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A COMPARATIVE INVESTIGATION OF METHANOL AND SILVER NANOPARTICLES EXTRACTION FROM CORIANDRUM SATIVUM SEEDS FOR ANTIDIABETIC POTENTIALITY – *IN-VITRO*

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Keywords:

Coriandrum sativum Linn, AgNP's, antioxidant activity, Anti-inflammatory activity, Alpha- amylase

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ABSTRACT: Botanical name of coriander is *Coriandrum sativum* Linn. *Coriandrum sativum* belongs to the family Umbelliferae which is a highly reputed ayurvedic medicinal tree commonly known as Dhania. Essential oils, flavonoids, fatty acids, and sterols have been isolated from different parts of *C. sativum* so far. The phytochemical analysis of both methanol and AgNP's extract was evaluated. Results revealed the presence of alkaloids, flavonoids, diterpenes, phenol, and phytosterol in the methanol extract, and AgNP's extract contained flavonoids, phenols, and phytosterols. The antioxidant activity of both the extract was measured by the ability to scavenging DPPH, phenols, flavonoids, metal chelating, FRAP and was compared with suitable standards. These assays showed that both the extracts exhibit significant antioxidant activity. The highest percentage of inhibition was given by methanol extract when compared to standards, while in DPPH assay, AgNP's showed the highest inhibition compared to methanol extract. The antibacterial activity of different concentrations of both extracts of *C. sativum* was determined against different bacterial strains. The methanol and AgNP's extraction showed maximum inhibition zone against all the tested bacterial strains at higher concentrations. AgNP's showed the highest inhibition zone compared to methanol extract. The anti-inflammatory activity of both extracts of *C. sativum* was evaluated using suitable standards. The methanol extract showed the highest activity than AgNP's. Alpha-amylase activity did not show any significant inhibition activity with both the extracts. The constituent components were separated in HPLC using a solvent system developed with the aid of TLC. The results of the study support the partial ethano-medical use of *C. sativum* as antibacterial agent.

INTRODUCTION: *Coriandrum sativum* Linn. has been credited with many medicinal properties. The essential oil obtained from its fruits at amounts ranging from approximately 0.5 to 2.5% is used both in flavours and in the manufacture of perfumes and soaps.

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The plant is grown widely all over the world for seed, as a spice, or for essential oil production. It is one of the earliest spices used by mankind. It has been used as a flavoring agent in food products, perfumes and cosmetics. It is used for various purposes such as for flavoring sweets, beverages, tobacco products, and baked goods and as a basic ingredient for curry powder. It has been used as an analgesic, carminative, digestive, anti-rheumatic, and antispasmodic agent. Coriander contains essential oils like borneol and linalool, which aid digestion and proper liver function.

Cineole, borneol, limonene, alpha-pinene, and beta-phellandrene have antibacterial properties. Linoleic acid, oleic acid, palmitic acid, stearic acid, and ascorbic acid (vitamin C) reduce the cholesterol deposition along the inner walls of the arteries and veins. Bio-nanotechnology is regarded as one of the most valuable nanotechnologies in the modern era. It is also an interdisciplinary area related to physics, electronic materials, biology, chemistry, and medicine, which uses these scientific fields to control matter at the molecular scale [1].

Study of Silver Nanoparticles: The importance of nanotechnology in a variety of fields such as food, health care, and feed, cosmetics, biomedical science, environmental health, chemical industries, drug and gene delivery, power science, electronics, mechanics, and space industries, has been already established. It is also extensively been used for the treatments of diabetes, cancer, allergy, inflammation, and infection. In recent years, the trend of the green synthesis of NPs is growing due to its numerous advantages over chemical synthesis. It is also compatible with biomedical and food applications, and this technique eliminates the use of high pressure, temperature, energy, and toxic chemicals. The growing need for environmentally friendly production of nanoparticles forced researchers to choose the greenway for their fabrication. Biological methods are more beneficial than the most popularly used photochemical reduction, chemical reduction, electrochemical reduction, heat evaporation, *etc*.

In biological methods, the plant extract has been used as a reducing and capping agent for the production of nanoparticles due to their reducing properties. The change in properties of the nano-particles, such as size, distribution, and morphology of the nanoparticles are clearly observed with biomaterial. Various nanoparticles like gold, silver, copper, iron, palladium, zinc, quantum dots (CdS, ZnS) are synthesized using a variety of biochemicals. Biosynthesis of silver nanoparticles (AgNP's) was successfully accomplished in the presence of *Prosopis farcta* fruit extract as a reducing agent. Proceeding of the reaction was assessed by using UV–vis spectroscopy [2]. Silver nanoparticles are selected for the study among the above-mentioned nanoparticles because of their several unbeatable properties such as optical, chemical, electronic, photoelectrochemical, catalytic, magnetic, anti-bacterial, and antimicrobial activity. Silver nano-particle acts as antimicrobial agent and can be used in medical applications such as blood collecting vessels, coated capsules, band-aids, biological labeling, *etc*.

Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging, sensing, targeted drug delivery, and gene delivery systems, and artificial implants. Hence, nanosized organic and inorganic particles are finding increasing attention in medical applications due to their amenability to biological functionalization. Based on enhanced effectiveness, the new age drugs are nanoparticles of polymers, metals, or ceramics, which can combat conditions like cancer and fight human pathogens. However, many of these routine methods are very costly and toxic to the environment. On the other hand, the biological method was reported as a clean, non-toxic, and environmentally acceptable route [3].

