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Biogenic synthesis of silver nanoparticles from bioactive *Cassia tora* plant for medical applications

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Abstract
The plant *cassia tora* is a long flowering plant, mostly found in the local area of post-Hajarmachi Karad taluka. Plant extract (root, leaves and stem) were obtained using a simple reflux method using methanol. Antimicrobial activity was carried out using the agar cup method with three microorganisms like *Escherichia coli* (*E.coli*), *Staphylococcus aureus* (*S. aureus*),and *Salmonella paratyphi A*. Phytochemical activity in the plant extracts using different tests wherein the extract shows the presence of secondary metabolites. The biosynthesis of silver (Ag) nanoparticles (NPs) was prepared from the plant extract and exhibited antimicrobial activity. Absorption spectra of leaves, stem, and root of *Cassia tora* were observed at 541, 342, and 254 nm respectively using UV-Vis spectroscopy. Thin layer chromatography (TLC) exhibited bioactive compound activity and their identification.

Keywords: *Cassia tora* plant extract, phytochemical analysis, silver nanoparticles, antimicrobial activity, TLC, UV-Vis spectroscopy

Introduction

This study was carried out with the objective to investigate the bioactive component of the plant "C*assia tora*" (tarvad) [1-3]. *Cassia tora* corresponds to herbaceous annual fetid herb and it is mainly obtained in the height of 30-90 cm with alternative pinnate leaves [4-6]. The root, stem, and leaves of *cassia tora* plant can be used in the advanced system of herbal medicines. *Cassia tora* is also a medicinal plant, which exhibits antimicrobial and antioxidant properties [7, 8]. This plant also contains secondary metabolites called bioactive substances [9]. Secondary metabolites are produced in the plants besides the primary biosynthesis and metabolic routes for compounds associated with plant growth and development. In this study, we used leaf and root, stem extracts of the plant [7, 8]. The extract was prepared using methanol as a solvent, methanol is a polar solvent. It is used for the extraction of various polar compounds like alkaloids, glycosides; terpenoids etc. for studying its microbial activity some standard test organisms like *staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and *salmonella paratyphi A* were used [10]. After this entire test phytochemical analysis of the plant, the product is carried out. Phytochemical is the chemical analysis of plant root, stem, and leaf, drugs are analyzed chemically as well as physically by qualitatively. Antimicrobials are among the most commonly used drug. An antimicrobial substance is an agent that kills microorganisms or inhibits their growth, usually, their antimicrobial agents are produced using various chemicals which are expensive and hazardous to nature, thus using plant extract of easily available plants are the best choice for the development of new drugs in order to decrease the infection caused by microorganisms. In the present article, we investigated the antimicrobial activity of the *Cassia tora* which shows the zone of inhibition [1]. The solvent methanol is used, which gives a proper band on TLC plates and medicinal value of *Cassia tora* that belongs to the bioactive phytochemicals flavonoids, phenolic substance, tannins, and essential oils which exhibits the physiological effect in the human being [2]. Moreover, Phytochemicals show protective or disease preventive properties. In the present article, the extracellular biosynthesis and antimicrobial activity of Ag NPs synthesis were carried out.

Materials and Methods

1. Collection of samples and Extraction

The present plant *Cassia tora* was collected from the local area of post-Hajarmachi, Karad taluka, and its extraction was carried out by methanol extraction using the reflux method. The leaves, stem, and root of the present plant were collected, washed, and dried in a vacuum oven for 3-5 days. The powder was obtained by grinding the leaves, stem, and root of the present plant and the obtained powder was employed for extraction. The obtained 10 gm (powder) from leaves was mixed with methanol solvent (100 ml). Then; the mixed powder was refluxed for 2h, filtration of extract and dried the obtained powder in the incubator at 37°C for 24 h. The dried extract was weighed and then dissolved in distilled water and preserved at 4°C. The same procedure was used for the root and stem of the *Cassia tora* plant.

2. Antimicrobial activity of plant extract

Sterile nutrient ager plate acts as growth media for the antimicrobial activity of the prepared samples along with the *Staphylococcus aureus*, *Salmonella paratyphi A*, *E.coli* was used as culture agent for said samples [3].

Culture suspensions of all the cultures were made in saline from nutrient agar slants. The antimicrobial activity was carried out using the agar cup method. 1 (ml) of each test culture is spread on sterile nutrient agar plates. A sterile cork borer of 5mm was used to make wells in the agar. Each well was then filled with the extract and then incubated at 37°C for 24 h. After incubation, the plates were checked for zones of inhibition.

3. Detection of phytochemicals

The following procedure was employed for the detection of phytochemical activity of extract.

