Insecticidal and antibacterial potential of *Syzygium aromaticum* (L.) merrill and perry

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ABSTRACT

The present study was designed to appraise the insecticidal and antibacterial potential of Clove, *Syzygium aromaticum* (L.) against some laboratory hosts and *Escherichia coli* respectively. The phytochemicals constituents were extracted with two solvents methanol and acetone. The result of the phytochemical analysis revealed that methanol extract has alkaloids, steroids, tannins, saponins, flavonids, cardiac glycosides, phenolic compounds and aromatic acids but in acetone extract all the constituents are similar as methanolic extract except aromatic acids. Results of the repellent activity observed against *Tribolium castaneum* and *Corcyra cephalonica* implies that silver based nanoparticles from methanol extract of 1% concentration showed maximum repellent activity of 71% against *T.castaneum*. Antibacterial efficacy of clove extracts tested against Gram negative bacterium *E. coli* revealed that 1% of methanolic extract of clove is found to have maximum growth inhibition (25mm). Results of the larvicidal activity tested against Bombyx mori recorded 100% mortality in 4% acetone extracts of clove.

Keywords: *Syzygium aromaticum, Tribolium castaneum, Corcyra cephalonica, Bombyx mori*, Repellent activity, Larvicidal activity

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INTRODUCTION

Synthetic pesticides are widely used to control the insect pests and phytopathogens. It may cause toxic residues in treated products and cause environmental pollution. Due to these problems, search is going on to discover new, eco-friendly management tools in integrated pest management (Ganga Visalakshy and Krishnamoorthy, 2009). *Syzygium aromaticum* (synonym: *Eugenia cariophylata*) commonly known as clove, cultivated at maximum altitudes of 200m above sea level in coastal areas. Essential oils of clove buds have been known to possess various antimicrobial and antioxidant properties (Fu *et al.*, 2007). Clove oil extract has shown eugenol acetate, eugenol and beta-caryophyllene as the major constituents, the last two constitutions are known to possess antibacterial and antifungal properties (Nassar *et al.*, 2007 and Ayoola *et al.,* 2008) and are effective in repellency of red imported fire ants *Solenopsis invicta* (Hymenoptera: Formicidae). Eugenol is the fastest acting compound clove oil is also an effective repellent of pestiferous social wasps *Vespula pensylvanica* (Saussure) and paper wasps mainly *Polistes dominulus* (Christ). Due to the significant role of cloves in the fields of medicine, microbiology and entomology, this study has been selected.

MATERIALS AND METHODS

Collection and Extraction of Cloves

Dried cloves were purchased from local market. To prepare 100% extract, 25gm of coarsely grinded clove was soaked in 100mL of methanol and acetone separately in sterile conical flask and kept in a shaker for nearly 24 hrs at room temperature and filtered out using Whatmann No.1 filter paper. The extract was then concentrated to 25 mL.

Preliminary phytochemical analysis

One per cent stock solution of methanolic and acetone extract of cloves was prepared by adding 1ml of extract with 99ml double distilled water and used for the preliminary

phytochemical analysis using standard methods (Selvaraj *et al*., 2018) and for the synthesis of silver based nanoparticles.

Experimental concentrations

Three different concentrations of methanolic and acetone extract of cloves *viz*., 0.25%, 0.5% and 1% were prepared and used for antibacterial and antifeedant studies. The experimental concentration for larvicidal bioassay was extended up to 4% (0.25%, 0.5%, 1%, 2% and 4%).

Preparation of silver nitrate solution

17mg of silver nitrate (10^{-3} M $AgNO_3$) was taken and dissolved in 100mL of distilled water (Selvaraj *et al*., 2018).

Synthesis of Silver nanoparticle

To induce the synthesis of silver nanoparticles, 10mL of silver nitrate solution was mixed with methanol and acetone extract of cloves in different concentrations *viz*., 25, 50 and 75µL. Appearance of brown colour was considered as an indication of synthesis of silver based nanoparticles (AgNPs) (Xavier *et al*., 2016).

