

# Comparative phytochemical and neuropharmacological profile of *Prunus domestica* and *Cymbopogon citratus*

Hina IMRAN<sup>1</sup>, Tehmina SOHAIL<sup>1</sup>, Nudrat FATIMA<sup>2</sup>, Syed Rafay YAQEEN<sup>3</sup>

<sup>1</sup>Pharmaceutical Research Centre, PCSIR Labs Complex, Karachi, Pakistan

<sup>2</sup>Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan

<sup>3</sup>Bay River College, Calgary, Alberta, Canada

Hina IMRAN **ORCID ID:** 0009-0001-3279-2087

Nudrat FATIMA **ORCID ID:** 0000-0001-8215-1897

## ABSTRACT

**Background and objectives.** There is a well-established link between the central nervous system (CNS) and antiemetic agents. In our previous studies, we reported significant antiemetic activity from *Prunus domestica* (plum) fruit and *Cymbopogon citratus* (lemongrass) leaves. This study was designed to further explore and compare their potential CNS effects.

**Materials and methods.** The ethanolic extracts of *P. domestica* fruit and *C. citratus* leaves were individually subjected to phytochemical and neuropharmacological analysis. To assess CNS effects, behavioral assessments and muscle coordination tests, including the rotarod and grip strength tests, were conducted on albino rats and mice. The extracts were administered at a dose of 500 mg/kg body weight, with diazepam (5 mg/kg) used as the standard reference drug.

**Outcomes.** Phytochemical analysis of both plant extracts revealed the presence of pharmacologically active compounds, including triterpenes, tannins, and saponins. Behavioral experiments showed that *P. domestica* did not significantly alter normal animal behavior, whereas *C. citratus* exhibited mild CNS depressant effects. Muscle coordination was assessed using the rotarod and grip strength tests, and the results indicated that *P. domestica* had more prolonged CNS stimulant effects compared with *C. citratus*. Animals treated with diazepam displayed the most significant CNS depressant effects across all tests. A *p*-value < 0.05 was considered statistically significant.

**Conclusions.** *P. domestica* demonstrated CNS stimulant activity, while *C. citratus* exhibited CNS depressant effects. Therefore, based on these comparative CNS effects, it can be concluded that *P. domestica* is a more potent antiemetic agent, as it does not significantly affect CNS function.

**Keywords:** *P. domestica*, *C. citratus*, phytochemical, neuropharmacological tests, rats and mice

## INTRODUCTION

Nausea and vomiting are common yet distressing conditions in which the stomach contents are forcefully

expelled through the mouth. While several antiemetic medications are available to alleviate these symptoms, they are associated with side effects and potential drug

Corresponding author:

Hina Imran

E-mail: dr\_hinaimran@yahoo.com

Article History:

Received: 10 October 2025

Accepted: 15 November 2025

interactions [1,2]. In contrast, herbal remedies have long been used to soothe nausea and vomiting [3,4]. Over the past few years, more than 45,000 medicinal plants have been documented for their bioactive compounds and medicinal properties. Among them, *Prunus domestica* L. (Rosaceae family) and *Cymbopogon citratus* (DC.) Stapf (Poaceae family) stand out for their potent medicinal properties. Both plants contain a rich array of bioactive compounds, making them valuable subjects for pharmacological research [5–8].

The central nervous system (CNS) plays a crucial role in regulating most bodily functions, including emesis (vomiting). The emetic response involves multiple neurotransmitters, such as histamine, dopamine, serotonin, acetylcholine, and neurokinin, all of which interact to trigger nausea and vomiting. Given this complex relationship, various drugs that target the CNS, including antiemetics, are used to control emesis [9,10]. In previous studies, we reported significant individual antiemetic activity of the ethanolic extracts of *C. citratus* (leaves) and *P. domestica* (fruit) [11,12]. However, despite these findings, the comparative central nervous system profiles of these two plants have not been systematically evaluated, and it remains unclear whether their neuropharmacological effects are compatible with safe antiemetic use. We hypothesized that *P. domestica* would exhibit minimal CNS depressant activity compared with *C. citratus*, thereby supporting its suitability as an antiemetic agent that does not adversely affect normal CNS function. Therefore, the present study was designed with three main objectives: (1) to characterize and compare the phytochemical composition of *P. domestica* fruit and *C. citratus* leaves; (2) to evaluate and compare their neuropharmacological effects using behavioral and motor coordination tests in rats and mice; and (3) to relate these CNS effects to their potential use in the development of a safe herbal antiemetic formulation.

