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Research Article

PHYTOCHEMICAL SCREENING AND GAS CHROMATOGRAPHY-MASS SPECTROSCOPY ANALYSIS OF BIOACTIVE COMPOUNDS AND BIOSYNTHESIS OF SILVER NANOPARTICLES USING SPROUT EXTRACTS OF *VIGNA RADIATA L.* AND THEIR ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY

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ABSTRACT

Objectives: The present study was aimed to investigate the facile synthesis of silver nanoparticles (AgNPs) using the green gram sprout extract (GGSE) of *Vigna radiata L.* and also *in vitro* studies of antioxidant and antimicrobial activities.

Methods: Gas chromatography-mass spectroscopy techniques have been used for the qualitative and quantitative evaluation of the phytochemicals present in the green gram seedlings. The antioxidant activity of AgNPs and GGSE was analyzed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. *In vitro* antibacterial activity was performed using the agar well diffusion method.

Results: The presence of various secondary metabolites such as flavonoids, steroids, terpenoids, alkaloids, amino acids, polyphenol, glycoside, and protein was found in samples. The major chemical compounds of *V. radiata* were n-hexadecanoic acid, stigmasterol, caffeine, hexadecanoic acid, cholest-5-en-3-ol (3.beta.)-, and cyclopentane. The percentage of DPPH activity was enhanced on increasing the concentration of AgNPs. *In vitro* antibacterial effect of the diverse concentrations of AgNPs was investigated against each Gram-negative (*Klebsiella aerogenes*, and *Escherichia coli*) and Gram-positive (*Bacillus substilis* and *Staphylococcus aureus*) bacterial strains.

Conclusion: The result suggests that biosynthesized AgNPs have good antibacterial and antioxidant activity and might be a potential for the bioactive components.

Keywords: *Vigna radiata*, Green synthesis, Silver nanoparticles, Gas chromatography-mass spectroscopy, 1,1-Diphenyl-2-picrylhydrazyl, Antibacterial activity.

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INTRODUCTION

Nanoscience research shows exponential growth, especially with potential applications in biomedical science and technology. Currently, metallic nanoparticles such as silver, gold, and platinum are focused much for specific optical, mechanical, and chemical properties than bulk metal [1]. Among these metal nanoparticles, silver plays a key role in fabric and pharmaceutical industries [2]. A wide spectrum of methods to synthesize AgNP is well documented. For example, distinct electrochemical method, sono-chemical, radiation, microwave assisted method, opposite micelles technique, segment transfer procedure, photochemical synthesis, organic strategies [3-10]. These methods involve the use of toxic substance and pose serious environmental and health problem. In recent years, metal nanoparticles protected by bioorganic ligand are involved in a great deal due to their widespread applications [11].

In current years, the facile synthesis of AgNPs with the aid of biologically active compounds has been extensively explored due to its easy availability and eco-friendliness [12]. Biosynthesized silver nanoparticle (AgNP) using *Eucalyptus chapmaniana* [13], *Terminalia bellirica* [14], *Acalypha indica* [15], and *Cynodon dactylon* [16] plant extracts has been acknowledged, and the applications of AgNPs in the detection of cancer, combating of microbes, bio-labeling, and drug transport are reported [17]. Biosynthesized nanoparticles are used in water filters, textile, and food industries due to their antimicrobial activities [18].

In the past few years, gas chromatography-mass spectrometry (GC-MS) has become a versatile tool for secondary metabolite profiling in both plant and non-plant species [19,20]. GC-MS analysis provides accurate results of analyte even at low concentrations. GC-MS analyses are extensively utilized in forensic science, elemental analysis, and pollution research [21]. In recent times, the bioactive additives tagged with non-polar, volatile substances, alkaloids, phenols, long chain and branched-chain hydrocarbons, alcohols, acids, esters, and different biologically energetic additives are being separated using GC-MS [22,23]. AgNPs prepared using green gram sprout extract (GGSE) (Leguminosae family) and characterized by UV-spectrum, X-ray diffraction, Fourier-transform infrared, standard error of the mean, and tested on antimicrobial activity using some microbes were published by our research group [24]. In this work, the author reported the phytochemical analysis, GC-MS, and antioxidant activity of AgNPs.

