Research Article

Green Synthesis of AgNP Using *Ficus benghalensis* Aerial Root and its AchE Inhibiting Property

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ABSTRACT

Aim: The aim of the study was to analyze the phytochemical components of adventitious roots of *Ficus benghalensis* and its biopotential ability on silver nanoparticle reduction. **Materials and Methods:** Aerial root was collected and its phytochemical was extracted with acetone and phytochemical detected by GCMS. AgNP is characterized by UV and SEM analysis. DPPPH antioxidant, and AchE inhibition were performed. **Results:** GCMS reveals 20 different peaks 19 different compounds predominantly with 2-Lauro-1,3-Didecoin, Alpha. Tocospiro A and 2h-1,2,3-Triazole-4-Carboxylic Acid, 2-(2-Fluorophenyl). The extract reduced silver at a 1:9 ratio and the time frame were recorded by 5 min to produce nanoparticles. The UV-vis adsorption spectra show the absorbance peak in the range of 430 nm and SEM reveals size of AgNP was 62 nm rectangle shapes. The free radical scavenging activity of hydroalcoholic extract of *F. benghalensis* root was determined and found to be more effective antioxidant activity than standard. AchE inhibition was moderate on root extract and significantly inhibited by AgNP. **Conclusion:** Green silver nanoparticle reduced by aerial root and its antioxidant potential along with inhibition of Acetylcholine esterase is still considered the main therapeutic strategy against Alzheimer's Disease. The study plant *F. benghalensis* will improve cognitive function and were screened for acetylcholinesterase inhibitory activity with nano drug delivery also a better candidate for future disease-modifying therapies against this devastating disease.

Keywords: Acetylcholinesterase, *F. benghalensis,* AgNP, UV-vis.

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INTRODUCTION

Ficus benghalensis comes under the Family of Moraceae and has been known for its vast number of species, consisting of more than 800 species in the form of trees, vines, shrubs, epiphytes, and hemiphytes. There are more than 800 species of *Ficus* that have been discovered. *Ficus* plants are generally known as figs or fig trees. The genus is distributed in various regions across the tropical and sub-tropical areas, mainly in Asia, America, Australia, and Africa.[1] In India, some of the species are considered sacred, especially *Ficus benghalensis*, which is referred to as India's National. The tree signifies spiritual knowledge and eternal life.[2] Some of the species are edible, while some are used as ornamental plants, especially *Ficuslyrata*, commonly known as the fiddle-leaf fig.[3] Banyan (*Ficus benghlensis* L. (FB) is a large plant and is a member of the family Mulberry (Moraceae). It has been used for thousands of years and has become an essential plant in the medicinal field. Banyan varies in morphology, growth habit, flower color, leaves, stems, and chemical