MATERIALS AND METHODS:

Methanol Extract of *Coriandrum sativum*: Fresh seeds of *C. sativum* were collected from general stores and washed thoroughly. They were shade dried at room temperature for 1-2 days. Then the dried samples were finely powdered and soaked in methanol and then kept for extraction for 24 h in a magnetic stirrer. After proper blending of the extract, it was filtered. The extract was collected and allowed to dry. The dried methanol extract was scraped and stored in screw cap bottles until further analysis.

Synthesis of Silver Nanoparticles Extract: Fresh seeds of *C. sativum* were collected from general stores and washed thoroughly. They were shade dried at room temperature for 1-2 days and soaked in 50 ml of pure deionized water for 24 h. This extract was filtered and centrifuged at 2000 rpm for 20 min. Then aqueous $AgNO_3$ solution (10^{-3}M) was prepared with 250 ml of deionized water. To 2.5 ml of concentrated coriander seed extract 25 ml, $AgNO_3$ solution was added dropwise kept for aging for binding of nanoparticles at room temperature. After aging, the extract was preserved for further analysis. The extraction further will be called

AgNP (silver nanoparticle) as there will be binding of $AgNO_3$.

FIG. 1: A. BEFORE AGEING, B. AFTER AGEING

Phytochemical Analysis: The extracts were further subjected for phytochemical screening.

Test for Alkaloids: To the 5 ml of both methanol and AgNP's extract were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). The formation of a red precipitate indicates the presence of alkaloids.

Test for Tannins (Ferric Chloride Test): To the 5 ml of both methanol and AgNP's extract, add few drops of 1% ferric chloride solution and note the color of reaction. The formation of a Green color precipitate indicates the presence of tannins.

Test for Saponins: About 5 ml of diluted methanol and AgNP's extracts were taken in a test tube and shaken vigorously, and kept for 5 min. The formation of a foamy layer indicates the presence of saponins.

Test for Glycosides: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of Benzene. The benzene layer was separated and treated with ammonia solution. The formation of rose-pink color in the ammoniac layer indicates the presence of glycosides.

Test for Flavonoids: Extracts were treated with few drops of sodium hydroxide solution (0.1N) solution. Formation of intense yellow color, which becomes colorless on addition of dil. HCl indicates the presence of flavonoids.

Test for Protein: The extract was treated with few drops of con. nitric acid. The formation of yellow color indicates the presence of proteins.

Test for Triterpenoids: The extract was treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. The appearance of golden yellow color indicates the presence of triterpenoids.

Test for Phenol: To the extracts, add 3-4 drops of 5% ferric chloride solution and observed the formation of dark blue or blackish color, which may indicate the presence of phenol in the extracts.

Test for Steroids: To the leaf extract, add few drops of acetic anhydride, warmed and cooled under tap water and add few drops of concentrated sulfuric acid and observe the color change violet to green color indicates the presence of steroids.

Test for Terpenoids: About 5ml of each leaf extract was taken, and add 2ml of chloroform and 3ml of concentrated sulfuric acid notice the formation of layer and color. A reddish-brown coloration of the interface confirms the presence of terpenoids.

Antioxidant Assay:

Determination of Total Phenol: The amount of total soluble methanol content in *C. sativum* seeds was determined according to the Folin-Ciocalteu method with slight modifications. Briefly, 10µL of extract solution from the stock solution was mixed with 100 µL of Folin-Ciocalteu reagent. After 10 min of incubation, 300 µL of 20% Na_2CO_3 solution was added, and the volume was adjusted to 1 mL using distilled water. The mixture was incubated in dark for 2 h, and the absorbance was measured at 765 nm using a U-Vis spectrophotometer against a blank sample. The total phenolic content was measured as gallic acid equivalents (mg GAE) /gram of dry weight (dw), and the values were presented as means of triplicate analysis.

Determination of Total Flavonoids Content: The content of flavonoids was determined by a pharmacopeia method using rutin as a reference compound. Briefly, 1 ml of aqueous extract in methanol (mg/ml) was mixed with 1 ml aluminum trichloride in methanol (20 g/l) and diluted with methanol to 25 ml. The absorption at 415 nm was read. Blank samples were prepared from 1 ml plant extract and 1 drop of acetic acid and diluted to 25

ml. A standard graph was constructed using rutin as the reference standard using the above method.

Metal Chelating Assay: The chelation of ferrous ions by extracts was estimated by the method of Dinis. Briefly, 50µl of 2mM $FeCl_2$, 1.6 ml of deionized water was added to 0.5 ml of the extract. The reaction was initiated by the addition of 0.1 ml of 5mM ferrozine solution.

The mixture was vigorously shaken and left to stand at room temperature for 10 min, and then it was observed at 562 nm. The metal chelating activity % was calculated by using the formula.

Ferric Reducing Antioxidant Power (frap) Activity: The FRAP assay was carried out according to Benzie and Strain. Briefly, the working FRAP reagent was prepared by a combination of 300mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in 10:1:1 ratio prior to use and heated to 37 °C in a water bath for 10 min. *Coriandrum sativum* seed extract of various concentration was allowed to react with 0.5 ml of the FRAP reagent.

The final volume of the reaction mixture was made up to 3 ml with DW. The reaction mixture was kept in the dark for 30 min. The readings of the colored product (ferrous tripyridyl-triazine complex) were taken at 593nm. The FRAP values were determined as optical density readings. Higher optical density indicated the higher ferrous reducing power.