METHODS

Preparation of GGSE

Quality seeds of *Vigna radiata* (green gram) obtained from the agro seed shop were washed and covered in a smooth moist cloth. The seeds began to sprout after germination time of 1–2 days. The sprouts were gathered and dried in the shade at room temperature (30°C). Electric blender was used to powder the dried sprouts. Cloth sieve is used to collect the fine powder of GGSE and is collected in airtight container for further studies.

Chemicals and reagents

Ascorbic acid, methanol, silver nitrate ($AgNO_3$), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) have been bought from Sigma-Aldrich. All reagents were of analytical grade.

Biosynthesis of AgNPs

About 5 g of dry sprout powder was boiled with 30 ml of deionized water for 30 min, then cooled and filtered using Whatman No 1 paper. This GGSE was stored in refrigerator for further use. The GGSE is used as reducing agent to reduce $AgNO_3$ to Ag. 0.1 mM $AgNO_3$ was prepared by dissolving 60 mg of $AgNO_3$ in 360 ml deionized water. To this solution, 40 ml of GGSE was added and stirred for 30 min using a magnetic stirrer. A color change from pale brown to reddish brown was observed within 2 h. The color change indicated the formation of AgNPs. The reddish-brown solution obtained was centrifuged (1200 rpm) for 15 min, washed the precipitate, and dried in Petri dish at 35°C. This dried powder was used for phytochemical screening, GCMS analysis, and antioxidant and antibacterial activities.

Qualitative phytochemical screening

Preliminary phytochemical screening of GGSE was made for the qualitative detection of various phytocompounds which include flavonoids, steroids, terpenoids, alkaloids, amino acids, polyphenol, glycoside, and protein. Preliminary phytochemicals were analyzed by conventional methods as described by Al-Owaisi *et al*. [25] and Sheel *et al*. [26].

Preparation of plant extract for GC-MS analysis

The freshly grown GGS was washed very well in tap water, shade dried, and powdered in a blender 50 g of sprout powder was mixed with methanol and stored inside the conical flask for 1 week. After 7 days, extract had been filtered with Whatman No. 1 filter paper. The filtrated methanol extracts are dried and used for GC-MS studies. GC-MS analysis was performed using Shimadzu QP 2010 plus mass analyzer. (injection mode: Normal, column oven temperature: 100°C, and injection temperature: 250°C). Each component was calculated by comparing the average peak area to the total area for relative peak area. The National Institute of Standards and Technology (NIST) database is used to identify >62,000 patterns of individual components. The unknown sample spectrum was compared with the spectrum of known component saved in the NIST library. The element name, shape, and molecular weight of the test sample had been ascertained.

DPPH free radical scavenging activity

The free radical scavenging (DPPH) activity for synthesized nanoparticles and GGSE was analyzed spectrophotometrically (517 nm) with the approach mentioned by Govindappa *et al*. [27]. *In vitro* antioxidant activity was measured in terms of standard ascorbic acid antioxidant equivalents. The percentage of antioxidant activity was calculated using the equation=[(a−b)/a]×100, where control [blank (a)] was prepared and b is the observance of the samples.

Bacterial source and antibacterial activity

Bacterial strains of *Klebsiella aerogenes* (MTCC 98), *Escherichia coli* (MTCC 443), *Bacillus subtilis* (MTCC 2295), and *Staphylococcus aureus* (MTCC 3160) were used and maintained in nutrient agar (Hi-Media, Mumbai) slants at 4°C. AgNPs and its antibacterial activity were analyzed using an agar well diffusion approach as reported by Gudikandula and Maringanti [28]. Gram-negative bacteria, namely *E. coli* (MTCC 443) and *K. aerogenes* (MTCC 98), and Gram-positive bacteria, namely *B. subtilis* (MTCC 2295) and *S. aureus* (MTCC 3160), were used. Mueller-Hinton Agar (Hi-Media, Mumbai) plates were prepared, sterilized, solidified, and swabbed uniformly. 6-mm length four wells roughly were made on the prepared plates using gel puncture. Different concentrations of AgNPs (10, 20, 30, and 40 µL) were loaded into the wells of all plates. The plates were analyzed for zone of inhibition after incubition time (24 h at 37°C).