1. Test for alkaloids: 1 (ml) of extract was added with the Wagner's reagent (2ml). The product of the reaction exhibited the reddish brown precipitate.
2. Test for flavonoids: 1 (ml) of extract was added with the 1 (ml) solution of lead acetate resulting in the yellow precipitate.
3. Test for phenolic compounds: 1 (ml) of extract was mixed with the 3-4 drops of ferric chloride solution, resulting the bluish black coloration.
4. Test for tannins: 1 (ml) of extract was added to 3 drop of ferric chloride causing the green black coloration.
5. Test for terpenoides: 1 (ml) of extract +2 (ml) of chloroform + 3 (ml) of concentrated sulphuric acid was mixed simultaneously to observe the reddish brown coloration.
6. Test for carbohydrates: 1 (ml) of extract was added to 1 (ml) of benedict's solution and heated the mixture for 5min causing the formation of green coloration.
7. Test for volatile oil: 2 (ml) of extract + 0.1 (ml) Sodium hydroxide (NaOH, 10%) + Hydrochloric acid (HCl) was mixed and found the white colored precipitate.
8. Test for glycosides: 2 (ml) of extract + 1 (ml) of HCL and heated and then neutralize using 10% of NaOH leading to observe brick red precipitation.

4. Synthesis of Silver (Ag) Nanoparticles (NPs)

The aqueous extracts were obtained by boiling the leaves, stem, and root powder in distilled water. The extract was then added to 0.001 molar solution of silver nitrate AgNO3 (50 ml) and the mixture was incubated at room temperature (RT) for 48 h. The leaves, stem and root, and genic extract reduce Ag^{+} ions into Ag NPs. Here, the extract acted as a reducing agent. Further, it caps NO^{3-} ions and deactivates their charge and activity.

Fig 1: (a-b) the color change for the leaves extract solution (leaves +$AgNO_3$ solution) after incubation of the solution, (a) The solution mixture before synthesis of Ag NPs and (b) the solution after synthesis of Ag NPs; (c-d) the color change for the STEM extract solution (extract + $AgNO_3$) after incubation of the solution, (c) The solution mixture before synthesis of Ag NPs, and (d) the solution after synthesis of Ag NPs, and (e-f) the color change for the ROOT extract solution (extract + $AgNO_3$) after incubation of the solution; (e) the solution mixture before synthesis of Ag NPs, and (f) the solution after synthesis of Ag NPs.

Result and Discussion

1. Collection of sample and extracts

The leaves, root, and stem extract were collected and prepared using the reflux method and extract stored at 4°C. The capture images of the source of extracts like leaves, stem, and root of *Cassia tora* plant are exhibited in Fig. 2 (a-c) respectively. Moreover, the obtained images of extract from leaves, stem, and root are also exhibited in Fig. 3(a-c) respectively.

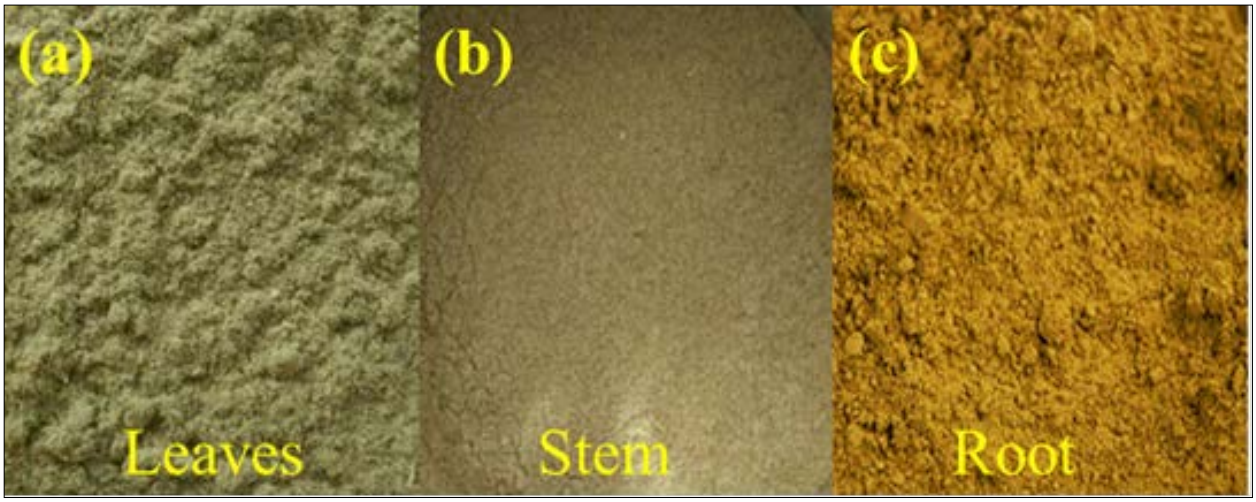

Fig 2: Sources of extract (a) leaves, (b) stem and, (c) root of *Cassia tora plant*

