FORMULATION AND EVALUATION OF A NATURAL SEDATIVE AND ANXIOLYTIC NASAL SPRAY USING THE ETHANOL EXTRACT FROM *Glinus oppositifolius* (EEGO) PAPAIT LEAVES

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ABSTRACT

The increasing prevalence of anxiety disorders and the adverse effects associated with conventional anxiolytic and sedative drugs highlight the urgent need for safer, more accessible alternatives. This study aimed to formulate and evaluate a nasal spray for sedation and anxiety using an ethanol extract of *papait* leaves. An experimental design was employed, involving the formulation of an ethanol extract of *Glinus oppositifolius* as a nasal spray at different concentrations, and the assessment of its anxiolytic and sedative effects through behavioral assays (hole-cross and open-field tests) in animal models, alongside pharmaceutical evaluation of the nasal spray. The results showed that the most effective dose concentration of the EEGO nasal spray was 10 mg/mL, which reduced locomotor activity. In contrast, the 15 mg/mL dose was suggested to be potentially effective as an alternative sedative nasal spray. The *papait* nasal spray reduced exploratory behavior and anxiety-like symptoms in test animals, supporting its potential as an effective sedative and anxiolytic agent. This study assessed the pH, clarity, and pump delivery of nasal solution concentrations B and C. Both concentrations had a pH of 5.7, a light yellow color, and showed minimal visible impurities, detectable only against a dark background. The pump delivered 10 sprays per 1 mL, indicating that it is effective and applicable. The findings support the potential of *papait* nasal spray as a plant-based alternative with sedative and anxiolytic effects, encouraging the long-term usage of plant-based remedies. Further research into the exact mechanisms of action, optimal dosing strategies, and possible clinical uses would be beneficial in developing this formulation as an alternative natural sedative and anxiolytic nasal spray.

Keywords: Hole cross test, intranasal, open field test, pump delivery, sedation

INTRODUCTION

Background of the Study

Sedatives are central nervous system depressants used to induce calmness, reduce anxiety, and promote sleep (Vitukade, 2024). Anxiolytics, on the other hand, are defined as medications or other interventions that inhibit anxiety (Witkin & Barrett, 2024). Although these drugs are potent agents in the treatment of medical and psychological issues, they do pose considerable side effects, making the door open for more efficacious and better-tolerated treatments. In this case, natural remedies could complement the existing therapy and offer better approaches towards the management of anxiety and decrease the intensity of pain, which most sedative drugs target.

Anxiolytics are medications or interventions that reduce anxiety (Witkin & Barrett, 2024) and are commonly prescribed for anxiety disorders, sedation, insomnia, and short-term relief of anxiety symptoms (Fuentes et al., 2014). According to the World Health Organization, about 4% of the global population experienced anxiety in 2019. Although synthetic anxiolytic drugs are effective (Moniruzzaman et al., 2016), they often cause adverse side effects, highlighting the need for safer, more tolerable alternatives. Natural remedies may serve as

complementary treatments, offering improved management of anxiety and pain reduction with fewer risks.

Researchers are continually exploring natural sources for therapeutic solutions. Several herbal plants, such as hops, valerian, passionflower, and kava, are known for their sedative properties (Muzammil et al., 2020), though these species are not common in the Philippines. Hence, studying local plants with similar effects is essential. One such plant is *Glinus oppositifolius* (papait), a native Philippine vegetable rich in alkaloids, flavonoids, glycosides, saponins, sterols, and tannins (Martin-Puzon & Rivera, 2015). Moniruzzaman et al. (2016) found that the ethanol extract of *G. oppositifolius* (EEGO) produced sedative and anxiolytic effects in mice, reducing exploratory behavior and motor coordination in a dose-dependent manner. These results validate its traditional use as a natural sedative and anxiolytic, likely due to its bioactive compounds—particularly flavonoids, which act on the GABAergic system to induce calming effects.

In the study of Moniruzzaman *et al*., (2016), it was designed to investigate the sedative and anxiolytic profiles of the *papait* extract. However, it did not explore formulations that could provide sedative effects. While numerous studies have highlighted the antioxidant benefits and traditional uses of *papait* leaves, there is limited clinical research addressing its sedative effects, particularly regarding its use as a nasal spray. Nasal spray formulations offer an effective route for local, systemic, and central nervous system drug delivery, with advantages including cost-effectiveness, ease of use, and high patient compliance (Thorat, 2016; Bhatt, 2017). It also demonstrated improved patient cooperation and satisfaction compared to nasal drops (Ljung & Andréasson, 2014).