## MATERIAL & METHODS

### Plant material and extraction

*P. domestica* fruit and *C. citratus* leaves were collected locally from Karachi and identified based on their macromorphological features and botanical profile. In brief, the dried plant materials were cleaned and chopped into small pieces. Each plant (1 kg) was extracted separately with 2.5 L of ethanol. Filtration was performed using filter paper, and the soaking and filtration process was repeated twice on the remaining

residue. The filtrates from each extraction were pooled and concentrated using a rotary evaporator at 50–60°C under reduced pressure.

### Phytochemical studies

For phytochemical studies, different chemical reagents were prepared and standard procedures were performed as reported by researchers [13–16].

#### (i) Test for terpenoids

**Liebermann–Burchard test:** Two grams of each sample were mixed with 2 mL chloroform and 3 mL concentrated  $H_2SO_4$  was added carefully with gentle mixing. A reddish-brown color indicates the presence of triterpenes.

#### (ii) Test for alkaloids

**Dragendorff test:** Two milliliters of plant extract were warmed with 2%  $H_2SO_4$ , and then a few drops of Dragendorff reagent were added. An orange precipitate indicated the presence of alkaloid components.

#### (iii) Test for tannins

**Lead acetate test:** Two milliliters of extract were treated with a few drops of 1% lead acetate. Formation of yellowish precipitates indicates the presence of tannins.

#### (iv) Test for saponins

**Froth test:** Two milliliters of plant extract were treated with 5 mL distilled water and shaken vigorously. The froth formed indicates the presence of saponins.

#### (v) Test for carbohydrates

**Molisch's test:** Two milliliters of aqueous extract were treated with 2 drops of alcoholic  $\alpha$ -naphthol solution in a test tube. One milliliter of concentrated sulfuric acid was run down the side of the tube without mixing. A violet-colored ring at the junction of the two liquids indicates the presence of carbohydrates.

#### (vi) Test for steroids

**Salkowski test:** Two milliliters of extract in a test tube were treated with 2 mL acetic anhydride, 1 mL chloroform, followed by 0.5 mL sulfuric acid. If the test solution shows a color change from violet to blue to green, it denotes the presence of steroids.

#### (vii) Test for proteins

**Biuret test:** Two milliliters of extract were treated with 10% sodium hydroxide solution and a few drops of 0.1% copper sulfate solution and observed for the formation of a violet/pink color.

## Neuropharmacological studies

### Animal selection and ethical approval

Male and female albino rats (150–200 g) and mice (17–25 g) were obtained from PCSIR Labs Complex, Karachi. All animals were housed in plastic cages. The animal rooms were maintained under standard experimental conditions with a 12 h light/dark cycle and free access to food and water. The experimental procedures were approved by the Ethical Committee (IEC/AN-10) for the use of experimental animals of PCSIR Laboratories Complex, Karachi.

### Behavioral studies

Previously, we reported the acute oral toxicity study of *P. domestica* and *C. citratus* [17–19]. Behavioral studies were carried out in mice as previously reported by Ahmad et al., 2011. Animals were divided into four groups (n = 5). The extracts of *P. domestica* and *C. citratus* were administered at a dose of 500 mg/kg (p.o.) to groups I and II. Groups III and IV received diazepam (5 mg/kg) as a standard drug and distilled water as a control. The animals were observed for behavioral changes, if any, at 30 min intervals during the first hour and at hourly intervals for the next 4 h after drug administration for the following parameters. The depressant action was scored as no effect (–), slight depression (+), moderate depression (++), strong depression (+++), and very strong depression (++++).

**a) Grip strength:** The animal was held by a pencil horizontally, and the time it took for the pencil to drop onto the table was recorded.

