

# The Local Anesthetic Activity of *Lavandula angustifolia* and *Eugenia caryophyllata* Essential Oils

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**Abstract**—Previous studies show that *Lavandula angustifolia* (lavender) and *Eugenia caryophyllata* (clove) essential oils can help relieve dental pain. Clove oil and lavender oil were tested as topical anesthetics in this study on rabbits, and their effects and likely mechanisms of action were analyzed. Clove oil and lavender oil were extracted by hydrodistillation using a Clevenger-setup apparatus. Topically applying lidocaine, clove oil, or lavender oil topically all significantly reduced corneal sensitivity. The sensitivity of the cornea was successfully reduced by treatments consisting of lidocaine at a concentration of 0.5%, 25 µg of clove oil, and 50 µg of lavender oil. When clove oil is applied topically to the cornea, it produces effects similar to those of a local anesthetic due to the involvement of the cholinergic system. To achieve the desired effect of producing local anesthesia in the cornea of the rabbit, lidocaine, clove oil, and lavender oil were applied topically to the animal. A noticeable local anesthetic effect was produced when sub-anesthetic doses of lidocaine were combined with sub-anesthetic doses of lavender or clove oil.

**Index Terms**—Clove oil, Corneal reflex, *Eugenia caryophyllata*, *Lavandula angustifolia*, Lavender oil, Lidocaine, Local anesthesia.

## I. INTRODUCTION

To evaluate anesthetic activity *in vivo* model, one uses the rabbit conjunctival reflex test. The antispasmodic qualities of certain herbal plants, such as “*Lavandula angustifolia* M, *Mentha piperita* M, *Salvia officinalis* M, *Eugenia caryophyllus* M, and *Foeniculum vulgare* M, support their historical use for digestive system diseases” (Koulivand, Khaleghi Ghadiri and Gorji, 2013; Tschiggerl and Bucar, 2010). The chemical phytoconstituents in essential oils are responsible for this biological process. Simpson (2019) reported using it to treat stomach cramps. Numerous *in vitro* investigations on the antispasmodic effects of essential oils derived from the aforementioned medicinal plants. They convert to prevent the contractions brought on by different

spasmogens by acting pharmacologically, which results in an unspecific antagonistic response. Essential oils’ non-polar components may interact well with the plasma membrane’s lipid bilayer. Essential oil blocks the neurotransmission by decreasing Ca<sup>2+</sup> inflow or stopping the increase in Na<sup>+</sup> permeability (Bikmoradi, et al., 2017).

The more than 100 lavender components include “linalool, perillyl alcohol, linalyl acetate, camphor, limonene, tannin, triterpene, coumarin, cineole, and flavonoids” (Wilson, et al., 2021). These chemicals work by dose-dependently binding to glutamate, a key central nervous system excitatory neurotransmitter. Linalool successfully lowers mouse motor activity. The hypnotic and anticonvulsant properties of lavender are attributed to GABA amplification. Menthol is an analgesic and local anesthetic, whereas eugenol stabilizes membranes (Zheljazkov, et al., 2013; Silva, et al., 2015).

These methods of anesthesia involve local anesthetics. Surface anesthetic anesthetizes skin, eyes, ears, and throat. Block or infiltration anesthesia: Nerve endings are sedated by subcutaneous injection of the drug. Nerve block, often called condition block, involves delivering drugs near a nerve. Drugs are injected into the subarachnoid area to provide spinal anesthesia. Epidural anesthesia involves injecting drugs outside the dura (Batiha, et al., 2023).