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composition. It is available throughout the year in different regions of the world. It grows in evergreen except in some dry areas where it remains leafless for a very short time due to dryness and shortage of water. The tree is known to be epiphytic when young, with petioles of 1.25 to 5 cm in length, ovate lamina sessile, and reddish hypanthodia upon maturation. The female flowers are pedicellate, elongated with about 3 to 5 mm in length. However, male flowers are absent in the same stalk.[4] The essential oil, which is extracted from FB has different compounds such as sesquiterpene and monoterpene, as well as α-cadinol, γ-cadinene, α-muurolene and germacrene-D-4-ol. FB also has tannins, sterols, phenols, ß-flavonoids and saponins in large amounts. Some compounds such as aromatic acids, triterpenoids, mucilage, carbohydrates, gums and volatile oils are totally absent in the leaf extract of this plant.[5] Traditionally, different plant parts of *Ficus benghalensis* are claimed to have several analgesic properties. It has also been recommended for rheumatism and skin disorders such as sores.[6] The bark is considered useful in burning sensation, ulcers, and painful skin diseases. It can be used in inflammation and toothache.[7] *Ficus* species is one of the largest genera of the plant kingdom, with promising phytoconstituents from various classes of compounds, including phenols, flavonoids, sterols, alkaloids, tannins, saponin, terpenoids, etc. The FB plant contain quercetin-3-galactoside, rutin, friedelin, taraxosterol, lupeol (Figure 1), β-amylin, psoralen, bergapten, β-sisterol, and quercetin-3-galactoside. The latex contains the caoytchoue, resin, albumin, cerin, sugar, and malic acid.[8] The bark of FB has 5, 7 dimethyl ether of leucopelargonidin-3-0-α-L rhamnoside and 5, 3 dimethyl ether of leucocynidin 3-0-α-D galactosylcellobioside, beta sitosterol-alpha-Dglucose, as well as meso-inositol. Earlier, glucoside, heptatriacontene10-one, tetratriaconthene-2-one, 6-heptatriacontene-10-one, βsitosterol-alpha- Dglucose, andmeso-inositol, leucodelphinidin derivative, bengalenoside, aglucoside, leucopelargonin derivative, leucocynidin derivative, and glycoside of leucopelargonidin.[9] Nanotechnology deals with particles which are less than 100 nm and have important roles in medicines, industries, drug-gene delivery etc. The size of the nanoparticles size is similar to most of the biological molecules and structures therefore the nanoparticles may be used for both *in vivo* and *in vitro* biomedical research and applications. Microbial synthesis of silver nanoparticles can be achieved either by intracellular scheme or extracellular scheme.[10]

MATERIALS AND METHODS

Plant material

Dried roots were powdered in a mixer grinder. The powder of roots was packed in paper bag and stored in air-tight containers until use.

Preparation of extract

Extraction of Plant Material

The aerial root of *Ficus bengalensis* was collected and dried for several days under shadow conditions. Powered material was extracted with acetone by the soxhalet method. The solvent phase was collected and evaporated in a hot air oven at 50°C over a night. The extract is then used for further antimicrobial assay.

Test for carbohydrate

1 mL of Fehling's reagent was taken in a separate test tube. A few drops of extract were added and kept in a boiling water bath.

Test for Tannins

10 mL of bromine water was added to the 0.5 g aqueous extract. Decoloration of bromine water showed the presence of tannins.

Test for Saponins

5.0 mL of distilled water was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was mixed with a few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

Tests for Flavonoids

Alkaline Reagent Test. 2 mL of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow color was produced, which became colorless when added 2 drops of diluted acid to the mixture. This result showed the presence of flavonoids.

Tests for Glycosides

A solution of glacial acetic acid (4.0 mL) with 1 drop of 2.0% $FeCl_3$ mixture was mixed with the 10 mL aqueous plant extract and 1 m LH_2SO_4 concentrated. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.

Test for Terpenoids

2.0 mL of chloroform was added with the 5 mL aqueous plant extract and evaporated on the water path and then boiled with 3 mL of H_2SO_4 concentrated. A grey color formed which showed the entity of terpenoids.

Test for Steroids

2 mL of chloroform and concentrated H_2SO_4 were added with the 5 mL aqueous plant crude extract. In the lower chloroform layer red color appeared that indicated the presence of steroids.

Detection of Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

Mayer's test

Filtrates were treated with Mayer's reagent. The formation of a yellow cream precipitate indicates the presence of alkaloids.

Detection of Phenols

Ferric chloride test

10 mg extracts were treated with a few drops of ferric chloride solution. The formation of a bluish-black color indicates the presence of phenol.

Standardization of reduction

50 mM of silver nitrate mixed with 2% aerial root extract at 9:1, 8:2, 7:3, 6:4 and 5:5. Changes in reduction were visually noted against time.

Green synthesis of silver nanoparticles

10 mL aerial root extract (2%) was mixed with 90 mL $AgNO_3$ (50 mM) in a 250 mL flask and observe the color of the solution. The color of the solution was changed from light yellow to dark brown color. These color changes indicated the conversion of silver nitrate ($AgNO_3$) into silver nanoparticles (AgNPs). Then 2 mL of this solution was taken and absorbance was recorded at 200-600 nm using UV-visible spectrophotometer. Then the solution was centrifuged at 5,000 rpm for 15 min and a silver pellet was collected. Further pellet was washed 3 times using 5 mL deionized water and centrifuged for 15 min. Then purified pellet was dried in a hot air oven (80°C) for 5 hr and subjected to SEM analysis.