**b) Alertness/awareness:** The animal's response was visually monitored by observing its ability to orient itself when placed in a new position, noting whether it could do so without bumping into objects or falling.

**c) Sound response:** Mice are usually silent and do not produce vocalizations, so this test was conducted to observe any vocal sounds, which could indicate the presence of a noxious stimulus such as pain, irritation, or fear.

**d) Touch response:** This response was observed by gently touching the animals with forceps at various body parts.

**e) Pain response:** Pain was graded when a small artery forceps was gently applied to pinch the tail of the mice.

**f) Pinna reflex:** This reflex was recorded by lightly touching the mouse's ear, as a healthy animal will move away in response to the irritation.

## Muscle coordination tests

The motor coordination test using the rotarod and the grip strength test were employed to assess CNS activity.

### (i) Rotarod test

The test was conducted as described previously [20]. Prior to the experiment, animals were placed on a rotarod (UGO Basile) for 3 minutes at a speed of 5 rpm. Only those animals that remained on the rotating rod without falling for the full 3 minutes were selected. Mice were divided into four groups (n = 5). Groups I and II received *P. domestica* and *C. citratus* extracts at a dose of 500 mg/kg (p.o.), while Group III was administered diazepam (5 mg/kg) as a standard drug. Group IV received distilled water as a control. Each mouse was allowed to rotate on the rod for 3 minutes, and the time spent on the rod was recorded at 30, 60, 90, 120, and 150 minutes following drug administration.

### (ii) Grip strength test

The effect of the drug on muscle strength was assessed using a grip strength meter (UGO Basile, Italy), where each rat was lifted by the tail and encouraged to grasp a rigid metal bar attached to a digital force gauge. Subsequently, the rat was gently pulled backward by the tail until it released the metal bar, and the tension reading was displayed digitally as the grip strength. The animals were divided into four groups (n = 5). Group I and Group II received *P. domestica* and *C. citratus* extracts at a 500 mg/kg dose, respectively. Group III was given diazepam (5 mg/kg) as the standard, while Group IV was given distilled water and served as the control group. Assessment of muscle strength was started at 15, 45, 75, and 105 min after the oral administration of test and standard drugs [20].

## Statistical analysis

Statistical analysis was performed using Student's t-test, and the values were considered statistically significant at  $p \leq 0.05$ .

## RESULTS

### Phytochemical studies

The qualitative phytochemical screening of *P. domestica* and *C. citratus* was performed by standard methods, and the results indicate the presence of valuable phytochemicals (Table 1).

TABLE 1. Chemical constituents of *P. domestica* and *C. citratus*

| Plant name          | Chemical constituents |         |         |          |              |         |         |           |
|---------------------|-----------------------|---------|---------|----------|--------------|---------|---------|-----------|
|                     | Triterpene            | Tannins | Saponin | Alkaloid | Carbohydrate | Protein | Sterols | Flavonoid |
| <i>P. domestica</i> | —                     | +       | +       | —        | +            | +       | +       | +         |
| <i>C. citratus</i>  | +                     | +       | +       | +        | +            | +       | +       | +         |

