EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF MOMORDICA DIOICA ALONG WITH ITS SILVER AND COPPER NANOPARTICLE SYNTHESIS

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Abstract-

Momordica dioica Roxb. Is a perennial climber that belongs to the Cucurbitaceae family. It is also known as bitter gourd or spiny gourd.They are well known for the bitter taste due to the presence of phytochemical (alkaloid) and have a wide range of medicinal values. The preliminary phytochemical tests result indicates the presence of Saponins, Flavanoid, Alkaloid and Glycoside. Antioxidant capacity of the aqueous and methanol extract was

investigated by different assay, namely DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay and Phosphomolybdate assay. The antimicrobial activities of the pure extract, aqueous extract , methanol extract were determined against two gram positive bacteria, two gram negative bacteria and two yeast with agar cup method(AST).The dry powder of Momordica Dioica methanol extract was the most active against the tested microorganisms in the study with higher inhibition zones and lower minimal bactericidal or fungicidal activities (MBC or MFC) than the other extracts. FTIR spectra of pure extract showed strong bands at 1653nm alkene group present.

Silver and copper nanoparticles were synthesized using the fruit extracts of Momordica dioica (Md-AgNPs) (Md-CuSO4). The structural characterization of(Md-AgNPs) (Md-CuSO4) was performed by UV–vis spectroscopy and Fourier transform infrared spectroscopy (FTIR).UV–vis recorded the absorbance spectra of Md—AgNPs at 300 nm and Md-CuSO4 at 300 nm. FTIR spectra of the synthesized silver strong bands at 2464nm indicates carbon dioxide and copper strong bands at 3765nm indicates alcohol.

The major outline of the project was-

- Extraction and screening of various phytochemical compounds present in plant.
- Determination of antioxidant potential of the extracts using various in vitro assay such as DPPH and Phosphomolybdate assay.
- Determination of antibacterial potential of extracts against common organisms namely *E.coli, S.Aureus, Strep. Pyogen, salmonella typhi, candida albicans and Aspergillus Niger.*
- FTIR analysis of sample
- Silver and copper nanoparticles synthesis using Momordica Dioica
  Characterisation- UV spectrophotometer
  FTIR

Introduction-

Momordica dioica Roxb. is a perennial climber that belongs to the Cucurbitaceae family. 80 species make to the Momordica genus. The most recent revision of Indian Momordica lists six distinct species, four of which are dioecious and two of which are monoecious. Although this genus originated in the Indo-Malayan region, it is currently known to grow in countries such as India, Bangladesh, Sri Lanka, Myanmar, China, Japan, South East Asia, Polynesia, Tropical Africa, and South America. Its cultivation up to an altitude of 1500 metres in Assam and the Garo highlands of Meghalaya is documented.While it is popularly known as spine gourd, teasel gourd, or small bitter gourd around the world, it is known as kakrol in Bangladesh and kankro, kartoli in India. The fruits, young twigs, and leaves of this crop are used as a vegetable or cooked as a vegetables. It is available in the forest of dry and moist deciduous in feeding months August to February.

The Teasle gourd is an important summer vegetable in Bangladesh and the Indian subcontinent. It has many advantages, like high market price, good nutritional value and keeps quality longer.

They are well known for the bitter taste due to the presence of phytochemical (alkaloid) and have a wide range of medicinal values (Jha *et.al.*,2017).

Momordica dioica contains alkaloids, steroids, triterpenoids, flavonoids, glycosides, saponins, vitamins, protein, carbohydrates, momordicin. It has been used in the treatment of malaria, inflammation, antioxidant, diabetes, hyperglycemic.

Plants that are shown to be nutritious also fulfil people's hunger and serve as a wonderful source of nourishment. Many tribal people have been seen to maintain their ancient dietary customs and live in unaltered forest environments. This may highlight the healthy nutritional value of wild edible plants that rural people eat as a primary source of nourishment or as a supplement. It has been noted that these tribes turn to various herbal edibles when food is scarce due to tragedies like drought, flood, or other calamities. The nutritional makeup of a significant percentage of wild (Salvi *et.al., 2015*).

Vegetables naturally contain tannins, which are polyphenolic substances. Their historical use has been influenced by their presence in nature in a variety of ways. Their additional modification with an eye toward an industrial use has been made possible by the reworking of their customary utilisation. Sometimes these alterations imply the addition of dangerous compounds like formaldehyde, which is a category B1 carcinogen (Fraga-corral *et.al.,* 2020).

Glycosides called saponins are found in abundance in plants. Their distinctive feature, also known as sapogenin, is the quantity of sugar chains affixed to the triterpene or steroid aglycone backbone. Although it has been established that the primary dietary sources of saponins are the primary non-food sources employed in industrial and medical uses, which are dicotyledons similar to legumes (Adiukwu *et.al.*,2013).

Widely present in fruits and vegetables, flavonoids are polyphenols that have been shown in numerous models to be effective antioxidants (Hidalgo *et.al.*,2010).

Alkaloids are one of the most diverse groups of secondary metabolites found in living organisms and have an array of structure types, biosynthetic pathways, and pharmacological activities.The phenylpropanoid pathway mostly produces plant phenols. They are potent antioxidants and may guard against oxidative damage to proteins, lipids, and macromolecules like DNA that contribute to chronic illnesses including cancer and cardiovascular disease.

Aqueous and methanolic extract was used for screening of antimicrobial and antioxidant properties.The screening of antimicrobial activity was done using reported agar cup method on common gram positive ,gram negative bacteria and fungus. Whereas DPPH (2,2-Diphenyl-1-Picrylhydrazyl) and phosphomolybdate method was used to evaluate antioxidant activities of extract.

Nanotechnology is promising as a rapidly growing field with its application in science and technology. Noble metal nanoparticles such as silver, gold and platinum are broadly applied in medicinal applications. Silver nanoparticles are important materials that have been studied widely. There is a growing need to develop an eco-friendly method for the synthesis of nanoparticles that does not utilize toxic chemicals. In general, nanoparticles are prepared by a variety of physical and chemical method which are not eco-friendly. Nowadays, green chemistry procedures are using various biological systems such as bacteria, fungi, yeast, and plant extract for the synthesis of nanoparticles. Among them, plant extract based green biosynthesis of metal nanoparticles especially gold and silver with controlled physicochemical properties have been reported by many researchers. Silver nanoparticles prepared by using biological materials have the properties of a high surface area, smaller in size and high dispersion. These prepared nanomaterials have many applications, including spectrally selective coatings for solar energy absorption, optical receptors, generation of intercalation materials for storage batteries catalysis in chemical reactions, biolabelling, and antibacterial agents. It is well known, that silver is an effective antibacterial agent and possesses a strong antibacterial activity ity against bacteria, fungi and viruses, even though the mechanism and the manner of action are still not well known. The high antibacterial activity of silver nanoparticles is a result of well developed surface, providing maximum contact with the environment(Nahar *et.al.*,2014).

Copper oxide nanoparticles (CuO NPs) have atttracted significant interest due to their wide variety of applications, including in catalysts, gas sensors, high-Tc superconductors, giant magnet resistance materials, solar energy devices, and in the preparation of organic-inorganic nanocomposites. Additionally, CuO is used as an antifungal, antibiotic and antimicrobial agent when introduced into textiles and coatings (Tang *et.al.,* 2017).

Medicinal plants are the richest bioresource of drugs for traditional systems of medicine therefore man has been using plant extracts to protect himself against several diseases and also to improve his health and life-style. The different phytocontituents present in medicinal plants are flavonoid, alkaloid, phenol and tannins, carboxylic acids, terpenes and amino acids and inorganic acids. These phytoconstituents give specific distinctiveness and properties to plants.Therefore, the analysis of these chemical constituents would help in determining various biological activities of plants. A variety of techniques can be used to determine and estimate the presences of such phytoconstituents in medicinal plants. Chromatography and spectroscopic techniques are the most useful and popular tools used for this purpose. The Fourier Transform Infrared Spectrophotometer (FT-IR) was perhaps the most powerful tool for identifying the types of chemical bonds/functional groups present in the phytochemicals. The wavelength of light absorbed was the salient feature of chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a compound can be determined. Moreover, FTIR spectroscopy is an established time-saving method to chracterize and identified functional groups.UV-VIS spectroscopic is simple, cost-effective and rapid tests for detecting phytocomponents. UV-visible spectroscopy uses light in the visible ranges or its adjacent ranges. The colour of the chemicals involved is directly affect the

absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic spectrum (kalaichelvi *et.al.*, 2014).

The bitter gourd Is also referred to as Momordica dioica Roxb. Ex. Willd (Cucurbitaceae). It has long been used as an astringent, febrifuge, antibacterial, anthelmintic, spermicidal, and for other conditions such bleeding piles and urinary infections as well as sedation. Studies show that it has hepatoprotective, analgesic, antibacterial, anti-inflammatory, anti-lipid peroxidative, and antioxidant activities (Bhavana et.al., 2010).

2. LITERATURE REVIEW-

2.1 SCIENTIFIC CLASSIFICATION OF SPINY GOURD-

Kingdom	Plantae
Subkingdom	Tracheobionata
Supedivision	Spermatophyta
Division	Magnoliphyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Violales
Family	Cucurbitaceae
Genus	Momordica
Species	Dioica

Table no.1. scientific classification of spiny gourd 2.2 VERNACULAR NAMES –

English- small bitter gourd, spine gourd, Teasle gourd

Hindi- kakora, parora, kantola

Marathi- kantoli

Bengoli- kartoli

Malayalam- venpaval

Tamil- paluppakkay

Telugu- Agakara, karkotaki

Kannada- Madahagala- kaya

Sanskrit - vahisi

Punjabi- Bharkarela

Assam- Batkarila

Gujarati - Katwal

Figure 1- Fruits of spiny gourd

2.2 BOTANICAL DISCRIPTION-

In India, kantola, or spiny gourd, is a common dish. Rajasthan is known for its love of the spiny gourd, or kantola, especially during the rainy season. Teasle gourd, another common name for the spiny gourd (Momordica Dioica). It is primarily grown in India's mountainous regions. Cucurbitaceous plants produce the spiny gourd. When unripe, they are a dark green colour that turns light green to yellow as it ripens. Spiny gourds typically have a diameter of 2-3 cm.

