of pH, concentation and time period. And the tests were completely checked through top affirmation, *Viola odorata* extract has shown best absorbance at 541nm which was afterward affirmed by literature (cytodiagnostics inc.introduction to gold nanoparticle characterization).

### B. *Nanoparticles optimization*

The best reaction was observed at different concentration, pH 7, for 24 hours at 80°C, and the best reaction for enemy GNPs was observed at 3mM at pH 8-10, which gave UV absorbance peaks at 375nm for *Viola* green nanoparticles, respectively, in comparison to their crude extracts noted at 420nm, which was in line with previous literary works and affirms previous findings (Das R. K., 2012). UV peak settings were made at 200 to 700 nm using a UV-Vis spectrophotometer, Shimadzu, UV 1601at IIUI (CIRBS), which revealed a reduction in NPs mixtures compared to their crude extracts and pure salt solutions, such results are also noted by (Foo YY, et al., 2017). Within 20-25 minutes of mixing 10 ml of aqueous extract of plant, with 90 ml of 1 mM HAuCl4 solution (in the case of *Viola*) responded to the change in colour from brown and yellow to purple violet due to reduction of Au3+ ions. comparative color changes from yellow to dull purple violet is becase of the excitation of AuNPs and surface plasmon resonance of gold nanoparticles synthesized by plant extracts.

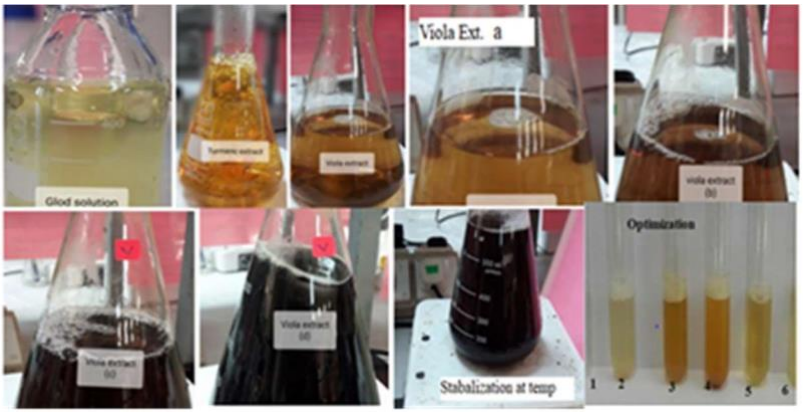



Figure 4.2: Showing color change of extract for nanoparticle synthesis

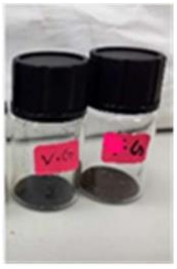


Figure: 4.3 Showing the nanoparticles synthesized.

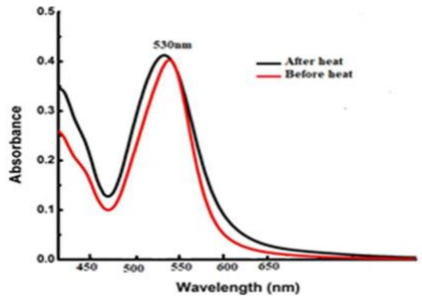



Figure 4.4: Showing V.GNPs heat treatments.

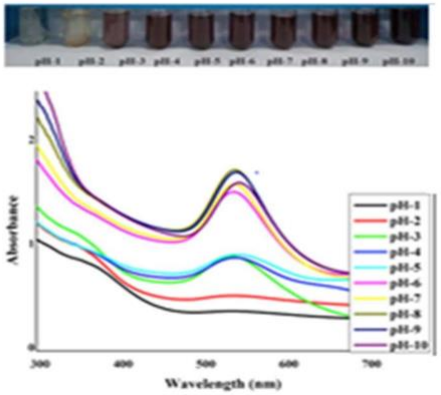



Figure 4.5: Showing results for different pH for VGNps .

