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ANTIOXIDANT PROPERTIES OF SILVER NANOPARTICLES AND METHANOL EXTRACT OF *CINNAMOMUM VERUM* BARK

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Abstract: This study was aimed at synthesizing silver nanoparticles of *Cinnamomum verum* and find their antioxidant properties in comparison with the Methanol extract. FTIR spectral measurements were carried out at a resolution of 4 cm^{-1} to identify the potential functional groups of biomolecules in the *Cinnamomum verum* extracts. The spectral analysis revealed presence of compounds such as flavonoids, phenols, terpenoids, and proteins, and could confirm that these biomolecules in the C*innamomum* were responsible for reducing, capping, and stabilizing of the AgNPs. The antioxidant activity of synthesized AgNPs was evaluated by DPPH and reducing power assay, L- ascorbic acid was used as a positive control. The antioxidant activity using DPPH showed Cinna. methanol higher antioxidative than the $AgNP_s$ at 500µg/ml and the reducing power of Cinna. $AgNP_S$ was evaluated to be higher than the Cinna methanol. Thus, the AgNPs synthesized from *Cinnamomum* could be promising candidates for use in nano medicine and beneficial in the nutraceutical industries.

***Keyword*:** Silver Nanoparticles ($AgNP_s$), Fourier Transform Infrared (FTIR), Cinnamomum (Cinna.), 2, 2-diphenyl-1-picryl-hydrazyl radical scavenging (DPPH), Silver Nitrate ($AgNO_3$)

Introduction

Cinnamomum verum, called true cinnamon tree or Ceylon cinnamon tree, is a small evergreen tree belonging to the family *Lauraceae*, native to Sri Lanka and Southern parts of India. The inner bark of several other *Cinnamomum* species is also used to make cinnamon, but *C. verum* has a subtler flavor that makes it preferred for certain recipes as spice (Adewole *et al.*, 2013). They have been therefore used as food additives. They improve the flavor, taste and color of food, as well as extending the shelf life of food by inhibiting the growth or decreases the food borne pathogens. Spices are known to be natural antimicrobials which have found relevance in the preservation of foods. Therefore, cinnamon, garlic, ginger, mint, etc. are used as substitute in health remedies.

Most spices show antioxidant and antimicrobial activity against bacteria, yeasts, and molds. The biological activity of spices is based on the phenolic compounds, so can be effectively applied as food preservatives. Spices can be classified according to their antioxidant and antimicrobial activities into three categories; the first classified as strong (cinnamon, clove, mustard), the second as medium (all spices, sage, bay leaf, caraway, coriander, cumin, rosemary, thyme, oregano), and the third as weak (black pepper, red pepper, ginger) Tarik *et al.*, 2016.

Nanotechnology is an emerging field, which utilizes nanoparticles (NPs) in various applications such as in food packaging, as preservatives, in cosmetics, as carriers of therapeutic agents in nanomedicine (Shalaby *et al.*, 2015; Al Sammarraie *et al.*, 2018). The biosynthesis of nanoparticles has received increasing importance in the last decade due to societal demand to develop environmentally friendly technologies in material synthesis. The biosynthesis method employing plant extracts has increased some attention as a simple and viable alternative to chemical procedures and physical method synthesizing metal nanoparticles only in recent years. Al Sammarraie *et al.*, 2018.

Nanotechnology is becoming increasingly important and popular in the food and health sectors providing promising results and applications in increasing nutritive value and drug delivery system through bioactive Nano encapsulation, biosensors to detect the quality pathogens, as well as moved resources for the evaluation and the development of newer, safer, and effective dry formulation. (Cos *et al.*, 2006). Among various metal used like gold, silver nanoparticles (AgNPs) have been applied as antibacterial, antifungal, antiviral, and anti- inflammatory, antidiabetic and catalytic activity due to its distinguishable physical, chemical, biological and prevention of biofilm (Gurunathan *et al.*, 2014). Chemical synthesis of silver nanoparticles mostly ends in aggregation as the storage time extends while biosynthesis of nanoparticles using plant extracts also known as synthesis is low-cost, environmentally friendly and produces stable nanoparticles (Banerjee *et al.*, 2014; Halawani *et al.*, 2017; Sharma *et al.*, 2014).