The formulation and evaluation of *papait* (*Glinus oppositifolius*) leaves as a natural sedative nasal spray is significant amid the rising demand for alternative treatments for anxiety and sleep disorders. This research aims to develop an accessible, cost-effective, and natural therapy that promotes mental health and well-being. It aligns with several Sustainable Development Goals—SDG 3 (Good Health and Well-Being) by supporting mental health, SDG 12 (Responsible Consumption and Production) through the sustainable use of herbal resources, SDG 9 (Industry, Innovation, and Infrastructure) by promoting healthcare innovation, and SDG 17 (Partnerships for the Goals) by integrating traditional knowledge and modern science. Furthermore, the study contributes to the global movement toward integrative health by bridging traditional and modern medical practices. It encourages further exploration of underutilized plant species, supporting sustainable plant-based therapeutics and biodiversity conservation.

Statement of the Objectives

This study aimed to formulate and evaluate the ethanol extract of Glinus oppositifolius (EEGO) as a natural sedative and anxiolytic in a nasal spray dosage form. This study was conducted from January to April 2025. Specifically, it aimed to:

1. Formulate the ethanol extract of *Glinus oppositifolius* (EEGO) in a nasal spray dosage form.
2. To assess the sedative and anxiolytic properties of EEGO nasal spray when compared to the control drug, diazepam, through the following tests:
    a. Hole Cross Test
    b. Open Field Test
3. Evaluate the pharmaceutical properties of the ethanol extract of *Glinus oppositifolius* (EEGO) nasal spray in terms of:
    a. pH
    b. Clarity

c. Pump delivery

Null Hypotheses

There was no significant difference in the sedative and anxiolytic properties of ethanol extract of *Glinus oppositifolius* (EEGO) nasal spray when compared to the positive control diazepam.

METHODOLOGY

Research Design

The research employed a comparative-descriptive experimental approach, using animal models to evaluate the sedative and anxiolytic effects of the formulated EEGO (Ethanol Extract of *Glinus oppositifolius*) nasal spray. It was compared to diazepam as a positive control. The quality of the formulated *papait* nasal spray was assessed based on pH, clarity, and pump delivery. Since this study involves an experiment to determine the efficacy of ethanol as a sedative and anxiolytic in Wistar rats, it is an experimental study.

Study Site and Sample Collection

The mature *papait* leaves were collected near a rice farm in Brgy. Wacal, Solano, Nueva Vizcaya. Formulation of the extract into a nasal spray and performance of test procedures were conducted at the SMU Center for Natural Sciences Research Laboratory.

Plant Identification

The plant specimen was identified and certified by a registered Agriculturist from Nueva Vizcaya State University.

Animal Identification

Twenty-four laboratory rats were used in the different test procedures. The laboratory rats were purchased at C and A Pet shop and Veterinary Services located in Doña Dadang Bldg., Saquing St., DTM, Bayombong, Nueva Vizcaya. The animals were inspected and certified by Dr. Aldrick T. Abiasen, DVM. Laboratory rats (*Rattus norvegicus*) were commonly used to test drug efficacy and safety.

Data Gathering Procedure

Collection and Preparation of Papait Leaves

The mature *papait* leaves were collected in Brgy. Wacal, Solano, Nueva Vizcaya. The collection process involved identifying and harvesting healthy *papait* leaves to ensure the integrity of the plant material for further analysis. The leaves were thoroughly washed with running tap water to remove any debris or contaminants, dried under the sheds, and then further dried in an oven at a temperature of fifty degrees Celsius (50°C).

Crude Extraction

For the ethanol extract preparation, 400g of the powdered, dried leaves were placed in a beaker and macerated with 1,560 mL of 95% ethanol, with occasional stirring at room temperature for 3 days. The filtrate was then collected and completely dried using a rotary evaporator. Finally, 9.14 g of crude extract (yield 2.28%) was obtained and used throughout the studies and in the formulation of the nasal spray.