UV Spectrometer Analysis of AgNPs

The silver nanoparticles formed at different concentrations of methanolic and acetone extract of cloves was analyzed in UV-vis Spectrophotometer (Shimatsu-1800) at 200 – 500nm and the results were documented (Selvaraj *et al.,* 2018)

Antibacterial activity of Clove extracts

The antibacterial activity of methanol and acetone extracts and silver based nanoparticles of cloves was studied at three different concentrations against gram negative bacterium, *Escherichia coli* using well diffusion method (Bauer *et al.,* 1996). Freshly prepared agar plates were inoculated with pure culture of test organism by pour plate technique. Four wells of 6mm diameter were made in each plate with the help of well puncture. The test sample with three different concentrations *viz*., 0.25%, 0.50% and 1% (methanol extract, acetone extract and Silver nanoparticles) and a control (methanol for methanolic extract, acetone for acetone extract and silver nitrate solution for Silver nanoparticles) was loaded in the respective wells and incubated at room temperature for 24 hrs. The zone of inhibition formed around the wells was observed and recorded.

Insect repellent activity of clove extracts

The repellent activity was examined by insecticide-impregnated filter paper, following the methods described by Jilani and Saxena (1990). The repellent activity of clove extracts and AgNPs of Clove extracts were observed against stored grain pest such as *Corcyra cephalonica* and *Tribolium castaneum*. Filter papers of 1×1" size were prepared and impregnated with methanolic extracts, acetone extracts and AgNPs of clove extracts. Repellency tests were carried out separately for each experimental solution as against control by placing both the filter papers on 9cm diameter round filter paper which was placed on a round wax coated metallic plate. In each experimental set up 10 insects were introduced and closed with petridish so that they could not escape. Similarly the repellent activity of methanolic extracts, acetone extracts and AgNPs of methanolic extracts and acetone extracts of clove were observed at three different concentrations *viz*., 0.25%, 0.5% and 1% with three replicates each for each concentration after 15 minutes. The results were observed and the repellent activity was calculated using the following formula.

API = Control – Experiment / Control + Experiment

Larvicidal activity of Clove

Larvicidal activity of clove extracts and AgNPs were carried out in silk worm, *Bombyx mori* considering it as a Lepidopteran insect model. Experiment was carried out with five different concentrations *viz.,* 0.25%, 0.5%, 1%, 2% and 4% with three replicates. Mulberry leaves were dipped in the experimental extracts and were air dried. The mulberry silkworm larvae were fed with the treated leaves and the mortality was observed for every 24 hrs for four days. The results observed and 50% Lethal Concentration (LC_{50}) were calculated for each clove extracts.

RESULTS AND DISCUSSION

Qualitative analysis of phytochemicals

Phytochemical analysis is carried out using standard procedure (Brindha *et al*., 1981) which showed that alkaloids, steroids, tannins, saponins, flavanoids, cardiac glycosides, phenolic compounds and aromatic acids are present in methanolic extract whereas in acetone extract except aromatic acids all the other secondary metabolites were reported (Table 1).

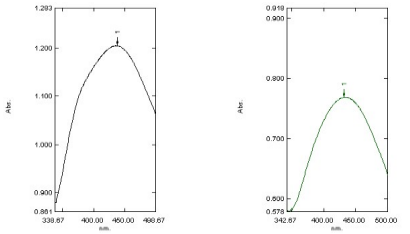
Table 1. Phytochemical analysis of methanol and acetone extract of cloves

Test(s)	Methanol extract	Acetone extract
Alkaloids	+	+
Steroids	+	+
Tannins	+	+
Pholobatannins	-	-
Saponins	+	+
Flavanoids	+	+
Terpenoids	-	-
Cardiac glycosides	+	+
Phenolic compounds	+	+
Aromatic acids	+	-
Xanthoproteins	-	-

Clove extracts are reported to possess alkaloids, steroids, tannins, saponins, flavanoids, cardiac glycosides, phenolic compounds and aromatic acids. The phenolic compounds are one of the largest and most prevalent groups of plant metabolites (Singh *et al.,* 2007). They possess various biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, inhibition of angiogenesis and cell proliferation activities (Han *et al.,* 2007). Flavonoids are hydroxylated phenolic substances synthesized by plants in response to microbial infection and they have been found antimicrobial substances against wide array of microorganisms in vitro. Steroids have been reported to have antibacterial properties (Raquel, 2007) and are very important compounds due to their relationship with compounds such as sex hormones. Cloves control nausea and vomiting, cough, diarrhea, dyspepsia, flatulence, stomach distension and gastro intestinal spasm, relieve pain, cause uterine contractions and stimulate the nerves (Sulieman *et al*., 2007; Tanko *et al*., 2008). In addition, cloves are highly anti-oxidant (Chaieb *et al.,* 2007), anti-ulcerogenic, anti-thrombotic, anti-fungal (Park *et al.,* 2007), anti-viral (Saeed and Tariq, 2008) and anti-parasitic.