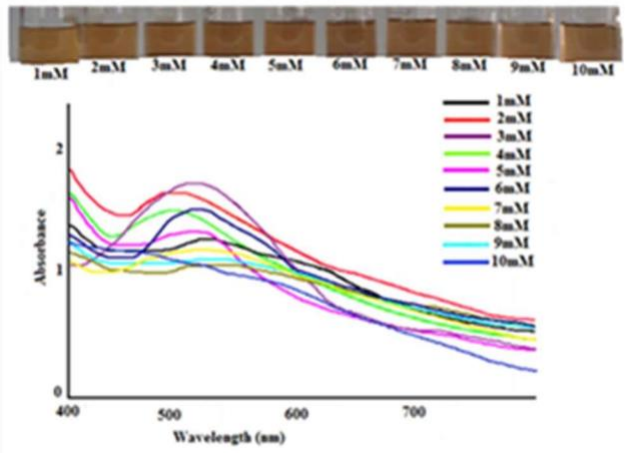



Figure 4.6: Showing results for different concentrations for VGNPs.

***C.FTIR***

FTIR as being a reliable method for characterization and identification of compounds. Which supports molecular vibrations to induce a change in the dipole moment of the molecules to produce IR bands. The technique arose from the study of energy quantum mechanics. The differences in absorbance are attributable to differences in the spectral profiles of the samples. *Viola* extract CO stretching vibrations of carboxylic acid are shown by small bands at 1200-1300 cm-1. Polyols (phenolic acid and flavonoids), terpenoids, and protein components are plentiful in viola extract, as shown by the bands 1550 and 1650cm-1. While obvious enhanced bands for C-N stretching vibrations of aromatic rings and strong bonding for amines can be seen in nanoparticles at 1550 and 1650cm-1. It also displays a distinct C-O band, indicating a strong acyl group. For the confirmation of alcohol and alkanes in nanoparticles while they are missing in extract solution, stretch between 3000-3001 stretching vibrations of aromatic rings and strong bond for hydroxyl groups O-H and C-H. V.GNPs shows strongly vibration extending at 3422 cm−1(O- H extending Liquor), 2940 cm−1(C- H stretching Alkane), 2845 cm−1(C- H extending Aldehyde), 1651 cm−1(N-H band Amine), 1393 cm−1(O H twisting Phenol), 1237 cm−1(C-Nstretching Fragrant Amine) and 1048 cm−1(C-O extending Alky A conceivable instrument o\ Inside 20 min of expansion of 10 mL fluid *Viola odorata* leaf extract to 90 mL of 1 mM $HAuCl_4$ solution, the response mixture exhibits and alter in color from yellow to purple violet due to decrease of Au3+. Writing reports comparative color changesfrom yellow to dim purple violet due to excitation of AuNPs surfaceplasmon. Fourier Change Infrared Spectroscopy (FT-Alky A conceivable instrument of gold nanoparticle arrangement likely included the carbonyl group/ionic amines together with a have of other utilitarian bunches provided by bioactive specialists, at first authoritative to gold particles (Au3+) to create complexes. Decrease happened to surrender seed particles with oxidation states of zero (Au0). Upon accumulation of a few of these seed particles, nucleation locales were shaped. Gold metal particles inevitably built up around these seeds in a prepare known as auxiliary development (B. Kumar et al., 2016). All of these distinct vibrational groups point to Au NP once again. The above-mentioned groupings have previously been seen ponders (You et al., 2012).

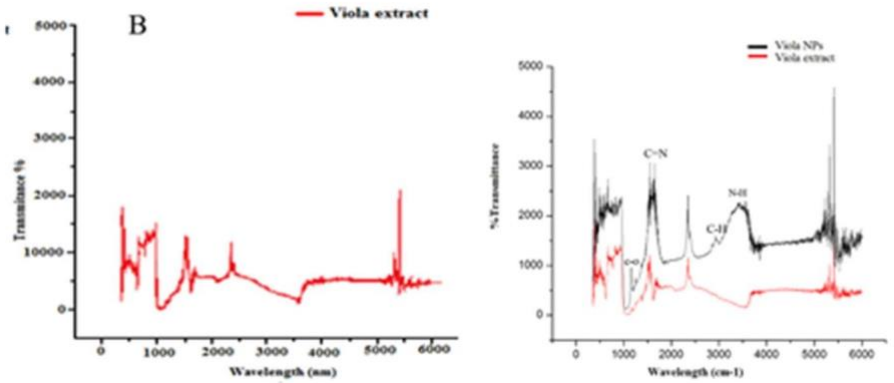



Figure: 4.7: FTIR of *Viola* extracts and its nanoparticles.