Radical Scavenging Activity using DPPH Method: The ability of the seed extracts to scavenge the stable free radical DPPH was assayed by the method of Mensor. DPPH (2,2-diphenyl-2-picryl hydroxyl), a stable free radical, when acted upon by an antioxidant, is converted into diphenyl-picryl hydrazine with a color change from deep violet to light yellow color. This can be quantified spectrophotometrically at 518 nm to indicate the extent of DPPH scavenging activity by the plant extracts.

Reagents: 1. DPPH, **2.** Methanol

In this DPPH assay, 2 mg of DPPH is dissolved in 100 ml of methanol, and it is considered as standard. To clean seven test tubes, add 1 ml of DPPH then add 8 µl, 16 µl, 24 µl, 32 µl, 40 µl, respectively. Incubate for 30 min and record OD at 517 nm using a spectrometer.

Alpha-Amylase Inhibition Assay: The inhibition assay was performed using the DNS method. The assay mixture consisted of methanol extract and made up to 1ml with distilled water. Add alpha-amylase enzyme and incubate for 30 min at room temperature. After incubation, a 1% soluble starch solution is added, and DNS Reagent contains alpha-amylase solution (1 U/mL) and methanol extract at a concentration of 200-1000µg/mL. Once again, the reaction mixture is incubated in boiling water for 5 min. The tubes were cooled, and the absorbance was measured at 540 nm. The reference sample included all other reagents and the enzyme with the exception of the test sample. The alpha-amylase inhibitory activity was expressed as percentage inhibition.

Anti-Inflammatory Assay: The anti-inflammatory assay was performed using phosphate buffer saline. The assay mixture consisted of 0.2 ml of bovine albumin in each tube, 2.8 ml of phosphate buffer saline, methanol extract with distilled water which was made up to 2ml, was added. This mixture was incubated at 37 °C for 15 min then again incubate this mixture at 70 °C for 5 min. Tubes were then cooled and measure OD under 660 nm.

Estimation of Protein by Lowry's Method: Pipette out the amount of 0.0, 0.4, 0.8, 1.2, 1.6, and 2 ml of the prepared working protein standard solution into the clean and dry test tubes. The volume was made up to 2 ml by adding the respective volume of distilled water. About 2 ml of the prepared reagent A was added to all the test tubes, mixed well, and allowed the tubes undisturbed for 10 min. After incubation, reagent B of 0.2 ml was added to all the test tubes. Mix the tubes thoroughly and was incubated in the dark for 30 min. After incubation, the absorbance of all the tubes was read at 660 nm against a blank.

Antibacterial Assay: The isolated phytochemical fractions were assessed for their antibacterial activity against the pathogenic bacteria.

Test Organisms: Of all, six bacterial strains were used throughout the investigation *viz*., *Klebsiella* sp., *Proteus vulgaris*, *E. coli*, *Enterobacter aerogenes*, *Bacillus cereus*, and *Staphylococcus*

aureus. Fresh young bacterial broth cultures were prepared before the screening procedure [4].

Preparation of Inoculums: Stock cultures were maintained at 4 °C on slants of nutrient agar. Active cultures for the experiment were prepared by transferring a loop full of cells from the stock cultures to a test tube of nutrient broth and were incubated for 24 h at 37 °C.

Disc Diffusion Method: Paper discs impregnated with specific antibiotics and the test substances are placed on the surface of the Nutrient agar medium inoculated with the target organisms. The plates are incubated and the zones of inhibition around each disc are measured.

Thin Layer Chromatography: The pre-coated silica gel thin-layer chromatogram sheet was used solely for TLC. The methanol and AgNP's extract was spotted at 2 cm from the edge of the sheet. The chromatogram is developed with a mixture of a suitable solvent system and dried at room temperature. The spots were visualized in an iodine chamber. R_f value of the colored spots was recorded.

A sample of 10 mg/ml was prepared; 2.5 µl of samples were spotted on a TLC plate and allowed to dry. A TLC plate is made up of a thin layer of silica gel 0.25mm with fluorescent indicator F_{254} with solvent system chloroform: ethanol (9.5:0.5) was used for TLC analysis. The strip or plate is then placed with this end dipping into the solvent mixture, taking care that the sample spot/zone is not immersed in the solvent. As the solvent moves towards the other end of the strip, the test mixture separates into various components. This is called the development of TLC plates. The separation depends on several factors, the plate is removed after an optimal development time and dried, and the spots/zones are detected using UV chamber, and the R_f value is calculated using

R_f = Distance moved by compound/distance moved by solvent

High-Performance Liquid Chromatography:

- Instrument: HPLC
- Column: C18 column 250 mm × 4.6 mm, 5u particle
- Mobile Phase: Linear
  - **A.** HPLC grade ACN (30%)
  - **B.** HPLC grade water (70%)
- Flow Rate: 1ml/min
- Injection volume: 10µl
- Absorbance: 276 nm

RESULTS AND DISCUSSION:

Phytochemicals: Natural phenolics exert their beneficial health effects mainly through their antioxidant activity. The importance of medicinal plants in drug development is known to us, and humans have used them for different diseases from the beginning of human history [5, 6]. These compounds are capable of decreasing oxygen concentration intercepting singlet oxygen, preventing 1st chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non-radical species, and breaking chains to prevent continued hydrogen abstraction from substances. Phenolic compounds contribute to the overall antioxidant activities of plant foods **Table 1** and **2**.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF *CORIANDRUM SATIVUM* SEEDS EXTRACT (METHANOL EXTRACT)

