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Research Article

The chemical composition, characterization, and combination effect of Ocimum *campechianum leaf* essential oils and bio-produced silver nanoparticles against *Listeria monocytogenes* and *Escherichia coli.*

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Abstract

Metallic nano-particles (NPs), particularly silver nanoparticles (AgNPs) are emerging as biological tools for assessing the antibacterial, antifungal, and antioxidant properties and to enhance the potential therapeutic efficacy of botanicals. Basil (Ocimum spp.), rich in various essential oils has been attributed with a wide range of antimicrobial properties. The aim of this study was to compare anti-bacterial activity of basil (Ocimum campechianum) essential oil, bioproduced silver NPs and their possible combinations against Listeria monocytogenes and Escherichia coli. The constituents of essential oil of Ocimum campechianum was analyzed by gas chromatography/mass spectrometry (GC-MS). The AgNPs were monitored by visualizing color changes and were characterized by using UV-Vis, FT-IR, SEM, and TEM. Further, antibacterial activity of basil EO extracts alone, bioproduced silver NPs alone, and their possible combination activity with basil EO extracts were assessed by measuring turbidity of growth rates of Listeria monocytogenes and Escherichia coli using BioScreen C analyzer. A total of 20 compounds were identified in the essential oil accounting for 99.6% of the composition. The oil of O. campechianum contained as main components, caryophyllane 4, 8- α-epoxy (59.5%), (E)-caryophyllene (11.7%), and (E)-methyl isoeugenol (6.3%), α-Cymene (5.6%). The presence of AgNPs was confirmed by the excitation of Surface Plasmon Resonance (SPR) using UV–visible spectrophotometer at 442nm. The FT-IR analysis showed the presence of possible biomolecules needed for reducing silver ions; SEM and, TEM analysis confirmed the spherical shape and the average particle size (5-50nm). The reduction in the growth of L. monocytogenes was 66% by EO extract, 42% by bio-produced AgNPs alone, and by 49% by the combined effect of (EO extract with bio-produced AgNPs). The EO extract of accession PI 652066 reduced the growth of E. coli by 62%, whereas combined EO extract with bio-produced AgNPs reduced the growth by 25%. However, there was no inhibition of the growth of E. coli by AgNPs alone. The results suggest that O. campechianum plant extract could be used as a proficient reducing agent for the bio production of AgNPs and, can be used to treat multidrug resistant microorganisms and other bio medical applications.

Keywords: *Ocimum essential oils (EO), Gas Chromatography, Silver nanoparticles (AgNPs), Characterization techniques, Human pathogenic bacteria.*

1. Introduction

Nanotechnology has assumed a great importance in recent years because of its possible applications in several areas, such as electronics, pharmacy, computers, catalysis, biotechnology (Chitte *et al*., 2012). The preparation of noble metal and inorganic nanoparticles such as gold, silver, and platinum have been widely used for many years for technological applications (Das, Gang, & Nath, 2011).

Among nanostructured noble metals, silver and silver nanoparticle possess unique physiochemical properties and metabolic activity due to their large surface area to volume ratio. These unique properties of AgNPs enable them to be used for sensing and imaging applications, medical biosensors, diagnosis, drug delivery, and medical device coating (Thawadi, Rasool, & Youssef, 2017). They are also used in medical industry as topical ointments to prevent infection of burn and open wounds (Sivaranjani & Meenakshisundaram, 2013), and antibacterial properties (Sondi & Salopek-Sondi, 2004). Biological methods for synthesis of NPs have emerged as an alternative to the conventional methods

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of using either chemical or physical synthesis (Ramteke *et al*., 2013). Ecofriendly plant extracts contain biomolecules, which act as both reducing and capping agents that can be utilized to synthesize stable and shape-controlled nanoparticles (Saha, Deb, & Bhattacharyya, 2015). Silver NPs are known to possess oligo dynamic properties, and can kill antibiotic-resistant microbes while exerting limited cytotoxicity against mammalian cells (Hsueh *et al*., 2015; Martinez-Castanon *et al*., 2008).