Silver nanoparticle, may be added in nontoxic concentration of food as several studies carried on the toxicity of silver nanoparticles (Ivask *et al.*, 2014) verified studies nanoparticles had no cytotoxicity to mammalian cells at 26.7mg/L. in another investigation recorded that AgNPs couldn't affect the mammalian cells morphology up to 6,500ng/ml concentration (Arora *et al.*, 2008). Nanoparticles have the ability to discover food spoilage and food pathogens through Nano sensors. Also, nanoparticles used in food packaging consisting of polymers in combination with Nano devices are known as smart packaging. Natural, edible Nano laminates can also carry antioxidants and antimicrobials. For extension of shelf-life (Ramachandraiah *et al.*, 2015).

According to the World Health Organization (WHO), 2019, more than 30% of the World's population relies on traditional medicine for their primary healthcare needs. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Duraioandiyan *et al*, 2006). Due to the adverse effects and multidrug resistance to the chemically synthesized drugs, researchers have returned to ethno-pharmacognosy. *Cinnamomum* has been used for generations as a spice, fragrance and for herbal treatment. The researches carried out *invitro* and *invivo* studies provides evidence that *Cinnamomum* has antimicrobial, anti-inflammatory, antioxidant, cardiovascular, cholesterol-lowering, immunomodulatory effects and antitumor activity. *In vitro* studies have demonstrated that cinnamon may act as an insulin mimetic, to potentiate insulin activity or to stimulate cellular glucose metabolism (Khan *et al.*, 2003). Furthermore, animal studies have demonstrated strong hypoglycemic properties. The use of *Cinnamomum* as an adjunct to the treatment of type 2 diabetes mellitus is the most promising area, but further research is needed before definitive recommendations can be made (Shen *et al.*, 2002), Anderson *et al*, (2004), Cao *et al* , (2007), Taher *et al*, (2004), Taylor *et al*, (2001).

Materials and Methods

Material collections:

Cinnamomum verum (inner barks) was purchased at Local Ayurvedic Shop, Nanded.

Raw Materials Preparation:

Cinnamomum verum (inner barks) were brought to the laboratory and the sample was subjected to treatment before experimental use. The modified method described by Adewole *et al*, (2013) was used accordingly. The particle size of the sample was determined manually by sieve analysis (Jillavenkatesa *et al.*, 2001). The fresh *Cinnamomum* was sorted and cleaned by washing in distilled water. It was allowed to dry for two days and then coarsely milled in waring blender and proceeded for Maceration/Hydro-distillation. The sample was stored in an airtight container at 4 ^{o}C and used for experimental analysis.

Extraction:

a) Methanol Extract

Cold maceration was the method of extractions for the methanol extract. The solvent used was methanol, the volume of the solvent was twice the physical size of the extract, and the sample was crushed out in a big conical flask, the solvent added, covered and kept at a room temperature for 24 hours, shaken at intervals. After maceration, the plant sample was filtered using muslin, the extract was put in a rotary-evaporator to reduce the volume in the extractant after which it was transferred into a stainless plate and put in a water bath for complete drying. The dried extract was collected (using a spatula) and put in airtight bottle containers, kept in a cool dry place for laboratory analysis.

b) Hydro Distillation of Essential Oil

The essential oil was extracted using the hydro-distillation process (Kehinde *et al.*, 2021). The 100g cinnamon powder was taken in a round bottom flask and 800 ml distilled water was added, then set up a Clevenger apparatus. The set was placed in a balloon heater attached to a refrigerator to ensure condensation of essential oils for 3 hours. At the end of the distillation, two phases were observed, an aqueous phase (aromatic water) and an organic phase (essential oil), less dense than water. The essential

oil was collected, dried under anhydrous sodium sulfate, and stored in an amber bottle in the dark, at 4°C, until used.

Synthesis of Silver Nanoparticles:

Cinnamomum AgNPs extract was synthesized by adopting the method described by Gloria *et al.*, 2017. 20g of the sample was weighed into a 250ml capacity conical flask and 100 ml of distilled water was added and boiled at 60°C in a water bath for 10 minutes respectively. Each extract was cooled, filtered using Whatman filter paper No. 1. Fifteen (15) ml of the extract was added into 45ml aqueous silver nitrate ($AgNO_3$) (0.1M solution) at room temperature and stirred continuously with a magnetic stirrer for 15 minutes so as to get a solution of extract and $AgNO_3$ in the ratio of 1:3. Each conical flask containing the respective extract was wrapped in aluminum foil and kept in the dark to prevent auto-oxidation of silver. After 24 hours, each extract containing silver Nanoparticle ($AgNP_s$) was centrifuged at 3000 rpm for 10 minutes and the resulting pellets were dried in an oven at 100°C for 24 hours. The resultant $AgNP_s$ of each extract was used for antioxidant assay.