S. no.	Phytochemicals	Observation	Result
1	Alkaloids	Formation of Green color	Presence of Alkaloids
2	Tannins	Formation of Green color	Absence of Tannins
3	Saponins	Formation of foamy layer	Absence of Saponins
4	Glycosides	Formation of rose-pink color in the ammoniacal layer	Absence of Glycosides
5	Flavonoids	Formation of intense yellow color	Presence of Flavonoids
6	Proteins	Formation of yellow color	Absence of Proteins
7	Diterpenes	Formation of emerald green color	Presence of Diterpenes
8	Phenols	Formation of dark blackish color	Presence of Phenols
9	Phytosterols	Formation of golden yellow color	Presence of phytosterol
10	Carbohydrates	Formation of orange red precipitate	Absence of Carbohydrates

By this phytochemical analysis, the methanolic extract of *C. sativum* seed showed the presence of alkaloids, flavonoids, diterpenes, phenol and phytosterols.

TABLE 2: PHYTOCHEMICAL ANALYSIS OF *CORIANDRUM SATIVUM* SEEDS EXTRACT (AGNPS EXTRACT)

S. no.	Phytochemicals	Observation	Result
1	Alkaloids	Formation of Green colour	Absence of Alkaloids
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9	Phytosterols	Formation of golden yellow colour	Presence of Phytosterols
10	Carbohydrates	Formation of orange red precipitate	Absence of Carbohydrates

By this phytochemical analysis, in the AgNPs extract of *C. sativum* seed contained flavonoids, phenols and phytosterols. Biological synthesis or green synthesis of nanoparticles is an alternative and eco-friendly method for production of nanoparticles [7, 8].

Antioxidant Assay:

Determination of Total Phenolic Content: Major role of phenols in scavenging the free radicals is due to the presence of hydroxyl groups. Anti-oxidant activity of the extract is proportional to the amount of phenol content present in the extract.

Several studies on polyphenolic compounds protecting from mutagenesis and carcinogenesis are reported. Phenolic content in the methonolic and AgNPs extract of *C. sativum* was assessed using gallic acid as standard.

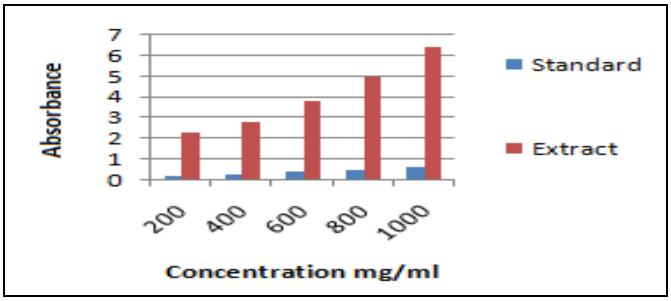


FIG. 2: TOTAL PHENOLIC ASSAY (METHANOL EXTRACT)

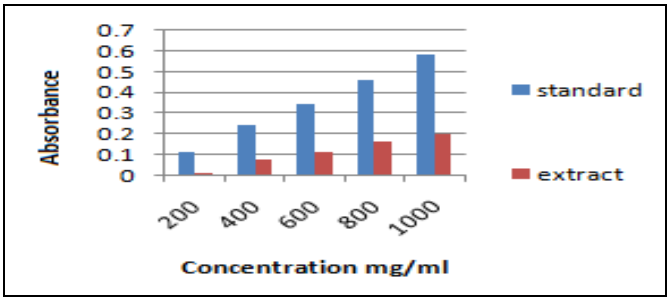


FIG. 3: TOTAL PHENOLIC ASSAY (AgNPs EXTRACT)

When compared to standard, methanolic extract showed high activity; whereas, AgNPs extract showed less activity compared to standard.

Determination of Total Flavonoids Content: Total flavonoid content was determined using $NaNO_2$ and $AlCl_3.6H_2O$, and result was expressed as mg quercetin equivalents/g both methanol and $AgNO_3$ extract content was determined using rutin reagent, and absorbance was recorded at 490 nm.

The total methanol and $AgNO_3$ extract content was then determined from a rutin standard curve, and result was expressed as mg methanol and extract equivalent/g DM.

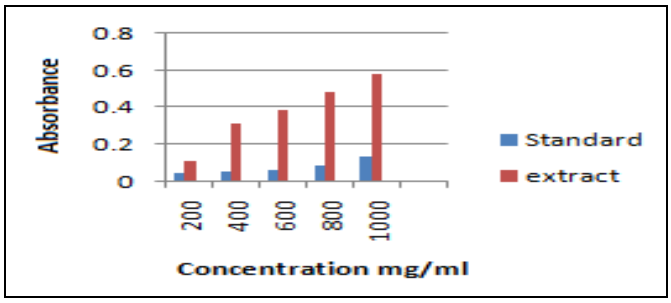


FIG. 4: TOTAL FLAVONOIDS (METHANOL EXTRACT)

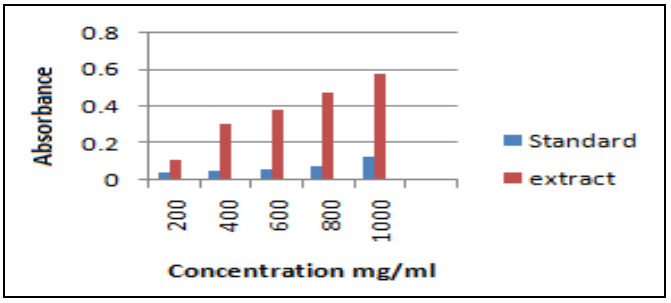


FIG. 5: TOTAL FLAVONOIDS (AgNPs)

In both the extracts, the extractions showed higher concentration than standard.