Hence, biological approaches employing plant extracts as bio-reductants for the synthesis of silver NPs have been explored. The fungal strain, *Aspergillus foetidus* MTCC8876 (Swarup, Mukherjee, Chakraborty, & Das, 2013), leaf extracts of plants such as *Ocimum tenuiflorum*, *Solanum tricobatum*, *Syzygium cumini*, *Centella asiatica* and *Citrus sinensis* extracts (Logeswari, Silambarasan, & Abraham, 2015), *Musa barbisiana*, *Ocimum tenuiflorum*, *Azadirachta indica* (Arumugam *et al*., 2017) have been used for synthesizing bioproduced silver NPs. In the present study, bioproduction of silver nanoparticles were carried out by using the leaves of *Ocimum campechianum* rich in essential oils and distinct aromatic compounds. Studies show that *Ocimum micranthum Wild (O. campechianum)* leaves contain saponins, flavonoids, tannins and essential oils (Charles & Simon, 1990). The *O. campechianum* is commonly sold for culinary purposes, aromatic baths, and as a medicinal plant (Zoghbi *et al*., 2007). A chemotype from North East-Brazil showed analgesic activities, anticonvulsant, antispasmodic and antifungal properties as per studies reported by (M. G. de V. Silva, *et al*., 2004), anesthetic, analgesic, and anti-inflammatory activity with potential application in traditional medicine (P. R. N. Vieira *et al*., 2014), is often attributed to predominant essential oil constituents, such as methyl chavicol, eugenol linalool, camphor, and methyl cinnamate (Simon, Quinn, & Murray, 1990). The antimicrobial properties of the leaf extracts of *O. campechianum* species in combination with or without silver nanoparticles have not been determined against common human pathogens to date. The objective of this study was to compare the antibacterial activity of essential oil and bio-produced silver NPs of O. *campechianum* accession PI 652066 against the growth of selected human pathogenic bacteria, *Listeria monocytogenes* and *Escherichia coli*.

2. Materials and Methods

2.1. Collection of plant material.

The seeds of *O. campechianum* accession PI 652066 were, planted in seed germination trays filled with a soilless potting mix (Pro-Mix - Premier Horticulture Inc., Quakertown, PA USA) in the greenhouse. The acrnymn, PI indicates plant introduction number. Six-week-old greenhouse grown seedlings were transplanted on to raised beds (50 cm wide, 15 cm high, 25 m long, and 2 m apart, covered with 4 mm black plastic with drip irrigation tubing underneath the plastic) at the Alabama A & M University Winfred Thomas Agricultural Research Station located in Hazel Green, AL (latitude 34°89'N and longitude 86°56'W). Soil at the experimental site is a Decatur silty loam (fine, kaolinitic, thermic Rhodic Paleudult). Fresh leaves from the plants were harvested at the peak vegetative phase early morning and placed immediately in a cooler with ice packs for transportation to the laboratory for further processing.

2.2. Extraction of Essential Oil from O. campechianum

The essential oils (EO) were extracted from 100g of fresh leaves for 3 hours using a Clevenger-type distillation apparatus, per the British Pharmacopoeia specification (Ajao, Olaniyi, Kudirat, Eunice, & Nusirat, 2017; Shadia, El-Aziz, Omer, & Sabra, 2007). The EO were collected and stored at 4°C until further analysis.

2.3. GC-MS Analysis

GC–MS analysis of the samples was carried out by using the Agilent GC 6890 series Mass Selective Detector, equipped with fused silica capillary HP-5MS column (30m X 250μm X 0.25 μm). Helium gas was used as the carrier gas and the initial oven temperature was set at 60 ^{0} C and held for 5 minutes. The final temperature of the oven was 280 ^{0} C with flow rate at 1.5mL/min. Mass spectra were recorded over 35-650 amu range with electron impact ionization energy pf 70 eV. The total running time for each sample was 78 minutes. The diluted oil in DCM (1.0 μL) was injected in splitless mode.

2.4. Compound Identification

The linear retention indices (RI) for the compounds were determined by the alkane standard mixture (C_{10}-C_{40}) all even series (Sigma- Aldrich USA). Retention indices (RI) values were measured from HP-5MS column and calculated. RI values for alkane mixture and *Ocimum* EO sample both were calibrated by using open chrom software. After calculation of RI values using open chrom, identification of the oil components was based on their retention indices determined by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007).

2.5. Bio- production of Silver NPs

Ten grams of fresh leaves were finely cut and boiled in 50 mL of sterile distilled water for 5 minutes. Three mL of the filtrated plant aqueous extract was added to a heated $AgNO_3$ solution (50 mL of 1 mM $AgNO_3$). The filtrate acts as a reducing and stabilizing agent to form AgNPs (Anuradha, Syama Sundar, Kumar, & Ramana, 2014; Okafor, Janen, Kukhtareva, Edwards, & Curley, 2013). The reaction was carried out for a period of 24h at 25 °C in dark. The nanoparticles obtained were used for further characterization.