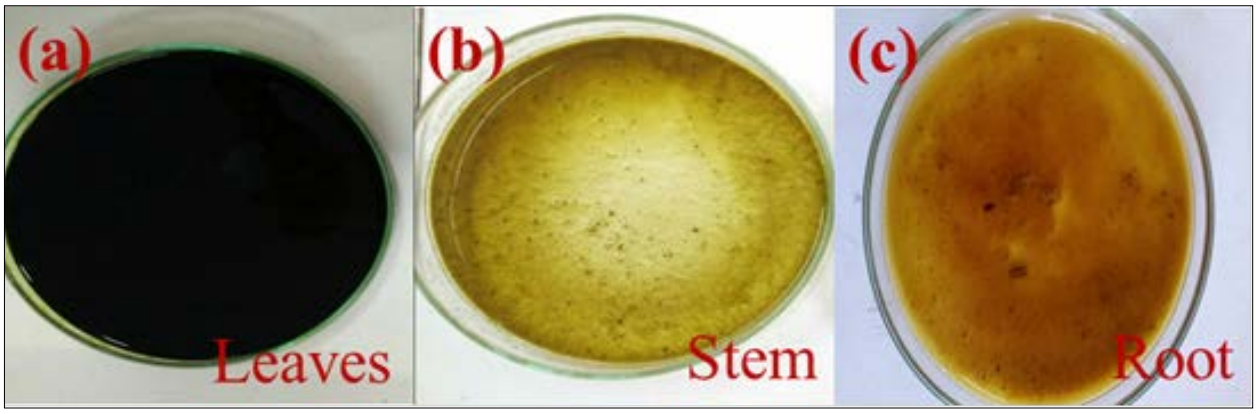

Fig 3: Extract of (a) leaves, (b) stem and, (c) root of *Cassia tora plant*

2. Antimicrobial activity of the leaves, stem and root extract

Fig 4: Antimicrobial activity of *E.coli* of (a) leaves, (b) stem, and (c) root of *Cassia tora* plant.

The antimicrobial activity of the extract was obtained using the diffusion agar-cup method. Inhibition zone of antimicrobial activity of leaves, stem, and root against *E. coli* have been exhibited in Fig. 4 (a), (b) and (c) respectively, where *E. coli* exhibits more inhibition zone of stem compared to leaves and root. Moreover, the antimicrobial activity of plant extract against the *S.aureus* was also exhibited in Fig. 5 (a-c). The root extract of *Cassia tora* plant exhibited a very high inhibition zone against *S.aureus* as compared to leaves and stem (Fig. 5c). Similarly, antimicrobial activity of *Cassia tora* plant against *Salmonela paratyphi A* was exhibited in Fig.6, where leaves extract shows the high inhibition zone compared to stem and root extract.

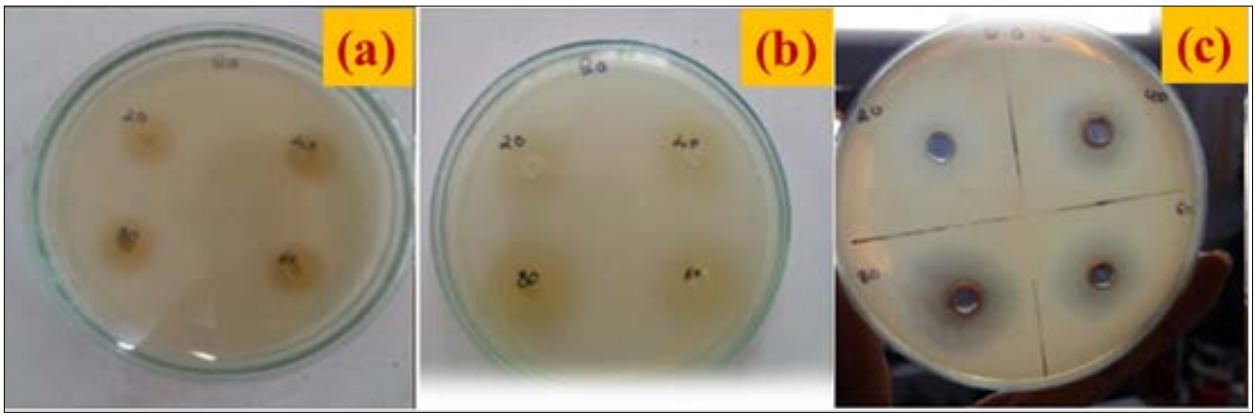

Fig. 5: Antimicrobial activity of *S.aureus* of (a) leaves, (b) stem, and (c) root of *Cassia tora* plant.