Characterization of silver nanoparticles:

The purified AgNPs were characterized using the following techniques. The formation of AgNPs was monitored by visual assessment of the color changes of the solutions. The reduction of silver was measured periodically at a wavelength range of 300–700 nm using a UV-Vis spectrophotometer (Shimadzu make). The UV-Vis spectra of AgNPs produced was plotted and recorded as a function of bio reduction time (15 min intervals) at room temperature at a resolution of 0.5 nm. Fourier Transform Infrared (FTIR) (Nicolet iS5, Thermo-scientific Berlin Germany) analysis was used to determine the possible biomolecules responsible for the reduction of silver ions to AgNPs. The samples were analyzed using a spectrometer. Spectra was collected from 50 scans at a resolution of 4 cm^{-1} in the range of 500-4000. The remaining pellet was used for AgNPs antioxidant in comparison with Methanol extract.

Antioxidant Assay:

The antioxidant activities of the *Cinnamomum verum* methanol extract and AgNPs was evaluated using 1,1-diphenyl-2- picrylhydrazyl (DPPH) and Reducing power.

a) Free Radical Scavenging Assays

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form of DPPH. This transformation results in a color change from purple to yellow, which is measured by a spectrophotometer. The disappearance of the purple color is monitored at 517 nm. The free radical scavenging activity can be measured by using 2, 2-diphenyl-1-picryl-hydrazyl. The reaction mixture consists of 0.1 ml of DPPH in methanol (0.3mM), 1.0 ml of the extract and 1.0ml of methanol. It is incubated for 10 min in dark, and then the absorbance is measured at 517 nm. In this assay, the positive control is ascorbic acid.

The percentage DPPH radical scavenging activity can be calculated using the formula:

% DPPH radical scavenging activity= {(A0-A1)/Ao} x 100

Where A0 is the absorbance of control and A1 is the absorbance of the test sample.

b) Reducing Power Assay (RP)

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of liquid per oxidation process, so that they can act as primary and secondary antioxidants.

The reducing power can be determined by taking 1.0 ml of extract with 2.5 ml of phosphate buffer (200 Mm, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50^0C for 20min. Thereafter, 2.5 ml of trichloroacetic acid (600 mM) is added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) is mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (6 mM) and absorbance is measured at 700 nm. Ascorbic acid can be used as positive control.

Results and Discussion

The methanol extract and Silver nanoparticles of *Cinnamomum* obtained were analyzed for antioxidant properties. *Cinnamomum verum* showed strong antioxidant activity by inhibiting DPPH, and reducing power activities when compared with standard L-ascorbic acid. The results of this study shows that the extract can be used as an easily accessible source of natural antioxidant. The IC50 of Cinna. methanol was found to be higher than the Cinna. $AgNP_S$ (**Table 1**).

Table 1: IC50 OF DPPH

Sr. No	Samples	IC50
1	Cinna. AgNPS	1595
2	Cinna. methanol	492.6

Fourier-Transform Infrared Spectroscopy

FTIR spectral measurements were carried out at a resolution of 4 cm^{-1} to identify the potential functional groups of biomolecules in the spice extracts responsible for reducing and capping the bio-reduced AgNPs (Sasidharan *et al*, 2011). The absorbance of the *Cinnamomum* sample as a function of wave number (cm^{-1}) was determined using FTIR (Nicolet iS5, Thermo-scientific Berlin Germany). The FTIR was carried out to identify the functional groups and the types of bonds occurring at the range of 500-4000 (cm^{-1}). The FTIR analysis revealed different stretches of bonds at different peaks for the spices. Some of the functional groups studied in this work belong to hydrocarbons, esters, alcohols, acids which are mostly (monoterpenes and sesquiterpenes of bioactive compounds. **Figure 1** below, presents the FTIR spectra of C*innamomum*. The bands observed denote stretching vibrations responsible for compounds such as flavonoids, phenols, terpenoids, and proteins, and could confirm that these biomolecules in the C*innamomum* were responsible for reducing, capping, and stabilizing of the AgNPs as reported by Liu *et al.*, 2007 and Khanna and Nair 2009.