Metal Chelating Activities: As excess free irons have been implicated in the induction and formation of free radicals in biological systems, we tested our medicinal plant extracts in a metal chelating assay. Tested in the concentration range of 0.5 to 1.5 mg/mL, which showed strong chelating activities in concentration-dependent manners. Here, the concentration of methanol and $AgNo_3$ extract (*C. sativum*) increased the percentage of metal chelating assay up to 50-75% but not as much as standard ferrozine.

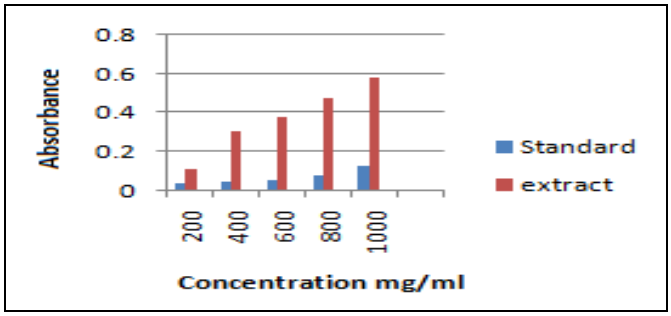


FIG. 6: METAL CHELATING (METHANOL EXTRACT)

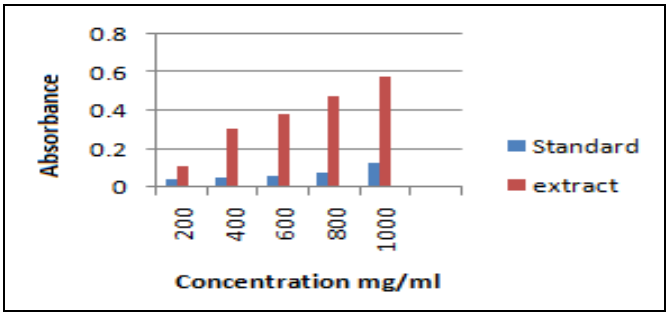


FIG. 7: METAL CHELATING (AgNPs)

In both the extracts, methanol extract, and AgNPs extract, the extractions showed higher concentration than standard.

Ferric Reducing Antioxidant Power (FRAP) Activity: The FRAP assay gives fast and reproducible results. The dose-response characteristics of different antioxidants showed different activities with methanol and $AgNo_3$ extract, but the dose-response of each individual antioxidant tested was linear, showing that activity is not concentration-dependent, at least over the concentration ranges tested in this study.

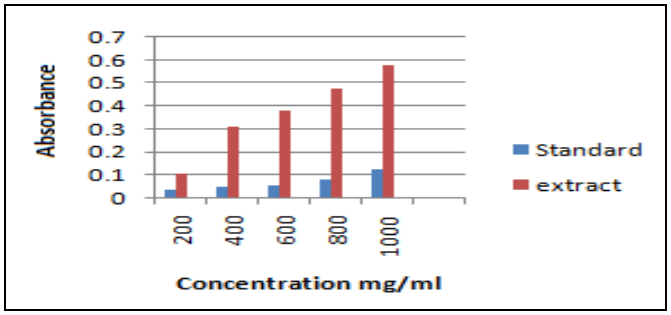


FIG. 8: FRAP (METHANOL EXTRACT)

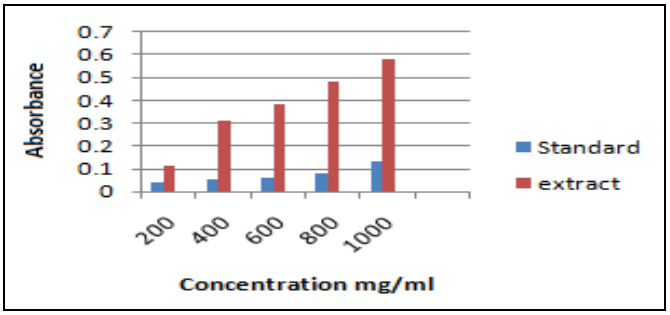


FIG. 9: FRAP (AgNPs)

Both the extracts were observed to show high absorbance when compared to standard.

DPPH Radical Scaven0ging Activity: In DPPH radical scavenging assay, all plant extracts exhibited scavenging activities in a concentration-dependent manner, in the range of 20-100 µg/mL. Here, the concentration of methanol extract increased the percentage of scavenging up to 20-90%.