Formulation of EEGO Nasal Spray

For the active ingredient, the EEGO, three concentrations were formulated: 5, 10, and 15 mg/mL. The optimized formulation according to Menaka et al. (2013) contains 0.02% benzalkonium chloride as a preservative, 0.02% disodium EDTA as an antioxidant, 1.36% potassium dihydrogen phosphate, and 3.58% disodium hydrogen phosphate as a buffer to maintain the formulation's pH. Normal Saline Solution was also added as a vehicle solvent.

Sedative and Anxiolytic Assay of EEGO Nasal Spray

Wistar rats were divided into three groups of four animals each for control and test treatments. The control group received diazepam (1 mg/kg, intraperitoneally), while test groups received *papait* nasal spray at concentrations of 5, 10, and 15 mg/mL intranasally. In the hole cross and open field tests, treatments were given immediately after pretreatment readings, while for other models, diazepam was administered 15 minutes and *papait* 30 minutes before testing (Moniruzzaman et al., 2016). Anxiety was induced by tail handling or isolation in a small box for 10 minutes (Howe & Bundell, 2023).

During dosing, awake rats were restrained using an intranasal grip, with the head held parallel to the floor. A pipette delivered the drug at a 45° angle to each nostril, releasing two small droplets per nostril about 2–3 seconds apart. Each nostril received 30 μL, and rats were held in position for 15 seconds to ensure inhalation (Hanson et al., 2013).

Hole Cross Test

A cage measuring 50 x 40 x 20 cm, with a fixed partition in the middle and a hole 5 cm in diameter, was used in this experiment. Initially, the Wistar rats were administered the control drug diazepam. At the same time, a separate group received *papait* and was allowed to cross the hole between the chambers. The animals were then observed for 3 min, and the number of passages was recorded before and at 30, 60, 90, and 120 min following the treatments (Moniruzzaman *et al.,* 2016).

Open Field Test

This test is a widely used model for evaluating the emotional behavior of animals, especially rodents. The open field apparatus consists of a wooden field measuring half a square meter, with a series of squares painted in alternating black and white. It has a wall of 50 cm height and was placed in a dimly lit room. The animals were placed in the middle of the open field to explore freely, and the number of squares they visited was counted for 3 minutes as a pretreatment reading. Immediately after the reading, the animals were treated with EEGO or diazepam and observed at 30, 60, 90, and 120 min after treatment (Moniruzzaman *et al.,* 2016).

Evaluation Parameters of the EEGO Formulation

The evaluation parameters were based on the factorial design developed and used by Bhuva & Patel (2018) to evaluate nasal spray solutions. A registered pharmacist further observed the pharmaceutical evaluation of the formulated nasal spray in the laboratory.

pH Test

The pH of the nasal formulation is very important, as it helps prevent nasal mucosal irritation and maintain normal physiological ciliary movement. Therefore, the formulation pH was adjusted to 4.5-6.5, and the pH of all prepared formulations was measured with a digital pH meter.

Clarity Test

The color and clarity were visually examined against black and white surfaces in the inspection booth. The test was performed to determine whether the colloidal dispersion is free of particulate matter. The dispersion in the test tube was observed against a black-and-white background under light using a clarity testing apparatus.

Pump Delivery Test

Pump delivery is a crucial parameter that is interrelated with spray content uniformity and affects product performance. The formulation was actuated 10 times in a pre-weighed bottle. The bottle was reweighed after 10 actuations, and the difference was calculated.

Treatment of Data

To determine the sedative and anxiolytic properties of *papait* nasal spray using HCT and OFT, the frequency of crosses was recorded and summarized. Moreover, the mean and standard deviation were computed. The t-test was used to evaluate the difference in the means of efficacy between the most effective dose of EEGO nasal spray and the controlled drug, diazepam. This statistical analysis helped determine whether there is a significant difference in the efficacy of EEGO nasal spray compared to the control drug, diazepam, when administered. To evaluate the pharmaceutical properties of pH, clarity, and pump delivery, pH measurements with a pH meter and physical evaluations were used, and the data obtained were compared with the standard values.

Ethical Considerations

This study was approved by Saint Mary's University Research Ethics Board (SMUREB) at 2nd Floor, Rev. John Van Bauwel Hall, SMU Main Campus, Ponce Street, Don Mariano Marcos, Bayombong, 3700 Nueva Vizcaya, Philippines (email: reb@smu.edu.ph; cellphone: 09177053041).

