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Studies on Essential Oil Extraction in *Plectranthusamboinicus* (lour.) leaves and Evaluation of Antioxidant and Antimicrobial Activities

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ABSTRACT

Essential oils are complex volatile compounds, naturally synthesized by various parts of the plant during the secondary metabolism of plants. A wide range plants having the medicinal properties have been explored and used for the extraction of essential oils worldwide due to their antimicrobial properties the bacterial, fungal, and viral pathogens. *Plectranthusamboinicus* (Lour.) Spreng. is a perennial herb belonging to the family Lamiaceae which occurs naturally throughout the tropics and warm regions of Africa, Asia and Australia. The various bioactive compounds present in *P.amboinicus* is used for various ailments.

In this work, the studies were carried out in *P.amboinicus* using agar diffusion method for screening the most effective essential oils and agar dilution to determine the inhibitory concentration of essential oil. Antibacterial activity of essential oil is proven against *Pseudomonas putida*, *Escherichia coli* and *Streptoccocus* sp. Antibacterial activity is done by disc diffusion method. The results were observed in terms of IZ around the disc caused by diffusion of antibacterial properties from essential oil impregnated disc into the surrounding medium. Antioxidant property is evaluated using impregnated disc of essential oil in the test tubes with solution a graph along with standard is plotted against it using the data of optical density and based on percentage of DPPH inhibition activity of the solution.

Keywords: *Plectranthus*, Lamiaceae, antioxidant, antimicrobial.

Introduction

Plectranthus is a large genus, with more than 300 species from the family of Lamiaceae. It is a perennial herb belonging to the family Lamiaceae which occurs naturally throughout the tropics and warm regions of Africa, Asia and Australia. It has a rich diversity of ethnobotanical and medicinal uses. Several species of the genus possess interesting medicinal properties such as the extract of *P. barbatus* is used for the treatment of stomachache and as a pugitive, nausea and gastritis and intestinal spasms in Brazil. *P.*

caninus, *P. laxiflorus* and *P. barbatus* are used in the treatment of teeth and gum disorders. It is also reported that *P. amboinicus* and *P. barbatus* are used to treat a wide range of diseases such as for the treatment of digestive system, skin conditions and allergies, infections and fever, genito-urinary conditions, pain, respiratory conditions and muscular-skeletal conditions (Lukhoba, 2006).

The production of essential oils and their utilization as potential natural sources for new phytomedicines could be of economic value (Khalid and Gohary, 2014). The leaves of the *P.amboinicus*are often eaten raw or used as flavoring agents, or incorporated as ingredients in the preparation of traditional food. The literature survey revealed the occurrence 76 volatiles and 30 non-volatile compounds belonging to different classes of phytochemicals such as monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, phenolics, flavonoids, esters, alcohols and aldehydes (Arumugam et al., 2016). The presence of various important constituents or secondary metabolites such as flavonoids, glycosides, phenols, tannins, and steroids, which have been identified through various spectroscopic methods. Chang et al., 2010 investigated therapeutic efficacy of *P. amboinicus* in treating Rheumatoid Arthritis (RA) using collagen-induced arthritis animal model.

There 26 compounds were identified by GC and GC-MS from the essential oil of *P. amboinicus* and studied its chemical composition and larvicidal potential against the malarial vector mosquito *Anopheles stephensi by* Senthilkumar and Venkatesalu, (2010). The major chemical compounds were carvacrol (28.65%) followed by thymol (21.66%). Goncalves et al., 2012 evaluated the antimicrobial activity of the essential oil, obtained from leaves of *P.amboinicus*, the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The MIC and MBC of the essential oil were 0.09 ± 0.01%.Leaf extract of *P. amboinicus* is also subjected to synthesis of nickel oxide nanoparticles using nickel nitrate as the precursor. Also, it is showed a moderate positive effect in antibacterial test and a positive reaction in the antifungal activity against the fungal strain Candida. Hasibuan and Ilyas (2013) studied about the antioxidant and cytotoxic activities of P. amboinicus (Lour.) Spreng. Extracts. The antioxidant activity was tested using DPPH and Beta Carotene-Linoleic Acid methods.This plant is also subjected to the preparation of silver nanoparticles (Ag NPs) using its leaf extract. The synthesized Ag NPs showed better antimicrobial property towards gram negative *E. coli* and towards tested *Penicillium* spp. than other tested microorganisms using disc diffusion method (Ajitha et al., 2014, Chiu et al., 2012).