### *E. X- Ray Diffraction (XRD)*

Powder X-ray diffraction is used to reveal the crystalline size and auxiliary characteristics of zinc nanoparticles. Cu Ka radiation (k = 0.1540 nm) was used in the XRD, with 2Theta values ranging from 200 to 800. The XRD design of bio-synthesized gold nanoparticles from *Viola* The same results have been recorded by (Arockiya F. et al., 2014). Both objects are crystalline in nature, as shown by the angle and constricted diffraction peaks. The particle's crystalline size (D) was calculated using the Scherrer equation and XRD line broadening estimate.

D = 0.89/(Cos)

Where the wave is

= The NPs line's total width half-most extreme (FWHM) or a specific diffraction at top and bottom.

= The angle of diffraction

Others have also reported the results of the same line (Ampritpal Singh et.al, 2012). The images clearly demonstrate the close proximity of auxiliary metabolites capping that are ascribed to bio.organic substances exhibited inside the plant extracts, as shown by the strong reflections in both tests' XRD spectrums. V.GNPs were synthesised with high-intensity crests at 38°, 44°, and 64°, respectively, corresponding to (111), (200), and (211) which can be seen in (Figure). Bragg reflections, which were the exact top locations as provided for facing the centre cubic cross section structure of gold. The peaks of AuCl4 were decreasing with 32°, 38°, 46°, and 57° since the pH of the solution rises, the breadth of the XRD data, indicating an increase in the size of produced AuNPs, which is consistent with the assimilation spectra of AuNPs at various pH. These results show similarity to that of (Alti D. et al., 2020). The average particle size determined by XRD design at Debye-Scherrer conditions was about 50 nm for V. GNPs synthesised using AuCl4 and 100 nm for T. NPs produced properly. The XRD examination may not be completed fast or concurrently with the mixture of extracts. As a result, the size of AuNPs estimated from XRD spectra is greater than the period of synthesis. These findings are consistent with (Simon, T., et al., 2018).

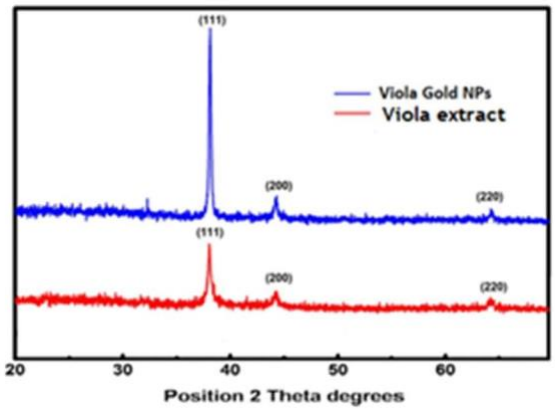



Figure 4.8: XRD results for VGNPs.

### *F. SEM Results*

Scanning electron microscopy (SEM) Demonstrate No. JEM 3145 LV, JOEL, Germany was used to examine the morphology (shape, estimate, and microstructure) of the green produced NPs. The

SEM images of synthesised VGNPs reveal a variety of normal forms, such as round, Furthermore, it seems that known circular and hexagonal morphology, as well as irregular and non-uniform circular and hexagonal (wurtzite) forms, are compatible with nanoparticle XRD design. Nanoparticles have a hexagonal form with r= 30 nm and a few with r= 25 nm, The Figure: seems to be a lump-like agglomeration to some extent because to the high concentration in testing. However, the surface immobilisation of biomolecules may not be seen in the images. The SEM image revealed that V. GNPs have a round to triangular form with r=25nm, implying a particle size of 50nm normal estimate, and the round shaped particles are examined in SEM image creating clusters or free as shown in Figure. For Au nanoparticles, the size is predicted to be about 100nm. (Tahvilian et al., 2019).