Statistical analysis

Experiments were performed in triplicates, and the results are expressed as mean±standard deviation. The statistical analysis was made with origin software (OriginPro evaluation, 2018).

RESULTS AND DISCUSSION

Biosynthesis of AgNPs and the usage of GGSE

The GGSE was blended with the aqueous extract of synthesized $AgNO_3$ at room temperature. The various stages of AgNP formation are shown in Fig. 1. After 30 min, the initial color change was observed and a brownish red coloration was obtained after 2 h as shown in Fig. 2. The final color change indicates the completion of AgNP synthesis process. The change in color from watery to brownish red color indicates the formation of AgNPs and this variation is attributed due to surface plasmon resonance of AgNPs within the reaction samples [29,30].

Qualitative phytochemical screening

The qualitative phytochemical screening of methanolic GGSE is shown in Table 1. Phytochemical screening showed the presence of

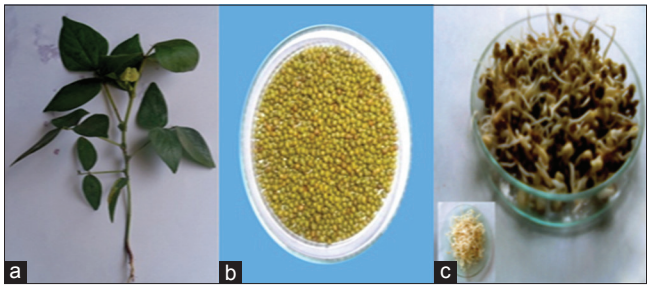

Fig. 1: Biosynthesis of silver nanoparticles using *Vigna radiate*. (a) Whole plant, (b) fine seeds, (c) sprouts

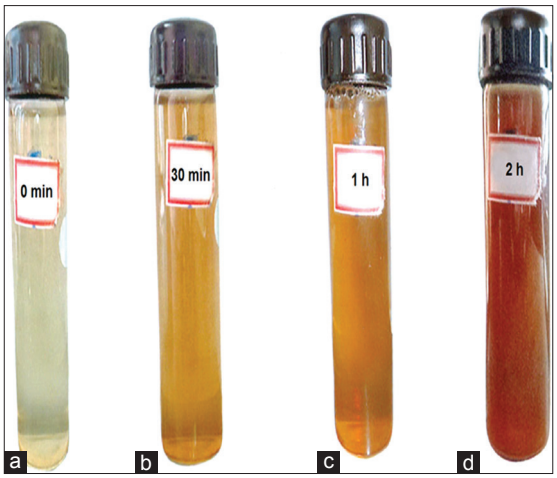

Fig. 2: Biosynthesis of silver nanoparticles using green gram sprout extract of *Vigna radiata*. (a) $AgNO_3$+sprout extract 0 min, (b) 30 min, (c) 1 h, (d) 2 h

Table 1: Phytochemicals screening results of green gram sprout extracts

S. No.	Phytochemical	Results (qualitative)
1	Tannin	−−−
2	Saponin	−−−
3	Flavonoids	++
4	Steroids	+++
5	Terpenoids	++
6	Alkaloids	+++
7	Amino acids	+++
8	Polyphenol	+++
9	Glycoside	+++
10	Protein	++

−: Absence, +: Presence, ++,+++: Indicates intensity of color

flavonoids, steroids, terpenoids, alkaloids, amino acids, polyphenol, glycoside, and protein. It is reported that the leaves of *D. montana* have steroids, flavonoids, and phenols [31]. Biologically active compounds might play a large role in forming, capping, and stabilization of AgNPs [32].