2.6. Characterization of bio-produced silver NPs

2.6.1. pH and color

The pH of the medium may play a crucial role in the biosynthesis of nanoparticles. The size of the silver nanoparticles was controlled by changing the pH values of the reaction system. The change in pH was determined using Digital pH meter Systronics and calibrated using buffers of 0.1 N HCl or 0.1 N NaOH (Behera & Nayak, 2013). UV-visible spectroscopy as well as visual observation of color changes were used to infer the production of silver NPs and as a result of reduction of Ag^+ to Ag^0.

2.6.2. UV-visible spectroscopy

UV-visible absorption spectra to monitor the formation of AgNPs was determined by using a UV-Visible spectrophotometer (Model CARY 3E, Varian Co.,) between the range of 350–700 nm.

2.6.3. The Fourier Transform Infrared Spectrometer (FTIR) analysis

The FTIR was carried out to determine the nature of the capping agents in the leaf extracts and the spectrum was recorded using (NICOLET IS10 FTIR, ThermoFisher, USA), within a scanning range of 4000-500 cm^{-1} at a resolution of 2 cm^{-1}.

2.6.4. Scanning Electron Microscopy (*SEM) analysis*

The bio-produced silver NPs was confirmed for morphological structure by scanning electron microscopy (JEOL JSM-6610LV) (JEOL JAPAN) performed at acceleration voltage of 15KV. Thin films of the sample were prepared on a silicon wafer by just dropping one drop of the sample, then let it dry by putting it under a fume hood. The samples were characterized by using the SEM at a voltage of 20 kV.

2.6.5. Transmission Electron Microscopy (*TEM) analysis*

The shape as well the diameter of the bio-produced silver NPs were determined using TEM. The TEM grids were prepared by placing a five µL of the AgNPs solutions on carbon-coated copper grids and dried under the heat of an incandescent lamp. The TEM 200 analysis software, JEM- 2010 electron microscope ((JEOL, Tokyo, Japan) was used in this study.

2.6. Antibacterial Assay

2.6.1. *Preparation of essential oils extracts*

For this purpose, the essential oil extracts of *O. campechianum were* reconstituted by dissolving them in 1% DMSO (Dimethyl sulfoxide). The mixture at a ratio of (1:1) of equal volumes of essential oils and bio-produced AgNPs were vigorously mixed by in a vortex and were then incubated in a ultrasonic bath for 20 min before experimentation (Bransonic 3510r-Dth) Branson Ultrasonic Corporation USA).

2.6.2. Antibacterial activity assay (BioScreen assay)

The strains of Gram-positive *L. monocytogenes* and Gram-negative bacteria *E. coli* cultures were grown overnight in MHI (Mueller Hinton Infusion) broth at 37 °C, and diluted in sterile MHI solution to provide a final concentration of approximately 10^5 colony-forming units per mL and adjusted according to turbidity at 0.5 McFarland tube scale (V. A. Silva *et al*., 2015).

Growth rate was determined by measuring turbidity using the BioScreen C (BioScreen C, Lab system, Helsinki, Finland) with a wideband filter between 420 and 580nm. The optical density (OD) curve was generated based on the turbidity measurements at regular intervals for 24h. The microbiological calculations were generated directly to MS Excel sheets. The measurements were then processed to generate microbiological growth curves by plotting turbidity vs. time ("Growth Curves USA, exclusive US distritutor of Bioscreen C Automated Microbiology Growth Curves Analysis System," 2015), (Wu, Griffiths, & McKellar, 2000). The percentage inhibition of the bacteria was calculated per the following equation (1):

$$Percentage\ of\ inhibition = \frac{(100\ x(sample\ mean\ (OD) - control\ mean\ (OD)}{\text{Control mean (OD)}} \quad (1)$$

3. Results

3.1. Chemical composition of Ocimum campechianum leaf essential oils

The leaf essential oil composition of O. campechianum accession PI 652066 is presented in Table 1. A total of 20 compounds were identified in the essential oil accounting for 99.6% of the composition. The major components of the leaf oil were Caryophyllene 4, 8- α-epoxy (59.5%), (E)-caryophyllene (11.7%), and (E)-methyl isoeugenol (6.3%), α-Cymene (5.6%), (Z)-Methyl isoeugenol (3.3%), and Germacrene D (3.2%).