*Eugenia caryophyllata* is also known as clove. It is a member of the *Myrtaceae* family and is 20 m tall. It is growing in “Madagascar, Tanzania, Sri Lanka, Brazil, and Indonesia” (Arung, et al., 2011). As an oral antibacterial and for treating toothaches, joint pains, and headaches, clove oil and its essential oil have long been utilized in aromatherapy (Chaieb, et al., 2007; Dalai, et al., 2014). The main component of clove essential oil, eugenol, also possesses a variety of biological activities, such as antiallergic (Chioca, et al., 2013), antifungal (Pinto, et al., 2009), and antioxidant (Gülçin, Elmastas and Aboul-Enein, 2012) capabilities. For fish and amphibians, the essential oil of *E. caryophyllata* and its principal constituent, eugenol, has been demonstrated to be a safe, effective, and inexpensive anesthetic (Rosenthal, Baran and Jacobs, 2009). In addition, eugenol’s analgesic activity in several pain models has been thoroughly demonstrated (Lionnet, Beaudry and Vachon, 2010; Daniel, et al., 2009; Park, et al., 2011; Properzi, et al., 2013).

One of the most often studied therapeutic herbs is lavender, also known as *Lavandula*, which belongs to the

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*Lamiaceae* family (Prusinowska and Śmigielski, 2014). The purple-blue flower of the shrub has been used for centuries to treat various illnesses. “*L. angustifolia*, *Lavandula latifolia*, *Lavandula stoechas*, and *Lavandula intermedia*” are among the most commonly utilized species of lavender (Tschiggerl and Bucar, 2010). It is grown worldwide for commercial use. It thrives in the Indian states of Himachal Pradesh, Uttar Pradesh, and Kashmir Valley. It is recognized to have calming, anti-inflammatory, anti-nociceptive, antibacterial, and antioxidant properties (Shaw, et al., 2007; Kritsidima, Newton and Asimakopoulou, 2010).

A phytochemical investigation found that lavender essential oils contain “linalool, linalyl acetate, 8-cineole-ocimene, terpinen-4-ol, and camphor.” This structure underpins attribute mechanisms (Jianu, Pop and Gruia, 2013). Herbal therapies such as lavender essential oils can treat antibiotic resistance, invasive procedures, side effects, and medication addiction. Due to these properties and medication tolerance, lavender is an effective therapeutic herb (de Rapper, Viljoen and Vuuren, 2016).

If pain comes from the cornea, it would be very strong and make it hard to do anything. Corneal nociceptor density is approximately 35 times that of dental pulp and 500 times that of skin (Rosenthal, Baran and Jacobs, 2009). Polymodal nociceptors react to high-threshold touch, chemicals, cold, heat, and protons. Dry eye, post-herpetic neuralgia, trigeminal neuralgia, polluted settings, contact lens wear, and recent refractive surgery can all induce eye pain (Hirata and Meng, 2010). Acute and chronic trigeminal neuralgia is difficult to treat due to a lack of knowledge (Miranda, Sierralta and Prieto, 2009).

When adverse effects are considered, herbal therapies outperform opioids and non-steroidal anti-inflammatory medications. Dentistry uses *E. caryophyllata* clove bud oil as a mouth antibacterial, topical anesthetic, and painkiller. In this study, we investigated the potential local anesthetic activity of the essential oils of *L. angustifolia* and *E. caryophyllata*. Linalyl acetate and linalyl linalool are the two main phytoconstituents in this lavender oil. Three major phytoconstituents include eugenol, eugenol acetate, and caryophyllene of clove oil. Two essential oils were investigated to determine their possible local anesthetic activity ratio. The corneal touch thresholds (CTT) and duration of the local anesthetic were also measured after topical instillation of clove oil and lavender oil alone and in combination with lidocaine (Ait Said, et al., 2015). Therefore, the aim of the study was to investigate the local anesthetic effects of clove oil and lavender oil on rabbit corneas.

## II. MATERIALS AND METHODS

### A. Animals

All study methods and animal care practices are approved by the current laboratory animal care recommendations (TIU/FP/2022/07, dated December 15, 2022) and ethical principles for researching conscious animal pain at the Department of Pharmacology Animal House at Tishk International University in Erbil (Pathan, et al., 2020). Randomly selected adult male rabbits weighing 630–660 g were kept in plastic

cages with free access to food and water over a 12-h cycle with temperature control of 22°C (three rabbits per group). All experiments were carried out between 10 am and 4 pm. Each rabbit received topical solution 3 days between trials.

