

Original Research Article

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## Analysis of Phyto-Constituents and Free Radical Neutralizing activity of *Cassia alata*—A Green Source for Ag Np's Synthesis

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### ABSTRACT

#### Keywords

Antioxidant activity, *Cassia alata*, Phytocomponents, Silver nanoparticles.

#### Article Info

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*Cassia alata* is a lesser exploited plant for its biological activity due to its greater ornamental value which always cover medicinal applications. In the present study, Nanoparticle fabrication ability of the leaf extract was exploited for the successful synthesis of silver nanoparticles. It was observed that the extract could mediate the synthesis nanoparticle in lesser time with a high synthesis rate. Major phytocomponents present in the extract was analyzed and high percentage of phenolic compounds were observed, however there were traces of tannins, saponins etc. phytochemicals were found to facilitate free radical scavenging ability of the extract. From the analyzed parameters such as H<sub>2</sub>O<sub>2</sub> scavenging activity, super oxide radical scavenging, lipid peroxidation inhibition activity and DPPH assay, it was evident that extract possess high degree of antioxidant capacity, which was found to be proportional to its concentration.

### Introduction

Since prehistoric time, medicinal plants have been exploited for their antimicrobial, antimalarial and analgesic activity (Chatterjee *et al.*, 2012). Studies conducted so far upon medicinal plants can be categorized into two: such as optimization of ancient knowledge about a plant and discovering novel applications of lesser known plants. Both this branches have their own importance, as former enlighten the scientific reason behind ancient medications and the later opens up new possibilities of phyto pharmacology. Interest upon plant based therapeutic agents have been increased now a days after observing their

prominent biocompatibility and lesser toxicity than chemically synthesized counterparts (Meenupriya *et al.*, 2014). This trend make research on medicinal plants and their derived compounds of great economic value.

Till date, numerous methods have been explored so far to synthesize nanoparticles such as reduction of solutions, chemical and photochemical reactions, thermal decomposition compounds, radiation assisted, electrochemical, sonochemical and microwave-assisted processes. Plant assisted fabrication of nanomaterials has

acclimatized for their vast applications, cost effectiveness and biocompatibility (Hasan, 2015). Silver nanoparticle is one among the most desired metal nanoparticle with maximum applications. They are comparatively cheap to synthesize and biological activity of silver enhance the activity of synthesized nanoparticles. Many plants such as Thulasi, Neem (Banerjee *et al.*, 2014), Tomato (Many *et al.*, 2014) and Garlic (White *et al.*, 2012) have been used for the synthesis silver nanoparticle successfully but the demand of improvisation urges to find out more methods and cost effective sources for the same.

Normally nanoparticles are used for binding and transporting drug molecule to their target site due to which ligand receptor interaction will be enhanced and which reduces the required amount of drugs. Hinge on the previous studies, it is assumed that if the carrier nanoparticles can be synthesized by the same plant that with desired activity that will increase the efficacy of drug-carrier conjugate. *Cassia alata* belongs to the family Caesalpiniaceae, widely distributed in tropical countries such as India, Brazil, Indonesia and Fiji. It is commonly called as “Candle Bush Plant” (Ogunwande *et al.*, 2010).

They are well known for their skin protective ability, it is also used against anemia, constipation, dermatitis, dyspepsia, fevers, liver problems, menstrual disorders, and as purgative, expectorant astringent, vermicide (Mohideen *et al.*, 2005). In the present study, the ability of *Cassia alata* to mediate the synthesis of silver nanoparticle was evaluated and to scrutinize its phytoconstituents and free radical scavenging activity. This enables to develop a drug-nanoparticle conjugate that with antioxidant property.

## **Materials and Methods**

### **Preparation of Plant Extract**

*Cassia alata* plants were collected from in and around Ranipet, Tamilnadu, India. Plants were identified and verified by authorized botanist and stored for further studies. Five gram of chopped leaf sample was dispersed in 50% methanol to retrieve extract. It was kept in a water bath at the temperature of 50°C - 60°C for 5- 6 hrs. After cooling, the mixture in the flask was allowed to pass through Whatman filter paper no. 1 and filtrate was collected. Filtrate was vaporized to get a film of plant residue. The extract was stored in screwed tubes in -4 ° C to prevent the loss of bioactive compounds until further use (Bhat *et al.*, 2016).

### **Screening the Ability to Synthesis Ag NP's**

Leaf extract of *Cassia alata* was used as a source for the synthesis of silver nanoparticles. To 5 ml filtrate, 10 ml of 0.025 M AgNO<sub>3</sub> was added and the arrangement was left at room temperature, until the color of the solution changes from light yellow to brown. The presence of Ag NPs in the solution will be confirmed by a dark brown color (Gaddam *et al.*, 2014).