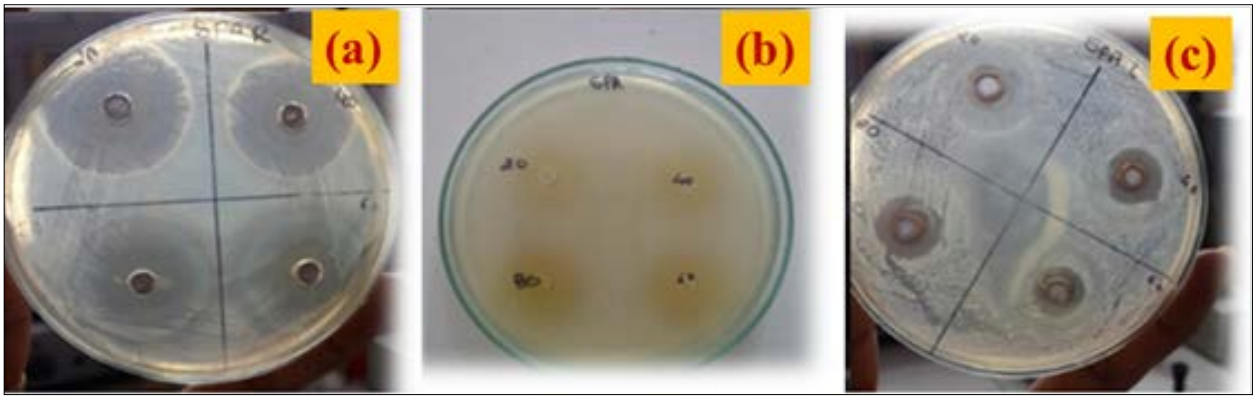

Fig. 6: Antimicrobial activity of *Salmonela paratyphi A* of (a) leaves, (b) stem, and (c) root of *Cassia tora* plant.

3. Phytochemical screening

Table 1: Results for phytochemical tests for Leaves extract.

Test	Expected observation	Observed	Result
Phenol Compound	Bluish black color	Bluish black color was observed	+
Volatile Oil	White ppt.	White ppt was observed.	+
Tannins	Blue color- garlic tannin Green color-Catecholic tannin	Greenish blue color was observed.	+
Terpenoides	Red color	No red coloration was shown.	+
Glycoside	Reddish brown color	No reddish brown color was observed.	_
Carbohydrates	Green color	Green color was observed.	_
Alkaloids	Reddish brown ppt.	No reddish brown ppt was observed.	_
Flavonoids	Yellow precipitate	No yellow ppt was observed.	_

Table 2: Results for phytochemical tests for STEM extract.

Test	Expected observation	Observed	Result
Phenol Compound	Bluish Black Color	Bluish black color was observed	+
Volatile Oil	White ppt.	White ppt was observed.	+
Tannins	Blue Color- Garlic Tannin Green Color- Catecholic Tannin	Greenish blue color was observed.	+
Terpenoides	Red Color	No red coloration was shown.	_
Glycoside	Reddish Brown Color	No reddish brown color was observed.	_
Carbohydrates	Green Color	Green color was observed.	+
Alkaloids	Reddish Brown ppt.	No reddish brown ppt was observed.	-
Flavonoids	Yellow precipitate	Yellow ppt was observed.	+

Table 3: Results for phytochemical tests for ROOT extract

Test	Expected observation	Observed	Result
Phenol compound	Bluish black color	Bluish black color was observed	+
Volatile oil	White ppt.	White ppt was observed.	+
Tannins	Blue color- garlic tannin Green color-Catecholic tannin	Greenish blue color was observed.	+
Terpenoides	Red color	Red coloration was shown.	+
Glycoside	Reddish brown color	No reddish brown color was observed.	_
Carbohydrates	Green color	Green color was observed.	_
Alkaloids	Reddish brown ppt.	No reddish brown ppt was observed.	_
Flavonoids	Yellow precipitate	Yellow ppt was observed.	+