RESULTS AND DISCUSSIONS

Section 1. Formulation of the Ethanol Extract of *Glinus oppositifolius* (EEGO) In A Nasal Spray Dosage Form

Figures 1 and 2 show the image of the formulated EEGO nasal spray.

Figure 1

Papait Nasal Spray Concentration B

The sedative and anxiolytic nasal spray was formulated using the optimal concentrations identified during the experiment. Concentration B (10 mg/mL) was selected and formulated as a sedative and anxiolytic nasal spray. Each 100 mL of this formulation contained the active ingredient: 1,000 mg of the crude extract of papait leaves, along with its excipients: 20 mg of benzalkonium chloride, 20 mg of disodium EDTA, 2.04 g of potassium dihydrogen phosphate to adjust the pH, and 3.58 mg of disodium hydrogen phosphate. Additionally, 100 mL of Normal Saline Solution was used as the vehicle solvent. Each pump delivered a dose of 1 mg.

Figure 2

Papait Nasal Spray Concentration C

Similarly, concentration C, at 15 mg/mL, was formulated as a sedative nasal spray, differing only in the amount of crude extract used: 1,500 mg. At the same time, the excipients remained the same as in concentration B. Each pump delivered a dose of 1.5 mg. The 15 mL of each formulated product was filled into clean amber nasal spray bottles, labeled, and packaged appropriately.

Section 2. Assessment of the sedative and anxiolytic properties of EEGO nasal spray when compared to the control, Diazepam, through the following tests

Tables 1 and 2 show the mean, standard deviation, and T-test of HCT.

Table 1

Hole Cross Test

		Number of Crossing				
	3 minutes before Drug Administration		**30 mins**	**60 mins**	**90 mins**	**120 mins**
Control Drug (Diazepam)	T1	1	6	3	0	0
	T2	6	6	0	0	0
	T3	5	2	0	0	0
Mean (SD)			***4.66 (2.65)***	***1 (1.22)***	***0 (0)***	***0 (0)***
5 mg/mL	T1	5	8	0	0	0
	T2	3	1	1	0	0
	T3	4	6	0	3	6
Mean (SD)			***5 (2.73)***	***0.33 (0.71)***	***1 (1.22)***	***2 (1.73)***
10 mg/mL	T1	4	1	0	0	1
	T2	5	3	2	0	0
	T3	5	14	4	0	0
Mean (SD)			***6 (3)***	***2 (1.73)***	***0 (0)***	***1 (0.71)***
15 mg/mL	T1	1	2	0	0	0
	T2	6	1	0	0	0
	T3	5	0	21	0	0
Mean (SD)			***1 (1.22)***	***7 (3.24)***	***0 (0)***	***0 (0)***

Table 1 shows that EEGO at 15 mg/mL consistently showed fewer crossings and sustained reduction in locomotor activity when compared to the control drug, with a mean of 1 crossing (SD 1.22) at 30 minutes and complete inactivity (mean 0, SD 0) at later time points. The 10 mg/mL dose showed decreased crossings but greater variability (mean 6, SD 3 at 30 minutes;

mean 2, SD 1.73 at 60 minutes), while the 5 mg/mL dose yielded variable, less reliable effects, indicating reduced potency at lower concentrations.

The hole-cross test is a well-established method for evaluating sedative effects in rats, in which a reduction in movement, such as fewer hole crossings, indicates sedation (Khan *et al*., 2014). Moreover, locomotor activity is considered to be an indicator of alertness, and a decrease in locomotor activity indicates a sedative effect (Sherman *et al*., 2015). In this test, a significant decrease in locomotor activity reflects a diminished curiosity or exploratory behavior towards a new environment, which is considered an indicator of sedation. Across the three trials, a reduction in crossings was observed in the test samples from concentration A to C, with the highest concentration (C, 15 mg/mL) showing the most notable sedative effect.

Hence, this indicates that the test compound may exhibit potential sedative efficacy close to the control drug Diazepam under the conditions tested.