UV Spectrometer analysis

UV-visible spectroscopic analysis was carried out at the range between 200-500nm Shimatsu UV-Spectrophotometer 1800. A characteristic peak was observed at 431.5nm with an absorbance of 0.769 in methanol extract and 437.5nm with an absorbance of 1.205 in acetone extract which confirmed the synthesis of silver based nanoparticles of clove (Fig 1).



Wavelength (nm)	Absorbance
437.5	1.205
431.5	0.769

Figure 1. UV – Vis spectrum of AgNP of Clove acetone and methanolic extracts

Antibacterial potential of clove extracts

The antibacterial potential of clove extracts were tested against gram negative bacterium, *Escherichia coli* and the following results were observed. A 1% concentration of methanolic clove extract was found to have a maximum inhibitory zone of 25mm and 1% of acetone clove extract is found to have a maximum inhibitory zone of 24mm (Table 2). However there is no remarkable zone of inhibition found in the silver based nanoparticles of both methanolic and acetone extracts of clove. Clove oil was found to be active against food borne gram positive bacteria *S. aureus, B. cereus, E. faecalis, L. monocytogenes* and gram negative bacteria *E. coli, Y. enterocolitica, S. choleraesuis, P. aeruginosa* (Gupta *et al.,* 2014). The antibacterial activity of plant extracts has been linked by many researchers due to the

presence of phytochemicals. The antimicrobial activity of clove essential oils could be associated with Eugenol (2 methoxy-4 allyl-phenol) (Gupta *et al*., 2008). Spices have been traditionally used and have been reported to have antiseptic and disinfectant properties. Eugenol is the main volatile compound extracted from clove bud.

Table 2. Antibacterial activity (%) of clove extracts against *Escherichia coli*

Concentration (%)	Methanol Extract	Acetone extract
0.25	19±1.4	19±1.5
0.5	21±1.4	21.5±2.1
1.0	25±0.6	24±0.5

and used in traditional medicine, as a bactericide, fungicides and anesthetic (Amanda *et al*., 2009). Even though Kim, *et al*., in 2011, studied the antimicrobial activities of silver nanoparticles with respect to Gram positive, *Staphylococcus aureus* and Gram negative, *E.coli* and suggested that Ag-NPs

could be used as an effective antibacterial material, the silver based nanoparticles of both methanolic and acetone extract wouldn't show significant inhibitory zone against the Gram negative bacterium, *E.coli*.

Repellent activity of clove extracts

The stored grain pests, *Corcyra cephalonica*, a lepidopteran insect and *Triboliun castaneum*, coleopteran insect were selected to estimate the repellent activity of clove extracts. The Access Proportion Index (API) was calculated for the methanol extract, acetone extract and its silver nanoparticles (Table 3). Uniformly, three concentrations (0.25%, 0.5% and 1.0%) were taken from each extract and silver nanoparticles. In methanolic extract, maximum repellent activity of 60% was observed in 1% concentration against

Table 3. Repellent activity (%) of clove extracts against *Tribolium castaneum* and *Corcyra cephalonica*

Concentration (%)	Pest(s)	Methanol Extract	Acetone extract	AgNP-Methanol	AgNP-Acetone
0.25	*Tribolium castaneum*	11±0.5	14±1.5	11±0.8	14±1.1
0.5		40±1.2	11±1.6	55±1.5	33±0.6
1.0		60±1.0	66±0.9	71±0.6	60±0.3
0.25	*Corcyra cephalonica*	11±0.8	16±1.6	33±2.0	20±1.0
0.5		20±1.5	40±2.0	55±1.0	33±0.2
1.0		60±0.8	60±1.6	66±0.8	66±0.3