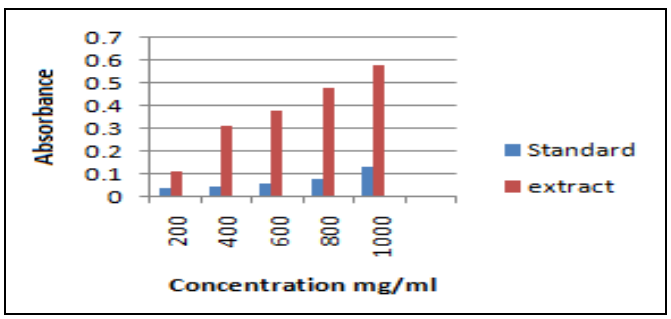


FIG. 10: DPPH (METHANOL EXTRACT)

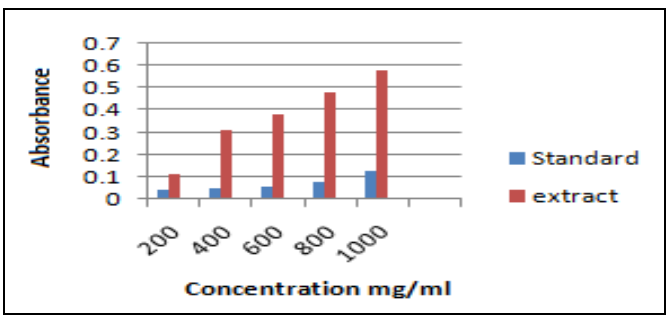


FIG. 11: DPPH (AgNPs)

Both the extractions showed higher concentration than standard.

Alpha-Amylase Inhibition Assay: The carbohydrates digestive enzymes are hydrolyzed by pancreatic α-amylase and liable for the breakdown of oligosaccharides and disaccharides into mono-saccharides suitable for absorption. The inhibition of these digestive enzymes is specifically useful for the treatment of non insulin diabetes because it will slow down the release of glucose in the blood. The results indicated that α-amylase was significantly inhibited in a concentration-dependent manner following incubation with various concentrations of methanol extract. With the increasing concentration of methanol extract level, the enzymatic activity level was not reduced. Since alpha-amylase is not inhibiting by methanol extract of *C. sativum* it is not used for the treatment of diabetes.

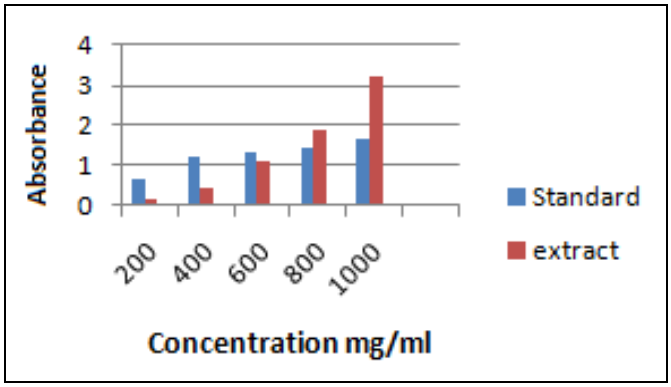


FIG. 12: α-AMYLASE INHIBITION ASSAY (METHANOL EXTRACT)

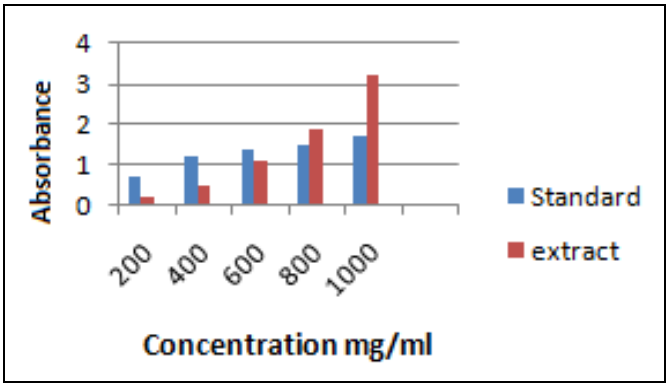


FIG. 13: α-AMYLASE INHIBITION ASSAY (AgNPs EXTRACT)

The results indicate that α- amylase was not significantly inhibited in a concentration-dependent manner following inhibition with various concentration of methanol and AgNPs. The increasing concentration of both extract levels did not reduce the enzyme activity level, and hence, these extracts are not approachable for diabetic care.

Anti-Inflammatory Assay: The anti-inflammatory effects of herbal extracts emphasized different assays that are frequently used to test the in-vitro anti-inflammatory activity of herbal constituents. Protein denaturation assays and membrane stabilization assays are frequently used to evaluate *in-vitro* anti-inflammatory activities.

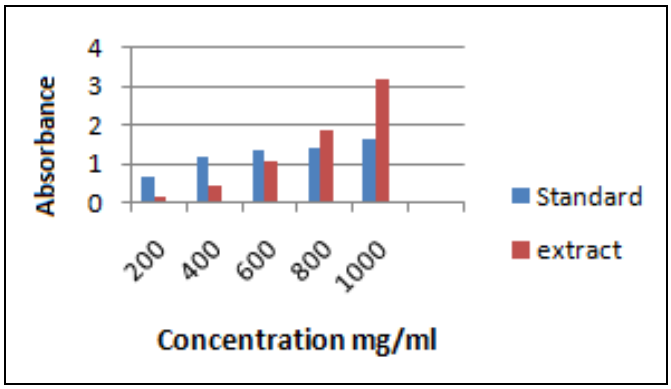


FIG. 14: ANTI-INFLAMMATORY ACTIVITY (METHANOL EXTRACT)

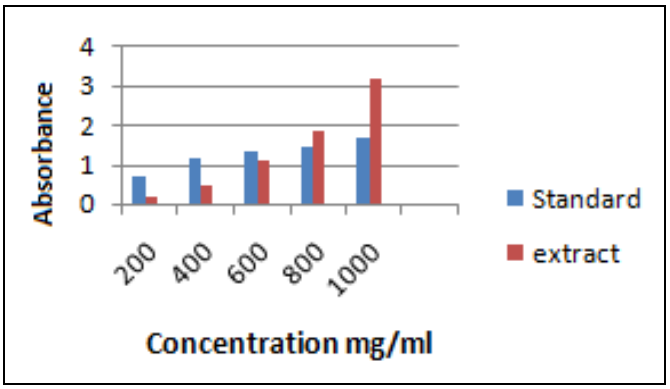


FIG. 15: ANTI-INFLAMMATORY ACTIVITY (AgNPs EXTRACT)

The methanolic extract shows the highest activity compared to AgNPs. Hence, methanol extract of coriander seeds approach for anti-inflammatory activity can be considered.