Table 1. Chemical composition of Ocimum *campechianum* leaf essential oils

RI[1]	RI[2]	Compound	Percent composition
869	871	(4Z)- Hexanol	0.7
928	928	α- Pinene	0.2
971	973	Hexanoic acid	0.6
975	975	*Trans*- pinene	0.3
989	991	Cineole dehydro 1, -8-	0.2
1025	1024	p-Cymene	0.6
1027	1029	Limonene	5.6
1035	1037	*Cis*-Ocimene	1.3
1045	1050	*Trans*-Ocimene	0.1
1186	1186	(E)-4-Octenoic acid, ethyl ester	0.3
1194	1096	Linalool	0.1
1356	1359	Eugenol	1.9
1388	1388	β -Cubebene	2.0
1414	1416	Caryophyllane 4, 8- α-epoxy	59.5
1417	1419	(E)-Caryophyllene	11.7
1450	1452	(Z)-Methyl isoeugenol	3.3
1477	1480	(E)- β-Ionone isomethyl	1.0
1482	1485	Germacrene D	3.2
1492	1492	(E)-Methyl isoeugenol	6.3
1501	1502	*Trans*- β -Guaiene	0.6
		Total Identified	99.6

KEY: RI[1]-Retention index determined with reference to a homologous series of n-alkanes on a HP-5MS column; RI[2] -Retention indices from Adams (2007) unless otherwise indicated.

3.2. Analysis of bioproduced silver NPs

3.2.1 pH and color

The individual colors of pure silver nitrate ($AgNO_3$), the aqueous basil extract, and the bio-produced silver NPs of *O. campechianum* accession PI 652066 are shown in Figure. 1.

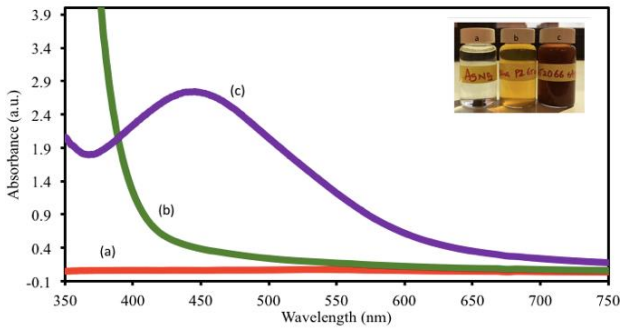

Fig. 1. UV-vis spectroscopy results showing comparison of control (a) $AgNO_3$, (b) aqueous basil leaf extract, and (c) bio produced silver NPs of *O. campechianum* PI 652066 accession

The $AgNO_3$ was a clear, colorless liquid, whereas aqueous extract of the basil, *O. campechianum,* was light yellow in color. The sangria colored solution was obtained when the bioproduced silver NPs were formed. This color change indicated the reduction of Ag^+ to Ag^0.

3.2.2. UV-vis spectra analysis

The bio produced leaf extract of *O. campechianum* PI 652066, showed a strong absorption between 415-465 nm (Figure 1.), corresponding to the resonance of silver NPs before gradually declining to almost the same as control $AgNO_3$ alone. The bioproduced silver NPs of basil showed absorbance at about 2.4 a.u. higher than control, because of the excitation of surface plasmon resonance.

3.2.3. FTIR analysis

Figure 2. shows bio produced silver NPs of *O. campechianum* accession (PI 652066) leaf extract act as

reducing and capping agent to stabilizing silver NPs due to presence of functional groups confirmed by FTIR analysis. There were two absorption peaks observed around 3345 cm^{-1} and 1636 cm^{-1}, indicating that presence of capping agents with the silver NPs. The peak around 3345 cm^{-1} corresponds to both functional groups of (N-H) and (O-H) stretching vibrations that represented to the amines, alcohol and phenol groups. The peak around 1636 cm^{-1} corresponds to carbonyl group (C=O) that was involved in the reduction of Ag+ to Ag^0.