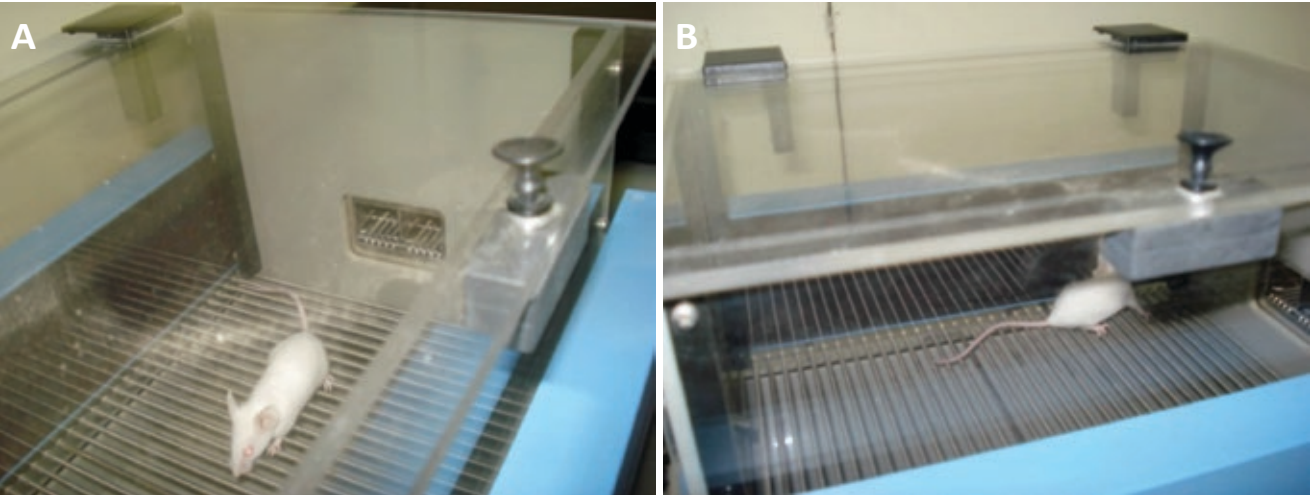


FIGURE 1 (A-B). Behavior studies of *P. domestica* and *C. citratus*

Neuropharmacological studies

Behavior studies

The behavioral findings are summarized in Table 2 and Figure 1. *P. domestica* administered at a 500 mg/kg dose was associated with normal animal behavior, with a strong grip maintained throughout the 4 h observation period. Reflex responses to pain, sound, touch, and pinna stimuli were also normal. In contrast, the animal group treated with *C. citratus* at the same dose showed a reduction in grip strength, suggesting mild central nervous system (CNS) depressant effects. This decreased activity was also reflected by lower performance in the rotarod and grip strength tests. The standard drug, diazepam (5 mg/kg), showed the lowest

grip strength and the highest depression scores across sound, pain, pinna, alertness, and touch responses over the observation period.

Muscle coordination tests

(i) Rotarod test

In the rotarod test, both *P. domestica* and *C. citratus* extracts at 500 mg/kg showed changes in motor performance over time, as indicated by the time spent on the rotating rod (Table 3 and Figure 2). In the *C. citratus* group, the mean time on the rotarod was 120.2 ± 11.1 s at 30 minutes and 130.3 ± 4.6 s at 120 minutes, whereas in the *P. domestica* group, the time remained close to 180 s throughout the 3 h observation period. The standard drug diazepam showed markedly lower

TABLE 2. Behavior studies of *P. domestica* and *C. citratus* extracts

| Sr. # | Treatments                     | Behavior type |                      |                |                |               |              |
|-------|--------------------------------|---------------|----------------------|----------------|----------------|---------------|--------------|
|       |                                | Grip strength | Alertness/ awareness | Sound response | Touch response | Pain response | Pinna reflex |
| 1.    | Group I<br><i>P. domestica</i> | —             | —                    | —              | —              | —             | —            |
| 2.    | Group II<br><i>C. citratus</i> | +             | —                    | —              | —              | —             | +            |
| 3.    | Group III<br>Diazepam          | ++            | ++++                 | +++            | ++             | ++++          | +++          |
| 4.    | Group IV<br>Distilled water    | —             | —                    | -              | —              | —             | —            |

No effect (—), slight depression (+), moderate depression (++), strong depression (+++), very strong depression (++++)

TABLE 3. Motor coordination activity of *P. domestica* and *C. citratus*

| Sr. # | Treatments                     | Time spent on rotarod, in seconds |               |              |               |               |
|-------|--------------------------------|-----------------------------------|---------------|--------------|---------------|---------------|
|       |                                | 30 min                            | 60 min        | 90 min       | 120 min       | 150 min       |
| 1.    | Group I<br><i>P. domestica</i> | 180.3 ± 0.08*                     | 180.4 ± 0.1   | 180.4 ± 0.1  | 180.4 ± 0.1   | 180.4 ± 0.1   |
| 2.    | Group II<br><i>C. citratus</i> | 120.2 ± 11.1*                     | 113.4 ± 11.1* | 105.8 ± 5.5* | 130.32 ± 4.6* | 140.4 ± 6.7*  |
| 3.    | Group III<br>Diazepam          | 52.84 ± 71.4*                     | 47.6 ± 36.3*  | 45.6 ± 39.7* | 121.06 ± 72.1 | 146.2 ± 49.98 |
| 4.    | Group IV<br>Distilled water    | 180.4 ± 0.1                       | 180.4 ± 0.1   | 180.3 ± 0.1  | 180.4 ± 0.1   | 180.3 ± 0.1   |

