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ANTIOXIDANT POTENTIAL OF SILVER NANOPARTICLES USING LEAVES OF "*CYMBOPOGAN FLEXUOSUS*"

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Abstract: Phytochemicals are multipurpose secondary metabolites of plants that have medicinal qualities. In present research work we examined the various phytoconstituents present in lemongrass (*Cymbopogan flexuosus*), its pharmacological potential including antioxidant potential. Lemongrass is well known for its hypoglycemic and hypolipidemic effects as well as its anti-oxidant, anti-microbial, anti-inflammatory, anti-hypertensive, anti-diabetic, anti-mutagenicity, and anxiolytic qualities. As a result, it is extensively utilized in the food, feed, cosmetics, and pharmaceutical industries. Strong bioactive components found in lemongrass include terpenoids, alkaloids, and phenolic metabolites, which include phenolic acids, flavonoids, stilbenes, and lignans. Lemongrass commonly used to enhance health by creating a balanced intestinal environment. At the industrial level, lemongrass is regarded as a fundamental food and feed ingredient because it doesn't have any toxicity or residual problems. By producing antimicrobial, anti-inflammatory, and antioxidant reactions, lemongrass powder and essential oils are utilized to modify the gut environment and improve the gut system's capacity to absorb nutrients.

Index Terms **-Silver Nano particles, Antioxidant potential, lemongrass**

1. INTRODUCTION

Lemon grass is commonly known as citronella grass belonging to family Poaceae. The grass is native to India, Shri Lanka, Pakistan along with found in America, Africa and Asia. It having hight about 3 metres, grows in bunches with strong base. The leaves are simple, tightly clasping at the base with sharp margin .Lemongrass is characterized by distinct lemon like odor. The aroma of the grass is due to presence of essential oil, citral monoterpenes [1]. Due to presence of aroma the grass is used in cooking, in preparation of soaps, teas, beverage, aroma therapy, and cosmetic and perfumery industry. Due to multiple bioactive it has been used as anti-fungal, anti-bacterial, anti-oxidant, anti-diarrhoeal, anti-inflammatory, anti-malarial, cardio protective and anti-cancer etc. [2]. All these reported pharmacological actions of the drug are due to presence of various secondary metabolites are Phyto steroids, phenolic compounds, amino acids, organic acids, and volatile compounds. The essential oil extracted from lemongrass contains citral as a chief constituent along with linalool, geraniol, limonene, myrcene, citronellal, etc. Among all geraniol and linalool has good anti-oxidant property.

The aldehydes present in lemon grass oil are citral, Geranial, and Neral which are used in aromatherapy while luteolin is a flavonoid which is responsible for anti-inflammatory and anti-oxidant property [3]. Considering all the applications, sliver nanoparticles synthesized using leaves of lemongrass will be utilized for multiple disorders. Utilization of all these bioactive constituents present in the lemongrass for synthesis of silver nanoparticles will becomes a cost effective and eco-friendly method [4]. Metal nanoparticles are useful due to its distinct physical and optical properties, whereas silver nanoparticles have been used for diagnostic purpose, biomedical device coatings or as a drug delivery carriers, implants [5]. Chemical, physical as well as plan mediated synthesis are well known methods for synthesize silver nanoparticles. Physical approaches include evaporation-condensation method while chemical approach includes grinding of metal by mechanical means or microwave assisted synthesis can be the alternative method [6].Chemical method of synthesis is associated with the adverse effects while physical method requires high input and it is costlier hence plant mediated synthesis is better option. Silver nanoparticles prepared from herbal material having multiple pharmacological effects. Plants secondary metabolites like flavonoids and phenolic compounds are commonly used to prepare silver nanoparticles due to its reducing, stabilization action along with they act as a good capping agent during conversion of silver ions to silver nanoparticles. Nanoparticles prepared from herbal material reported to have applications in healthcare, food, and textile and cosmetic industry [7].

2. MATERIAL AND METHOD

2.1 Plant Material

Fresh lemongrass leaves were harvested from Chikhali village from Kolhapur district. The collected leaves were shade dried for five days to reduce moisture. The dried plant material is then converted into small pieces for extraction.