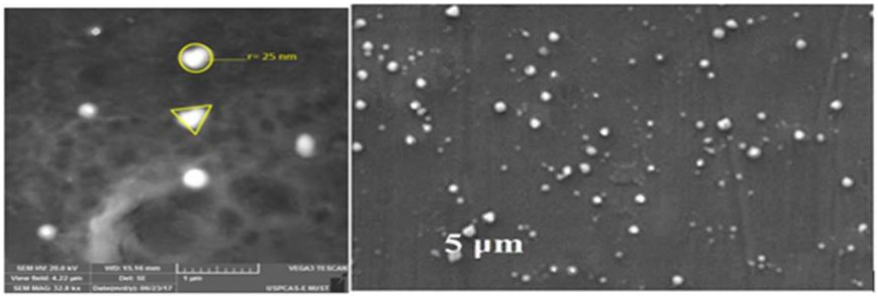



Figure 4.9: Showing SEM images of V.GNPs.

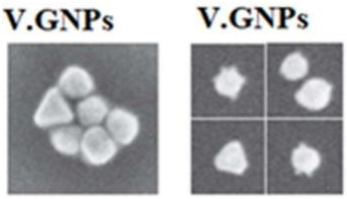



Figure 4.10: Showing shapes of VGNPs at low resolution of SEM

**4.3 Bioactivity analysis**

***a) Anticancer Activity***

Green synthesized Viola nanoparticle at different concentration were tested against 10Ug/ml, 5Ug/ml, 2.5Ug/ml, 1.25Ug/ml, 0.625Ug/ml, 0.15625Ug/ml,0.078125Ug/ml (MCF-7 breast adenocarcinoma cells and vero cell lines.

In case of MCF-7 breast adenocarcinoma cell activity has increased in all concentrations of gold nanoparticle with Viola extracts (Ext-V) which were observed as 92.64.98-111.41%, 83.65111.40% respectively. However, in ZnNP-T have shown very less with 64.70-68.45% activity in comparision to other treatments. For AuCl4 the activity was stimulated an was in the range of 80.34-90.34% at all concentrationsas shown in figure. Similar results were reported by Rajeshkumar, (2016). The anticancer activities of the gold nanoparticles were performed with different concentrations against HepG2 and A549 cell and increased cell death was obseved. In future this synthesis approach, is novel in biomedical applications. Gold nanoparticles (AuNPs) with *Marsdenia tenacissima* plant extracts as the most excellent anticancer theraptic. In vitro anticancer activity against lung cancer cell lines (A549). MTT assay revealed that AuNPs produce toxicity based on the dose-dependent A549 and cause cells growth inhibition by AuNPs treatment

activates. It has been well documented that the AuNPs from *M. tenacissima* extract are apposite stabilizing agents, which serve as an effective anticancer agent against lung cancer cell lines (A549) (Sun et al., 2019).

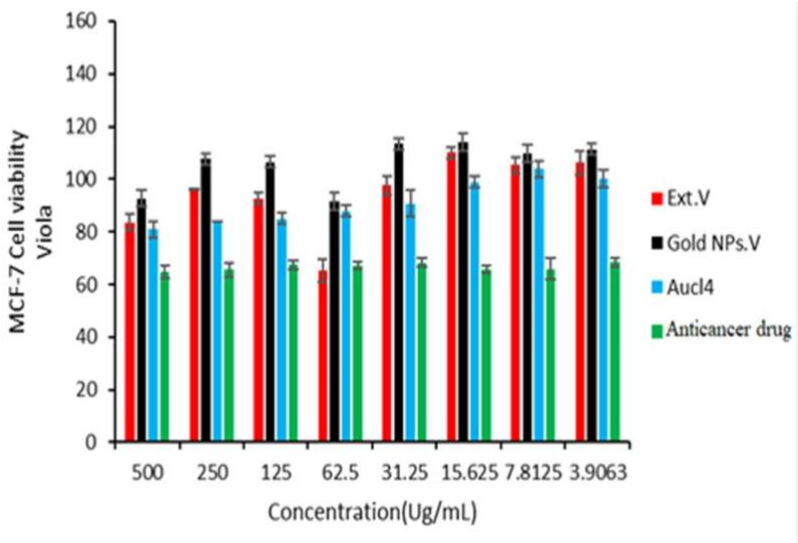



Figure: 4.11: Anticancer activity of Viola (MCF-7 viable cell) at concentrations at 5003.9063Ug/ml.