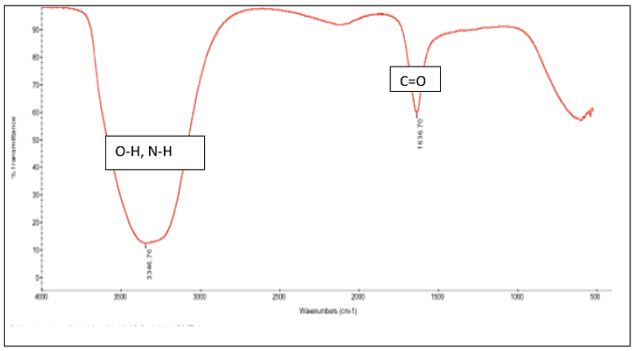

Fig. 2. FTIR spectra of bio produced silver NPs of *O. campechianum* PI 652066 accession

3.2.4. SEM analysis

The SEM image of *O. campechianum PI 652066* accession is shown in Fig. 3. The nanoparticles formed were relatively spherical in shape and varied between 5 and 50 nm in size.

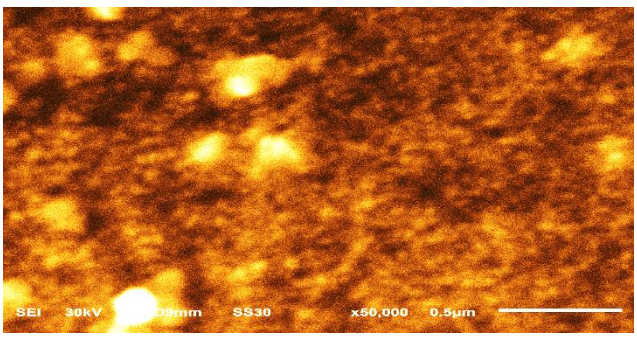

Fig. 3. SEM image of bio produced silver NPs of *O. campechianum* PI 652066 accession

3.2.5. TEM analysis

The TEM image of bio produced silver NPs synthesized by *O. campechianum* leaf extract is shown in Figure 4. The bio produced basil AgNPs were identified as roughly spherical shaped with smooth edges, nano sized between 5 and 50 nm and well dispersed.

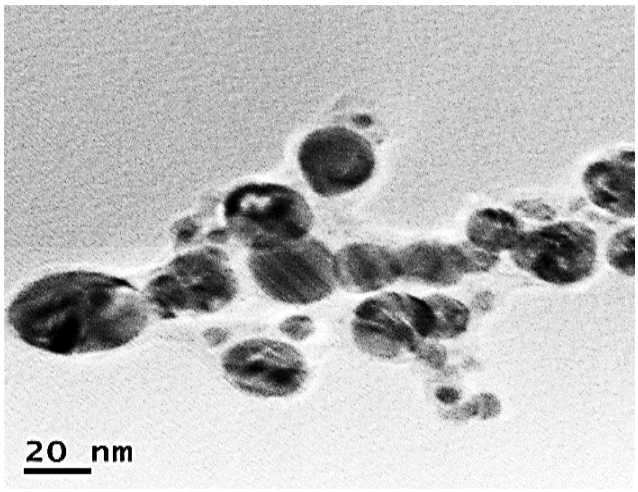

Fig. 4 TEM micrograph of bio produced silver NPs of *O. campechianum* PI 652066 accession

3.2.6. Antibacterial activity

The growth reduction of *L. monocytogenes* in response to basil EO extract, bioproduced silver NPs, and combined activity of EO extract with silver NPs, are shown in Figure 5A. The EO extract of *O. campechianum* (PI 652066 accession) reduced bacterial growth by 66%, while bioproduced silver NPs, and (combined extracts of basil EO extract with silver NPs) reduced bacterial growth by 42% and 49%, respectively. The OD mean absorbance units were 0.25, 0.42, 0.37, respectively. The OD for *L. monocytogenes* alone was 0.72. Both EO extract alone and in combination with silver NPs showed greater antimicrobial activity than bioproduced silver NPs alone.