Acetylcholinesterase inhibition assay (*in vitro*)

AChE activity was measured within a 96-well microtitre plate based on the Ellman method. Forty µL of plant extracts at concentrations of 200, 150, 100 and 50 µg/mL were mixed with 50 µL of 3 mM DTNB, 50 µL of AChE of brain homogenate (prepared at 10% (w/v), and 35 µL of 50 mMTris-HCl (pH 8.0) containing 0.1% BSA, and samples incubated for 5 min at 37°C. The reaction was initiated by the addition of 25 µL of 15 mM ATCI resulting in the production of a 5-thio-2-nitrobenzoate anion read at 412 nm every 5 sec for 10 min using a Spectramax microplate reader (ThermoFisher, Stafford, UK).

The percent inhibition was calculated using the formula:

$$\frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

DPPH assay

The free radical scavenging activities of the target ethanol were determined using the DPPH free radical scavenging assay. A fresh methanolic solution of DPPH (20 µg/mL) was prepared and stored at 10°C in the dark. PHE were dissolved in water (v/v). The 0.5 mL extract was added to the freshly prepared methanolic DPPH solution (1.0 mL) and stirred. After reacting for 5 min, discoloration was recorded at 517 nm. The absorbance of the DPPH radicals without antioxidants was also measured as a control, and 95% methanol was used as blank. The absorbances were compared with those of the blank control. The reactions were performed in three replicates and averaged. Antioxidant activity was calculated as follows:

$$\% \text{ Antioxidant activity} = \left[\frac{\left(\begin{array}{l}\text{control absorbance} - \\ \text{sample absorbance}\end{array}\right)}{\text{control absorbance}}\right] \times 100\%$$

RESULTS

Phytochemistry of studied plant

Root sample was collected from the Chennai region and processed after air drying (Plate 1). Phytochemicals present in the root were extracted by the soxhlet method. The presence of phytochemical (Plate 2) was qualitatively determined and results are given in Table 1. Out of 9 phytochemicals, 6 was positive and 3 were found to be negative. The qualitative test showed the presence of flavonoids, phenol, tannins, glycoside, alkaloids and saponins. The qualitative analysis of the *F. benghalensis* prop roots crude extract revealed the absence of Tannins, glycosides, terpenoids and reducing sugars. Figure 1 represented major peaks detected in GCMS revealing elution of 19 different compounds. The major peak was 2-LAURO-1,3-DIDECOIN(RT 37.2 min/21.2%) followed by 10% of Dodecanoic acid, 2,3-dihydroxypropyl ester(RT 37.4 min) and 8-Bromooctanoic acid, ethyl ester(RT 37.6), 10% alpha.-Tocospiro A(RT 38 min), 8% of N-[3-(1-hydroxy-2,2-Dimethylpropyl) phenyl] pivalamide were predominantly detected in GCMS. In addition to that Pentadecanone, Heptadecanone, Hexadecanoic acid,

Table 1: Phytochemical Test.

Sl. No	Test	Result
1.	Barfoeds Test	Negative
2.	Tannins	Positive
3.	Saponins	Positive
4.	Flavonoids	Positive
5.	Glycoside	Positive
6.	Sterols	Negative
7.	Terpenoids	Negative
8.	Alkaloids	Positive
9.	Phenol	Positive

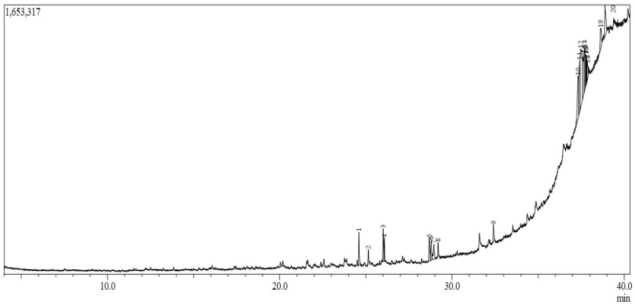

Figure 1: GCMS spectrum of aerial root.

