

# Comparative Evaluation of Immobilization Activity of Clove Bud Extract with Lignocaine Hydrochloride

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**Abstract**— Clove (*Syzygium aromaticum*), a member of the family Myrtaceae, is a traditionally valued medicinal spice whose principal bioactive constituent, eugenol, is reported to possess local anaesthetic-like activity through reversible blockade of voltage-gated sodium channels. The present study was undertaken to prepare an ethanolic extract of clove buds by Soxhlet extraction, to screen it for preliminary phytochemical constituents, and to evaluate and compare its immobilization activity with the standard local anaesthetic lignocaine hydrochloride using the earthworm (*Lumbricus terrestris*) model. Ethanolic extract at 0.5%, 1% and 2% w/v concentrations, along with a 2% lignocaine hydrochloride standard and a distilled-water control, were tested for immobilization and recovery time. Qualitative phytochemical screening revealed the presence of carbohydrates, alkaloids, tannins, flavonoids and resins in the extract, with a percentage yield of 61.9% for the ethanolic extract. The 2% clove extract produced the fastest immobilization ( $4:09 \pm 0:53$  min) among the test groups, while the standard drug produced the slowest immobilization but the longest recovery time ( $18:16 \pm 0:54$  min and  $45:10 \pm 0:44$  min respectively), confirming a dose-dependent immobilization effect of the extract. These findings support the traditional use of clove as a natural local anaesthetic and indicate its potential as an economical, plant-based alternative worthy of further pharmacological investigation.

**Key Words:** *Syzygium aromaticum*, Clove bud extract, Lignocaine hydrochloride, Immobilization activity, Earthworm model, Eugenol, Local anaesthetic, Phytochemical screening

## 1. INTRODUCTION

Clove (*Syzygium aromaticum* (L.) Merr. & L.M. Perry), a member of the family Myrtaceae, is a fragrant spice widely cultivated in Madagascar, Sri Lanka, Indonesia and southern China. Traditional medicine, relying largely on herbal remedies, continues to serve as the primary source of primary health care for nearly 80% of the world's population, owing to the perceived lower toxicity and comparable efficacy of natural products relative to synthetic drugs [1].

Clove has historically been used to treat nausea, gastrointestinal ailments, and to relieve toothache and oral pain. Dental pain is among the most common forms of orofacial pain, often necessitating rapid and effective local anaesthesia. Lignocaine (lidocaine) hydrochloride, an amide-

type local anaesthetic that reversibly blocks voltage-gated sodium ( $\text{Na}^+$ ) channels in nerve membranes, remains the clinical gold standard for such applications because of its rapid onset and favourable safety profile [2], [9].

The principal constituent of clove bud oil, eugenol (comprising approximately 70-90% of the essential oil), has been reported to exhibit local anaesthetic-like activity by reversibly inhibiting nerve conduction through modulation of sodium, calcium and transient receptor potential (TRPV1) channels [3], [5], [6], [7], [8]. The earthworm (*Lumbricus terrestris*) immobilization model is a simple, economical, and widely accepted in vitro screening method for evaluating neuromuscular depressant or local-anaesthetic-like activity, as earthworms possess a well-developed nervous system with measurable, reproducible locomotor responses [4].

In the present study, an ethanolic extract of clove buds was prepared and its immobilization activity was evaluated and compared with lignocaine hydrochloride in the earthworm model, in order to assess the potential of clove bud extract as a natural, economical alternative source of local anaesthetic activity.

### 1.1 Aim and Objectives

The aim of the study was to analyse the phytochemical constituents of *Syzygium aromaticum* bud extract, and to evaluate and compare its immobilization activity at different concentrations with the standard local anaesthetic lignocaine hydrochloride using the in vitro earthworm model. The specific objectives were: (i) to collect and authenticate *Syzygium aromaticum* buds; (ii) to prepare the ethanolic extract by Soxhlet extraction; (iii) to perform preliminary phytochemical screening of the extract; (iv) to evaluate the immobilization activity of the extract; and (v) to compare this activity with that of lignocaine hydrochloride.

## 2. MATERIALS AND METHODS

Materials used in the study included a Soxhlet apparatus, Petri dishes, earthworms (*Lumbricus terrestris*), clove buds (*Syzygium aromaticum*) and ethanol as the extraction solvent. *Syzygium aromaticum* buds were procured from a local market, dried and reduced to a coarse powder. Healthy, fully grown *Lumbricus terrestris* earthworms (110-220 mm in length) were collected from agricultural land.