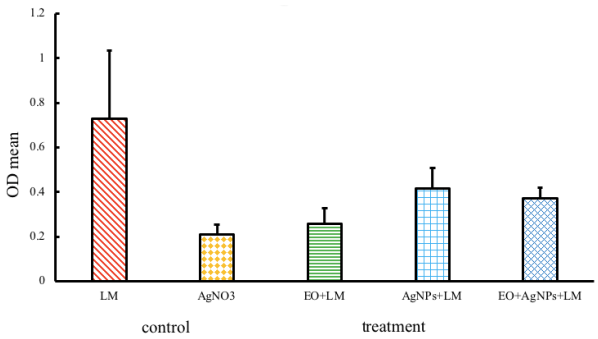

Fig. 5A

Figure 5B. shows the growth response of *E. coli* to basil EO extract, bioproduced silver NPs, and combined EO extract with bioproduced silver NPs. The EO extract of *O. campechianum* (PI 652066) had higher growth reduction of 62%, while the EO extracts with bioproduced silver NPs reduced the growth of bacteria by 25%. The mean absorbance units were 0.25 and 0.47, respectively. However, the bioproduced silver NPs did not show any inhibition of the growth of test bacteria E. *coli*. The OD mean absorbance units of AgNPs was 0.66 after 24h. This OD was slightly higher than that of *E.coli* (the control) which had OD of 0.65. The OD units for the

negative control, silver nitrate ($AgNO_3$ solution) was 0.20.

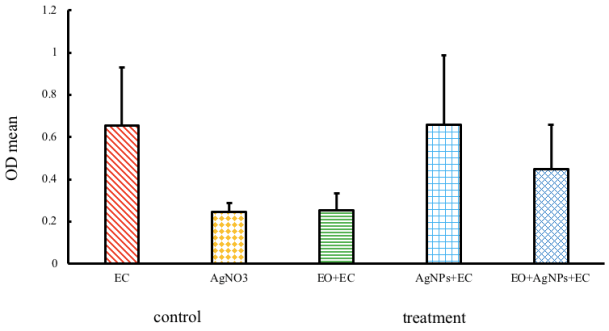

Fig. 5A

Fig. 5A and 5B. Comparison of optical density mean growth reduction over time of (A) *L. monocytogenes* (B) *E. coli* treated with EO extracts alone, bioproduced silver NPs alone and EO extracts plus bioproduced silver NPs from *O. campechianum* (PI 652066) accession.

KEY: EO+EC-essential oil *with E. coli;* AgNPs+EC- AgNPs with *E. coli* ; EO+AgNPs+EC- essential oil and AgNPs with *E. coli*; EO+LM-essential oil with *L. monocytogenes*; AgNPs+LM - AgNPs with *L. monocytogenes;* EO+AgNPs+LM - essential oil and AgNPs with *L. monocytogenes;* Negative control $AgNO_3$.
Error bar signifies the ±1 standard deviation from the sample means at OD 420–580.

4. Discussion

Our results based on GC-MS analysis indicated that essential oil (EO) composition of Brazil basil, *O. campechianum* accession (PI 652066) had extensive diversity in terms of the chemical constituents. However, "caryophyllane 4, 8- α-epoxy" had the highest percentage (~ 60%) in comparison to the other constituents (Table 1.). Therefore, the chemotype for O. *campechianum* in this study is "caryophyllane, 4, 8- α-epoxy". This is based on the statements of Grayer *et al*. (1996); Vina & Murillo. (2003), the major constituents of essential oil above 20% constitutes as its chemotype. It is common for differences in chemotypes to be in response to environmental changes, agronomic practices, time of harvest as well as soil type (Joshi & Hoti, 2014; Sims, Juliani, Mentreddy, & Simon, 2014; R. F. Vieira & Simon, 2000).

The characteristic sangria color solution is consistent with the bio production of silver NPs. This color change is due to the presence of stabilizing compounds such as phenolics, terpenoids, proteins etc. in the leaf extract medium. The phenolic compounds with low pH of 5.0 probably act as capping agents for silver NPs. The silver nitrate ($AgNO_3$) solution by itself had a higher pH of 6.4. This is in agreement with the study of Gurunathan *et al*., 2014; Perumal Samy, Manikandan, & Al Qahtani, 2013. These authors indicate that secondary metabolites of leaf extracts provide stability to bioproduction of colloidal silver nanoparticles. The UV-Vis spectra showed a strong absorption between 415-465 nm which is characteristic of silver NPs showing surface plasmon resonance. This absorption spectra is due to a plasma wave of free electrons on the silver surfaces which are set into oscillation when electrical field is applied through the medium. A similar surface plasmon resonance observed in this study was also reported by Kumar, Kulriya, Pivin, & Avasthi, 2011; Vilas, Philip, & Mathew, 2014; Vollath, 2013. In addition, we observed that there was a 2.4 higher absorbance units (a.u.) for the bioproduced silver NPs of basil in comparison to the control (silver nitrate solution) (Fig 1). In FTIR analysis we confirmed that secondary metabolites with functional groups are responsible for a strong binding ability, capping and stabilization of bioproduced silver NPs; these prevent agglomeration of the nanoparticles by coating layers on the surface of the silver NPs. Similar results were reported by Patel, Channiwala, Chaudhari, & A. A. Mandot, 2015; Rónavári *et al*., 2017; Tejaswi, Rao, & Chakara, 2013. There are two peaks, the first peak around 3345 cm^{-1} is due to (O-H) structural polymeric association) and (N-H) stretching vibration are the functional groups of alcohol, phenol and amines. The second peak at 1636 cm^{-1} is assigned due to the (C=O) stretching vibration for amide I derivative for proteins that is involved in the reduction of silver (Fig 2.). This is similar to the study by Arumugam *et al*., 2017; Bhattacharya, 2015. Further validation of silver NPs production, their nano morphology and particle size distribution were determined by SEM and TEM analysis.