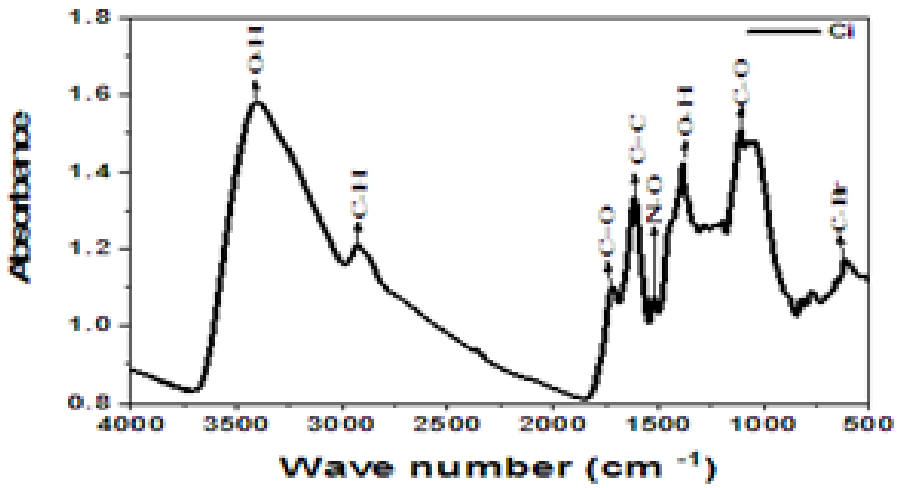

Figure1: FTIR spectra of *Cinnamomum verum bark*

Table 2: Vibrational frequencies and wave number of *Cinnamomum verum*

Sr. No.	Wave number (cm^{-1})	Functional group compounds	
1	3404.76	O-H stretching	alcohol (strong)
2	2925.42	C-H stretching	alkene (medium)
3	1719.76	C=O stretching	conjugated acid (strong)
4	1612.32	C=C stretching	α,β-unsaturated ketone (strong)
5	1518.74	N-O stretching	nitro compound (strong)
6	1384.58	O-H stretching	carboxylic acid (strong)
7	1108.47	C-O stretching	aliphatic ether (strong)
8	616.27	C-Br stretching	halo compound (strong)