2.2 Extraction method

Hydro-distillation method was used to separate the lemongrass oil from the plant material where plant material is heated with water to produce steam, the essential, oil is collected through condenser and oil-water mixture was collected and is separated by decantation [8].

2.3 Phytochemical screening

Phytochemical screening of plant material was done for presence of secondary metabolites. The standard protocol was utilized for analysis of the extract for the presence of secondary metabolites [9, 10].

2.3.1 Test for phytosterols

Salkowski's test: To 1ml of filtrate add few drops of con. Sulphuric acid. Shaken well and allowed to stand. Formation of red color in lower layer indicates presence of phytosterols.

Sulphur test: To the required extract add pinch of sulphur powder. If sulphur sinks at the bottom indicates presence of phytosterols.

Hesse's response: To the 5 ml extract add 2ml chloroform and 2ml con. Sulphuric acid. Formation of pink ring or red colour in lower chloroform layer indicates presence of phytosterols.

2.3.2 Test for alkaloids

Mayers test: To 1 ml of extract add 1ml of conc. HCl followed by few drops of Mayers reagent. Formation of green or white precipitate indicated presence of alkaloids.

2.3.3 Test for flavonoids

Lead acetate test: To 1 ml of extract add 1ml of 10%laed acetate solution. Formation of yellow color precipitate indicates presence of flavonoids.

2.3.4 Test for tannins

Gelatine test: Extract is dissolved in required quantity of water to this add 1% gelatine solution and 10%sodium chloride. Formation of white color precipitate indicates presence of tannins.

2.3.5 Test for saponins

Foam test: To 1ml of extract add 1ml of distilled water and shake it vigorously. Formation of foam indicates the presence of saponins.

2.3.6 Test for Terpenoids

Ferric chloride test: To 1ml of extract add 2ml of water and 1ml of 10% ferric chloride solution. Formation of intense color indicates presence of terpenoids.

2.4 FTIR spectroscopy of essential oil

The required quantity of sample of essential oil was placed in cleaned surface in contact with the ATR at ambient temperature. All spectra were background corrected by air spectrum. Spectrum were recorded in the rage of 600-4000cm-1.

2.5 Preparation of silver nanoparticles

The required quantity of leaf extract was mixed with the 0.2M solution of silver nitrate. The resulting solution is mixed using magnetic stirrer at 10,000rpm and heated up to 400C for 2hrs. The solution was incubated in dark condition for 24 hrs until colour change to dark brown. The residual was transferred into centrifuge flask and centrifuged at room temperature of 933 pm subsequently the black deposits obtain. Then the sediment layer obtain after centrifugation can be sonicated by using ultrasonicator [11].

2.6 Evaluation of silver nanoparticles

2.6.1 UV - Spectroscopy

The prepared silver nanoparticles were analyzed using UV visible spectroscopy to identify surface Plasmon resonance peek. Further nanoparticle size and shape effects on surface Plasmon resonance was determined and concentration and stability were assessed.

2.6.2 Zeta potential

Suspension of resulting nanoparticles was prepared which was used to determine zeta potential by electrophoretic light scattering (ELS) method.

2.6.3 Size Distribution

Suspension of resulting nanoparticles was prepared and analyzed by using Dynamic Light Scattering (DLS) method to determine size of the prepared silver nanoparticles.

2.6.4 Determination of Anti-Oxidant Activity

Antioxidant activity in the sample compound was estimated for their free radical scavenging activity by using DPPH (1,1-Diphenyl-2Picryl-Hydrazyl) free radicals 100 microliters of 0.1% methanolic DPPH was added over the samples at different concentration (1000micro gm/ml) & incubated for 30 min in dark condition. The samples were then observed for discoloration; from purple to yellow & pale pink were considered as strong & weak positive respectively. Plate was read on Elisa plate reader at 490nm Radical scavenging activity was calculated by the following equation.