Benzenepropanoic acid, octadecadienoic acid, Methyl stearate and 1,2-Dihydroanthra[1,2-d]thiazole-2,6,11-trione were detected in the range of 1-4%. The retention time and area percentage of GCMS are given in Table 2.

Reduction and synthesis of silver nanoparticle

Green Synthesis of silver nanoparticle followed by standardization of ratio of the reaction mixture (Figure 2). All the ratios showed immediate Np reduction capability. The initial visual confirmation of silver nanoparticle production was performed through visual observation as color change. Initially, colourless $AgNo_3$ solution turns to yellow and then within 5 min changed into dark brown followed by the addition of *Ficus benghalensis* at different ratios. Figure 3 reveals the UV spectrum green mediates silver nanoparticle absorption. The UV-vis spectrum shows broad absorption bands of 3.015 observed at 370 nm could be related to the presence of silver nanoparticles. The morphology of silver nanoparticles was analyzed by Scanning Electron Microscope (SEM). The monodispersed rectangle-shaped nanoparticles were reduced by a phytochemical-mediated approach (plate 4). In the absence of any surface coating, chemical-mediated particles have hydrophobic surfaces with a large surface area-to-volume ratio. Due to hydrophobic interactions between the particles, they tend to agglomerate forming large clusters. The scanning electron micrograph given in

Table 2: NIST Library matched compounds.

Peak	Retention Time	Area %	Name
1	24.596	4.05	2-Pentadecanone, 6,10,14-Trimethyl-
2	25.142	1.71	9-Heptadecanone
3	26.007	4.09	Hexadecanoic Acid, Methyl Ester
4	26.075	2.32	Benzenepropanoic Acid, 3,5-Bis(1,1-Dimethylethyl)-4-Hydroxy-, Methyl Ester
5	28.682	3.41	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester
6	28.786	2.65	9-Octadecenoic Acid, Methyl Ester, (E)-
7	28.95	2.83	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]- (T-Phytol)
8	29.193	1.59	Methyl Stearate
9	32.41	1.91	4,8,12,16-Tetramethylheptadecan-4-Olide
10	37.32	10.11	Dodecanoic Acid, 2,3-Dihydroxypropyl Ester
11	37.415	8.19	N-[3-(1-Hydroxy-2,2-Dimethylpropyl)Phenyl]Pivalamide
12	37.47	21.2	2-Lauro-1,3-Didecoin
13	37.665	10.16	8-Bromooctanoic Acid, Ethyl Ester
14	37.705	6.89	2h-1,2,3-Triazole-4-Carboxylic Acid, 2-(2-Fluorophenyl)-
15	37.76	1.58	Cyclododecasiloxane,
16	37.783	2.46	-Dinorgibberell-1(10)-Ene-7,19-Dioic Acid,19,2-Lactone
17	37.82	1.74	Silicone Grease, Silicon Fett
18	37.87	2.43	1,2-Dihydroanthra[1,2-D]Thiazole-2,6,11-Trione
19	38.624	10.60	Alpha.-Tocospiro A

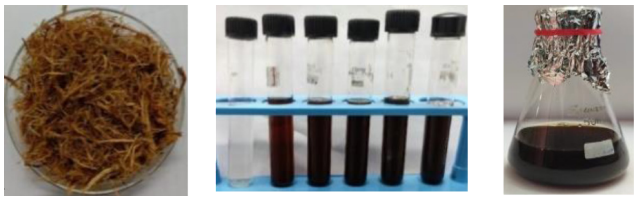

Figure 2: Synthesis of silver nanoparticle.