Table 2

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T-test for HCT

	treatment	Mean	Std. Deviation	Test Statistic
initial	Control drug (Diazepam)	4.00	2.646	t (4) =0, p=1
	15 mg/mL	4.00	2.646	
After 30 minutes	Control drug (Diazepam)	4.67	2.309	t (4) =2.524, p=.065
	15 mg/mL	1.00	1.000	
After 60 minutes	Control drug (Diazepam)	1.00	1.732	t (4) =-.849, p=.444
	15 mg/mL	7.00	12.124	
After 90 minutes	Control drug (Diazepam)	.00	.000[a]	
	15 mg/mL	.00	.000[a]	
After 120 minutes	Control drug (Diazepam)	.00	.000[a]	
	15 mg/mL	.00	.000[a]	

[l]t cannot be computed because the standard deviations of both groups are 0
*Significant at 0.05

The table shows a statistical comparison of the sedative effects between Diazepam and EEGO at 15 mg/mL. Initially, both groups had identical mean values (M = 4.00, SD = 2.646) with no significant difference (t(4) = 0, p = 1.000). After 30 minutes, the EEGO group showed a lower mean (M = 1.00) than the control (M = 4.67), but the difference was not significant (p = 0.065). At 60 minutes, EEGO displayed a higher mean (M = 7.00) than Diazepam (M = 1.00), though again not significant (p = 0.444) due to high variability. From 90 minutes onward, both treatments showed no activity (M = 0, SD = 0).

Overall, no statistically significant differences were observed between the control drug Diazepam and EEGO at a concentration of 15 mg/mL at any time point.

Table 3

Open Field Test

		Squares Visited				
		3 minutes before Drug Administration	**30 mins**	**60 mins**	**90 mins**	**120 mins**
Control Drug (Diazepam)	T1	56	14	1	0	0
	T2	41	12	0	0	0
	T 3	13	0	0	0	0
Mean (SD)			***8.66 (7.57)***	***0.33 (0.58)***	***0 (0)***	***0 (0)***
5 mg/mL	T1	21	12	0	8	1
	T2	47	56	0	0	0
	T3	13	8	0	0	0
Mean (SD)			***25.33 (26.63)***	***0 (0)***	***2.66 (4.62)***	***0.33 (0.58)***
10 mg/mL	T1	40	6	0	0	0
	T2	32	9	7	0	0
	T3	35	2	0	0	0
Mean (SD)			***5.66 (3.51)***	***2.33 (3.70)***	***0 (0)***	***0 (0)***
15 mg/mL	T1	64	7	10	0	0
	T2	26	24	4	0	0
	T3	76	67	0	0	0
Mean (SD)			***32.66 (30.92)***	***4.66 (5.03)***	***0 (0)***	***0 (0)***

The table demonstrates that the concentration B at 10 mg/mL showed the most consistent and significant reduction in locomotor activity, with a mean of 5.66 crossings (SD 3.51) at 30 minutes and 2.33 (SD 3.70) at 60 minutes, followed by complete inactivity at 90 and 120 minutes (mean 0, SD 0). On the other hand, 5 mg/mL (concentration A) and 15 mg/mL (concentration C) showed higher and more variable activity at 30 minutes, with their means of 25.33 (SD 26.63) and 32.66 (SD 30.92), respectively. With only partial or delayed reductions at later time points. Thus, the results in concentration B were consistent with the three trials performed.

Along with the result, the behavioral activity of the rats can be affected by several intrinsic and extrinsic factors. Although identical in lineage (breed), sex, age, and conditions of housing, they can individually differ significantly in terms of behavior and physiology. According to Tatem *et al*. (2014), the open field activity measurements vary depending on the animal strain, age, sex, and circadian rhythm. In accordance with our observation, the lower concentration (10 mg/mL) nasal spray outperformed a higher concentration (15 mg/mL) may reflect the drug titration paradox, as described by Minto *et al*. (2023), where increasing a drug's dose does not necessarily enhance its effect and may even reduce efficacy due to physiological or pharmacodynamic mechanisms.

In addition, environmental factors such as room temperature, humidity, lighting, noise, and even odor can affect assessment outcomes. This is further supported by several studies, which showed that rats exposed to acute noise stress showed significant behavioral alterations like increased fear and anxiety (Arjunan, A. 2019), while extremes in temperature and humidity can cause stress and impact animal behavior, leading to inconsistent data. Rats prefer slightly warmer environments (26°C - 30°C) than typical room temperatures. Deviations can cause changes in aggression and lethargy (Heijningen, 2024).

Hence, this indicates that the test compound may exhibit potential sedative and anxiolytic efficacy close to the control drug under the conditions tested.