### B. Drugs

Normal saline (0.9% of sodium chloride) was used to dissolve all drugs lidocaine (xylocaine) eye drops, each unit contains approximately 0.5 mL eye drops solution of lidocaine hydrochloride 4% w/v (20 mg), and samples (clove and lavender oil isolated pure essential oil from pharmacognosy laboratory) of oil used in topical studies. Tween 80 (more lipophilic nature) and saline (0.5% v/v) were used as a solvent to create an emulsion of clove oil and lavender oil. Tween 80 is a non-ionic surfactant and emulsifier that emulsifies clove oil and lavender in saline. The pH of each solution was adjusted to 7.4. Before apply.

### C. Extraction of Plant Material and Essential Oil (Figs. 1 and 2)

The buds of *E. caryophyllata* (clove) purchased from local market and *L. angustifolia* (lavender) leaves collected from a TIU campus garden in Erbil, Iraq, and authenticated by Dr. Javed Ahmad, a Pharmacognist, Faculty of Pharmacy, Tishk International University, Erbil, Iraq. The dried clove buds and the fresh lavender leaves were used to extract the essential oil. Hydrodistillation of clove buds and lavender leaves in a Clevenger apparatus for 3 h resulted in a 3 % (v/w) yield of essential oil. The extracted essential oil was dried over anhydrous sodium sulfate until all traces of water were removed and then stored in dark glass bottles at 4°C (Ali, Rozhan and Subasini, 2023).

### D. Methodology

Handle rabbits with caution. Remove the lashes from both eyes. To infuse medications into the eye, use the pouch approach. Make a tiny pouch by pinching the lower eyelid. Using a dropper, add one to two drops of saline/drug. Keep the lower eyelid in contact with the conjunctiva for 1–2 min by pulling it upward. After giving the drug, press the medial canthus for 5 s. Maintain one eye as the control (right or left) and the other as the test. Use saline in the control eye and



Fig. 1. Clove fruits.

medication in the test eye. The criteria include pupil size, light reflex, touch reflex, and corneal reflex (Farazifard, et al., 2005).

### E. Pupil Size Evaluation

Each pupil's diameter should be measured. This can be measured with a pupillometer made of hard paper. Cut a firm piece of paper to the length of a 5-inch ruler. Then, at the lowest to highest of millimeter diameter, cut holes in it. It could be started with 1 mm and work our way up to 10 mm and measured with a simple scale (Fig. 3a). It was tough to force the rabbits' eyes open. As a result, measuring diameter becomes simple with a pupillometer (Fig. 3b). Place the pupillometer in close to the rabbit's eye so it can see the pupil. Match the hole on the pupillometer to the size of the pupil and record the pupil's diameter in millimeters on the corresponding hole (Zagon, et al., 2014).

### F. Examining the Light Reflex

A torch was used to test light reflex. This experiment required the use of a pencil torch. Always bring the light from the side (behind) to the front. The light was not placed from the front side of the rabbits. When light was flashed into the eye, the diameter of the pupil changed. There is no decrease or increase in pupillary diameter, noted down the completed three readings (Kim, et al., 2013).

### G. CTT Evaluation

Fine cotton filaments or wick was used to test it so that no projecting cotton component in the manufacture of the filaments. With the tip of a cotton filament, touch the cornea's periphery. Always bring the cotton filament from the side forward (back). Cotton filament and our hands should not be visible to rabbits. During the testing period, the rabbits were held and restrained by hand with care. Using a set of calibrated cotton filaments, animal withdrawal reactions to corneal touch, such as blinking and head withdrawal (abrupt head movement), were counted as positive. In five tests on the control and test eye, the CTT was characterized as the filament force that triggered at least three withdrawal responses (Zagon, et al., 2014; Kim, et al., 2013).