GC-MS analysis

The outcomes of the GC-MS evaluation confirmed that 24 compounds (Fig. 3) have been present in GGSE. These compounds have been diagnosed using GC-MS. The mass spectra of those compounds matched with the ones found within the NIST/NBS spectral database and the information are given in Table 2. The important compounds of GGSE (retention time 30.027) were found to be stigmast-5-en-3-ol, (3-beta), stigmasterol, pregnane, silane derivative trimethylsilyl ester of tetracosanoic acid, 9-octadecenoic acid (z), n-hexadecanoic acid, mome inositol, and ethyl alpha-d-glucopyranoside stated to own antioxidant, antibacterial, anticancer, hepatoprotective, anti-inflammatory, and antimicrobial and inhibition of parasitic increase [33].

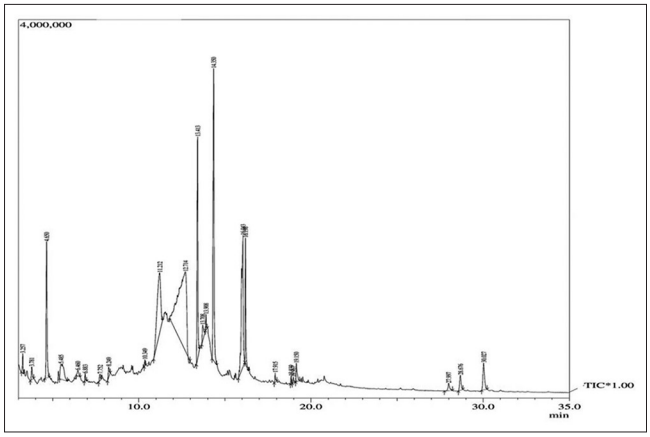

Fig. 3: Chromatogram of green gram sprout sample

Antioxidant activity

The free radical scavenging capabilities of biosynthesized AgNPs and GGSE were made using the DPPH assay. In DPPH assay, the antioxidant activities of AgNPs and the GGSE were found increased as the concentration of samples used for assay changed from 20 to 100 µg/mL (Fig. 4). In general, GGSE-mediated biosynthesized AgNPs have the good antioxidant capacity as that of the standard (ascorbic acid). This is due to the presence of phytocompounds that might have radical scavenging activity, particularly polyphenols which are a potential hydrogen atom donor [34]. The presence of antioxidant outcomes indicated that free radical scavenging activity of AgNPs has increased concentration-dependent way.

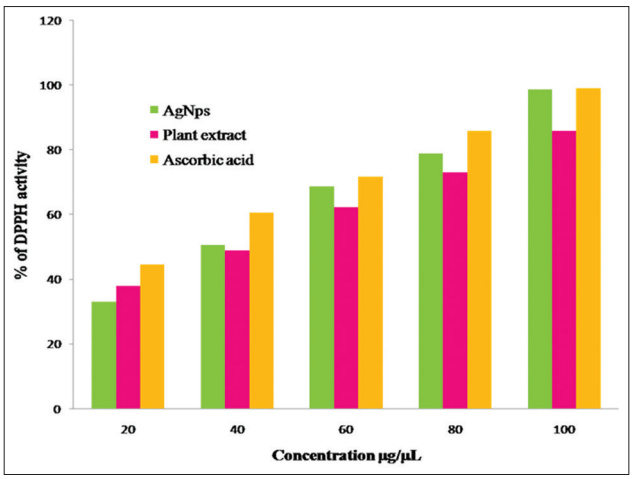

Fig. 4: 1,1-Diphenyl-2-picrylhydrazyl antioxidant activity of silver nanoparticles and green gram sprout extract of *Vigna radiata*