2.3 CLIMATE CONDITION REQUIRED FOR SPINY GOURD FARMING-

Kantola is a warm-weather crop with low humidity. It is possible to grow this vegetable in both tropical and subtropical climates. Good sunlight is necessary for this crop's growth and productivity. For its cultivation, a temperature range between 27° C to 32°C is ideal.

2.4 HARVESTING OF SPINY GOURD-

After 75 to 80 days from the date of sowing, these veggies will be ready for harvest. In their second year, they will be ready for plucking in 35 to 40 days. When a fruit is in its sensitive stage, it is harvested. To prevent over-mature veggies, alternate days are advised for picking. It is possible to carry out hand harvesting without upsetting the vine. If you are growing these for seed production, let the fruits on the vine until they are fully ripe. They typically change colour from green to orange. Mature seeds are easily recognised when the pulp inside the fruit turns red in colour.

2.5 SOIL REQUIREMENT AND YIELD OF SPINY GOURD-

Spiny gourd can be grown on sandlylaom to clay soils with pH values 5.5-7.0. soils with well drainage and good organic matter best suited for its cultivation.

An average yield of 75-100 quintals/ hecter can be obtained with good management practices.

2.6 EXTRACTION METHODS-

Extraction is the main process by which bioactive compounds may be obtained from biomass materials. The objective of extraction process is to maximize the amount of target compounds and to obtain the highest biological activity of these extracts. The extraction yield and biological activity of the resulting extract is not only affected by the extraction technique but also by the extraction solvent. Many solvents, including methanol, ethanol, acetone, and water, have been used for extracting bioactive compounds from the plant material. Due to the variety of bioactive compounds contained in plant materials and their differing solubility properties in different solvents, the optimal solvent for extraction depends on the particular plant materials, and the compounds that are to be isolated (Nguyen *et.al.,* 2019).

A. Aqueous extract - Aqueous extracts of plants are a simple, economical and eco-friendly alternative to use as a source of antifungal activity. Here is a protocol for extracting any type of plant material (leaves) with water using a simple blender to grind the dried leaves with water and then a series of centrifuges to eliminate the solid waste, finally a series of filtrations to have an aqueous extract sterile. This aqueous extract can be stored at 4 ° C and depending on the plant the activity can be maintained for a fixed period of time. After this process the aqueous extract is ready to be used in bioassays of antifungal activity

B. Methanol extract- It is also polar in nature, miscible with water, and could extract polar secondary metabolites.

Advantages. It is self-preservative at a concentration above 20%. It is nontoxic at low concentration, and as small amount of heat is required for concentrating the extract.

Disadvantages. It does not dissolve fats, gums, and wax; it is flammable and volatile (Abubakar *et.al.*,2020).

C. Crude extract- The crude extract is the freshly obtained product acquired from the extraction process of the natural substance. According to the crude extract definition, the obtained extract is formed in liquids, semi-solids, and powder form. In the pharmaceutical industry, the crude extract formation of the drug is obtained from the natural plant, which has a higher concentration of active agents by the extraction process.

2.7 PHYTOCHEMICALS STUDIES-

Extracts were subjected for phytochemical studies to find out phytoconstituents present (Harborne *et al.*, 1998).. Studies revealed the presence of flavonoids, glycosides, phenolic compounds, alkaloids, carbohydrates, proteins, saponins and amino acids in Plants.

The fruit of Momordica dioica contains ashes: 9.1%, crude protein: 5.44%, crude lipid: 3.25%, crude fiber: 22.9%, and carbohydrate: 59.31%. Its fruit has high energy value (288.25 kcal/100 g) in dry weight. Its mineral ranges (mg/100 g dry weight,) are: potassium (4.63), sodium (1.62), calcium (7.37), iron (5.04), and zinc (3.83) [14]. In another investigation, its nutritional value of per 100 g edible fruit is reported to contain 84.1% moisture, 7.7 g carbohydrate, 3.1 g protein, 3.1 g fat, 3.0 g fiber and 1.1 g minerals and small quantities of essential vitamins like carotene, thiamin, riboflavin and niacin (Talukdar *et.al., 2014).*

2.8 ANTIOXIDANT STUDIES-

Recently antioxidants and secondary metabolites are attracting a great deal of attention for their effects in (hamissou *et.al.,* 2013).preventing diseases due to oxidative stress.which leads to degeneration of cell membranes and leads to many pathological diseases. Antioxidants and secondary metabolites play a major role in preventing disease due to oxidative stress, which leads to degeneration of cell membranes and many pathological disease (Pehlivan et al. 2021).

Moreover, recent investigations have shown that the antioxidants with free radical scavenging properties (Nagulendran *et.al., 2007).* plant origin could have great importance as therapeutic agents in ageing process and free radical mediated diseases (Hamissou *et.al., 2013).*

2.9 FTIR-

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of alcohol, phenol, alkanes, aldehyde, aromatic compound, secondary alcohol, aromatic amines and halogen compound(Sahu *et.al.*,2013).

Fourier Transform Infrared spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional Groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By Interpreting the infrared absorption Spectrum, the chemical bonds in a molecule can be determined(Ashok Kumar *et.al.*,2014).

2.10 SILVER AND COPPER NANOPARTICLES SYNTHESIS-

The synthesis of nanoparticles is in the spotlight in modern nanotechnology. In recent years, the development of competent green chemistry methods for synthesis of metal nanoparticles (NPs) has become a main limelight of researchers. Biological synthesis of nanoparticles using plant extract is currently under exploitation. The first time in this paper we have reported the green synthesis of silver nanoparticles (AgNPs) by reduction of silver nitrate, using fruit extracts of Momordica charantia (bitter melon);

commonly found plant in south East Asia. The reaction process for the synthesis of silver nanoparticles is simple, cost-effective, novel, rapid and ecofriendly route using fruit extract of M. charantia plant, which acted as a reducing and stabilizing agent simultaneously at room temperature. Formation of the nano silver was confirmed by surface plasmon spectra using UV-Vis spectrophotometer and absorbance peak at 440 nm. Different silver ion concentration and contact times were experimenting in the synthesis of silver nanoparticles. The prepared nanoparticles properties were characterized by UV-Vis (Nahar *et.al.,*2015).

The use of environmentally benign materials such as plant extract for the synthesis of silver nanoparticles (AgNPs) offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications (David *et.al.,*2014).

Copper oxide nanoparticles (CuO NPs) have attracted significant interest due to their wide variety of applications, including in catalysts, gas sensors, high-Tc superconductors, giant magnet resistance materials, solar energy devices, and in the preparation of organic-inorganic nanocomposites. Additionally, CuO is used as an antifungal, antibiotic and antimicrobial agent when introduced into textiles and coatings (Tang *et.al.*,2016).

XRD analysis was performed using a bruker D8 Advance diffractometer with Cu-Kα radiation (λ = 1.54 Å) and a step size of 0.02° over the range from 10–80° at a scan rate of about 4°/min. FTIR analysis of the prepared CuO NPs was carried out with a Jasco system to evaluate surface capping. A purified sample was dried and mixed well with KBr powder for pellet preparation for FTIR measurements. The surface morphology and size of the synthesized CuO NPs were analyzed using a highresolution transmission electron Microscope (JEOL JEM 2100 HR-TEM). The sample for TEM analysis was prepared by placing a diluted CuO nanocolloid on the copper grid surface, which was later dried under a vacuum. Additionally, the surface charge and size distribution of the CuO NPs were examined using a Horiba Scientific SZ-100 Nanoparticle Analyzer. Samples for zeta potential analysis were prepared by diluting the CuO NPs with double distilled water (Tang *et.al.*,2015).

UV spectrophotometer

3.MATERIALS AND METHODS-

3.1 PLANT MATERIAL COLLECTION

The plant material *Momordica dioica* was bought from Ganeshpuri village in August 2022. The sample washed 2-3 times for removing dust particles. After washing the sample, the seeds were separated and removed the foreign material. Then the fruits (Pericarp) were used for experimental analysis study.

A. **Pure extract-** The fuits were kept in grinder to collect fresh extraction (juice) was stored in tight-seal dark container in refrigerator until needed.

B. **Aqueous extract –** The remaining materials (filtrate) were added in a beaker and add 300 ml distilled water and kept it about 90 minutes. After, extract were filtered using muslin cloth and stored in tight seal dark container in refrigerator until needed.

C. **Dry powder (methanol) extract -** fruit pieces were air dried for 5 days and made into powder by using electrical grinder. 5 g powder add in 10 ml methanol and stored in tight-seal dark container at 25° C until needed.

D. **Crude extract-** aqueous extract of Momordica Dioica fruit were taken in crucible and evaporate in electric boiling water bath for 90 minutes. Supernatant was collected and solvent was evaporated to make the volume of one fifth of the original volume. It was stored in air tight container at room temperature (Parekh *et.al.*, 2005).

3.2 CHEMICALS USED:

0.1Mm DPPH(Analytical grade), 20%NaOH, Chloroform (Analytical Reagent Grade),Methanol (Analytical Reagent Grade), Mayer's reagent, 2%H2SO, 10% Ferric chloride, Copper sulphate, Sudan indicator, con. H2SO4 , dil. HCl, Ascorbic acid (Analytical Reagent Grade)

#Preparation of Mayer's Reagent-

Mercuric chloride-1.36g

Potassium iodide-5g

100 ml distilled water

3.3 INSTRUMENTS:

Hot air oven, incubator, weighing balance machine, electric water bath, magnetic stirrer, water bath.

3.4 PHYTOCHEMICAL SCREENING FOR FRESH EXTRACTION (JUICE)

Extracts were tested for the presence of active principles such as Terpenoids, Saponins, Alkaloids, Flavonoids, Tannins, Phenol, and Lipids.

Following standard procedures were used(Palve, Shetty *et.al.,2015).*

1. Test for Tanins (Braymer's test):

1 ml of filtrate add in distilled water for diluting and then add 2 drops of ferric chloride. A transient greenish to black color indicates tannins present.