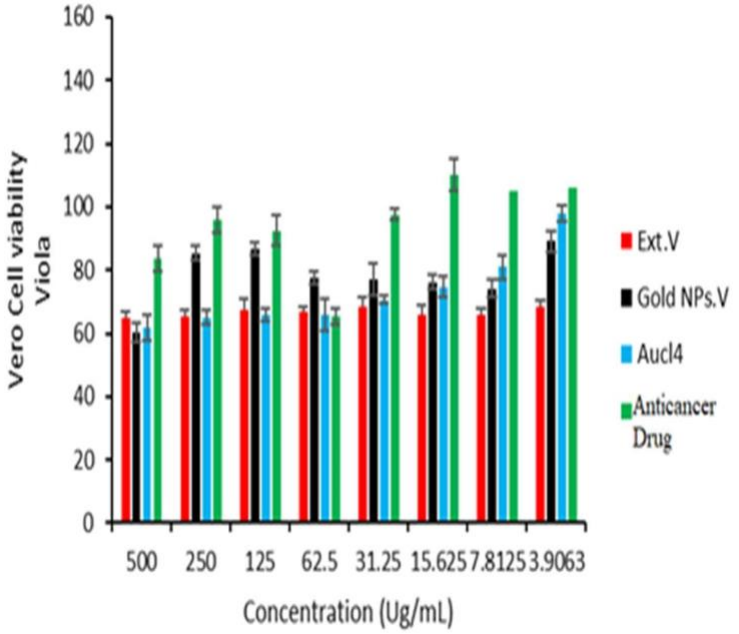



Figure: 4.12: Anticancer activity of *Viola* (Vero viable cell ) at concentrations at 5003.9063Ug/ml.

Treatments name (Ext-v=*Viola* extract, VGNP= Gold nanoparticles with *Viloa* extract, $Aucl_4$= Tetrachloroaurat). The vertical bars indicate the standard error, and the values are the averages of three replicates. At P0.05, all treatments are substantially different from one another.