4. UV-visible Spectroscopy of Ag NPs

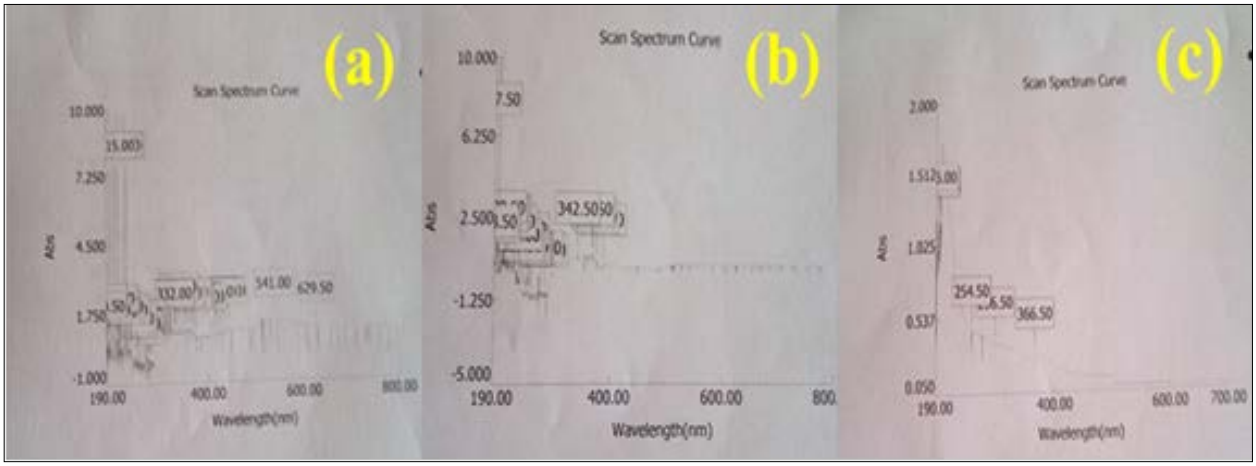

Fig 7: UV-Visible spectroscopy images of Ag NPs extracted from (a) leaves, (b) stem, and (c) root respectively

The absorption spectra of leaves, stem, and root of *Cassia tora* plant is exhibited in Fig.7 (a-c) respectively. The observed absorption spectrum was obtained at 541, 342, and 254 nm respectively using UV-Vis spectroscopy for leaves, stem, and root of the *Cassia tora* plant. The blue shift in the absorption spectra of *Cassia tora* plant indicates the increase in optical band gap which leads to decrease in the particle size of Ag NPs.

5. Antimicrobial activity of Ag NPs:

Culture suspension of all the cultures was made in saline from sterile NA slants. The antimicrobial activity was carried out using the disk diffusion method. 0.1ml of culture was spread on sterile nutrient agar plates. The disk was dipped in Ag nanoparticles solution and placed on the surface of the agar [11]. The plate was incubated at 37°C for 24 h. After incubation, the plates were checked for the zone of inhibition.

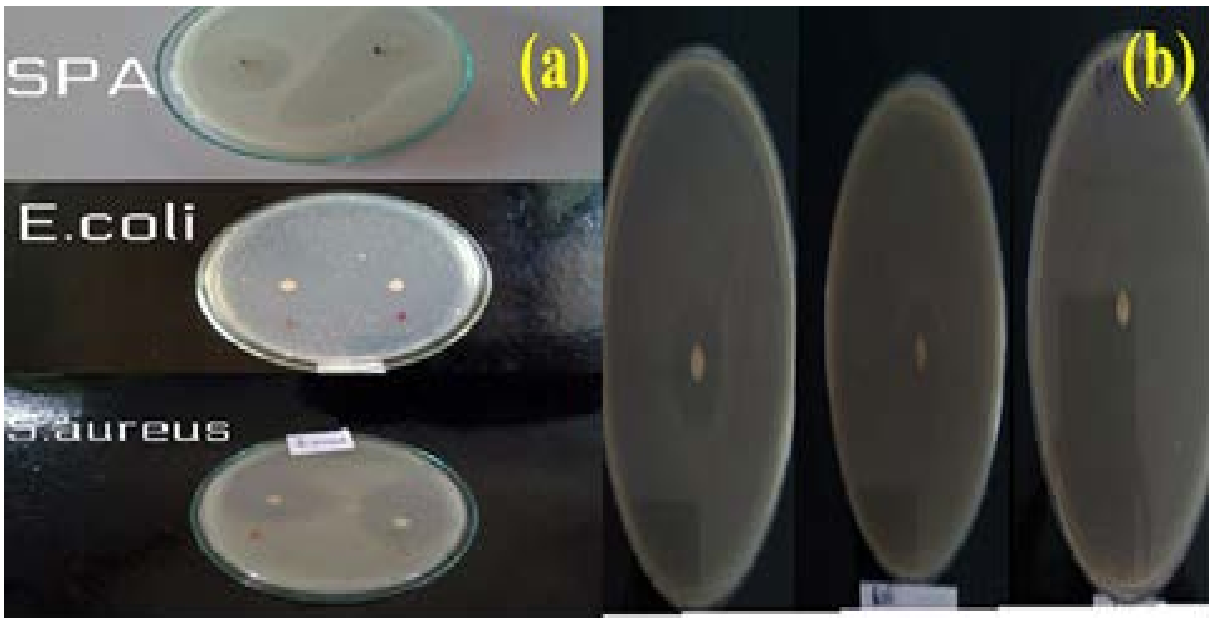

Fig 8: (a) Inhibition zone of synthesized Ag NPs (leaves and root), and (b) Inhibition zone of synthesized Ag NPs (stem extract).