2. Test for Saponnis (foam test):

The small amount of extract was diluted about 4 ml of distilled water. The mixture was shaken vigorously. If the foam resist about 10 minutes than the saponnins is present.

3. Test for flavonoids(NaOH test):

The extract has to be treated with few drops of sodium hydroxide solution. Formation of intense yellow color which become colorless on addition of acid indicates presence of flavonoid.

4.Test for phenol(ferric chloride test):

Extract have to be treated with a few drops of ferric chloride solution. Formation of bluish black color indicates presence of phenol.

5.Test for alkaloids (Mayers reagent):

Take 1 ml of filtrate add 2% sulphuric acid and warmed for 2 minutes. Then filtered and add few drops of Mayer's reagent. A creamy white colored precipitation indicates positive.

6.Test for glycosides:

1 ml of aqueous extract was mixed with chloroform and then add concentrated sulphuric acid. After shaking color change was observed (Maitra *et.al.,2017).*

3.5 ANTIOXIDANT ASSAY:

3.5.1DPPH ASSAY

Crude extract, Ascorbic acid (Standard), Methanol (solvent), 0.3mM DPPH (2,2-Diphenyl-1-picrylhydrazyl) solution [O.D- between 2-3], Varian Cary 50 UV spectrophotometer. Miscellaneous- Eppendorf tubes, Micropipette, Micropipette tips etc. (All Chemicals used were of analytical Grade)

DPPH scavenging potential of crude extract was measured according to the method described by Brand-Williams et al with slight modifications (W. Brand-Williams et al., 1995). Reaction mixtures were prepared as follows.

Different Concentrations of Crude extract was prepared in methanol and used. 0.5ml of Crude extract solution was added to 0.5ml of DPPH solution (0.3mM prepared in methanol). The reaction mixture was incubated under dark conditions for 30 min at room temperature and then the absorbance of the incubated solutions was measured at 517 nm using UV spectrophotometer.

$$\text{DPPH radical scavenging activity} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100\%.$$

BHA was used as standard. Different Concentrations of BHA were prepared in methanol and used. Different Concentrations of Standard solution was added to 1ml of DPPH solution (0.3mM prepared in methanol). The reaction mixture was incubated under dark conditions for 30 min at room temperature and then the absorbance of the incubated solutions was recorded at 517nm using UV sspectrophotometer.

Ascorbic acid was used as standard. Different Concentrations of ASCORBIC ACID were prepared in methanol and used. 0.5ml of Standard solution was added to 0.5ml of DPPH solution (0.3mM prepared in methanol). The reaction mixture was incubated under dark conditions for 30 min at room temperature and then the absorbance of the incubated solutions was recorded at 517nm using UV spectrophotometer.

Value of IC50 was determined from the plot of scavenging activity, drawn according to the concentration of the extract, which is defined as the total antioxidant activity necessary to decrease by 50% the initial DPPH radical concentration. The experiments were carried out in triplicate. Butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA) were used as standard antioxidants (Ceylan *et.al.,2015).*

3.5.2 PHOSPHOMOLYBDATE ASSAY-

One can assess the sample's antioxidant capacity. In a 4 ml vial, an aliquot of 0.1 ml sample solution and 3 ml reagent solution were mixed (0.6M sulphuric acid, 28mM sodium phosphate, 4mM ammonium molybdate). Following capping, the vials were incubated in a water bath at 95 ° C for 90 minutes. The mixture's absorbance at 765 nm was measured in comparison to a blank after the sample had reached room temperature. Ascorbic acid equivalents in milligrammes per gramme of sample are the results that are provided (Selvaraj *et.al.,2015*).

3.6 ANTIMICROBIAL ACTIVITY:

3.6.1 PREPARATION OF COTTON SWABS

A supply of cotton wool swabs on wooden applicator sticks was prepared. They were sterilized in culture tubes in the autoclave (Manikandan *et.al.,* 2020).

3.6.2 EXPERIMENTAL PROCEDURE:

Under aseptic conditions, the sterilised nutritive medium (20 ml) was put onto sterilised Petri plates, where it was left to harden on a flat surface. All of the extracts were first warmed to room temperature before being employed in the Agar cup method of a bioassay test against bacteria. By dipping a sterile swab into inoculums, test cultures of *Escherichia coli*, *staphylococcus aureus*, *streptococcus pyogens*, *salmonella typhi*, *Aspergillus Niger* and *candida albicans* were added to the sterile agar plates. By pressing and rotating the swab hard against the tube's side, above the liquid's level, extra inoculums were eliminated. Three times, the medium's surface was streaked with the swab, and after each application, the plate was rotated via a 60o angle (Palve *et.al.,2015).*

Finally, the agar surface's edge was cleaned with the swab(*Salih et.al.,*2016). The inoculation was dried for a short period of time at room temperature with the lid closed. A stainless steel cork borer with an 8mm diameter that has been flame sterilised was used to create four wells after drying. Then, using a micropipette, four wells were filled with 80 l of each of the four extracts and maintained in a refrigerator at 40C for 45 to 1 hours for diffusion. The plates were then placed for incubation at the appropriate temperature, which was 37 ° for 24 to 48 hours for *Escherichia coli*, *staphylococcus aureus*, *Sterptococcus pyogen*, *salmonella typhi*, and *Candida albicans.* The zone's diameter is measured and documented in millimetres (mm).

Sr no.	Name of the culture	Growth media	Temperature
1.	*Escherichia coli*	Nutrient agar	37° C
2.	*Staphylococcus aureus*	Nutrient agar	37° C
3.	*Streptococcus pyogen*	Nutrient agar	37° C
4	*Salmonella typhi*	Nutrient agar	37° C
5.	*Aspergillus niger*	Sabouraud agar	37° C
6.	*Candida albicans*	Sabouraud agar	37° C

3.7 FTIR SPECTRUM ANALYSIS

About one drop of Momordica Dioica fruit extract was loaded onto FTIR spectrum and the spectroscopic results were recorded on computer using OPUS software.

3.8 SILVER AND COPPER NANOPARTICLES SYNTHESIS-

Aqueous solution (1mM) of silver nitrate was prepared. For the green synthesis of silver nanoparticles (AgNPs), 1.8ml of fruit extract was mixed to 50mL of prepared silver metal ion solution and stirring continued for 4 min at room temperature. The reduction takes place rapidly as indicated by brown-yellow colour solution was formed after 30min which indicating the formation of silver nanoparticles. The effects of reaction conditions such as the silver ion concentration and reaction time were also studied.The biosynthesis of the silver nanoparticles were characterized by using a UV-Vis spectrophotometer. UV-Vis spectra were recorded on double beam spectrophotometer (PerkinElmer Lambda 25) 200,300 and 400 nm at a resolution of 1 nm. The distilled water was used as a blank. (Nahar *et.al.,*2015).

An amount (10 mL) of 0.01 M CuSO4 solution was added to 30 mL of aqueous C. auriculata extract and mixed well with mechanical shaking. The solution was then heated in a water bath at 80 °C for 1 h. The gradual change of color of the reaction solution from brownish yellow to dark brown indicated the formation of CuO NPs (Tang *et.al.,* 2016).

Both samples silver and copper nanoparticles are used to perform FTIR spectrum analysis.

4.Result

1.PHYTOCHEMICAL TEST- Phytochemical screening of plant extract of Momordica Dioica showed the presence of various constituents. The preliminary phytochemical tests result indicates the presence of Saponins, flavanoid, Alkaloid and glycoside. The qualitative test of phytochemicals was initially done by biochemical tests.

Phytochemical	Result
Saponin	Positive
Tannin	Negative
Flavanoid	Positive
Phenol	Negative
Alkaloid	Positive
Glycoside	Positive

Figure 1. presence of phytochemicals using preliminary tests.

Figure 2. Phytochemicals tests for Momordica Dioica plant extract.

Figure 3.Test for saponin (foam test) Persistence of foam for 10 minutes indicates presence of saponin.

Figure 4. Test for Tannins(Braymer's test) transient greenish to black colour absence indicates absence of tannins.

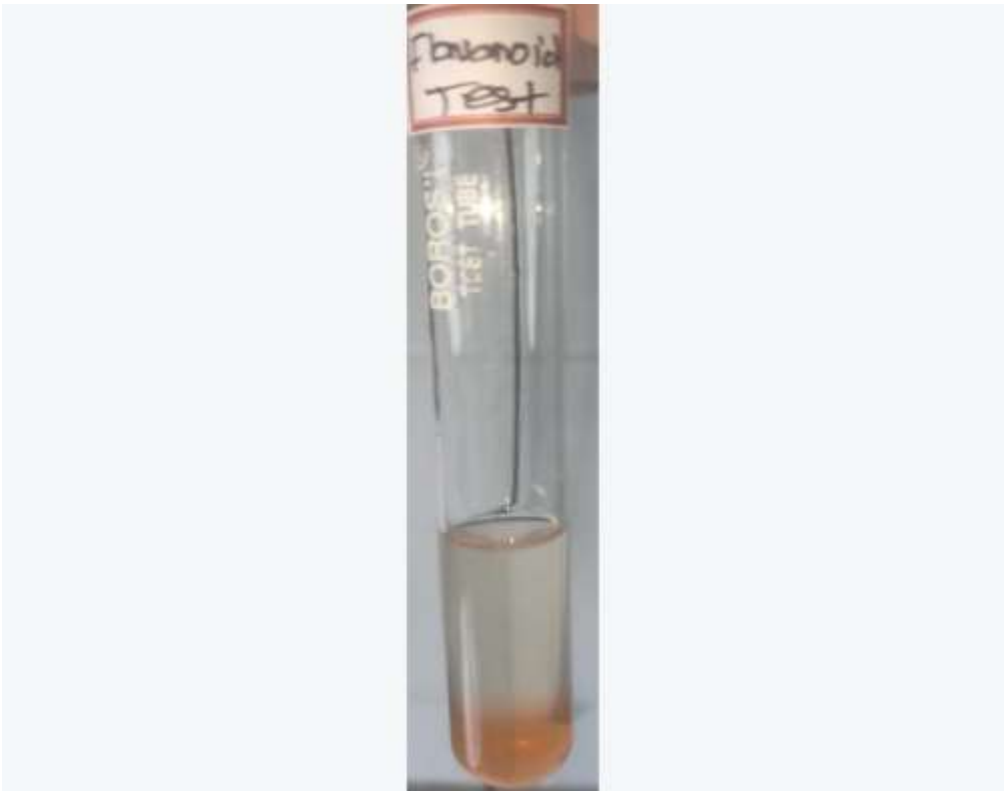


Figure 5. Test for Flavanoid (NaOH Test) formation of intense yellow colour which becomes colourless on addition of acid indicates presence of flavanoid.