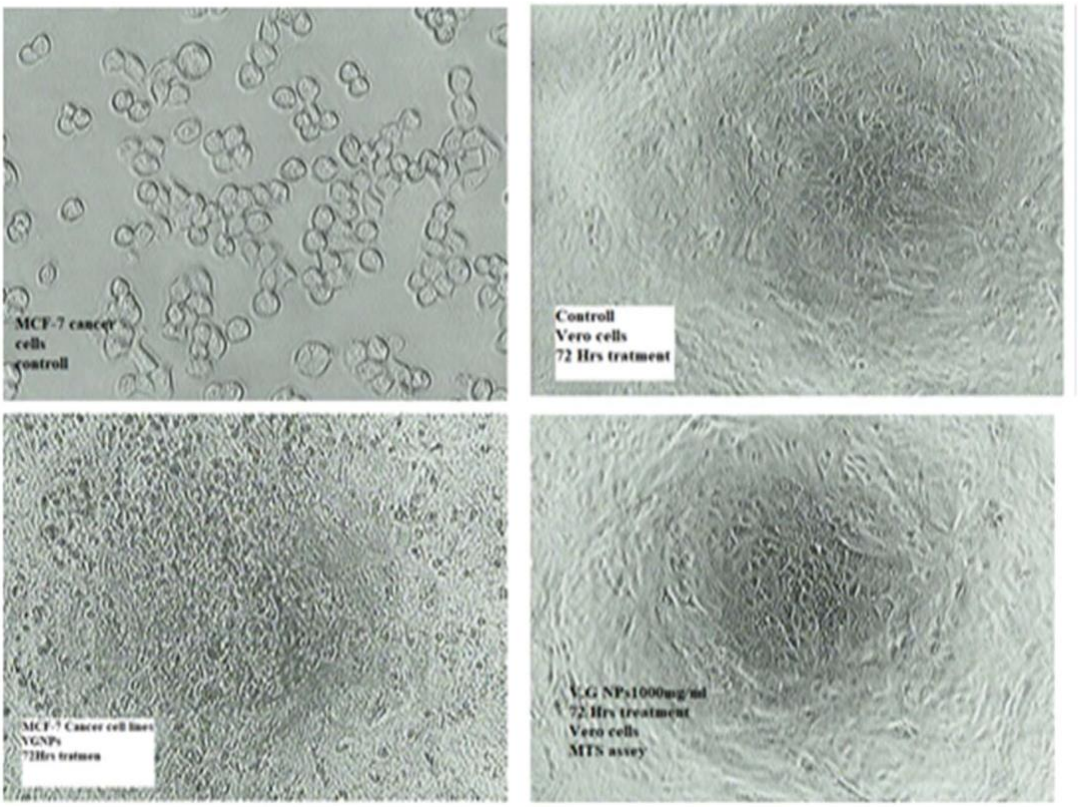



Figure 4.13: Anticancer activities of different VGNPs for MCF-7 breast cancer and Vero cell lines.

***b) Haemolysis and cytotoxicity analysis***

Cytotoxicolgical study of nanoparticles have opened new avenue for nanotechnological applications in medicine. It is important in two ways wether they kill pathogens and if applied on human cells how much they are safe and promising for a good health, In the present investigation, nanoparticles synthesized by green route gold salt with viola was checked for toxicological studies on human blood cells and they proved for being safe at specific concentrations. According to the results maximum haemolysis values were observed for VGNPs even from 250 µg/ µl it has 72% haemolysis values it means if in some ways it could be best microbial or antibiological ageant like for pest control etc. but overall the results have shown that the synthesized green NPs are safer at their specific concentartions similar findings were found while green synthesis of gold nanoparticles using medicinal plants which were found to be toxic against most of the pathogens but safe for humans and are also in line with haemolysis assay reported by some other researchers. The ecotoxicology and chemistry of manufactured nanoparticles was tested against environment by. And similarly Toxicity of Ag, CuO nanoparticles to selected environmentally relevant organisms and mammalian cells.

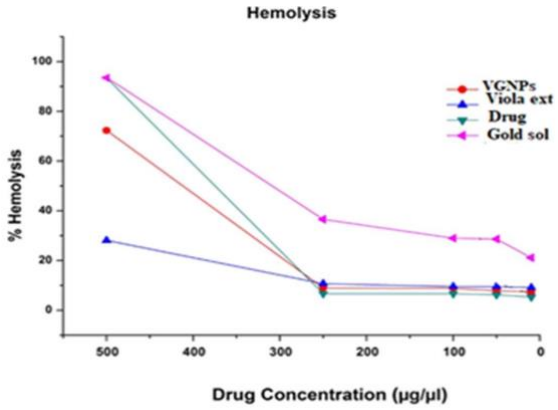



Figure: 4.14 : Cytotoxicity analysis, haemolysis of VGNPs.

***c) Antileishmanial activity***

The antleishmanial activity of different *Viola* nanoparticles were determined against *L. tropica KWH23* at different concentration 10Ug/ml, 5Ug/ml, 2.5Ug/ml, 1.25Ug/ml, 0.625Ug/ml, 0.15625Ug/ml, 0.078125Ug/ml. On the strain of Leishmania *tropica KWH23,* the anti-leishmanial effectiveness of biologically produced GNPs of *Viola* were investigated. The impact of VGNPs on promastigotes' metabolic activity was studied at various doses. At a magnification of 40x, viable cells were counted microscopically in a Neubaur chamber. Both the control and test groups had their promastigotes cells viability tested. The metabolic activity of the promastigotes was reduced when they were exposed to LED light.

The results of the study have revealed that *Viola* has potent antileishminal properties for having 100% killing abilities. Good results were observed against Leishmanial promastigotes at all concentration of gold nanoparticle with extract. The promastigotes cells were killed 2.01-10.05% at the range 10Ug/ml-0.078125Ug/ml in *Viola* extract. Same inhibition effects were observed in, Tetrachloroaurate. The wide-range assessment showed that the MSNPs at different concentrations have signifi-cantly more potent inhibitory effects as compared to the other treatments on the promastigote growth. (Awad et al., 2021; Soflaei et al., 2014; Ahmad et al., 2019).