From the TEM analysis, the surfaces of silver NPs were smooth and circular shaped, edges of nanoparticles mainly lighter than the centers surrounded by a thin film of plant material which might be due to the capping of bio molecules of leaf broth. Our study indicated that there was about 75% of nanoparticles size ranges from 5 to 50 nm of the total particles observed (Fig 3. & Fig 4.). A similar kind of particle size was also observed in studies conducted by Bindhani & Panigrahi, 2015; Padalia, Moteriya, & Chanda, 2015.

Our study showed that the *O. campechianum* EO extracts alone had higher activity against both Gram-positive (*L. monocytogenes)* and Gram-negative (*E. coli)* bacteria. Our study from GC-MS analysis (Table 1.), indicated that major chemical components of EO extracts of *O. campechianum* were caryophyllane 4, 8- α-epoxy, (E)-caryophyllene, (E)-methyl isoeugenol, α-Cymene , (Z)-Methyl isoeugenol, and Germacrene D may be attributed for their antibacterial activity against test bacteria. For instance, β-caryophyllene, has been reported as having strong antibacterial effect against test bacterial strains *(B. cereus, B. subtilis, S. aureus, E. coli, K. pneumoniae and P. aeruginosa)* with MIC values that ranged from 3 to 14 µM (Dahham *et al*., 2015). The antimicrobial action of EO components with their lipophilicity hydrocarbon skeleton and the hydrophilicity of their major functional groups such as (phenols, aldehydes, ketones) contributes to their antibacterial activity (Roldán, Díaz, & Duringer, 2010).

The bioproduced silver NPs alone had more pronounced antimicrobial activity on Gram-positive *L. monocytogenes* than on Gram-negative bacteria, *E. coli.* The mechanism of silver NPs alone was known to be as permeabilization of bacterial cell wall and destabilization of the peptidoglycan layer and negatively charged teichoic acids found in Gram positive bacteria enables the nanoparticles to bind to the positively charged silver NPs leading to their cell lysis. A similar observations was reported by Peiris *et al*., 2017. However, in the case of Gram-negative bacteria (*E. coli*), no growth reduction was observed by bio produced silver NPs alone in this study. This is may be due to a cationic surfactant on the bacterial suspension as well as coagulation of nanoparticles via a charge neutralization mechanism. A similar mechanism was suggested by Pal, Tak, & Song. (2015), using *E. coli* ATCC 10536 cells. However, Dakal, Kumar, Majumdar, & Yadav. (2016), indicated that low colloidal stability of the silver NPs alone in liquid system may have limited its antibacterial activity. In our study, bioproduced silver NPs combined with basil essential oil extract showed slightly higher bacterial growth reduction on both Gram positive and Gram-negative bacteria. However, there was no synergistic effect observed with this combination. Overall, our study indicates that EO extracts of *O. campechianum* had higher growth reduction of both test bacteria.

Conclusions

The study showed clearly that the *O. campechianum* leaf extracts can be used effectively to synthesize nanosized silver NPs and this was validated using different techniques. The antibacterial activity of essential oil extract had high percentage of growth reduction than the bioproduced silver NPs alone against both human pathogenic bacteria used in this study. We conclude that lowered concentrations /dose of EO extracts of basil (*O. campechianum*) in combination with silver NPs, is a potent natural antimicrobial agent that could serve as an alternative to antibiotic use.

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