Figure 6. Test for phenol (ferric chloride test) formation of bluish colour indicates presence of phenol.

Figure 7. Test for Alkaloid (mayer's test) creamy white coloured precipitation indicates positive.

Figure 8. Test for Glycoside – Colour change was observed and indicates presence of glycoside.

2. DPPH ASSAY

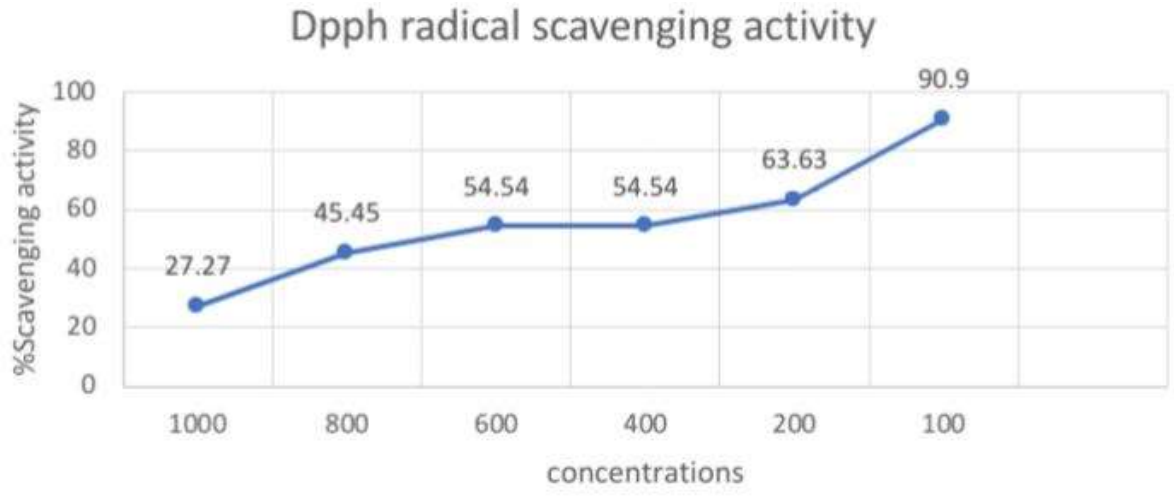
OD of Control – 0.11

Wavelength -530 nm.

Standard – BHA and Ascorbic acid **BHA**

Conc.	Stock (ml)	Diluent (ml)	DPPH (ml)	Total volume (ml)	OD	%RSA
1000	2	-	1	3	0.07	27.27
800	1.8	0.2	1	3	0.06	45.45
600	1.6	0.4	1	3	0.05	54.54
400	1.4	0.6	1	3	0.05	54.54
200	1.2	0.8	1	3	0.04	63.63
100	1	1	1	3	0.01	90.90

Table 1. calculation of Antioxidant activity of BHA



IC50 VALUE:

Y= mx+c

Where, m = slope = -0.055

c = intercept = 84.6931

50= -0.055x + 84.6931

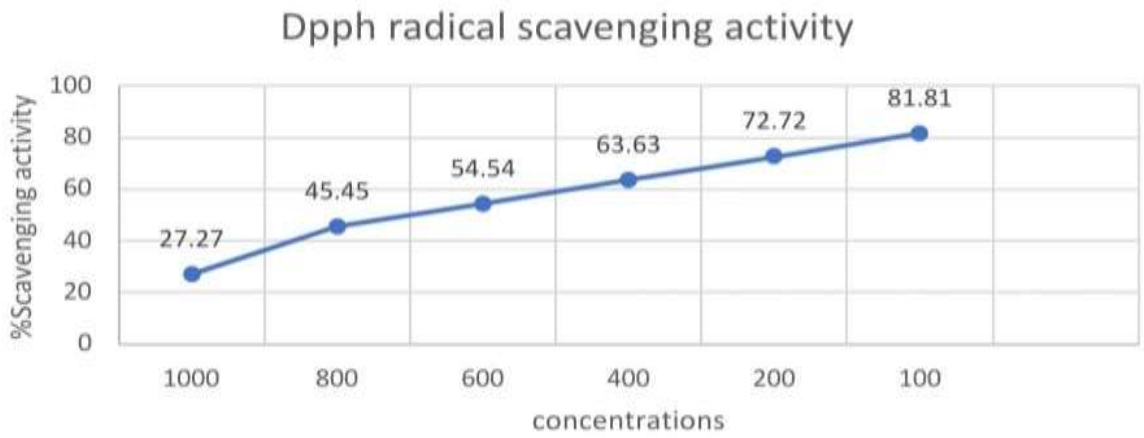
Therefore, x= 630.72

Thus, 50% antioxidant (DPPH radical scavenging) activity of BHA is seen at 630.72 µg/ml concentration.

Ascorbic acid

Conc.	Stock (ml)	Diluent (ml)	DPPH (ml)	Total volume (ml)	OD at 530 nm	%RSA
1000	2	-	2	4	0.07	27.27
800	1.8	0.2	2	4	0.06	45.45
600	1.6	0.4	2	4	0.05	54.54
400	1.4	0.6	2	4	0.04	63.63
200	1.2	0.8	2	4	0.03	72.72
100	1	1	2	4	0.02	81.81

Table 2.Calculation of Antioxidant activity of Ascorbic acid.



Slope-

Y= mx + c

Where, m = slope =- 0.55

C = intercept = 86.39

50= - 0.55 x+ 86.39

Therefore, x= 661.63

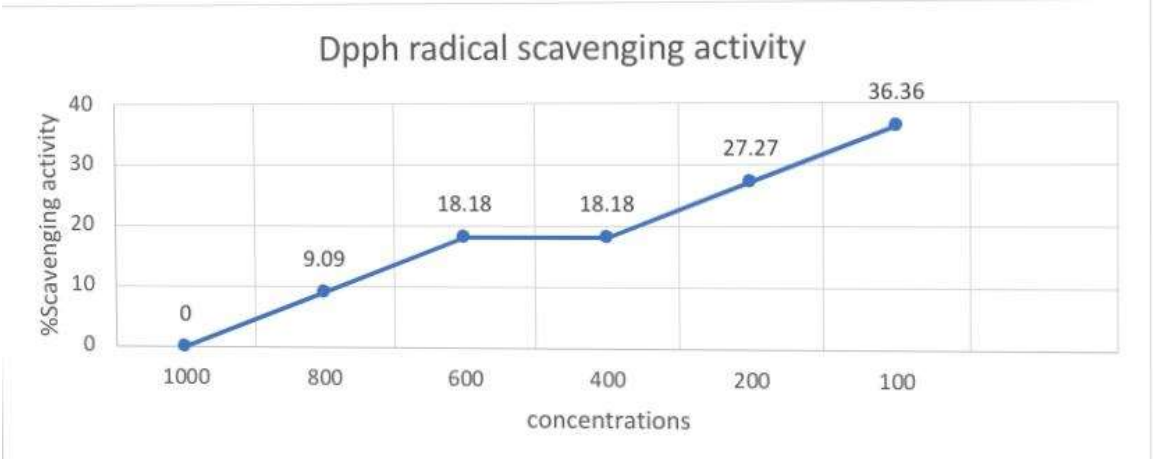
Thus, 50% antioxidant (DPPH radical scavenging) activity of Ascorbic acid is seen at 661.63 µg/ml concentration.

Crude weight+ diluent= stock

Diluent – Methanol

Conc.	Stock (ml)	DPPH (ml)	Total volume (ml)	OD at 530 nm	%RSA
1000	2	2	4	0.14	-27.27
800	2	2	4	0.12	-9.09
600	2	2	4	0.09	18.18
400	2	2	4	0.09	18.18
200	2	2	4	0.08	27.27
100	2	2	4	0.07	36.36

Table 3.Calculation of Antioxidant (DPPH radical scavenging) activity using Momordica Dioica.



IC50 VALUE:

Y= mx + c

Where, m = slope =- 0.065

C = intercept =44.7028

50= - 0.065x + 44.7028

Therefore, x= 81.4953

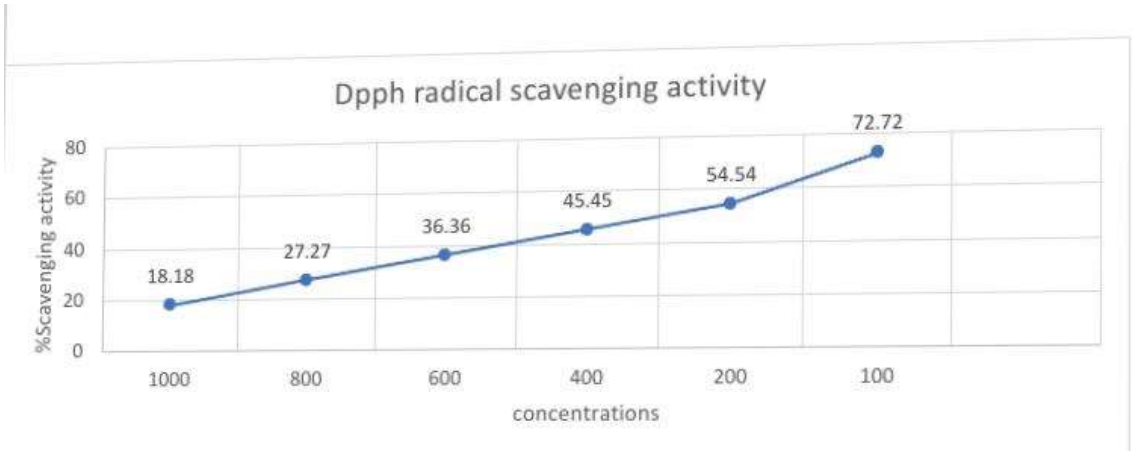
Thus, 50% antioxidant (DPPH radical scavenging) activity of Momordica Dioica is seen at - 81.4953µg/ml concentration.