### **Phytoconstituent Analysis of *Cassia alata***

The presence of alkaloids, phenol, flavonoids, saponins, cardiac glycosides and tannins were analysed by the method described by Mahmood and Doughari (2008). Anthraquinones, steroids and terpenoids were analysed by modifying the methods described by Senthilkumar *et al.* (2013). Quantitative analysis of Alkaloids, Phenols, Flavanoids and proanthocyanidins were also analysed (Mir *et al.*, 2013).

### Free Radical Scavenging Activity of Extract

Ability of the bioactive compounds to neutralize free radicals were analysed by DPPH assay, super oxide scavenging, inhibition of lipid per oxidation and hydrogen peroxide neutralization assay. DPPH assay was carried out by following the method proposed by Aksoy *et al.* (2013). Ability of the extract to inhibit lipid peroxidation was determined by the method of Ćetković *et al.* (2007). Super oxide freeradical scavenging assay (Hazara *et al.*, 2008) and finally hydrogen peroxide assay (Geetha *et al.*, 2013) were also carried out understand the antioxidant ability of methanolic extracts of *Cassia alata* .

### Results and Discussion

#### Extraction of Bioactive Compounds

A significant yield was obtained by methanolic extraction of *Cassia alata* leaves. Similar extraction was done by Sherikar and Mahanthesh (2015). Elevated yield might be due to the polarity property of methanol (Parekh *et al.*, 2005).

#### Biofabrication of Silver Nanoparticles by Leaf Extracts

Leaf extracts of *Cassia alata* could successfully mediate the synthesis of silver

nanoparticle by reducing AgNO<sub>3</sub>. Change of color from light yellow (which is characteristic color of methanolic extract of *Cassia alata*) to dark brown proved the reduction and presence of silver nanoparticle. This pattern of color change has been previously reported in nanoparticle synthesis mediated by *Solanum nigrum* and *Clitoria ternatea* (Krithiga *et al.*, 2015). The reduction of silver is assumed to be mediated by phytochemicals (Ahmed *et al.*, 2016) that in turn could be mediated either by a single compound or by a group of compounds present in extract.

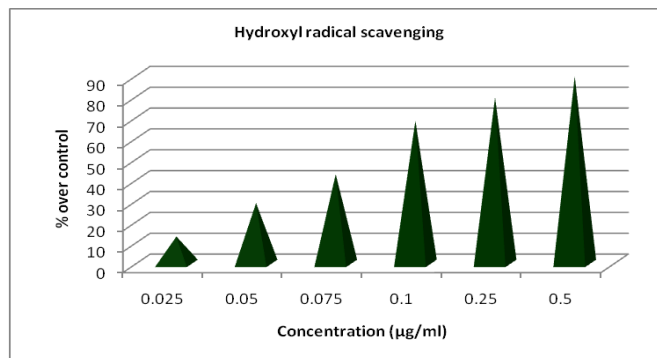
#### Phytoconstituent Analysis of the Extract

This study revealed that the methanolic extract of *Cassia alata* contained numerous phytochemicals such as alkaloids, flavonoids, saponins, tannins, steroids, cardiac glycosides, terpenoids etc (Table 1). Major component was found to be alkaloids followed by flavonoids. It is assumed that the medicinal properties of a plant are attributed by the phyto constituents present in it. Terpenoids purified from *Rhizoma Curcumae* have been reported for their anticancer activity (Lu *et al.*, 2012). Alkaloids have been studied for their effect on central nervous system (Patel *et al.*, 2012), whereas flavonoids are used for free radical scavenging (Meng *et al.*, 2012).

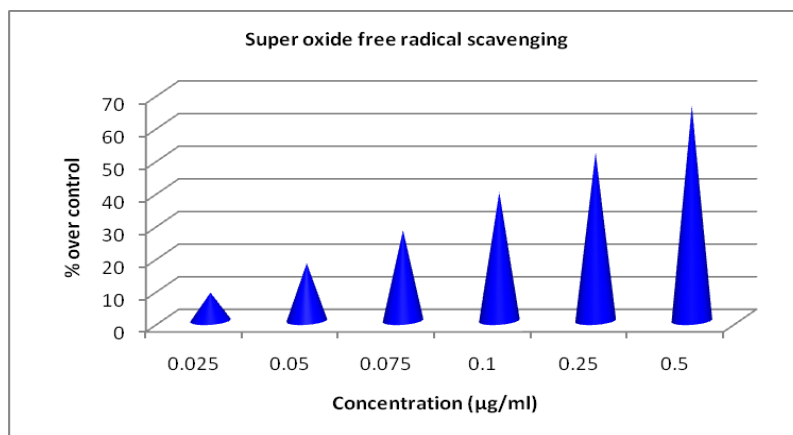
**Table.1** Phytochemical Composition of Methanolic Extract of *Cassia alata*