Materials and Methods

Fresh leaves of *P. amboinicus* are collected from Vallathol Nagar Grama Panchayat Cheruthuruthy, Thrissur. *P. amboinicus* commonly known Indian borage and it is a medicinal plant which comes under Lamiaceae. It is a fleshy, succulent herb with a distinctive taste and scent. It is known as panikoorka in malayalam and have other lesser known names, it shows fungicidal, insecticidal, antitumourous, antiinflammatory, antidiabetic along with antimicrobial and antioxidant activities. The leaves were cut into fine pieces using scissors and then they are weighed 100gms by using weighing machine. Plant collection was authenticated and avoucher specimen no. VCTBH0024 has been deposited at Vimala College (Autonomous), Thrissur.

Fig.1.*Plectranthusamboinicus*and extraction of essential oil

3.2. Essential oil distillation:

Extraction of essential oil from *P. amboinicus* is subjected to hydrodistillation method using clevenger apparatus for 3 hours. Plant material (leaves) was immersed directly in a round bottom flask filled with water. The pooled organic phases were anhydride sodium sulphate was used to remove water after the extraction. Essential oil was stored in air tight container in the refrigerator at -25^{0}C in sealed glass vials.

Antibacterial activity assay

Microorganisms used:

The following food-borne pathogens were used in their in the antibacterial test. *Pseudomonas putida, Escherichia coli* and *Streptococcus* sp. The strains are obtained from St. Mary's college, Thrissur. *E.coli* is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium that is commonly found in the lower intestine of warm-blooded organisms. It causes food contamination. *P. putida* is a Gram-negative, rod-shaped, saprotrophic soil bacterium. The *in vitro* antibacterial activity of essential oil from

*P.amboinicus*was evaluated using an Agar disc-diffusion method against selected two gram-negative pathogenic bacteria (*Escherichia coli*, *Pseudomonas putida*) and one gram positive bacteria *Streptococcus* sp.

The instruments used for the antibacterial activity were autoclaved at 121^0 C at 15 ibf pressure.1.3 gram of nutrient broth were weighed out, then dissolve broth in 100ml. 5 test tubes were inoculated with the strain of bacteria to this under aseptic conditions. Added 2.8 gram in 100 ml nutrient agar this nutrient agar and again autoclaved at 121^0 C at 15 ibf pressure. By using laminar air flow pour the nutrient agar solution to the sterilised petriplates. Next day inoculated each bacterial strain in to the petridishes by using an inoculation loop. On next day the disc were impregnated with the essential oil by dipping the extract followed by the drying the normal temperature.

A sterile (6mm diameter) impregnated with different concentrations of essential oil, was placed on the surface of each plate, and incubated for 24 hours at 37^0 C for bacteria. The essential oil were placed on disc by using a micropipette and firmly placed on to the inoculated agar ensuring even distribution to avoid overlapping of zones. The disc is impregnated with the antibiotic amoxicillin trihydrate were used to control sensitivity of the test organisms. The process is repeated in strains of bacteria. After 24 hours antibacterial activity was evaluated by measuring the diameter of the zones of inhibition against tested bacteria. The results of agar diffusion assays were evaluated by measuring the inhibition zone diameter (in mm), after incubation. All the experiments were carried out in triplicate and average and standard deviation (SD) were calculated for the inhibition zone diameters.

Determination of antioxidant activity

2, 2-diphenyl-2-picrylhydrazyl hydrate assay (DPPH assay)

Radical scavenging activity of essential oil against stable DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate, Sigma-Aldrich Chemie, Steinheim, Germany) was determined by using a spectrophotometer. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour were measured at 515 nm on a UV/visible light spectrophotometer. Radical scavenging activity of extracts was measured by slightly modified method of Brand-Williams et al., (1995), as described below. 1 ml of methanolic solution containing 0.198 mM of DPPH (2, 2-diphenyl 1-1- picrylhydrazyl) was added to 1 ml of various concentrations of the extracts and standard ascorbic acid. The absorbance of the mixture was measured at 517 nm with Labtronics NT 290 spectrophotometer after 30 min of incubation time at room temperature in the dark. The

experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

$$1(\%) = (1-(As/Ac)) \times 100$$

Where Ac is the absorbance of the negative control and As is the absorbance of the sample. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily.

Result and discussion

The use of essential oils with antimicrobial and antioxidant properties to increase the shelf life of food is a promising technology, and the essential oils of the Lamiaceae family, such as rosemary, thyme, and sage, have been extensively studied with respect to their use as food preservatives.

Antimicrobial activity

Antimicrobial studies, carried out with the agar disc diffusion test, showed that the essential oils of *P. amboinicus* exihibited activity against gram negative bacteria tested. The test provided positive result for two species of gram negative bacteria (*P.putida*, *E. coli*).The inhibitory zone diameter is maximum for *E.coli* (20cm). The result of agar diffusion assays were evaluated by measuring the inhibition zone diameters (in cm), after incubation. All the experiments were carried out in triplicate and average and standard deviation were calculated for the inhibition zone diameters. Antimicrobial studies, carried out with agar diffusion test,showed that the essential oils of *P. amboinicus* exihibited significant activity against gram negative bacteria of two different species with highest Escherichia coli.