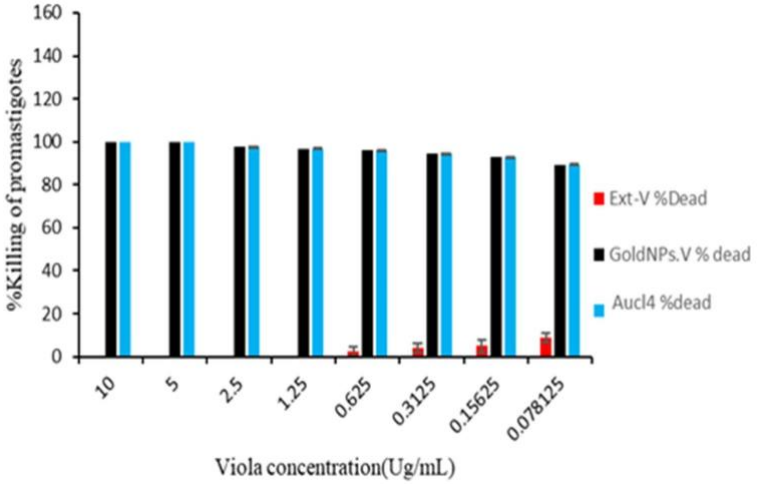



Figure: 4. 15: Antileismanial activity of *Viola* at 10-0.078125Ug/ml.

Treatment names (Ext-v=Viola extract, VGNP= Gold nan particle with *Viloa* extract, $Aucl_4$= Tetrachloroaurat). The vertical bars indicate the standard error, and the values are the averages of three replicates. At P0.05, all treatments are substantially different from one another.

## 5. Conclusions

The drug-resistant of microorganisms is an incredible challenge for therapeutic practitioners, and unused drugs are getting focus to treat numerous diseases. Inventive progresses in Nano medicine offer an easy way of bio-imaging techniques for early discovery frameworks. Green metallic nanoparticles synthesis is growing in to an important branch of nanotechnology, moving away from typically synthesized harmful and flammable chemical medications. The present study reports about the green synthesized gold nanoparticles with strong anti-microbial properties. The nanoparticles were optimized by distinctive parameters and found stabilized at pH 7, whereas for gold nanoparticles have greatest peaks in between 9-10 higher temperature ranges proved best for nanoparticles stabilization, in terms of different concentrations 3mM in case of gold nanoparticles had sharp peaks. UV-vis spectrophotometer and FTIR affirmed the morphology as whereas 50nm round or triangular for *Viola* gold green nanoparticles. The FTIR results have revealed phenols flavonoids, alkanes and a few amines are included in capping of these nanoparticles. The nanoparticles have affirmed for distinctive for antilasminial anticancer ativities, haemolysis and cytotoxic studies. While *Lashminia tropica KWH23,* cancer cell lines MCF-7 breast adenocarcinoma cell (ATCC® HTB-22) and african green monkey kidney VERO cell (ATCC®CCL81) were used for activity check. Overall the nanoparticles appeared distinctive activities but VGNPs demonstrated best than all for bioactivities analysis. *Viola* green nanoparticles were found promising and safe. In case of lashminiasis the plant pure extracts performed best and their exercises was ceased in nanoparticles formation which suggests capping of nanoparticles bound the free secondary metabolites of the crude extracts. Cytotoxic findings have demonstrated that both types of the nanoparticles are secure at lower concentrations. Overall the studies prove that gold influences the translation of important molecules, especially cation transporters. Numerous of these transporters are known to be included within the uptake of metals, especially overwhelming metals. This backhanded study proposes that gold is taken up by the plant, by means of particular metal transporters. An enormous number of transporters reacting to gold, in couple with the wide extend in cation substrate specificity means that there can be a great degree of useful cover in transporters with activity towards gold.

## Scope of study

This study guides about a simple biosynthesis approach for the synthesis of gold nanoparticles, many physical and chemical variables control their size, shape, capping and binding reactions. Assisted shape control can be presented in future like other with metallic salts of gold might be checked for more potency in comparison, due to the expanded significance of nanoparticle shape in controlling their optical properties. Plants special secondary metabolites of interest also could be addressed. These compounds can be used as metal ion precursors.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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