DPPH radical scavenging activity (%):

[(Absorbance of control - Absorbance of test sample)/ (Absorbance of control)] × 100

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening

Table 1: Results of phytochemical screening

Phytoconstituents	Qualitative test	Observation
Phytosterols	Salkowski's Test	Red color
	Sulphur test	Sulphur sink in bottom
	Hesse's response	Pink /Red ring (in lower chloroform layer)
Test for alkaloids	Mayers test	White precipitate
Flavonoids	Alkaline reagent Test	An intense yellow color becomes colorless on addition of diluted acid
	Lead acetate test	A yellow fluorescence
	Ferric chloride test	A green precipitate
	Pew's test	A red color
	Conc.H2SO4 test	An orange color
Tannin	Gelatin Test	A white precipitation
Terpenoids	2ml chloroform+5ml plant extract(evaporated on water bath)+3ml conc. H2SO4(boiled on water bath)	Grey color solution

3.2 FTIR spectroscopy of essential oil

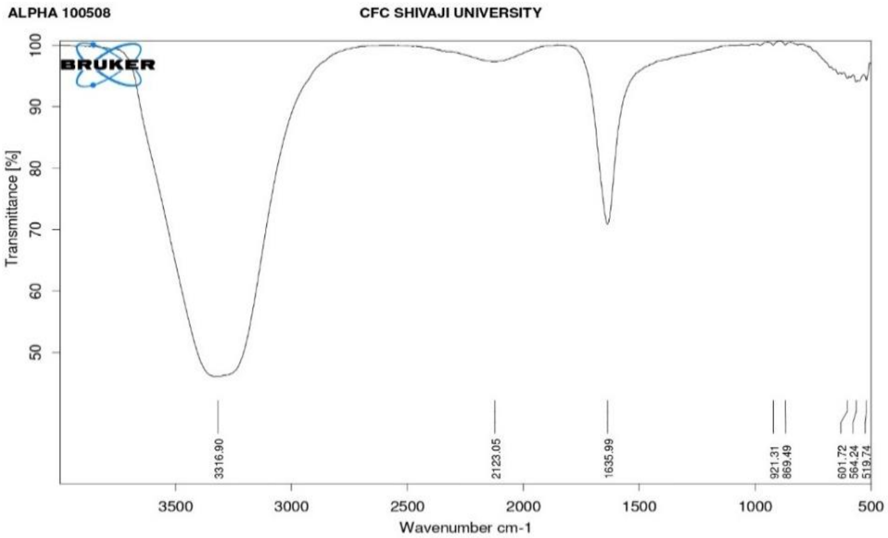