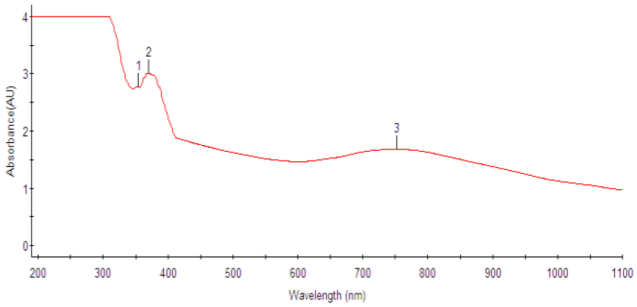

Figure 3: UV-visible spectrum of silver nanoparticle.

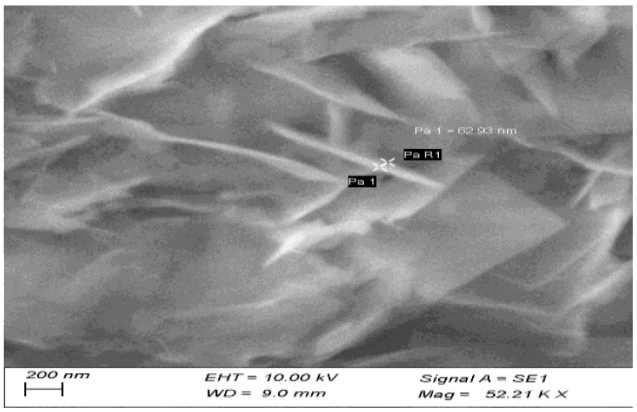

Figure 4: SEM analysis of reduced silver nanoparticles.

Table 3: Percentage of antioxidant.

Sample code	Extract	AgNP
STANDARD	73	
25	16.6	18
50	29	28
75	51.3	58
100	66.3	72
200	80.6	89
IC_{50}	72.08	72.20

Figure 4 reveals that the size of the silver nanoparticles showed that they were approximately uniform rectangle shape with 62 nm in size.

Bio potential of AgNP

The present study was carried out to investigate the antioxidant activity of the extract of *F. bengalensis* (Table 3). The free radical scavenging activity of the hydroalcoholic extract of aerial root of *F. benghalensis* was determined according to the DPPH radical scavenging method. According DPPH method, a compound with high antioxidant activity effectively binds with the radical hence preventing its propagation and the resultant chain reaction was noted at 200µg (80%) followed by 66% at 100 µg whereas AgNP showed 89% at 200 µg and 72% at 100 µg found to equal to 1000µg ascorbic acid. Different concentrations of samples, that is, hydroalcoholic extract show half of its maximal inhibitory effect (IC_{50}) 72.08 µg/mL for extract and 72.20 µg/mL among AgNP. Further, the activity on enzyme inhibition was performed on AchE inhibition. The most potent extracts and NP percentage AChE inhibition is given in Figure 5. The ethanol extracts of *F. bengalensis* were evaluated for inhibition of acetylcholinesterase (AChE) activity showed moderate activity and AgNP has shown potent activity. The descending order of AChE inhibitory

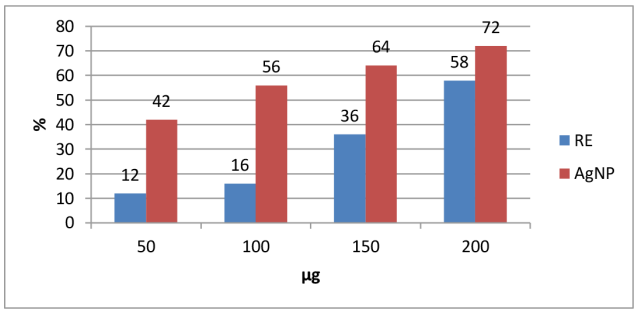

Figure 5: AchE inhibition by extract and AgNP.

potency of root extract was 58≥36≥16≥12 (200, 150, 100, 50 µg) and the AgNP exhibited 72, 64,56 and 42%. AChE IC_{50} inhibitory concentrations for root extracts were significantly inversely correlated to the activity of AgNP. This suggests that the agent(s) responsible for the AChE inhibitory activity contain phenolic and flavonoid compounds as capping agents.