T. castaneum and *C. cephalonica.* In acetone extract, the maximum repellent activity of 66 % was observed in 1% concentration against *T. castaneum*. In $AgNO_3$, maximum repellent activity of 71% was observed in methanol extract against *T. castaneum* and the least repellent activity of 11% was observed. Essential oil from *Syzygium aromaticum* (clove oil) possesses many compounds with biological activity, and it is used to control insects, fungus, mildews in stored grains (Shang and Cai, 2007). Among the secondary metabolites reported in this experiment, tannins and steroids are well known for their insecticidal properties (Suganthy, 2000; Sundararajan and Kumuthakalavalli, 2000). Clove essential oil at 5% possesses 100% of repellent activity against the chigger *Leptotrombidium imphalu* which could be a safer and cheaper alternative to synthetic repellents commonly associated harmful side effects (Eamsobhana *et al*., 2009). The maximum repellent activity of 71% and 66% is found in silver based nanoparticles of methanol extract against *T. castaneum* at 1% and silver based nanoparticles of both methanol and acetone against *Corcyra cephalonica* at 1% respectively. In this study, nanoparticles have a significant role in the repellent activity against stored grain pests.

Larvicidal activity of clove extracts

The silkworm, *Bombyx mori* was used as a lepidopteran model insect to study the larvicidal activity of clove extracts. Five different concentrations *viz*., 0.25%, 0.5%, 1%, 2% and 4% were used for the study. Cent percent mortality was observed in 4% acetone clove extract at 48 hrs. The lethal

concentration (LC_{50}) was calculated using SPSS software and it was found to be 234.186, 156.397, 147.940 and 109.198 for 96 hrs of methanol, acetone, Silver nanoparticles of methanol extract and silver nanoparticles of acetone extract respectively (Table 4).

Table 4. Chi square and LC_{50} values of different clove extracts

Clove Extracts	Hours	Chi-Square	LC_{50}
Methanol Extract	24	-	-
	48	0.013	433.426
	72	19.321	420.689
	96	121.062	234.184
Acetone Extract	24	15.016	383.584
	48	0.076	207.432
	72	0.045	179.773
	96	0.061	156.397
Methanol-$AgNO_3$	24	39.490	408.373
	48	27.543	253.485
	72	9.780	181.326
	96	6.036	147.940
Acetone-$AgNO_3$	24	24.921	472.403
	48	47.508	363.485
	72	39.010	205.996
	96	0.094	109.198

The clove essential oil may also be employed as insecticide (Diego *et al*., 2014). In this experiment, highest larvicidal activity is reported in the silver nanoparticles of acetone extract of clove 96 hrs with LC_{50} value 109.198 followed by silver nanoparticles of methanolic extract of clove with LC_{50} value 147.940.

REFERENCES

Amanda, L., Santos, G. O., Chierice, K. S., Alexander, A. R. and Ellen, M. 2009. Characterization of the raw essential oil eugenol extracted from *S. aromaticum*. *Journal of Thermal and Analytical Calorimeter*. **96**: 821- 825.

Ayoola, G. A., Lawore, F. M., Adelowotan, T., Aibinu, I. E., Adenipekun, E., Coker, H. A. B. and Odugbemi, T. O. 2008. Chemical analysis and antimicrobial activity of the essential oil of *Syzygium aromaticum* (Clove). *African journal of Microbiological Research*. **2**: 162-166.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., and Turck, M., 1996. Antibiotic sensitivity testing by a standardized single disk method. *American Journal of Clinical Pathology*. **45**: 493-496 .

Chaieb, K., Hajlaoui, H., Zmantar, T., Nakbi, K.A.B., Rouabhia, M., Mahdouani, K. and Bakhrouf, A. 2007. The chemical composition and biological activity of essential oil, *Eugenia cryophyllata* (*Syzygium aromaticum*, L. Myrtaceae): *Phytotherapy Research*. **21**: 501-506.

Diego Francisco Cortes-Rojas, Claudia Regina Fernandes de Souza, and Wanderley Pereira Oliveira., 2014. Clove (*Syzygium aromaticum*): a precious spice. *Asian Pacific Journal of Tropical Biomedicine*, **4**(2): 90–96.

Eamsobhana, P., Yoolek, A., Kongkaew, N., Parsartvit, A., 2009. Laboratory evaluation of aromatic essential oils from thirteen plant species as candidate repellents against *Leptotrombidium chiggers* (Acari : Trombiculidae), the vector of scrub typhus. *Experimental and Applied Acarology*. **47**(3): 257-262.

Fu, Y., Zu, Y., Chen, L., Shi, X., Wang, Z. Sun, S. and Efferth, T. 2007. Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytotherapy Research*. **21**: 989-994.