6. Thin Layer Chrometography (TLC)

TLC of *Cassia tora* extract of leaves, root, and stem with various solvents such as chloroform, methanol, and water were tested for antibacterial activity against three bacteria. Phytochemicals of the extracts such as tannins, alkaloids, total phenolic substance, and total flavonoids were measured. The present results of the plant extracts showed that it is a non-sequential method, which can be for performing the antimicrobial activity of the present samples [12]. For that purpose, chloroform and methanol can be suitable to extract the bioactive elements from *Cassia tora* (leaves, root, and stem).

TLC was done using all three samples. 1st TLC plate was taken which is coated with silica 60. On a plate, all 3 samples were spotted or a thin band was drawn. According to phytoconstituents, different solvent systems were prepared. Then, plates were kept in solvent chamber until the 3-4th part of the silica plate was not run. After that, the plate was taken out and kept for drying. Three colored spots were developed on the stationary phase and their distances were measured from the spotted area. Retention factor R_f, usually describes the chromatographic behavior of sample solutes and is commonly used for the measurements of the distance of the spots [12].

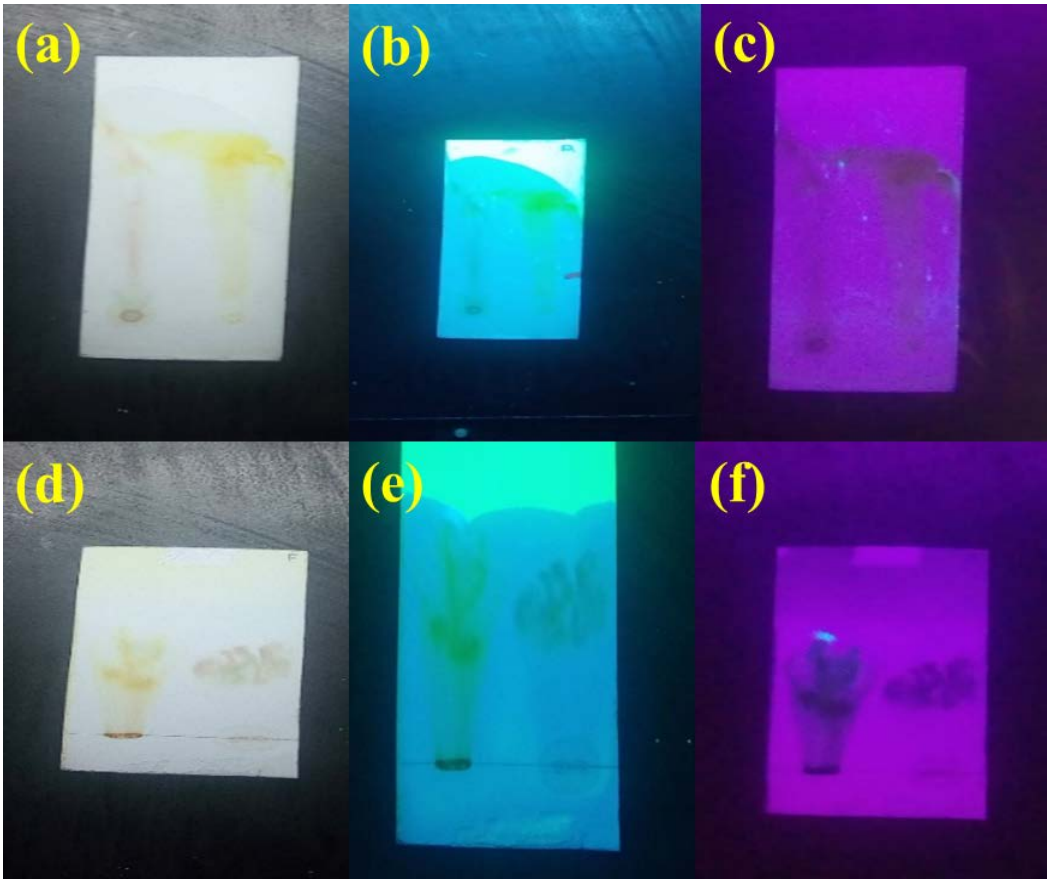

Fig 9: TLC run of root, leaves extracts for alkaloids (a-c) and flavonoids (d-f). The retention factor (R_f) values for alkaloids and flavonoids in the various TLC runs is observed. It indicates the TLC run for alkaloids is different than flavonoids. This imparts the presence of alkaloids and flavonoids in root and leaves extract. The observation is conducted at visible, 254 and 366 nm.