S.No	Phytochemicals	Result
01.	Alkaloids	Positive
02.	Phenol	Positive
03.	Flavonoids	Positive
04.	Saponins	Positive
05.	Cardiac glycosides	Positive
06.	Anthraquinones	Positive
07.	Terpenoids	Positive
08.	Steroids	Positive

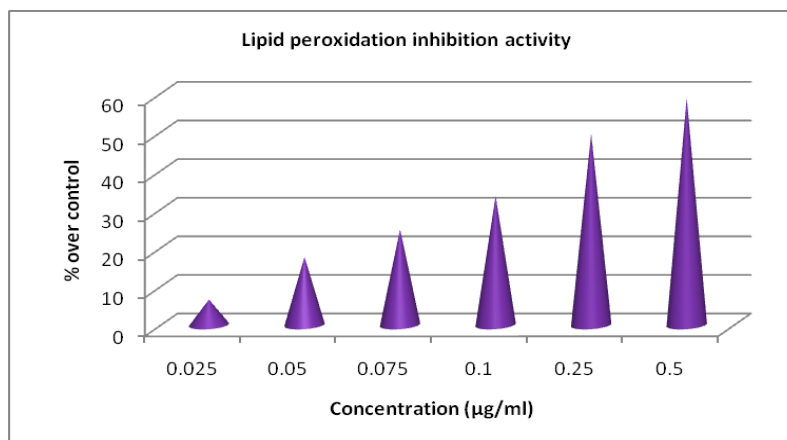
**Fig.1** Hydroxyl Radical Scavenging Activity of *Cassia alata*



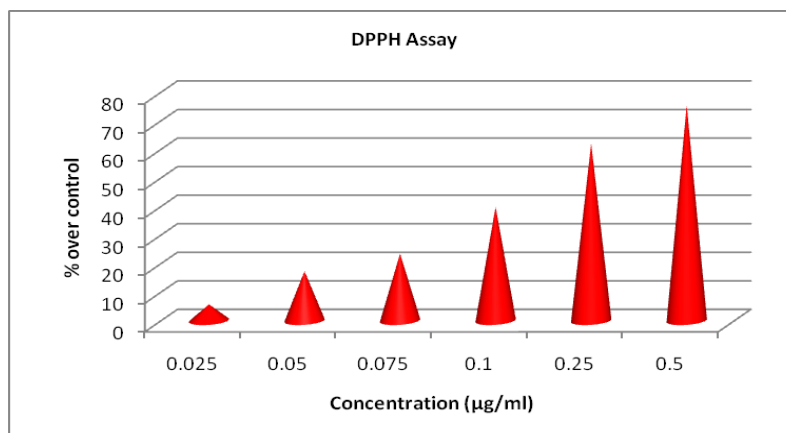
**Fig.2** Super Oxide Free Radical Scavenging Activity of *Cassia alata*



**Fig.3** Lipid Peroxidation Inhibition Activity of *Cassia alata*



**Fig.4** DPPH Assay of *Cassia alata*'s Leaf Extract



### Free Radical Scavenging Activity of the Extract

In order to evaluate the scavenging activities of the plant extracts, parameters like hydrogen peroxide test, superoxide analysis, lipid peroxidation inhibition and DPPH assay were studied. Extract exhibited significant free radical scavenging activity against selected radicals (Figure 1-4). Activity was found to be directly proportional with concentration of the plant extract. Maximum activity was found with the concentration of 0.5 µg/ml; whereas no reaction was found with the concentration of less than 0.025 µg/ml. Antioxidant property of *Cassia alata* extract is been majorly complimented by the bioactive components present in the extract such as alkaloids and flavonoids (Sagnia *et al.*, 2014), however phenol mediated free radical scavenging activities also have been reported by Sharma and Vig (2013).

*In conclusion* *Cassia alata* leaf extracts was found to be an effective agent for the synthesis of silver nanoparticles. Silver nanoparticles were synthesised in a notable time with impeccable rate. Antioxidant activity of extract found very promising as it could modulate free radical scavenging upto 90% compared that with the absence of

extract. It is assumed that the biotechnological activities of leaf extracts are complimented by its phytocomponents. Phenols, alkaloids and flavonoids constitute major part of extract. The results observed from this study can be taken forward to develop a nanoparticle bound antioxidant with high degree of activity.

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