DISCUSSION

The data of phytochemicals of aerial root extract indicates the absence of Carbohydrate, Sterols and Terpenoids. Phenol, flavonoids and alkaloids were the most frequently reported phytochemical in many studies. The data of our phytochemical was correlated with the finding of previous research data of some authors[11] and found similar fingerprint of phytochemical compounds. Phytochemical screening of *Ficus benghalensis* have maximum level of polyphenols have also been reported from other species of *F. religinosa* L. and *F. carica.*[12] The presence of antioxidants has been studied extensively in Ficus species using DPPH antioxidant assays found to be effective. *F. benghalensis* root's aqueous extract was reported to possess the highest scavenging activity and reducing power compared to its methanolic and ethanolic extracts.[13] The phytochemical screening of the *F. benghalensis* root revealed the presence of steroids, flavonoids, tannins, phenolic compounds, and anthraquinone glycoside as its major constituents.[14] The phenolics in medicinal plants possess significant pharmacological activities such as antibacterial, antiviral, antitumor, anthelmintic and antioxidant. The antioxidant potential is due to the presence of various functional groups such as hydroxyl, ketonic, methoxy and double-bond conjugation.[15] Detection of significant phenolic contents in *F. benghalensis* proposes it as an endless source of natural antioxidants. Babu *et al.*[16] reported the methanol extract of bark showed the presence of flavonoids, saponins, steroids, wax, terpenoids, cardiac glycosides and tannins. Our results were in well agreement with earlier findings of Okafor *et al.*[17] who

studied the *in vitro* micropropagation of a high-value endangered medicinal plant species, in order to explore its biogenic potential in the biomimetic synthesis of antimicrobial AgNPs. When root extract was combined with a silver nitrate aqueous solution, a 100% reduction of silver ions was seen after 30 min of interaction. It has been already documented that visual colour shifts were seen during the reduction of silver ions into silver nanoparticles in the reaction mixture, generating a dark brown colour with a UV peak at 437.5 nm as reported by Patave *et al*.[18] Mariselvam *et al*.[19] prepared aerial root tip-mediated silver nanoparticles. Further, our extract and AgNp displayed significant AchE inhibition. Inhibitory constituents bengalensinone (22β-hydroxylup-12,20-dien-3-one; 1), a new lupanetriterpene displayed inhibitory potential against enzyme cholinesterase from *Ficus bengalensis* was reported by Riaz *et al*.[20]

CONCLUSION

Ficus benghalensis also known as the Indian banyan tree, the bark of plant is used in Ayurvedic medicine for the treatment of diabetes. The immense potential of this tribal plant is being reviewed to explore its medicinal importance. The UV-vis adsorption spectra show the absorbance peak in the range of 430 nm size of 62 nm rectangle shape. The plant *F. benghalensis* improving cognitive function was screened for acetylcholinesterase inhibitory activity with nano drug delivery also a better candidate for future disease-modifying therapies against this devastating disease.

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Authors have no competing Acknowledgements.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AchE: Acetylcholine esterase; **AgNP:** Silver nanoparticle; **SEM:** Scanning electron microscope; **GCMS:** Gas chromatography mass spectrum; **AD:** Alzheimer's Disease; **NaOH:** Sodium hydroxide; **H_2SO_4:** Sulphuric acid; **UV:** Ultra violet.

SUMMARY

Aerial root mediated silver nanoparticle synthesized by one step reduction method and reduction confirmed by UV and color changes in the medium. The morphology of silver nanoparticle was thin and rectangular 62 nm in size. The aerial root has been positive on tested all phytochemical and GC reveals 19 different volatile compounds. Both extract and AgNP have significant free radical scavenging activity and AchE enzyme inhibition.

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