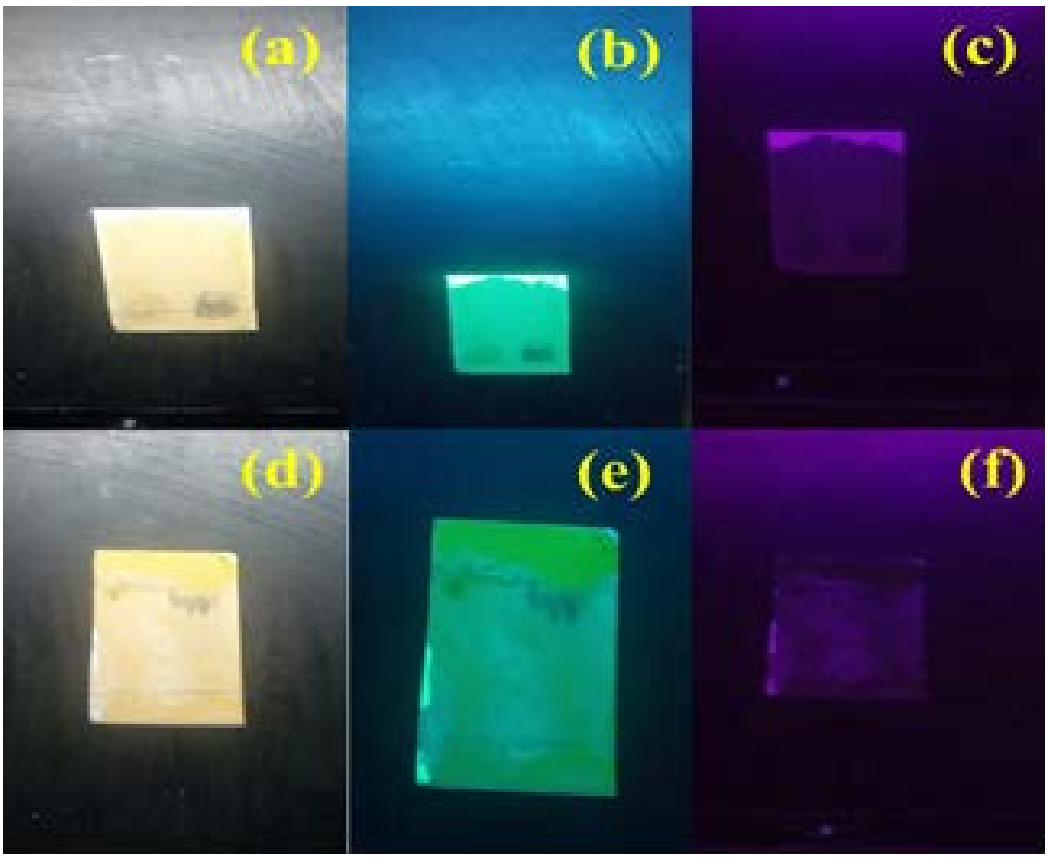

Fig 10: TLC run of root, leaves extracts for phenols (a-c) and tannins (d-f). The retention factor (R_f) values for phenols and tannins in the various TLC runs is observed. It indicates the TLC run for phenols is different than tannins. This imparts the presence of phenols and tannins in root and leaves extract. The observation is conducted at visible, 254 and 366 nm.