### 2.1 Preparation of Ethanolic Extract

Fifty grams of coarse clove bud powder was packed in a thimble and extracted with 250 mL of ethanol using a Soxhlet apparatus maintained at 65 °C for 6-8 hours. The extract was

concentrated by evaporation and dried for 2-3 days to prevent fungal contamination during storage. The percentage yield was calculated as the weight of dried extract divided by the weight of dried clove bud powder used, multiplied by 100. A yield of 61.9% (30.95 g from 50 g) was obtained with ethanol as the extraction solvent.



**Fig-1: Soxhlet extraction of clove buds with ethanol**

## 2.2 Preliminary Phytochemical Screening

The ethanolic extract was subjected to standard qualitative tests for carbohydrates (Molisch's, Benedict's, Fehling's tests), proteins (Xanthoproteic, Biuret tests), lipids (solubility, glycerol, Sudan III tests), alkaloids (Mayer's, Wagner's, Dragendorff's tests), tannins (gelatine, lead acetate, ferric chloride tests), flavonoids, resins, sterols (Salkowski test), cardiac glycosides (Keller-Kiliani test), triterpenes and anthraquinones, following established phytochemical screening protocols.

## 2.3 Immobilization Activity

Immobilization activity was assessed in *Lumbricus terrestris* earthworms, five per treatment group. Five groups were maintained: Group I - control (distilled water); Group II - clove extract 0.5% w/v; Group III - clove extract 1% w/v; Group IV - clove extract 2% w/v; and Group V - standard lignocaine hydrochloride 2% w/v. Twenty millilitres of the respective test solution was placed in labelled Petri dishes and a single earthworm was introduced into each. The stopwatch was started on introduction, and the earthworm was observed continuously; immobilization time was recorded as the time at which the worm showed no spontaneous movement and failed to respond to gentle mechanical stimulation with a glass rod or forceps. The earthworm was then rinsed with distilled water and transferred to a fresh Petri dish containing normal saline, and the recovery time - the time taken to regain normal spontaneous movement and respond to stimulation - was recorded.

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical Screening

Qualitative screening of the ethanolic extract revealed the presence of carbohydrates, alkaloids, tannins, flavonoids and resins, while proteins, lipids, sterols, triterpenes and anthraquinones were absent (Table-1).

#### Test for carbohydrates:

**(a) Molish's test:** 1 ml of plant extract was added to 0.4 ml Molisch's reagent. Afterwards, 1 ml of conc. Sulphuric acid was added along the side of the test tube. A purple colour indicates the presence of carbohydrates.

**(b) Benedict's test:** 1 millilitre of plant extract and 1 millilitre of Benedict's reagent were heated for 5mins. The formation of orange precipitate indicates the presence of carbohydrates.

**(c) Fehling's test:** Boiling and filtering 1 ml of plant extract with 2 ml of purified water. Then, 2 ml of Fehling's reagent were added to 2 ml of filtrate, which was then heated. Reddish brown precipitate indicates the presence of carbohydrates.

#### Test for proteins:

**(a) Xanthoproteic test:** 0.25 ml of nitric acid was applied to 1 ml of plant extract. Appearance of white precipitate indicated the presence of proteins.

**(b) Biuret test:** 1 ml of plant extract was taken in a test tube. Then add 4% NaOH and 1% CuSO<sub>4</sub>. Violet pink colour development indicated the presence of proteins.

#### Test for lipids:

**(a) Solubility test:** 1 ml of plant extract was evaporated to dried powder. Few drops of petroleum ether were poured into the test tube and shaken well. Complete dissolution of extract identified the presence of lipids.

**(b) Glycerol test:** 1ml of 1% CuSO<sub>4</sub>.5H<sub>2</sub>O solution, 5 drops of plant extract. The add 5 drops of 10% sodium hydroxide solution. Blue colour solution was obtained which indicates the presence of glycerol.

**(c) Sudan III test:** 1 ml of plant extract, few drops of sudan III solution were added. Appearance of red colour indicated the presence of lipids.

#### Test for alkaloids:

**(a) Mayer's test:** 1ml of extract, 2 drops chloroform, and 2 drops of mayer's reagent. A positive alkaloids reaction resulted in the production of white deposits.

**(b) Dragendorff's test:** 2 ml of reagent was added to 1 ml of plant extract. Formation of orange white precipitate indicated alkaloids.

#### Test for tannins:

**(a) Lead acetate test:** To the filtrate 5ml of 10% lead acetate solution was added. Formation of white precipitation indicates the presence of tannin.