Fig No.1: FTIR spectroscopy of essential oil

3.3 Evaluation of silver nanoparticles

3.3.1 UV-Spectroscopy

Table 2: Conc. and absorbance

Sr. No	Conc.	Abs.
1	0.2	0.0009
2	0.4	0.0012
3	0.6	0.0038
4	0.8	0.0042
5	1	0.0052

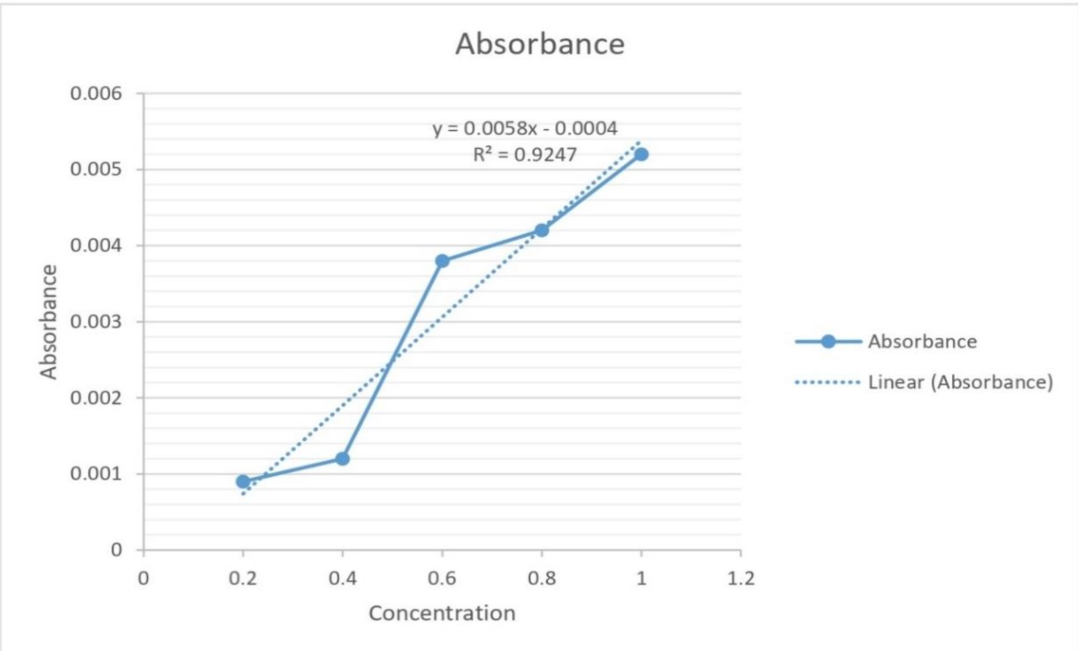

Fig. No.2: UV Spectrum of silver nanoparticles of *Cymbopogan flexuosus*

3.3.2 Zeta potential

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-23.4	Peak 1:	-23.4	100.0	7.71
Zeta Deviation (mV):	7.71	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.421	Peak 3:	0.00	0.0	0.00
Result quality :	Good				

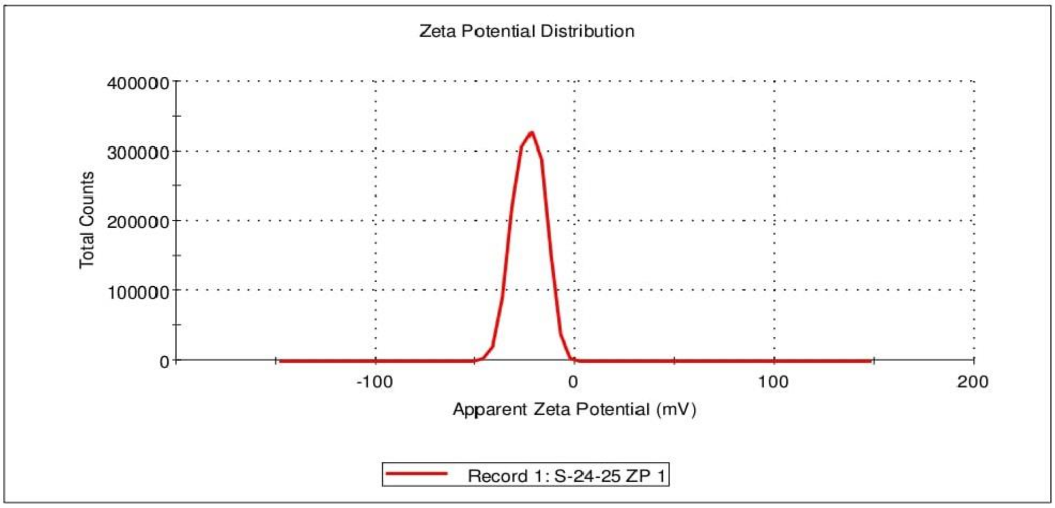

Fig No.3: Zeta Potential of silver nanoparticles of *Cymbopogan flexuosus*

3.3.3 Size Distribution

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	139.1	Peak 1:	147.6	88.2	35.67
PdI:	0.447	Peak 2:	0.9880	6.5	0.05468
Intercept:	0.827	Peak 3:	5021	5.3	596.2
Result quality :	Refer to quality report				