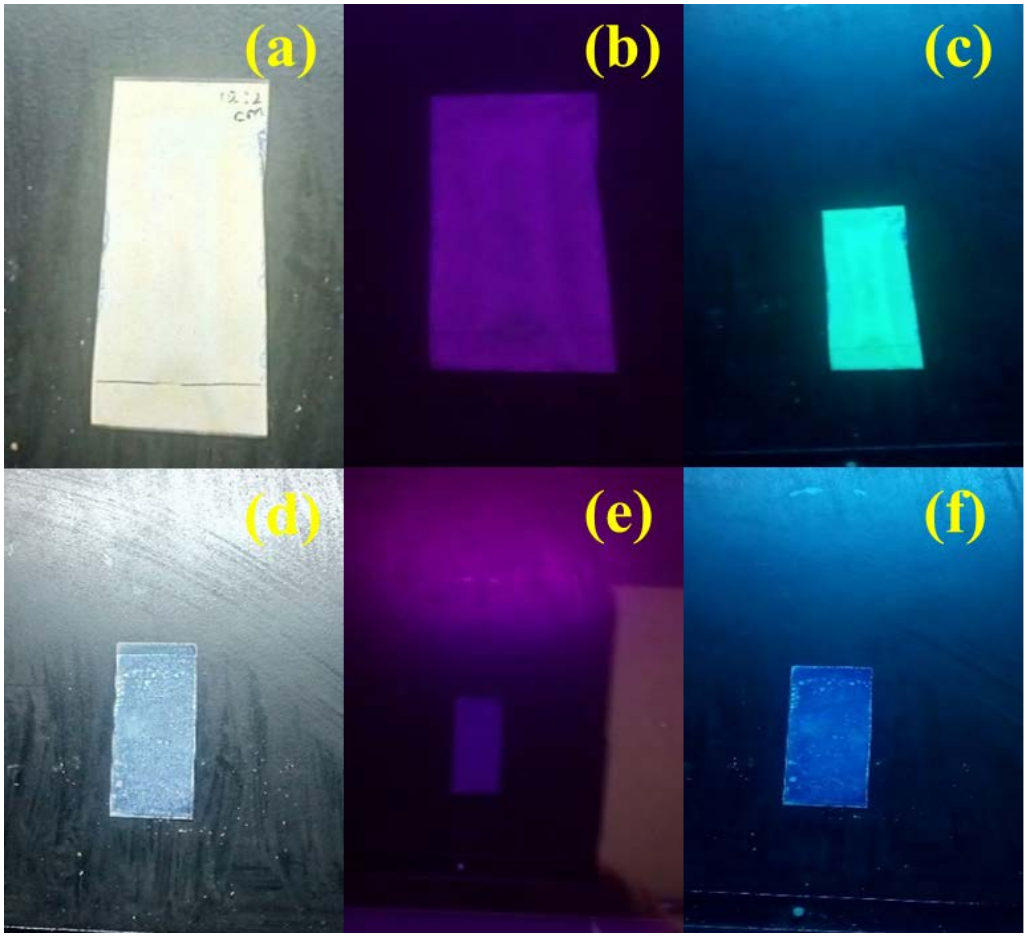

Fig 11: TLC run of stem extracts for alkaloids (a-c) and (d-f) phenols. The retention factor (R_f) values for alkaloids and phenols in the various TLC runs is observed. It indicates the TLC run for alkaloids is different than phenols. This imparts the presence of alkaloids and phenols in stem extract. The observation is conducted at visible, 254 and 366 nm.

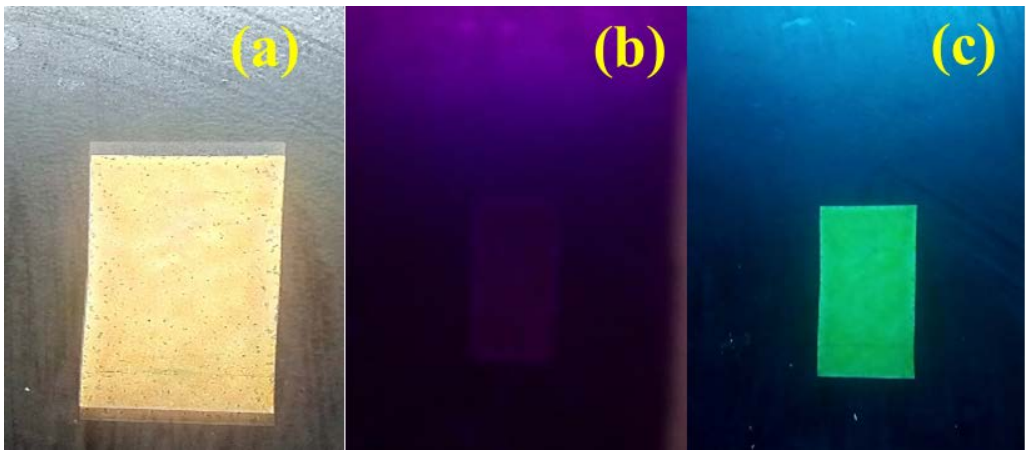

Fig 12: TLC run of stem extracts for tannin (a-c). The retention factor (R_f) values for Tannin in the various TLC runs is observed. This imparts the presence of tannin in stem extract. The observation is conducted at visible, 254 and 366 nm.

Conclusions

The plant sample is collected and extracted using suitable solvent methanol with the help of reflux apparatus. Antimicrobial activity is observed after performing the ager cup method which shows the zone of inhibitions. The plant extract is successful in the treatment of bacterial infection. The prepared Ag NPs from the *Cassia tora* plant can be used in the medical industry as a topical ointment to prevent infection against burn and open wounds. TLC was performed which indicate the presence of secondary metabolites. Secondary metabolites are observed in the plants besides the primary biosynthesis and metabolic routes for compounds associated with plant growth and development. Secondary metabolites can be used for signaling and regulation of primary metabolic pathways. The presence of secondary metabolites was carried out by different mobile phases and different spraying reagents.

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