Crude weight + diluent= stock

Diluent – Aqueous

Conc.	Stock (ml)	DPPH (ml)	Total volume (ml)	OD at 530 nm	%RSA
1000	2	2	4	0.09	18.18
800	2	2	4	0.08	27.27
600	2	2	4	0.07	36.36
400	2	2	4	0.06	45.45
200	2	2	4	0.05	54.54
100	2	2	4	0.03	72.72

Table 4.Calculation of antioxidant (DPPH radical scavenging) activity using Momordica Dioica



IC50 VALUE:

Y= mx + c

Where, m = slope =- 0.054

C = intercept = 70.7276

50= - 0.054x + 70.7376

Therefore, x= 383.70

Thus, 50% antioxidant (DPPH radical scavenging) activity of Ascorbic acid is seen at 383.70 µg/ml concentration.

3. Phosphomolybdate assay

Absorbance of mixture was measured at 765nm against blank.

Mean value – 0.13.

4.Antimicrobial activity

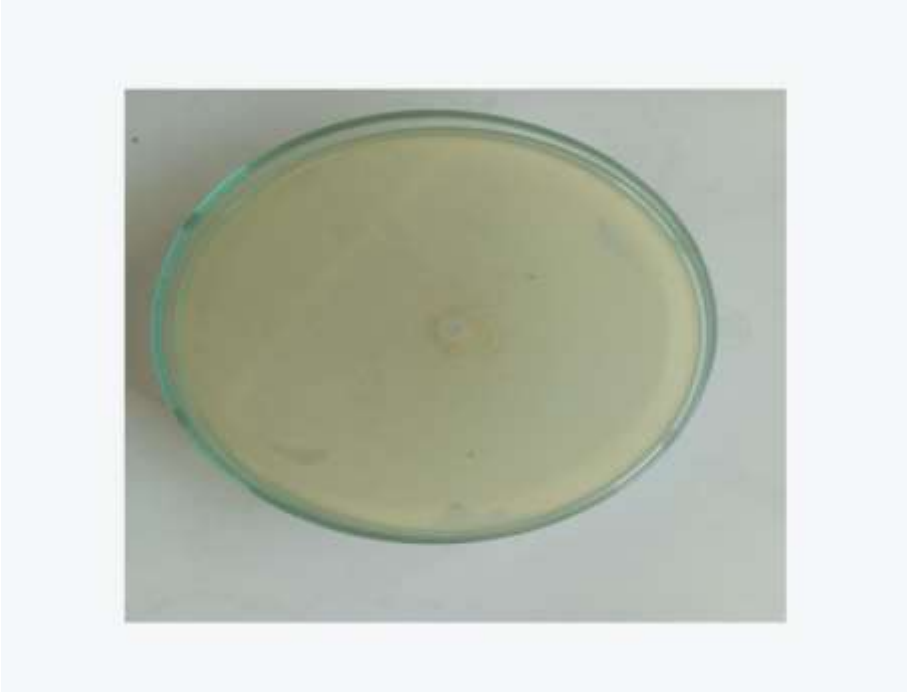


Figure 1. Zone of inhibition of bioassay of aqueous extract of Momordica Dioica on *e.coli.*

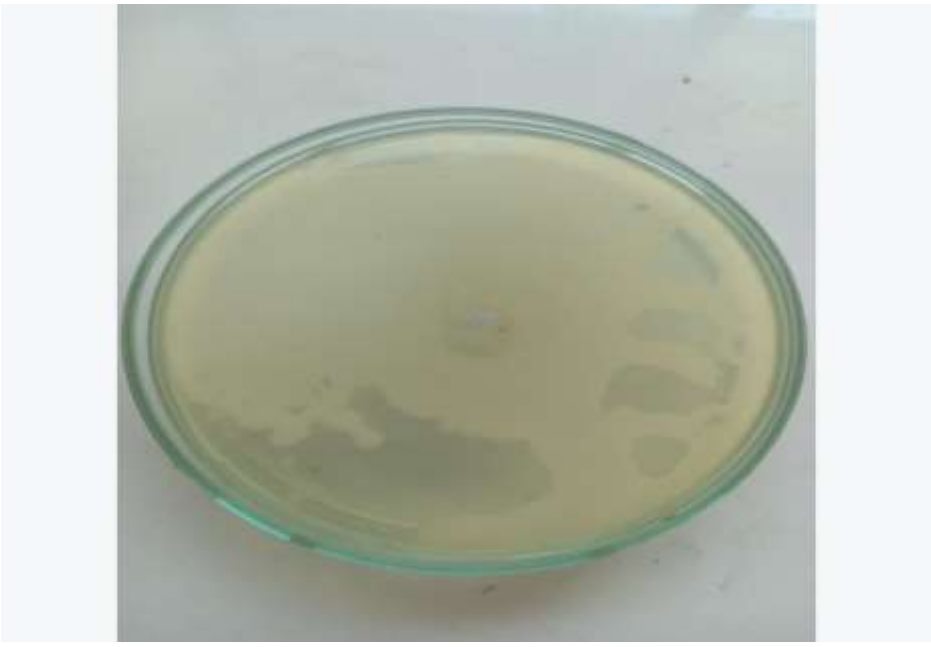


Figure 2. Zone of inhibition of bioassay of aqueous extract of Momordica Dioica on *S.Aureus.*

Figure 3. Zone of inhibition of bioassay of aqueous extract of Momordica Dioica on *streptococcus pyogen.*

Figure 4. Zone of inhibition of bioassay of aqueous extract of Momordica Dioica on *Salmonella typhi.*

Figure 5. Zone of inhibition of bioassay of aqueous extract of Momordica Dioica on *Aspergillus Niger.*

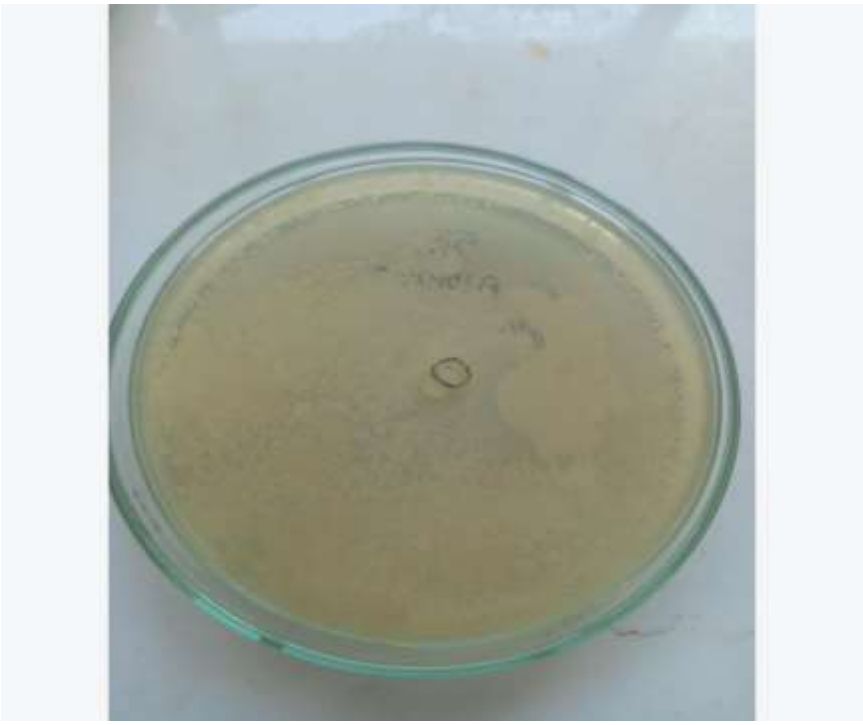


Figure 6. Zone of inhibition of bioassay of aqueous extract of Momordica Dioica on *Candida albicans.*

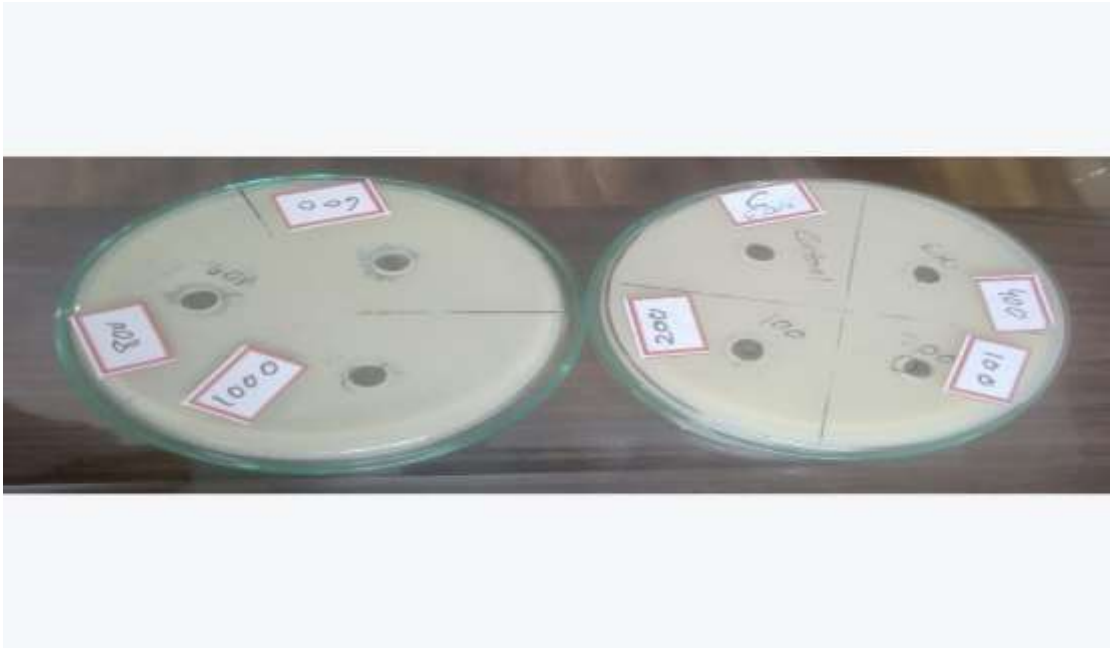


Figure 7. Zone of inhibition of bioassay of different concentrations of methanol extract of Momordica Dioica on *E.coli.*