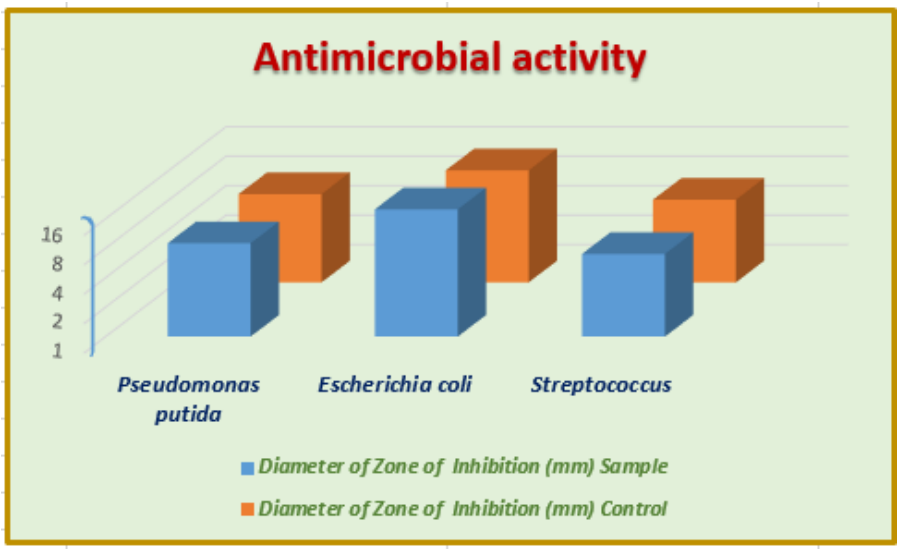

Fig.2. Antimicrobial activity of essential oil of *P. amboinicus*

These results showed that *P.amboinicus* can be used in traditional medicine. Literature shows that there are many reports regarding the studies of antimicrobial potential of medicinal plants. For example, the essential oil of *P. neochilus* (PN-EO) which is hydrodistillated using essential oil of *P.neochilus* displays promising antimicrobial activity against some cariogenic bacteria, including *Streptococcus mutans*, which is one of the main causative agents of dental caries. In the present study it reveals that the essential oil from *P.amboinicus* showed maximum activity against *E.coli* (20 mm) and the least activity towards the gram positive bacteria *Streptococcus* (7mm). Taken together, our results suggest that this essential oil might be promising for the development of new oral care products. This confirms that Lamiaceae species are recognized by the presence of terpenoids with antifungal, antibacterial, and insecticidal actions.

Antioxidant activity

The DPPH radical is a widely used model to evaluate the antioxidant property of plant extracts. DPPH is a stable nitrogen-centered free radical, the colour of which changes from violet to yellow upon reduction by either the process of hydrogen or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers.

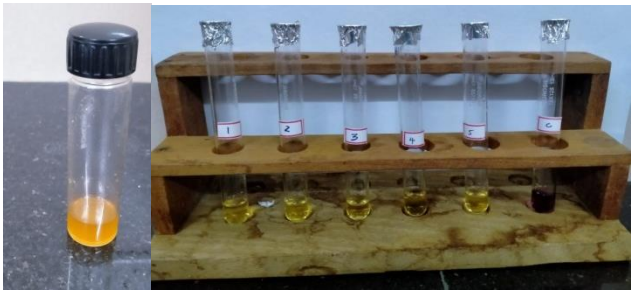

Fig.3.The extracted oil from *P. amboinicus*and its antioxidant assay

The radical scavenging activity of the extracts of *P. amboinicus* at different concentrations is shown in table.1. In DPPH assay the ability of compound to act as donor for hydrogen atom or electron was measured spectrophotometrically. Reduction capacity of DPPH radical is obtained by decrease in its absorbance at 515nm.

SL NO	CONCENTRATION	OD VALUE
1	1	0.667
2	2	0.266
3	3	0.13
4	4	0.073
5	5	0.074

Table.1. DPPH free scavenging activity of essential oil from *Plectranthusamboinicus*

DPPH Assay

In DPPH assay the ability of compound to act as donor for hydrogen atom or electron was measured spectrophotometrically. Reduction capacity of DPPH radical is obtained by decrease in its absorbance at 515nm. The percentage inhibition of DPPH radicals were increased as concentration of extracts was increased [Table.1 and Fig.4.]. The results showed that essential oil extracts of *P. amboinicus* had significant antioxidant activity. So the antioxidant potential may be due to these polyphenols. Hence the further studies are needed to evaluate the in-vivo antioxidant activity of the plant in various animal models.