Estimation of Protein By Lowry's Method: Lowry's method enhances the sensitivity of the biuret method. Protein reacts with FC reagent to give a blue-colored complex. The color so formed is due to the reaction of alkaline copper with the protein as in the biuret test and the reduction of phospomolybdic and phospohengestic components in FC by the amino acids tyrosine and tryptophan present in the protein. The intensity of the blue color is measured calorimetrically at 660 nm. The intensity of the color depends on the amount of these aromatic acids present.

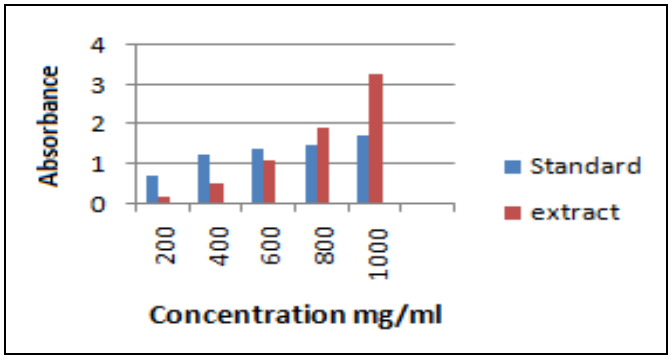


FIG. 16: PROTEIN ESTIMATION (METHANOL EXTRACT)

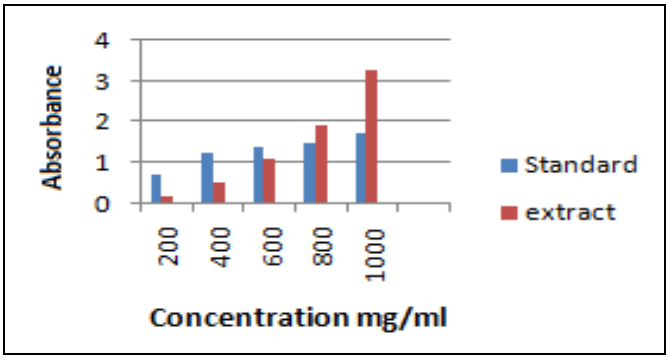


FIG. 17: PROTEIN ESTIMATION (AgNPs EXTRACT)

Phenols were present in higher concentrations in 200, 400, and 800µl for both methanol and AgNPs extracts showed less absorbance; whereas, for 800 and 1000 µl concentration extracts showed high absorbance.

Antimicrobial Activity of Two Extract of Coriander Seeds:

Disc Diffusion Method: In disc diffusion method, the methanolic extract of *C. sativum* was tested for its antimicrobial activity. The antimicrobial was determined by the disc diffusion method. There was the formation of inhibition zone against the test organisms like *S. aureus*, *E. coli* and *P. vulgaris.*

TABLE 3: ANTIMICROBIAL ACTIVITY IN METHANOL EXTRACT BY DISC DIFFUSION METHOD

Bacterial Strains	Inhibition zone diameter in cm				
	0.2 ml	0.4 ml	0.6 ml	0.8 ml	1 ml
S.aureus	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-
E. aerogens	-	-	-	-	-
Bacillus cereus	-	-	-	-	-
Escherichia coli	-	-	-	-	-
Klebsiella sp.	0.5	-	1	-	-

FIG. 18: *KLEBSIELLA* SP.

TABLE 4: ANTIMICROBIAL ACTIVITY IN AgNPs EXTRACT BY DISC DIFFUSION METHOD

Bacterial Strains	Inhibition zone diameter in cm				
	0.2 ml	0.4 ml	0.6 ml	0.8 ml	1 ml
Staphylococcus aureus	0.6	0.5	0.5	0.6	0.6
Proteus vulgaris	0.2	0.3	0.3	0.2	0.1
E. aerogens	0.2	0.3	0.3	0.3	0.4
Bacillus cereus	0.4	0.3	0.3	0.4	0.3
Escherichia coli	0.6	0.7	0.6	0.6	0.5
Klebsiella sp.	0.3	0.2	0.2	0.2	0.2