Values are expressed as mean ± SD, \*p < 0.05 when compared to control.

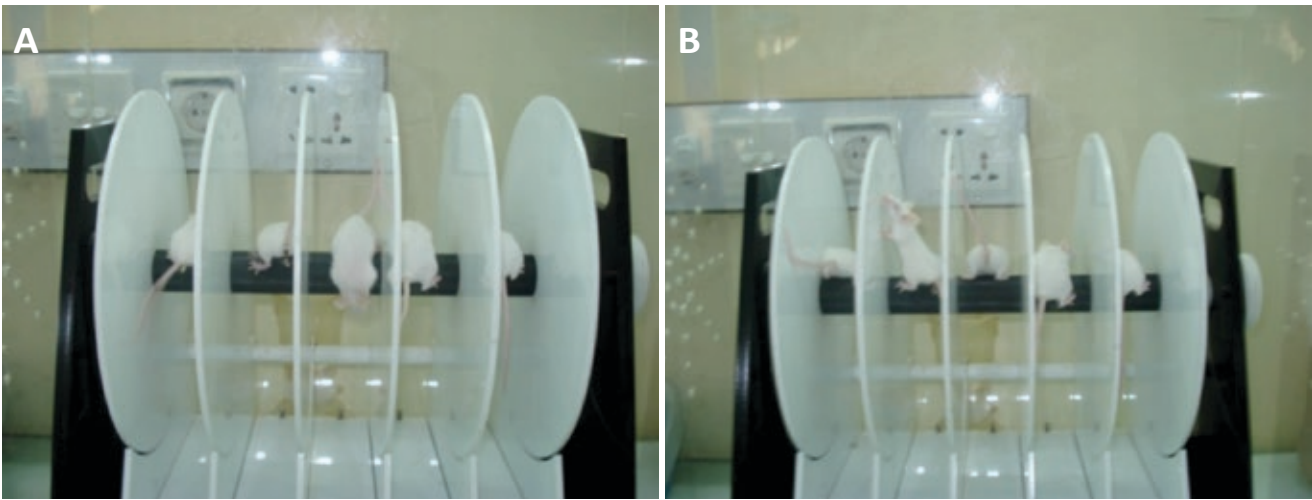


FIGURE 2 (A-B). Motor co-ordination activity on rotarod apparatus

TABLE 4. Grip strength test of *P. domestica* and *C. citratus*

| Sr. # | Treatments                     | Grip strength (g) |              |              |             |              |
|-------|--------------------------------|-------------------|--------------|--------------|-------------|--------------|
|       |                                | 0 min             | 15 min       | 45 min       | 75 min      | 105 min      |
| 1.    | Group I<br><i>P. domestica</i> | 37.0 ± 8.72       | 38.6 ± 10.13 | 45.2 ± 8.87  | 46.2 ± 8.81 | 40.4 ± 9.39  |
| 2.    | Group II<br><i>C. citratus</i> | 38.0 ± 0.61       | 33.2 ± 5.21  | 32.6 ± 4.39  | 32.4 ± 2.88 | 30.2 ± 5.49  |
| 3.    | Group III<br>Diazepam          | 36.2 ± 9.06       | 30.6 ± 10.31 | 27.8 ± 10.05 | 26.2 ± 9.23 | 24.0 ± 7.48* |
| 4.    | Group IV<br>Distilled water    | 37.6 ± 9.44       | 37.2 ± 8.25  | 37.8 ± 7.94  | 38.2 ± 7.75 | 38.0 ± 6.51  |

\*p < 0.05 when compared to control

rotarod times across most time points compared with distilled water.