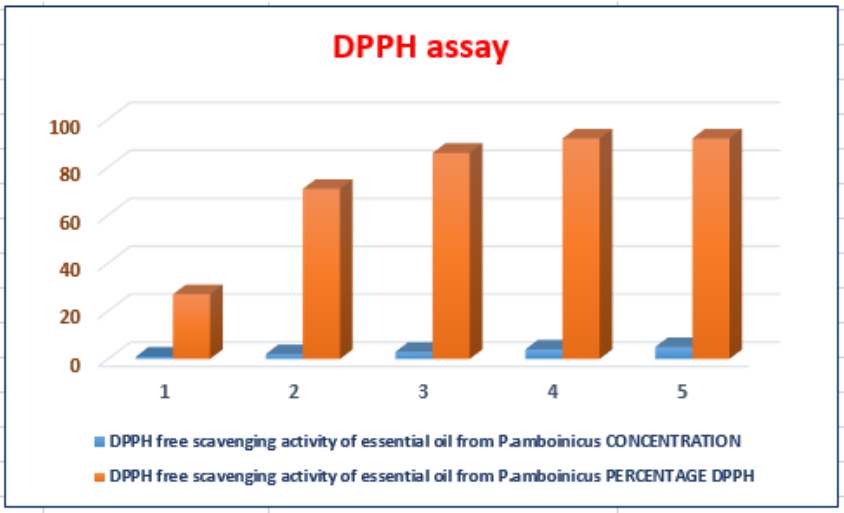

Fig.4. % of DPPH free scavenging activity of essential oil from *P.amboinicus*

Conclusion

The present study was undertaken to investigate the *P. amboinicus* is an important aromatic medicinal herb packed with many bioactive constituents and nutrients, which are important for maintaining good health. This study on essential oil from*P.amboinicus*shown high antimicrobial activity in *P. putida, E.coli* and *Streptococcus sp.cultures* and also showed positive antioxidant activity *which reveals it has significance in pharmacology*. Apart from its bioactive properties, it can be used for other purposes which cater to future needs in medicine.

References

1. Lukhoba, C. W., Simmonds, M. S., & Paton, A. J. (2006). Plectranthus: A review of ethnobotanical uses. *Journal of ethnopharmacology*, *103*(1), 1-24.
2. Arumugam, G., Swamy, M. K., &Sinniah, U. R. (2016). *Plectranthusamboinicus* (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance. *Molecules*, *21*(4), 369.

3. Khalid, A. K., & El-Gohary, A. E. (2014). Effect of seasonal variations on essential oil production and composition of *Plectranthusamboinicus* (Lour.) grow in Egypt. *International Food Research Journal*, *21*(5), 1859.
4. Chang, J. M., Cheng, C. M., Hung, L. M., Chung, Y. S., & Wu, R. Y. (2010). Potential use of *Plectranthusamboinicus* in the treatment of rheumatoid arthritis. *Evidence-Based Complementary and Alternative Medicine*, *7*(1), 115-120.
5. Senthilkumar, A., &Venkatesalu, V. (2010). Chemical composition and larvicidal activity of the essential oil of Plectranthusamboinicus (Lour.) Spreng against Anopheles stephensi: a malarial vector mosquito. *Parasitology research*, *107*(5), 1275-1278.
6. Goncalves, T. B., Braga, M. A., de Oliveira, F. F., Santiago, G. M., Carvalho, C. B., e Cabral, P. B., ... & Nagao-Dias, A. T. (2012). Effect of subinihibitory and inhibitory concentrations of Plectranthusamboinicus (Lour.) Spreng essential oil on Klebsiellapneumoniae. Phytomedicine, 19(11), 962-968.
7. Hasibuan, P. A. Z., &Ilyas, S. (2013). Antioxidant and cytotoxic activities of Plectranthusamboinicus (Lour.) Spreng. extracts. *International Journal of Pharmacy Teaching & Practices*, *4*(3), 1-3.
8. Ajitha, B., Reddy, Y. A. K., & Reddy, P. S. (2014). Biosynthesis of silver nanoparticles using *Plectranthusamboinicus* leaf extract and its antimicrobial activity. *SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy*, *128*, 257-262.
9. Chiu, Y. J., Huang, T. H., Chiu, C. S., Lu, T. C., Chen, Y. W., Peng, W. H., & Chen, C. Y. (2012). Analgesic and antiinflammatory activities of the aqueous extract from *Plectranthusamboinicus* (Lour.) Spreng. both in vitro and in vivo. *Evidence-based complementary and alternative medicine*, *2012*.
10. Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 28(1), 25-30.