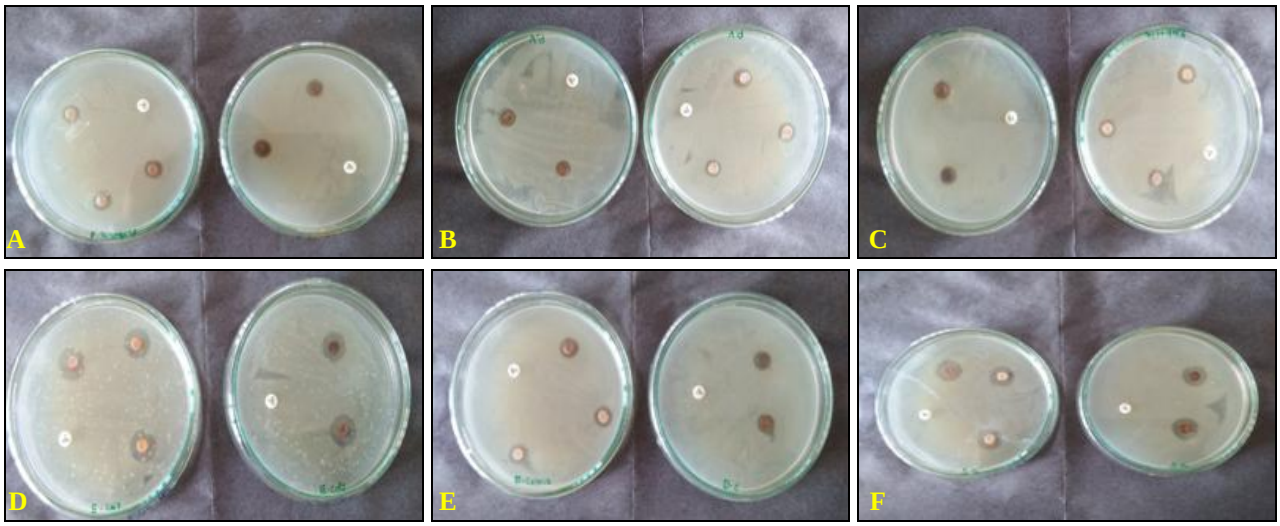


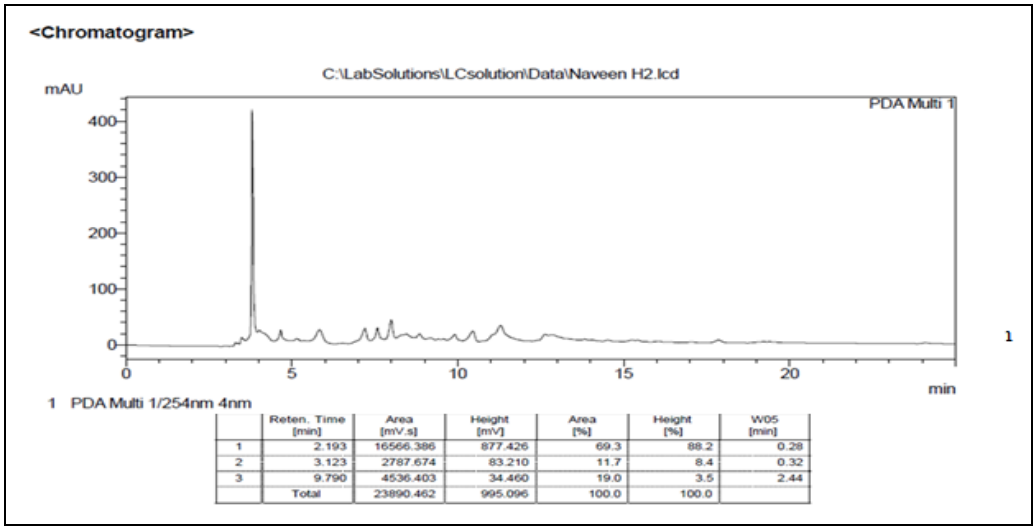
FIG. 19: A. *E. AEROGENS*, B. *PROTEUS VULGARIS*, C. *KLEBSIELLA* SP., D. *ESCHERICHIA COLI*, E. *BACILLUS CEREUS*, F. *STAPHYLOCOCCUS AUREUS*

In these studies, methanol extract showed slight inhibition for *Klebsiella* sp. and silver nanoparticles showed maximum inhibition against all the tested bacterial strains at higher concentrations **Table 3** and **4**.

Thin Layer Chromatography: TLC plate shows the band at 366 nm, indicating the presence of the unknown compound in methanol extract. Retention factor is a parameter that determines the polarity of compound moved by mobile phase, in range on R_f value 0.55 of one compound and another compound is 0.49 **Fig. 20**.

FIG. 20: TLC CHROMATOGRAM AT 366 WAVE CRUDE, (LANE 1- METHANOL EXTRACT), (LANE 2 – AgNP EXTRACT)

High-Performance Liquid Chromatography:



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.193	16566.386	877.426	69.3	88.2	0.28
2	3.123	2787.674	83.210	11.7	8.4	0.32
3	9.790	4536.403	34.460	19.0	3.5	2.44
	Total	23890.462	995.096	100.0	100.0	

FIG. 21: HPLC CHROMATOGRAM OF AgNP EXTRACT

Based on the HPLC analyses, one of the phenolic compounds show the highest purity in this sample, *i.e*., 69% at 2.29 min and area mVs showing 16566 **Fig. 21**.

CONCLUSION: Several investigations so far have been directed towards *C. sativum* uses as a therapeutic agent. This study attempted to compare the efficacy of both methanolic extract and AgNPs by performing phyto-chemical, antioxidant, α-amylase activity, and anti-inflammatory protein estimation and also antibacterial activity by well diffusion method. Results revealed that both the extracts had almost similar efficacy; specifically in α-amylase activity, none of the extracts showed any reduction of the enzyme. A probable aspect can be that these extracts cannot completely be targeted for diabetic cure therapy or further intensive work needs to be done. But in antibacterial activity, AgNP showed an inhibition zone against all the test organisms, and HPLC showed a peak for the same. Summing up, an intense study is required to develop the extracts for therapeutic use.

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CONFLICTS OF INTEREST: We declare that there is no conflict of interest.

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