(ii) Grip strength test

The muscle strength of the rats after receiving *P. domestica* and *C. citratus* extracts (500 mg/kg) was evaluated using the grip strength test (Table 4). In the *P. domestica* group, the baseline grip strength was 37.0 ± 8.72 g, increasing to 38.6 ± 10.13 g at 15 minutes and 45.2 ± 8.87 g at 45 minutes, with 46.2 ± 8.81 g recorded

at 75 minutes after drug administration. In the *C. citratus* group, the baseline reading was 38.0 ± 10.61 g, which decreased to 33.2 ± 5.21 g at 15 minutes and 30.2 ± 5.49 g at 105 minutes. In the diazepam group, grip strength decreased from 36.2 ± 9.06 g at baseline to 30.6 ± 10.31 g at 15 minutes and 24.0 ± 7.48 g at 105 minutes. Grip strength values in the distilled water group remained relatively stable throughout the observation period.

## DISCUSSION

Nausea continues to be ranked first on the list of troublesome and distressing symptoms experienced by patients. Previously, we reported the significant antiemetic activity of *P. domestica* and *C. citratus*. It is well reported that there is a direct connection between the CNS and antiemetic agents [21,22]. Therefore, to verify the comparative effects of these plants on the CNS, this study was planned. The ethanolic extracts of *P. domestica* and *C. citratus* were studied individually for phytochemical and neuropharmacological properties. To verify neuropharmacological effects, behavioral studies and muscle coordination tests were performed using the rotarod and grip strength tests.

The phytochemical studies confirm that both plant materials possess major phytochemical constituents like tannins, saponins, carbohydrates, proteins, sterols, and flavonoids (Table 1). Several researchers have also reported these compounds in *P. domestica* and *C. citratus* [5,23–25].

The behavioral studies demonstrate that *P. domestica*-treated animals maintained normal behavior during the observation period. There was a strong grip up to the 4 h period of observation, while pain, sound, touch responses, and pinna reflex were also found to be normal. *C. citratus*-treated animals showed slight CNS depressant effects, with a reduction in grip strength and pinna reflex. The standard drug diazepam (5 mg/kg) showed marked depressant effects. The animals lost their grip soon after administration of the drug, with moderate depression in sound response, strong depression in pain response, pinna reflex, and alertness, and very strong depression in touch response. The parameters observed and results noted are listed in Table 2 and Figure 1.

The rotarod results indicate that animals fed with 500 mg/kg *P. domestica* extract remained on the rotating rod at approximately  $180.3 \pm 0.08$  seconds from 30 minutes after drug administration to 3 hours post-drug. In contrast, animals fed with *C. citratus* (500 mg/kg) fell from the rotating rod at  $120.2 \pm 11.14$  seconds 30 minutes after drug administration. After 2 hours, the time on the rod increased to  $130.2 \pm 4.65$  seconds and after 3 hours to  $140.4 \pm 6.76$  seconds. The standard drug diazepam showed a highly significant CNS depressant effect, with  $52.8 \pm 71.45$  seconds at 30 minutes, gradually increasing up to  $146.22 \pm 49.98$  seconds after 3 hours. Statistical differences were found to be significant at  $p \leq 0.05$  when compared to the control group (Table 3 and Figure 2).

Another test performed to evaluate neuropharmacological effects was the grip strength test. The results indicate that animals administered *P. domestica* had a baseline reading of  $37.0 \pm 8.72$  g, which gradually increased at different time intervals up to  $46.3 \pm 8.18$  g after 75 minutes and  $40.4 \pm 7.34$  g after 105 minutes. *C. citratus* showed CNS depressant effects 15 minutes after administration, with readings of  $33.1 \pm 9.25$  g, which were reduced to  $30.2 \pm 11.14$  g after 105 minutes. Diazepam showed more depressant effects, with  $30.6 \pm 11.93$  g at 15 minutes, followed by  $24.0 \pm 8.19$  g after 105 minutes post-drug administration. Statistical differences were found to be significant at  $p \leq 0.05$  when compared to the control group (Table 4).