**(b) Ferric chloride test:** Five drops of a 5% ferric chloride solution were added. Formation of blue green colour indicated the presence of tannin.

#### Test for flavonoids:

To 0.5 ml of plant extract, 5ml dilute ammonia was added followed by the addition of 1 ml conc. Sulphuric acid. A yellow colouration that disappeared on standing indicated the presence of flavonoids.

#### Test for resins:

0.5 ml of acetic acid and 2 drops of conc. Sulphuric acid were added. It was clear that resins were present because a purple colour that turned violet in about 10 mins.

#### Test for sterols:

2 ml of conc. Sulphuric acid and 2 ml of chloroform were added with 0.5 ml of plant extract. The test tube was shaken gently for few mins. The development of red colour chloroform layer indicated the presence of sterols.

#### Test for triterpenes:

Few ml of plant extract was mixed with 5 ml of chloroform and warmed for 30 min at 40 degree. Few drop of sulphuric acid are mixed well. The appearance of red colour indicated the presence of triterpenes.

#### Test for anthraquinones:

2 ml of plant extract was shaken with 10ml benzene, and 5ml of 10% ammonia solution was added. The mixture was shaken in order to obtained the colour anthraquinones. The pink colour indicated the presences of anthraquinones.

**Table-1: Preliminary phytochemical screening of ethanolic clove bud extract**

| S.No | Phytoconstituent | Ethanolic Extract |
|------|------------------|-------------------|
| 1    | Carbohydrates    | +                 |
| 2    | Proteins         | -                 |
| 3    | Lipids           | -                 |
| 4    | Alkaloids        | +                 |
| 5    | Tannins          | +                 |
| 6    | Flavonoids       | +                 |
| 7    | Resins           | +                 |
| 8    | Sterols          | -                 |
| 9    | Triterpenes      | -                 |

| S.No | Phytoconstituent | Ethanolic Extract |
|------|------------------|-------------------|
| 10   | Anthraquinones   | -                 |

### 3.2 Immobilization and Recovery Time

The immobilization and recovery times recorded for the control, three concentrations of clove bud extract, and the lignocaine hydrochloride standard are summarised in Table-2. A clear, dose-dependent reduction in immobilization time was observed with increasing extract concentration, while recovery time increased proportionally.

**Table-2: Comparative immobilization and recovery time of clove bud extract and lignocaine hydrochloride**

| Group | Treatment            | Immobilization Time (min) | Recovery Time (min) |
|-------|----------------------|---------------------------|---------------------|
| I     | Control              | -                         | -                   |
| II    | Clove extract (0.5%) | 8:06 ± 0:40               | 6:42 ± 0:19         |
| III   | Clove extract (1%)   | 6:12 ± 0:56               | 14:56 ± 0:35        |
| IV    | Clove extract (2%)   | 4:09 ± 0:53               | 21:07 ± 0:30        |
| V     | Standard drug (2%)   | 18:16 ± 0:54              | 45:10 ± 0:44        |



**Fig-2: Earthworm immobilization assay - standard drug (lignocaine hydrochloride) treatment group**

Comparison of the two treatments shows that lignocaine hydrochloride produced markedly slower onset of immobilization but a substantially longer recovery period than the clove extract at all tested concentrations, indicating a more prolonged neuromuscular blocking action of the standard drug. In contrast, the 2% clove bud extract achieved the most rapid immobilization among the extract groups, though its overall action was of shorter duration. The immobilization

activity of the extract is attributed principally to eugenol, which blocks voltage-gated sodium channels in nerve membranes in a manner mechanistically similar to lignocaine, thereby preventing the initiation and propagation of nerve impulses. The presence of accompanying flavonoids, tannins and resins may further contribute to this activity through synergistic effects.

Overall, the standard drug demonstrated higher immobilization efficacy and a longer duration of action than the ethanolic clove extract; however, the extract exhibited clear, concentration-dependent immobilization activity, most pronounced at the 2% level, supporting its potential as a natural local-anaesthetic-like agent.

#### 4. CONCLUSION

The ethanolic extract of *Syzygium aromaticum* buds exhibited significant, dose-dependent immobilization activity in the earthworm model, attributable to the presence of secondary metabolites such as alkaloids, tannins, flavonoids and resins, and principally to eugenol acting as a fast, sodium-channel-blocking agent. While lignocaine hydrochloride remained more efficient in terms of immobilization onset and duration of action, the comparative data obtained in this study lend scientific support to the traditional use of clove as a natural local anaesthetic and highlight its potential as an economical, plant-based alternative meriting further pharmacological and phytochemical investigation.

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