Antibacterial activity

Antibacterial activity of synthesized AgNPs showed positive results toward the Gram-positive and Gram-negative microorganism. The antibacterial activity was found to be maximum for *K. aerogenes,* followed by *E. coli*, *B. subtilis,* and *S. aureus* (Fig. 5). From the results, it is obvious that the inhibition activity of AgNPs toward Gram-negative bacterial traces was higher than toward the Gram-positive bacteria. This

Table 2: Peak report derived from the chromatogram of the green gram sprout

Peak#	R. Time	Area	Area %	Name
1	3.257	588627	0.69	1-Butanamine, 2-methyl-N-(2-methylbutylidene)
2	3.781	564406	0.67	Cyclopentane, 1-acetyl-1,2-epoxy-
3	4.650	5036211	5.94	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl
4	5.485	2425030	2.86	1-Deoxy-d-arabitol
5	6.460	388872	0.46	2-Ethylhexyl pentenoate
6	6.883	231882	0.27	1-Heptanol, 2-propyl
7	7.752	295200	0.35	3-(4-Aminobutyl) piperidine
8	8.249	493866	0.58	Unknown
9	10.349	144343	0.17	1H-Pyrrole, 2-(2,4,6-cycloheptatrienyl)
10	11.212	9582380	11.31	Ethyl alpha-d-glucopyranoside
11	12.714	26132753	30.83	Mome inositol
12	13.413	7436423	8.77	Caffeine
13	13.708	895705	1.06	1H-Purine-2,6-dione, 3,7-dihydro-3,7-dimethyl
14	13.908	335443	0.40	Hexadecanoic acid, methyl ester
15	14.350	12098730	14.27	n-Hexadecanoic acid
16	16.043	9915501	11.70	Unknown
17	16.198	3239788	3.82	Octadecanoic acid
18	17.915	308432	0.36	9-Octadecenoic acid (Z)
19	18.839	197121	0.23	Trimethylsilyl ester of tetracosanoic acid
20	18.967	200087	0.24	Pregnane, silane derivative
21	19.150	896508	1.06	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester
22	27.997	488264	0.58	Cholest-5-en-3-ol (3-beta)-, carbonochloridate
23	28.676	979558	1.16	Stigmasterol
24	30.027	1886587	2.23	Stigmast-5-en-3-ol (3-beta)
Total		84761717	100.00	

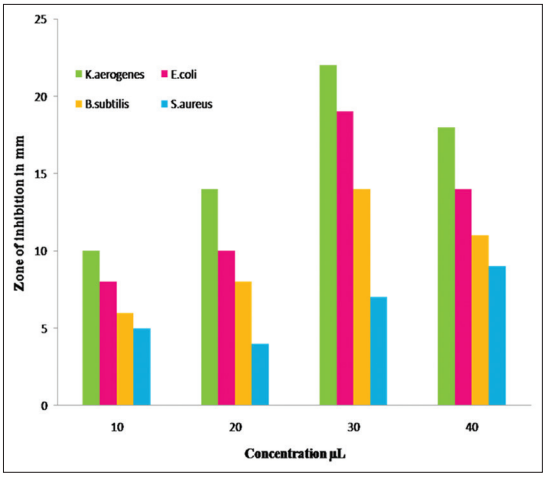

Fig. 5: Zone of inhibitions of silver nanoparticles against Gram-positive and Gram-negative bacteria through continuous culture method

could be attributed to the fact that the Gram-negative microorganisms have a thin peptidoglycan layer, but the Gram-positive microorganisms have a thick peptidoglycan layer [35].

CONCLUSION

The GGSE-assisted biosynthesis of AgNPs was found to be a convenient green route and cost-effective method. Phytochemicals present in the sprout extract act as a strong reducing and capping agent for the formation of AgNPs. The GGSE-assisted biosynthesis of AgNPs has shown potential antibacterial and antioxidant activity and proved to be an effective antibacterial material.

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AUTHOR'S CONTRIBUTIONS

The idea is mine, I have designed the work, experiments were carried out by me, and the paper is also written by me.

CONFLICTS OF INTEREST

There are no conflicts of interest in this article.

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