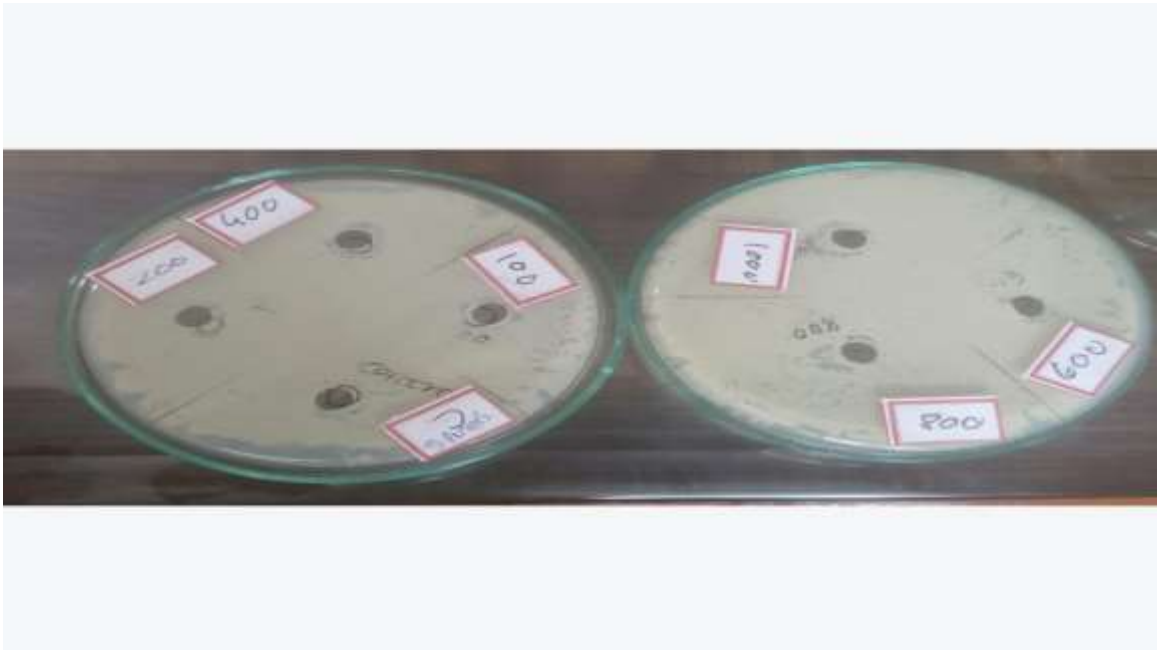


Figure 8. Zone of inhibition of bioassay of Different concentrations of methanol extract of Momordica Dioica on *S.Aureus.*

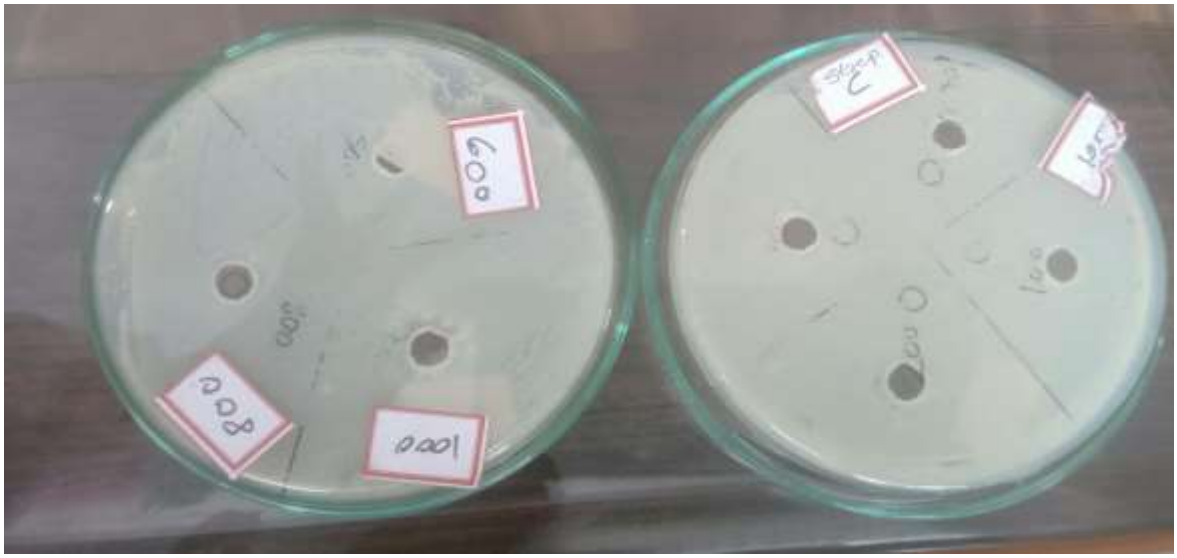


Figure 9. Zone of inhibition of bioassay of different concentrations of methanol extract of Momordica Dioica on *streptococcus pyogen.*

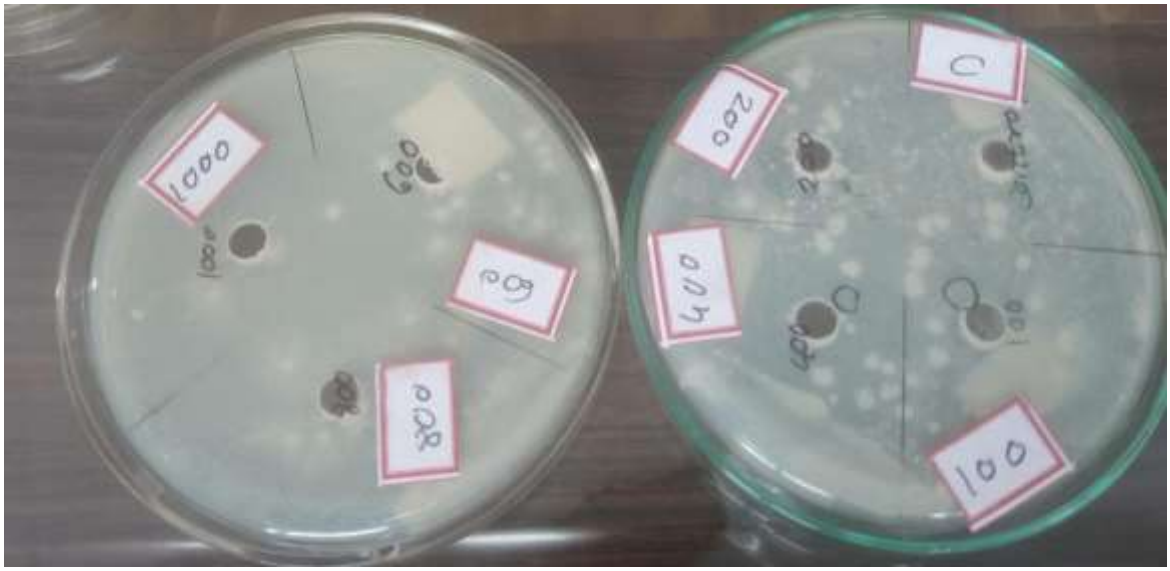


Figure 10. Zone of inhibition of bioassay of different concentrations of methanol extract of Momordica Dioica on *salmonella typhi.*

Figure 11. Zone of inhibition of bioassay of different concentrations of methanol extract of Momordica Dioica on *Aspergillus Niger.*

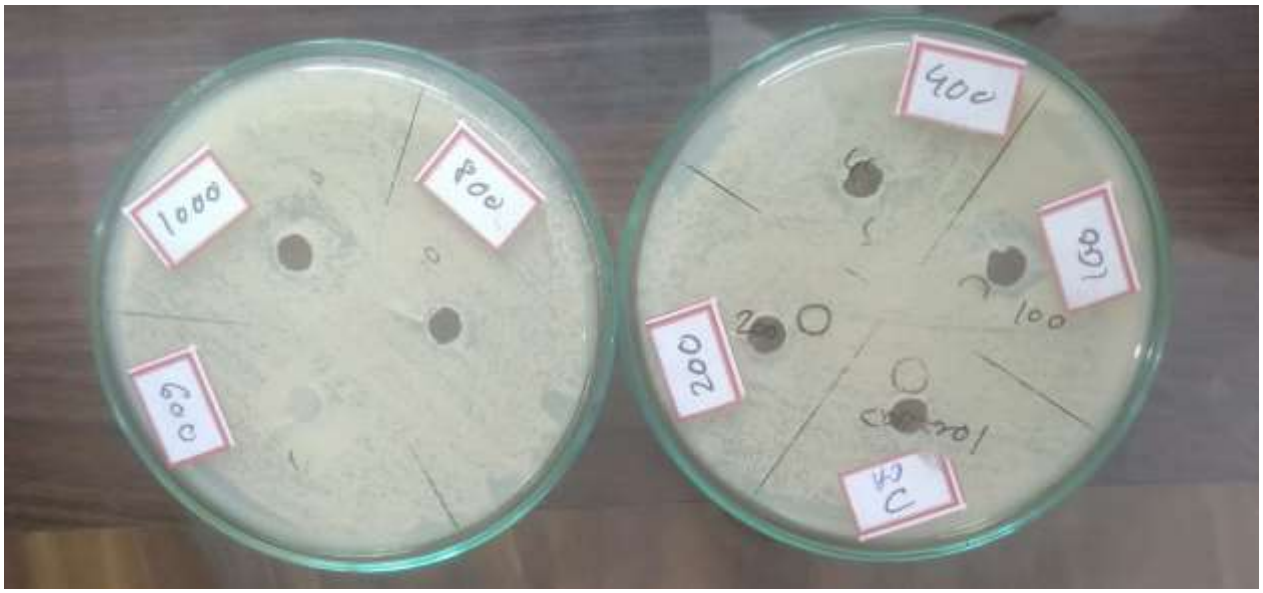


Figure 12. Zone of inhibition of bioassay of different concentrations of methanol extract of Momordica Dioica on *Candida albicans.*