### H. Dose-dependent study of CTT

The experiment was carried out using modified approach of Pathan, et al. (2020). For the evaluation of corneal

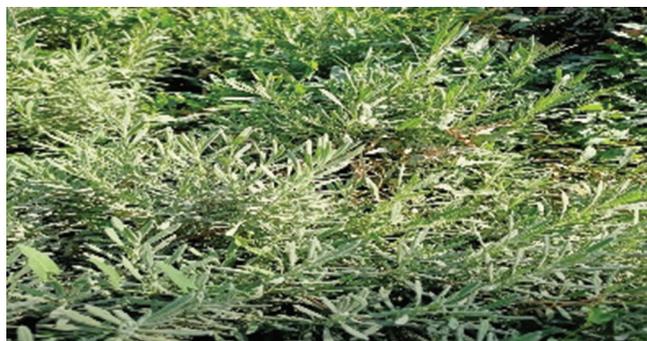


Fig. 2. Lavender leaves in TIU campus garden.

mechanical sensitivity, behavioral corneal responses were used to lower corneal sensitivity in a dose-dependent manner. In brief, a conjunctival response was elicited by stimulating the rabbit eye's external side with a corneal touch. The increased number of stimuli required to cause palpebral closure indicates that the medicines deposited in the rabbit eye sac have local anesthetic activity. Vehicle (Tween 80, 0.5% dissolved in saline), clove oil (50, 100  $\mu\text{L}/\text{eye}$ ), lavender oil (50 and 100  $\mu\text{L}/\text{eye}$ ), lidocaine (0.5% and 2%, 40  $\mu\text{L}/\text{eye}$ ), and combination of clove oil (25  $\mu\text{L}$ ) or lavender oil (25  $\mu\text{L}$ ) with lidocaine (0.5%, 40  $\mu\text{L}/\text{eye}$ ) were topically applied to the cornea in two consecutive 20  $\mu\text{L}$  volumes and rabbits gently restrained by hand to prevent wiping of solution for 1 min in each application. After corneal solution instillation, corneal reflexes were assessed 5–8 min later (Bates, et al., 2010).

### I. Study Depending on the Time of the Duration of Local Anesthetic

In the time-dependent study, the most prominent cotton filament (2  $\mu\text{L}$ ) was used to evaluate the duration of corneal anesthesia induced by clove oil (100  $\mu\text{L}$ ), lavender oil (100  $\mu\text{L}$ ), lidocaine 2%, and combinations of clove oil (25  $\mu\text{L}$ ) or lavender oil (25  $\mu\text{L}$ ) with lidocaine. Five minutes



Fig. 3. Observations for clove oil and lavender oil topical applications. (a) Rabbits gently restrained by hand. (b) Instill one to two drops of a drug in it using a dropper (essential oil). (c) Applications of saline for the control eye. (d) Assessing corneal touch threshold. (e) Evaluation of the length of the corneal anesthesia induced by clove and lavender oils. (f) Application of lidocaine drug. (g) Observed how the diameter of the pupil changed when light was shone into the eye. (h) There is no change in pupillary diameter of any kind.

before, 5 min after, 10 min after, 15 min after, 25 min after, and 40 min after applying each solution topically, corneal touch tests were performed. Caution: Avoid touching the cornea's central portion, since it might cause corneal ulcers or opacities. This can cause blindness, as the central part of the cornea is primarily responsible for vision (Tamaddonfar, et al., 2008).

*J. Statistical Analysis*

All experimental results are given as the Mean ± Standard Deviation. GraphPad - "One-way analysis of variance followed by Dunnett's multiple comparisons test was performed using GraphPad Prism version 10.0.  $p < 0.05$  was considered statistically significant.

III. RESULTS AND DISCUSSION

The iris of the eye is made up of circular and radial muscle fibers. The parasympathetic cholinergic nerve system sends nerve impulses to the circular fibers, which then make the constrictor pupillae contract. The sympathetic adrenergic nerve system sends nerve signals to the radial and dilator pupillae. When sympathetic and parasympathetic nerves are stimulated, mydriasis and miosis happen. However, when these nerves are paralyzed, the opposite effect happens. The above effects can be induced by drugs that affect autonomic nervous systems (Kim, et al., 2013).