Ganga Visalakshy, P.N. and Krishnamoorthy, A. 2009. Biological control of thrips of horticultural importance in India: Present status and Future scope. *Ecofriendly insect pest management*. (Ignacimuthu, S. and David, B.V. eds). 89-98.

Gupta, C., Garg, A. P. and Uniyal, R. C. 2008. Antibacterial activity of Amchur (dried pulp of unripe *Mangifera indica*) extracts on some food borne bacteria. *Journal of Pharmocological Research*. **1**: 54-57.

Gupta, N., Mittal, M., Parashar, P., Mehra, V., Khatri, M. 2014. Antibacterial Potential of *Elletaria cardamomum*, *Syzygium aromaticum* and *Piper nigrum*, their synergistic effects and phytochemical

determination. *Journal of Pharmocological Research*, **8**(8):1091-1097.

Han, X., Shen, T., and Lou, H. 2007. Dietry polyphenols and their biological significance. *International Journal of Molecular Science*s, **8**(9): 950–988.

Jilani, G., and Saxena, R. C., 1990. Repellent and feeding deterrent effects of turmeric oil, sweetflag oil, neem oil and a neem-based insecticide against lesser grain borer (Coleptera: Bostrichidae). *Journal of Economic Entomology*, **83**: 629-634.

Kafle, L., Shih, C. J. 2013. Toxicity and repellency of compounds from clove (*Syzygium aromaticum*) to red imported fire ants *Solenopsis invicta* (Hymenoptera: Formicidae). *Journal of Economic Entomology*, **106**(1): 131-135.

Kim., Soo-Hwan., Hyeong-Seon Lee, Deok-Seon Ryu., Soo-Jae Choi and Dong-Seok Lee. 2011. Antimicrobial activity of silver nanoparticles against *S.aureus* and *E.coli*. *Korean Journal of Microbiology Biotechnol*ogy **39**:77-85.

Nassar, M. I., Gaara, A. H., El- Ghorab, A. H., Farrag, A. R. H., Shen, H., Hug, E. and Mabry, T.J. 2007. Chemical constituents of Clove (*Syzygium aromaticum*, Fam. Myrtaceae) and Their Antioxidant Activity. *Revista Latinoamericana de Quimica*, 121-122.

Park, M. J., Gwak, K. S., Yang, I., Choi, W. S., Jo, H. J., Jeung, E. B. and Choi, I. G. 2007. Antifungi activities of the essential oils in *Syzygium aromaticum*. *Journal of Microbiology*, **45**: 460 – 465.

Raquel, F. E. 2007. Bacterial lipid composition and antimicrobial efficacy of cationic steroid compounds. *Biochemica et Biophysica Acta*, 2500-2509.

Saeed, S. and Tariq, P. (2008). In vitro antibacterial activity of clove against Gram negative bacteria. *Pakistan Journal of Botany*, **40**(5): 2157-2160.

Shang, J. F. and Cai, J.P. 2007. Inhibiting action of clove extract on mildews in stored grains. *Journal of Henan University of Technology* (Natural Science Edition), **28**:12-14.

Singh, R., Singh, S.K. and Arora, S. 2007. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. *Food and Chemical Toxicology*. **45**: 1216-1223.

Suganthy, M. 2000. Efficacy of different plant protection options on the oviposition preference of gram pod borer, *Helicoverpa aemigera* (Hubner) in chickpea. *Indian Journal of Plant Protection,* **28**(1):61-63.

Sulieman, A. M. E., Boshra, I. M. O. and El Khalifa, E. A. A. 2007. Nutritive value of clove (*Syzygium aromaticum*) and detection of antimicrobial effect of its bud oil. *Research Journal of Microbiology*, **2**: 266-2711.

Sundararajan, G. and Kumuthakalavalli. 2000. Effects of leaf extracts of selected plants against the larvae of *Helicoverpa armigera* (Hubner). *Environment and Ecology*. **18**(1):10-12.

Tanko, Y., Mohammed, A., Okasha, M. A., Umar, A. H. and Magaji, R. A. 2008. Anti nociceptive and anti inflammatory activities of ethanol extract of *Syzygium aromaticum* flower bud in wistar rats and mice. *African Journal of Traditional, Complementary and Alternative Medicines*. **5**: 209-212.

Zhang, Q. H., Schneidmiller, R. G. and Hoover, D. R., 2013. Essential oils and their compositions as spatial repellents for pestiferous social wasps. *Pest Manag Science*. **69**(4): 542-552.

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