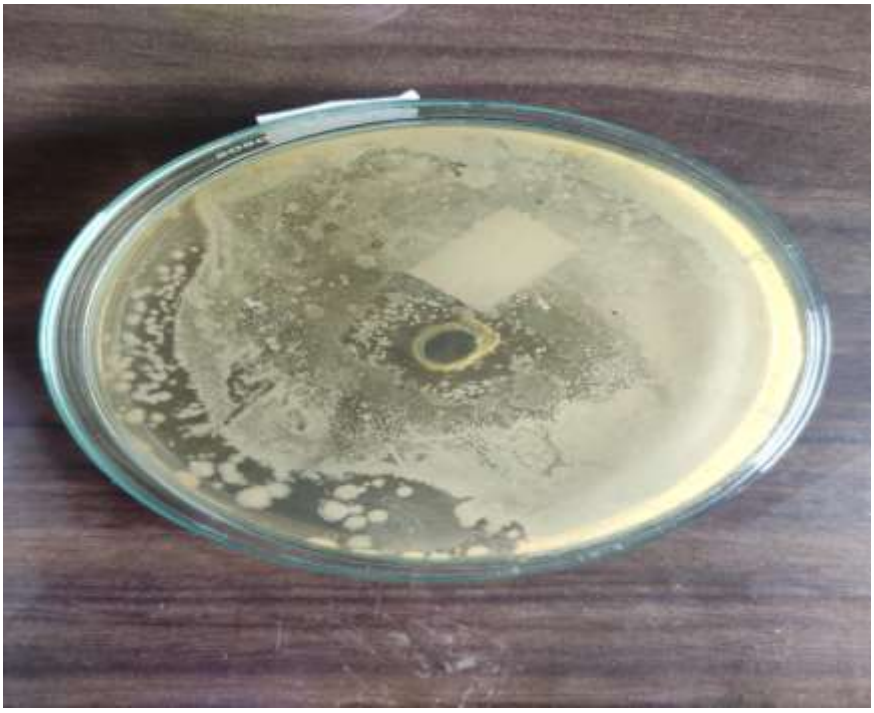


Figure 13..Zone of inhibition of bioassay of methanol extract of Momordica Dioica powder *on E. Coli.*

Figure 14.Zone of inhibition of bioassay of methanol extract of Momordica Dioica powder on S.aureus

Figure 15. Zone of inhibition of bioassay of methanol extract of Momordica Dioica powder on *streptococcus pyogen.*

Figure 16. Zone of inhibition of bioassay of methanol extract of Momordica Dioica powder on *salmonella typhi.*

Figure 17. Zone of inhibition of bioassay of methanol extract of Momordica Dioica powder on *Aspergillus Niger.*

Figure 18. Zone of inhibition of bioassay of methanol extract of Momordica Dioica powder on *Candida albicans.*

The antibacterial activity of Momordica Dioica fruit extract against test organisms is shown in picture.

Results obtained in the present study revealed that the extracts of fruit Momordica Dioica possess potential antibacterial activity against E.coli, S. Aureus, S. Pyogens, S. Typhi, A. Niger and C.albicans. when tested by agar cup method, The dry Momordica Dioica powder in methanol extract showed highest antibacterial activity against all organisms, Momordica Dioica methanol extract showed significant antibacterial activity, Pure Momordica Dioica extract showed lowest activity against all organisms and aqueous extract of Momordica Dioica did not showed any activity against all six organisms.

5.FTIR

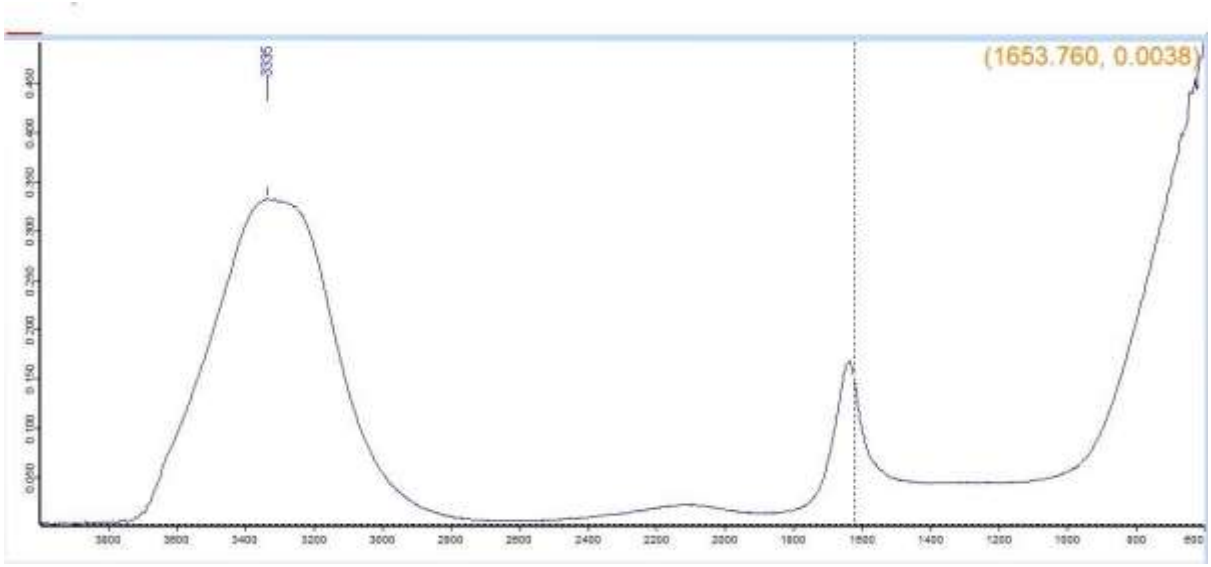


Figure 19. FTIR analysis of Momordica Dioica fruit extracts.

FTIR spectra of pure extract showed strong bands at 3335 nm and alkyne group is showed.

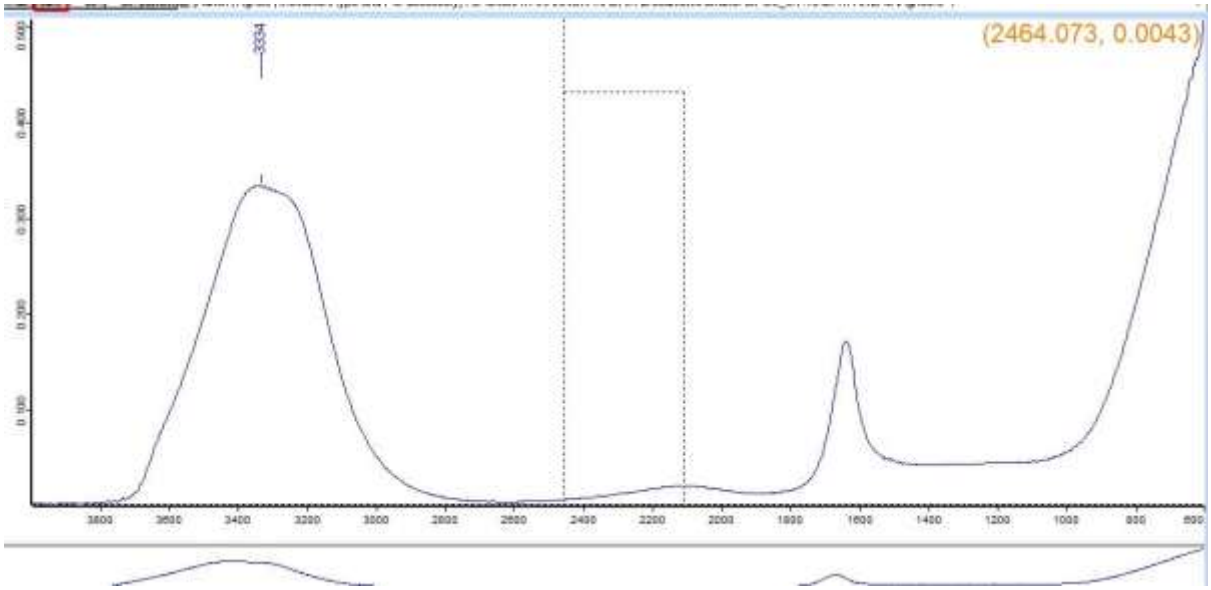


Figure 20. FTIR analysis of Synthesis of silver nanoparticles using Momordica Dioica fruit extract.

FTIR spectra of the synthesized silver strong bands at3334 nm .Silver nanoparticles were synthesized using the fruit extracts of Momordica dioica. And alcohol group showed.