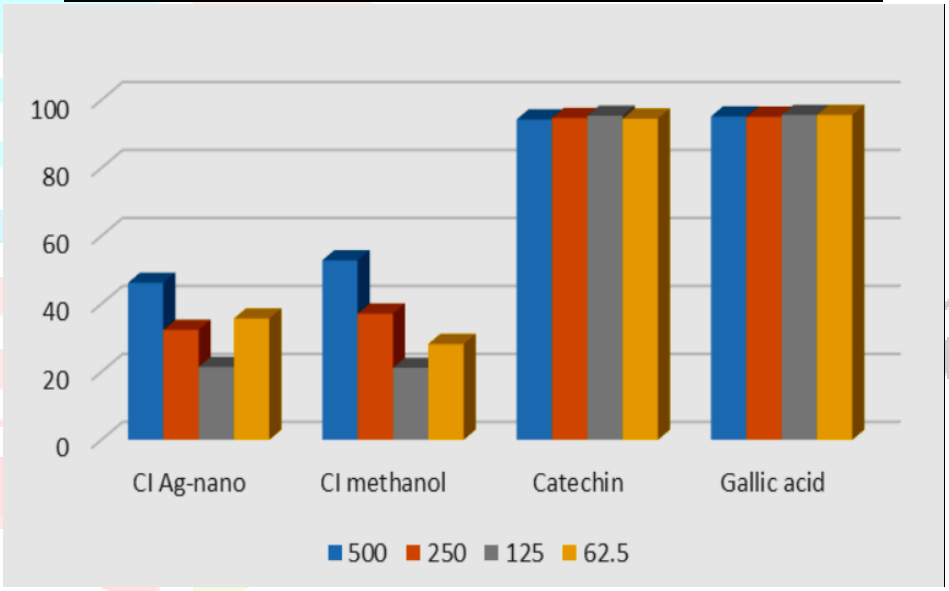

Figure 2: DPPH radical scavenging activity of *Cinnamomum verum*

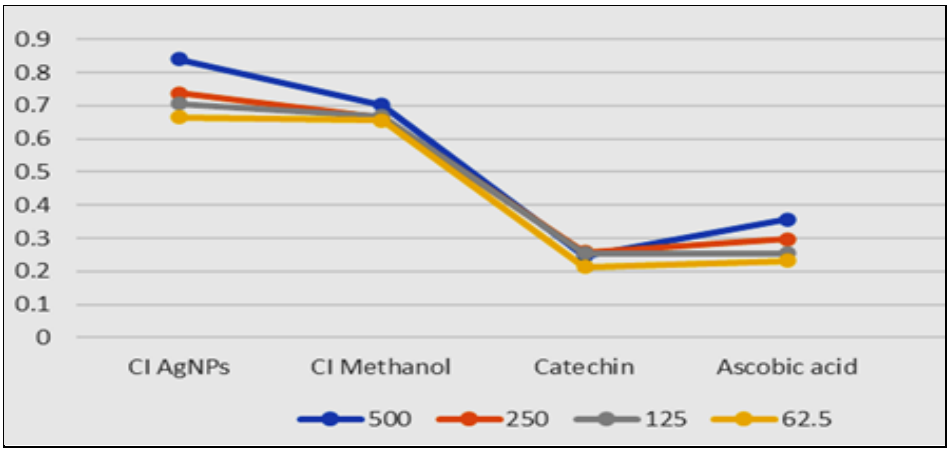

Figure 3: Reducing Power of *Cinnamomum verum*

Antioxidant Activity

Antioxidants control oxidative reactions by inhibiting, delaying or hindering the oxidation of the biomolecules (Kumar *et al.*, 2011). Non enzymatic antioxidants can also neutralize radicals for example water soluble substances such as Vitamin C, glutathione or fat-soluble substances such as Vitamin E, β-carotene (Trombino *et al.*, 2004). In recent years there has been an increase in the search for effective, non-toxic, natural compounds with antioxidative activity. Some nanomaterials have been seen to exhibit strong antioxidant properties.

In this study, *In vitro* antioxidant activity of the synthesized AgNPs and corresponding methanol extract of *Cinnamomum* were studied by analyzing antioxidant capacities which are indicative of the antioxidant potential of the synthesized AgNPs (Vivekanandhan *et al*, 2012) as reported.

DPPH is a stable organic free radical that has been used for investigating the free radical activities and thus antioxidant activity of various natural products (Rushender *et al.*, 2012). The DPPH was considered to be a model of lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid auto-oxidation. Being a stable free radical, DPPH is regularly used to determine radical scavenging activity of natural compounds. In its radical form, DPPH absorbs at 517 nm and its absorbance decreases upon reduction with an antioxidant (Lateef *et al.*, 2015). The IC50 values for DPPH scavenging activity of synthesized AgNPs were presented in **Table 1** above. **Figure 2** shows the dose response for the DPPH scavenging activity of the synthesized AgNPs and methanol extract. The AgNPs synthesized from the methanolic extract are potential free radical scavengers with effective inhibition activity in a dose dependent manner. The varying concentration of the AgNPs significantly scavenged DPPH, however, these activities are less than that of Ascorbic acid and Catchen, the standard reference used.

The Reducing Power of a compound is related to its electron transfer ability and therefore may serve as a significant indicator of its potential antioxidant activity (Gülçin *et al.*, 2003). The reducing power of the samples increased with increasing the concentrations. The reducing property of the extracts implies that it is capable of donating hydrogen atom in a dose dependent manner **Figure 3**. Our results are consistent with the data published previously (Gloria *et al.*, 2017). Here, we assume that the antioxidant activity and reducing power capacity of the extracts was likely due to the presence of polyphenols, which can act as free radicals scavenger by donating an electron or hydrogen.

Conclusion

Biologically active pure compounds are better than crude extract. Here, we examined the inner bark of *Cinnamomum verum* and found that methanolic extract of *Cinnamomum verum* (bark), which contains large amounts of phenolic and flavonoid compounds, exhibited the highest antioxidant and free radical scavenging. These in vitro assays indicate that *Cinnamomum verum* (bark) are a significant source of natural antioxidants, which could help to prevent the progression of various diseases caused by free radicals, such as certain cancers. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigation is needed to isolate and identify the antioxidant compounds present in the plant extract. The essential oil shows that the *Cinnamomum verum* has a good oil yield and aroma. This essential oil has several bioactive compounds and has a lot of health benefits. Methanol extract and the synthesized $AgNP_S$ of *Cinnamomum verum* could be used in nanomedicine, nutraceuticals and in food packaging industries (Yu *et al.*, 2018).

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