It manifests either by interfering with the parasympathetic supply to the eye by activating the sympathetic system excessively. Alpha ( $\alpha_1$ ) selective action causes pupil dilation by stimulating the dilator muscles. They are used to reduce painful ciliary spasms, diagnose Horner's syndrome, examine the retina and deeper structures, and treat chronic simple glaucoma, and corneal ulcers, and retinoscopy in children and elderly patients in particular. Constrict pupil,  $M_3$ -specific action by tightening iris sphincter muscles or by preventing the activity of the enzyme acetylcholinesterase, facilitates the action of acetylcholine at transmitter sites (Uthirapathy, 2023; Zagon, et al., 2014). These mechanisms improve drainage of the aqueous humor and lower intraocular pressure. These medications treat wide-angle glaucoma, xerostomia, and xerostomia during cataract and anterior chamber surgeries. Khalilzadeh, Hazrati, and Vafaei Sayah (2014) reported that the effects of miotics, which are parasympathetic drugs such as pilocarpine, physostigmine, and atropine, and mydriatics, which are sympathetic drugs such as amphetamine, epinephrine, and phenylephrine. When compared to saline

solution, local anesthetics such as cocaine and lignocaine have different effects on corneal reflexes and light perception, according to Hirata and Meng, (2010). Local anesthetics primarily block  $Na^+$  channels, which affects depolarization, and stops the initiation and propagation of action potentials (Dohi, Terasaki and Makino, 2009). Membrane stabilizing effects influence the depolarization process, and subsequently, the failure of the propagation potential. When a cotton wick is touched to the cornea of a rabbit's eye, the corneal reflex takes place. A rabbit's eyelids closing is referred to as a positive corneal reflex, and keeping them open is called to as a negative corneal reflex (Park, et al., 2009).

*A. The Local Anesthetic Activity of Topical Clove Oil, Lavender Oil, and Lidocaine*

Table I shows that the CTT was noticed to be 0.3 g of calibrated force in the cornea that had received a vehicle treatment (Fig. 3c). In the dose-dependent study, clove oil increased at doses of 50  $\mu$ L/eye (Table I,  $p < 0.05$ ) and CTT at 0.5 g of calibrated force. At doses of 50 and 100  $\mu$ L/eye, clove oil significantly produced a local anesthetic effect compared to the vehicle group (Table I,  $p < 0.001$ ) (Fig. 3d). Lavender oil raised the CTT at doses of 50 and 100  $\mu$ L/eye to 1 and 2 g calibrated force, respectively (Fig. 3e). In contrast to the vehicle group, topical lidocaine application at a dose of 2% (40  $\mu$ L/eye) but not 0.5% resulted in a highly effective local anesthetic effect (Table I,  $p < 0.001$ , Fig. 3f). Furthermore, when low doses of lavender or clove oil were mixed with low doses of lidocaine, there was a significant (Table I,  $p < 0.001$ ) local anesthetic effect for all forces tested. However, this effect was insignificant in control group.

Clove oil and lavender oil were used as local anesthetic because the test drug's corneal reflex loss indicates a corneal sensation loss (Fig. 3g and h). In addition, for all forces tested, a significant (Table I,  $p < 0.001$ ) local anesthetic effect was generated, when low doses of lavender or clove oil were combined with low doses of lidocaine (Dohi, Terasaki and Makino, 2009). The same doses of lavender oil and clove also allow an increase in the number of stimuli to induce conjunctival reflex (Zagon, et al., 2014). This was in contrast to the vehicle group. Clove oil and lavender oil were used as a local anesthetic because the test drug's loss of corneal reflex indicates a loss of corneal sensation.