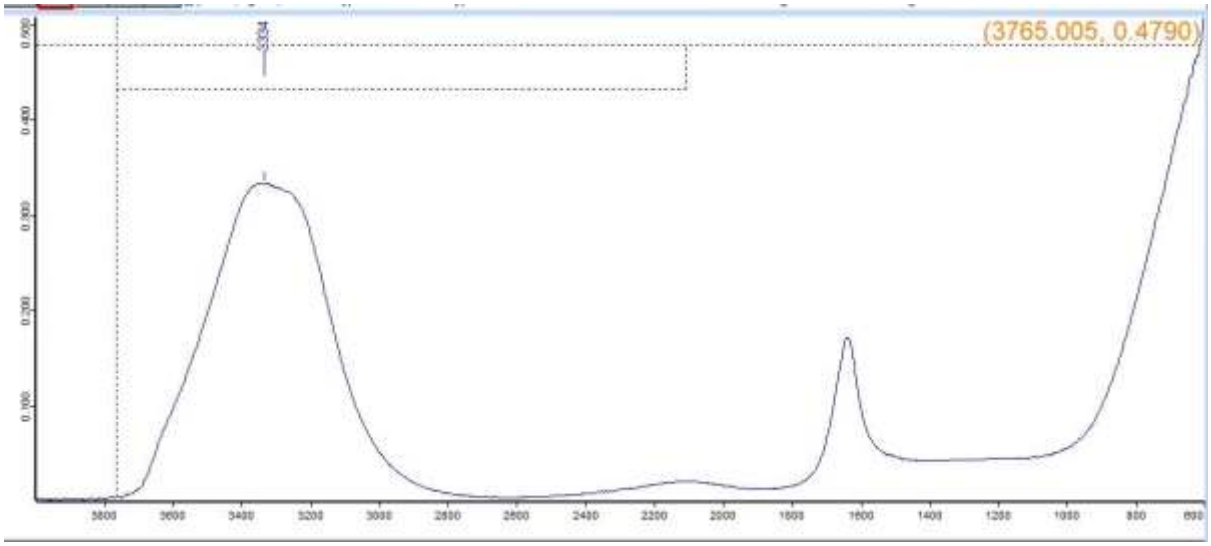
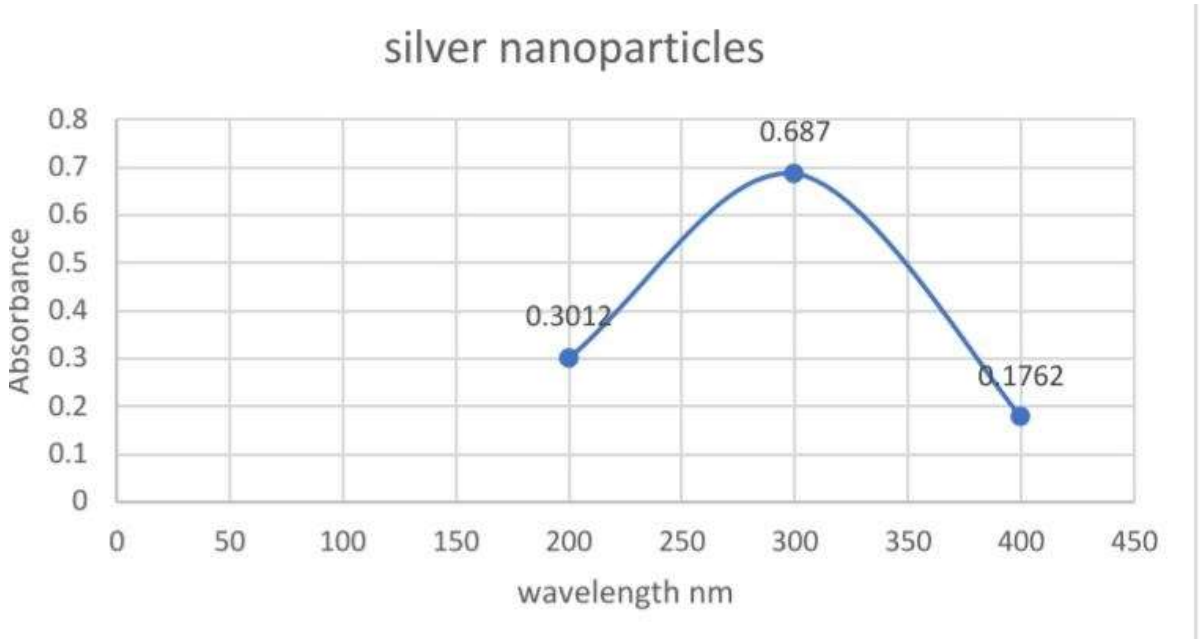


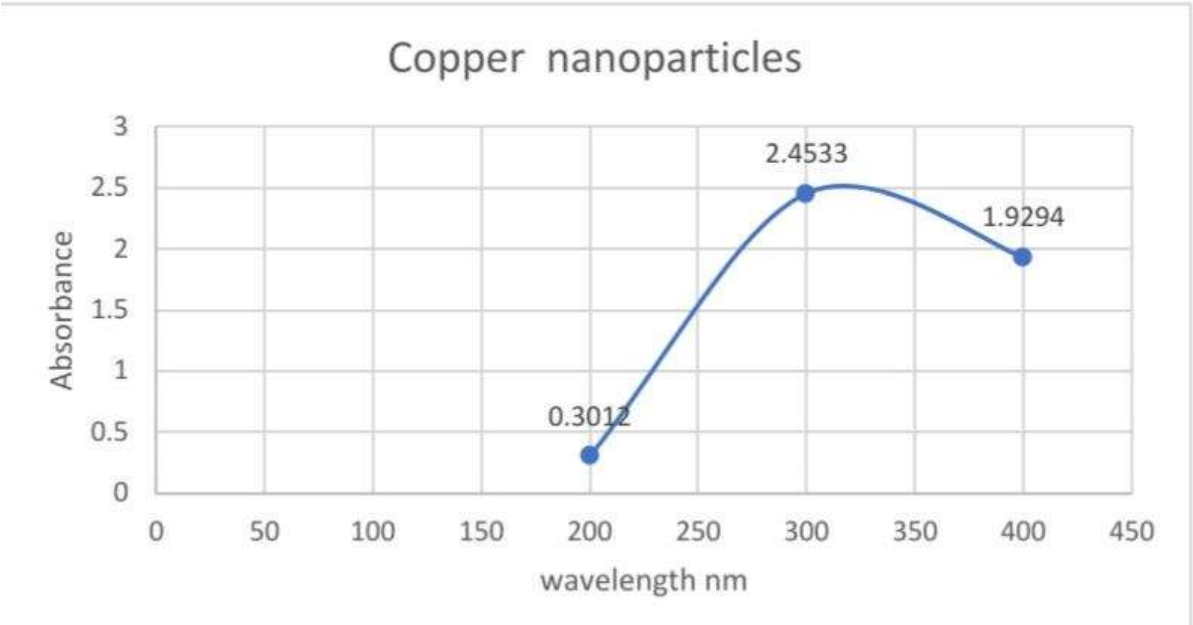
Figure 21. FTIR analysis of Synthesis of copper Nanoparticles using Momordica Dioica fruit extract

FTIR spectra of the synthesized copper strong bands at 3765nm.copper nanoparticles were synthesized using the fruit extracts of Momordica dioica. And it indicates O-H streching group that is alcohol is present.

UV spectrophotometer

Nanoparticles	Absorbance	Reading
Md—AgNPs	**200**	**0.3012**
	300	**0.687**
	400	**0.1762**
Md-CuSO4	**200**	**0.3012**
	300	**2.4533**
	400	**1.9294**





7. Discussion

Momordica dioica Roxb. Is a perennial climber that belongs to the Cucurbitaceae family. 80 species make to the Momordica genus.It is available in the forest of dry and moist deciduous in feeding months August to February. The Teasle gourd is an important summer vegetable in Bangladesh and the Indian subcontinent. It has many advantages, like high market price, good nutritional value and keeps quality longer.Medicinal plants are the richest bioresource of drugs for traditional systems of medicine therefore man has been using plant extracts to protect himself against several diseases and also to improve his health and life-style. The different phytocontituents present in medicinal plants are flavonoid, alkaloid, phenol and tannins, carboxylic acids, terpenes and amino acids and inorganic acids. These phytoconstituents give specific distinctiveness and properties to plants.Therefore, the analysis of these chemical constituents would help in determining various biological activities of plants.

Scavenging activity for free radicals of 1.1-diphenyl-2-picrylhydrazyl (DPPH) has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources.The DPPH radical scavenging activity obtained in a study on Momordica Dioica showed that the methanolic extract had approximately a DPPH radical scavenging activity with IC50= -81.4953 □g/ml and with aqueous extract IC50= 383.70.So, the Momordica Dioica showed significant Antioxidant activity with IC50.

Formation of silver nanoparticles from 1mM solution of AgNO3 was confirmed by using UV–Vis spectral analysis. Metal nanoparticles such as silver has free electrons, which give rise to surface plasmon resonance (SPR) absorption band down to the combined vibration of electrons of metal nanoparticles in resonance with

the light wave. Surface plasmon spectra were obtained for brown–yellow coloured silver solutions in the range of 200-400nm. FTIR spectra was found using FTIR opus software.

The formation of CuO NPs was indicated by the presence of a dark brown precipitate in the bottom of the flask. The Momordica Dioica extract filtrate containing 0.5 M CuSO4 solution began to change color 30 min into the reaction and turned completely dark brown after 1 h. A control experiment performed without the addition of plant extract did not show color change or evidence of CuO NP formation. The FTIR spectrum of the CuO NPs showed the existence of C-O and Hydroxyl functionalities on the NP surface.

8.Conclusion

On the basis of the results of the study, Momordica Dioica extract have significant antioxidant activity. Even though antioxidant activity was low than the standard, its considerably significant. The antioxidant activity may be attributed to flavonoids and other compounds. However, the exact components responsible for the antioxidant activity of both extracts are currently unclear. Therefore, it is suggested that further work be performed on the isolation and identification of these antioxidant components. The antioxidants have potential for application in food, pharmaceuticals and cosmetics.

Results obtained in the present study revealed that the extracts of fruit Momordica Dioica possess potential antibacterial activity against E.coli, S. Aureus, S. Pyogens, S. Typhi, A. Niger and C.albicans. when tested by agar cup method, The dry Momordica Dioica powder in methanol extract showed highest antibacterial activity against all organisms, Momordica Dioica methanol crude extract showed significant antibacterial activity, Pure Momordica Dioica extract showed lowest activity against all organisms and aqueous extract of Momordica Dioica did not showed any activity against all six organisms.

In this work, the AgNPs were synthesized using aqueous extract of M. Dioica fruit. The AgNPs characterized by UV–vis spectroscopy and FTIR. The biosynthesis of AgNPs using green resources like M. Dioica is a good method over chemical synthesis because this method is environment-friendly. M. Dioica extract was prepared and successfully employed for the development of silver nanoparticles. The results showed that the formation of AgNPs were strongly dependent on the process parameters such as silver ion concentration and interaction time of the solution. This simple, low cost and greener method for development of silver nanoparticles may be valuable in biotechnological, biomedical and environmental applications.

An eco-friendly, low-cost biosynthetic method for the preparation of CuO NPs using Momordica Dioica extact is presented in this work. The CuO NPS characterized by uv- vis spectroscopy and FTIR.

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