Halim et al. [26] reported that plants containing flavonoids, saponins, phenols, and tannins are useful for combating many CNS disorders. Our results indicate the presence of all these medicinally important constituents in both plants. A number of scientific reports also indicate that triterpenoids and alkaloids produce CNS depressant actions [27,28]. Our phytochemical studies showed that triterpenoids and alkaloids are absent in *P. domestica* but present in *C. citratus*. Thus, the differing neuropharmacological effects of *P. domestica* and *C. citratus* may be associated with their phytochemical composition. Therefore, our results correlate with reported studies [29].

The same Halim et al. [26] also mentioned that one of the most popular methods for evaluating a drug's action on the CNS is to conduct behavioral studies in experimental animals. Another researcher reported that in the rotarod test, a reduction in the time spent on the rotating rod due to loss of grip implies skeletal muscle relaxation. This effect also indicates an induced neurological deficit, including a calming effect, which further depicts CNS depressant activity [30]. Chen et al. [31] reported that the forelimb grip strength test in animal models measures changes in neuromuscular coordination, muscle strength, and total functional capability. The loss of grip strength is considered a measure of muscle relaxant activity. All these reported data validate our findings. The overall neuropharmacological results, including behavioral studies and muscle coordination tests (rotarod and grip strength), indicate that *P. domestica* has CNS stimulant effects compared with *C. citratus*.

A study conducted by Dudhgaonkar et al. [32] reported CNS depressant effects of *C. citratus* essential oil using the forced swimming test in mice. They also reported

that a possible mechanism of the antidepressant action of lemongrass is the attenuation of oxidative stress by monoterpenes and polyphenols present in it. Another study [33] reported CNS depressant effects of *C. citratus* leaves at a dose of 200 mg/kg in the plus-maze test. One more study on essential oil of lemongrass at 5 and 10 mg/kg showed that *C. citratus* has significant CNS antidepressant activity [32]. In our study, animals treated with *C. citratus* also showed muscle relaxation and reduced motor activity. As mentioned earlier, the absence of triterpenoids and alkaloids in *P. domestica* may be related to its CNS stimulant profile, while the presence of these compounds in *C. citratus* may contribute to CNS depressant effects. Hence, our results are justified.

Previously, we reported antiemetic effects of *P. domestica* and *C. citratus*. We reported more significant antiemetic effects of *P. domestica* (61%) compared with *C. citratus* (26.4%) [11,12]. Other authors [34] reported that flavonoids are key antiemetic constituents. Thus, we can assume that the more potent antiemetic effect of *P. domestica* may be due to the presence of more flavonoids than in *C. citratus*. This enhancement in *P. domestica* can also be due to a shared mechanism of action or synergistic effects on different pathways involved in nausea and vomiting, including its phytochemical composition, acting centrally (on the chemoreceptor trigger zone) as well as peripherally (on the gastric mucosa) to exert antiemetic effects. By focusing on all these points and in correlation with our previous study, it can be summarized that *P. domestica*

is a more potent candidate for antiemetic therapy and does not significantly affect the CNS. Further study is needed to clarify the underlying mechanism.

## CONCLUSIONS

On the basis of the comparative phytochemical and neuropharmacological studies on extracts of *P. domestica* and *C. citratus*, it is concluded that *P. domestica* exhibits CNS stimulant effects, whereas *C. citratus* shows CNS depressant activity. *C. citratus* produced greater behavioral depression, more muscle relaxation, and reduced motor activity, and the phytochemicals associated with CNS depression (such as triterpenoids and alkaloids) were confirmed in *C. citratus* but absent in *P. domestica*.

By focusing on these findings and correlating them with our previous antiemetic study, it can be summarized that *P. domestica* is a more suitable candidate for antiemetic therapy because it does not significantly affect CNS function. These results suggest that *P. domestica* may offer safer antiemetic benefits with minimal neuropharmacological interference.

Further studies are required to clarify the precise underlying mechanisms, isolate the active constituents, and evaluate dose–response relationships in additional experimental models.

**Source of funding:** none

**Conflict of interest:** none

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