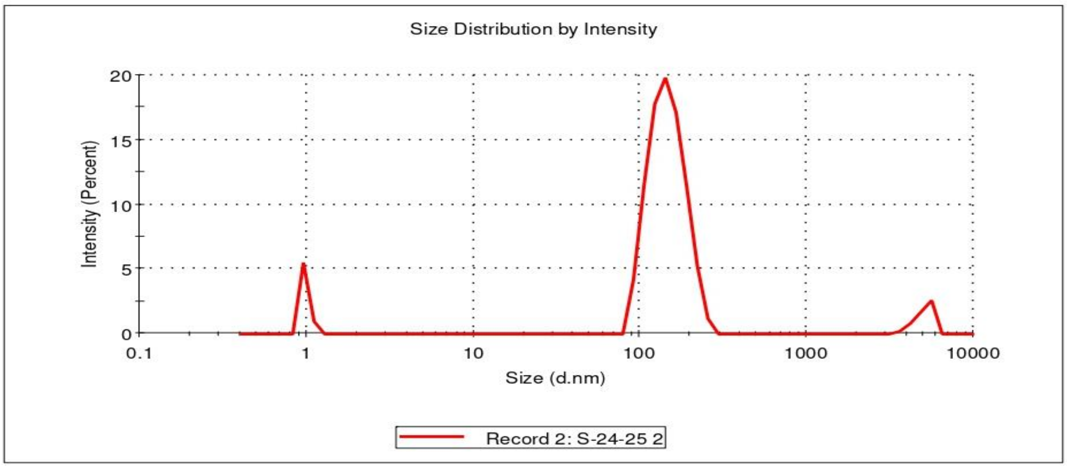

Fig No.4: Zeta Potential of silver nanoparticles of *Cymbopogan flexuosus*

3.3.4 Determination of Anti-Oxidant Activity

ASCORBIC ACID

Table 3: Conc. Vs % inhibition

Concentration	Sample Readings			Average	% Inhibition
20	0.775	0.753	0.778	0.769	22.59147
40	0.711	0.721	0.742	0.725	27.02249
60	0.615	0.66	0.684	0.653	34.23967
80	0.555	0.579	0.585	0.573	42.29607
100	0.5	0.516	0.516	0.511	48.57334

CONTROL SAMPLE

Table 4: Conc. Vs % inhibition

Concentration	Sample Readings			Average	% Inhibition
1:1	0.435	0.427	0.494	0.452	54.48136
1:2	0.314	0.312	0.304	0.310	68.78147
1:3	0.231	0.236	0.232	0.233	76.53575
	0.111	0.111	0.109	0.110	88.88888

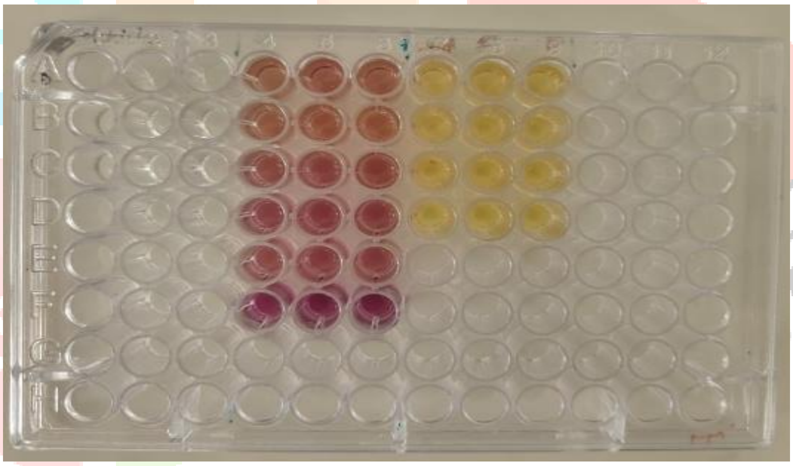

Fig No.5: Inhibition

4. CONCLUSION

Cymbopogan flexuosus is a plant used in folk medicine for its fragrance, flavoring, and antispasmodic properties. It also has antibacterial, antidiarrheal, and antioxidant properties. The plant contains phytoconstituents like flavonoids, phenolic compounds, terpenoids, and essential oils, which could be used to synthesize novel therapeutic agents. This study aims to provide a comprehensive understanding of the antioxidant effect of *Cymbopogan flexuosus.* The finding of this study can be used to optimize the method of preparation of silver nanoparticles with better antioxidant property. Also, these findings may support that silver nanoparticles could enhance its therapeutic potential and overcome problems related to utilization of herbal drugs.

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