Thin A-delta and C-type fibers on the corneal surface respond to painful chemical, mechanical, and thermal stimuli (Hirata and Meng, 2010). Clove oil's ability to relieve acute

TABLE I  
EFFECT OF DRUGS ON RABBITS EYE

Drugs	Light reflex		Corneal reflex		Pupil size mm (mydriasis)	
	Right (control) eye	Left (test eye)	Right	Left	Right	Left
Clove oil	+++	---	+++	+	11	14.2
Lavender oil	+++	---	+++	+	10.7	13.5
Lidocaine	+++	++	+++	---	No change	No change
Clove oil+lidocaine	+++	+	+++	---	11	15.6
Lavender oil+lidocaine	+++	+	+++	---	12	13.7

corneal pain depends on its cholinergic activity because the muscarinic cholinergic receptor antagonist atropine completely blocks its effect. *In vitro* studies have shown that clove oil and eugenol have potent anti-acetylcholinesterase properties (Dohi, Terasaki and Makino, 2009). Clove oil and eugenol's anti-acetylcholinesterase activity may also be in line with the mechanisms underlying their antinociception, as it has been shown that cholinergic system activation by muscarinic agonist or cholinesterase inhibitors has analgesic effects (Pan, et al., 2011; Uthirapathy and Tahir, 2021).

The findings of the present study demonstrated that lidocaine (as a positive control) and clove oil applied topically decreased the corneal mechanical sensitivity of rabbits. Clove oil and lavender were applied in high doses to produce corneal anesthesia patterns comparable to lidocaine's anesthetic pattern. When a low dose of lidocaine was combined with a low dose of clove oil or lavender oil, it worked well as a local anesthetic. When clove oil or lavender oil with lidocaine is given at the same time, it appears that a concur effect is what causes their local anesthetic effects. In contrast to the combination of lidocaine and a low dose of lavender oil, the lidocaine sub-anesthetic dose and clove oil generated an enduring anesthesia (Kajjari, Joshi and Hugar, 2022); the presence of other substances alongside eugenol in clove essential oil may be cause of this effect.

In the composition of clove oil, Eugenol (54.86%) and  $\beta$ -caryophyllene (20.19%) were exceptionally high in clove oil (Khalilzadeh, Hazrati and Vafaei Sayah, 2014). Eugenol, a phenylpropane derivative, is a popular local anesthetic, analgesic (Properzi, et al., 2013; Park, et al., 2011), and anti-inflammatory (Nardarajah, Dhanraj and Jain, 2018). Eugenol may modulate analgesia and local anesthesia by inhibiting voltage-gated Na<sup>+</sup> channels (Park, et al., 2006). Eugenol may inhibit pain by activating rodent and human trigeminal ganglion neurons' transient receptor potential ankyrin subtype 1 (TRPA1) receptors (Chung, et al., 2014). The effects of local anesthetics like lidocaine, like eugenol, inhibit voltage-gated Na<sup>+</sup> channels and activate TRPV1 (Leffler, et al., 2008). In the rabbit conjunctival reflex test, topical caryophyllene had a strong local anesthetic effect (Ahmed, Altaei and Ahmed, 2020). *L. angustifolia* essential oil has antimuscarinic effects. In the neuromuscular junction of the rabbit, linalool has been shown to shorten the time that the channel is open and inhibit acetylcholine release. These findings suggest that the local anesthetic properties of lavender and clove oils may result from their phytochemicals' capacity to block Na<sup>+</sup> and/or Ca<sup>+</sup> channels.

#### IV. CONCLUSION

The current data suggest that clove oil and lavender oil provided local anesthetic effects through cholinergic mechanisms in acute corneal reflexes. Lidocaine, clove oil, and lavender oil were applied topically to the cornea to diminish corneal sensitivity. However, the maximal anesthetic effect and duration differed according to the essential oils presented, including linalool, linalyl acetate, and eugenol.

Clove or lavender oil was excellent local anesthetics when paired with a sub-anesthetic concentration of lidocaine.

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