Table 4

T-test for OFT

	treatment	Mean	Std. Deviation	Test Statistics
initial	Control drug (Diazepam)	36.67	21.825	t (4) =.078, p=.942
	10 mg/mL	35.67	4.041	
After 30 minutes	Control drug (Diazepam)	8.67	7.572	t (4) =.623, p=.567
	10 mg/mL	5.67	3.512	
After 60 minutes	Control drug (Diazepam)	.33	.577	t (2.082) =-.849, p=.482
	10 mg/mL	2.33	4.041	
After 90 minutes	Control drug (Diazepam)	.00	.000[a]	
	10 mg/mL	.00	.000[a]	
After 120 minutes	Control drug (Diazepam)	.00	.000[a]	
	10 mg/mL	.00	.000[a]	

[a]t cannot be computed because the standard deviations of both groups are 0
*Significant at 0.05

The table presents a statistical comparison of the effects of the control drug (Diazepam) and the EEGO at a concentration of 10 mg/mL across multiple time points. At the initial time point, there was no significant difference in the mean response between the control drug (M=36.67, SD=21.825) and the EEGO at 10 mg/mL (M=35.67, SD=4.041), as indicated by a t-test result of t(4) = 0.078, p = 0.942. After 30 minutes, both showed a marked decrease in mean values (8.67 for the control drug and 5.67 for the treatment). Still, the difference remained statistically insignificant (t (4) = -0.623, p = 0.567). By 60 minutes, the mean value further decreased, with the control drug at (M=0.33, SD=0.577) and the EEGO 10 mg/mL was slightly higher at M=2.33, SD=4.041; however, this difference also did not reach statistical significance (t (2.082) = -0.849, p = 0.482). At 90 and 120 minutes, both had mean values of 0.00 with standard deviations of 0.000, making statistical comparisons infeasible due to the absence of variance in the data.

Overall, while both Diazepam and the 10 mg/mL treatment led to a progressive reduction in the measured parameter, no statistically significant differences were observed between the groups at any time point.

Section 3. Evaluation of the pharmaceutical properties of the ethanol extract of *Glinus oppositifolius* (EEGO)

The nasal sprays prepared at concentrations of 10 mg/mL and 15 mg/mL underwent pharmaceutical assessment. As shown in Table 5, the evaluation included parameters such as pH, clarity, and pump delivery of the formulated nasal sprays.

Table 5

Pharmaceutical Evaluation

Property	Concentration B (10 mg/mL)	Concentration C (15 mg/mL)
pH	5.7 pH	5.7 pH
Clarity on a White background	Light yellow color, free from black particles	Light yellow color, free from black particles

Clarity on a Black background	Small white particles are minimally present	Small white particles are minimally present
Pump Delivery	10 pumps/1 mL	10 pumps/1 mL

The pH of concentrations B and C was 5.7. For clarity, the color was observed to be light yellow, with concentration B paler/lighter than concentration C. The white background showed no black particles, whereas the dark background showed black particles. However, it appears to be a clear solution, with small white particles minimally present. Lastly, for the pump delivery test, the obtained result was 10 pumps per 1 mL.

Based on the results, both concentrations B and C exhibited a pH of 5.7, which falls within the accepted standard range, 4.5 to 6.5 (Patil *et al*., 2012). This indicates their suitability according to established pH protocols. Clarity estimates the levels of all known and significant impurities and contaminants in the drug substance under evaluation (Savale, 2018). The particles on the black background indicate impurities associated with the filtering process. Lastly, for the pump delivery, the volume administered per nostril should not exceed 1 mL (ideally 0.2 to 0.5 mL) to avoid runoff and swallowing (Rech *et al*., 2017). With the obtained results, the actuations of 10 pumps/1 mL are effective and applicable. The evaluation was observed and guided by a Registered Pharmacist.

The evaluation of the formulated nasal spray across various concentrations showed that the product complies with the standard pharmaceutical characteristics expected of nasal sprays.

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

This study formulated and evaluated the ethanol extract of *Glinus oppositifolius* (EEGO) *papait* leaves as a natural sedative and anxiolytic in a nasal spray dosage form. Evaluation of its sedative and anxiolytic properties revealed that a concentration of 10 mg/mL was the most effective in reducing anxiety-like behavior in the Open Field Test. In contrast, the 15 mg/mL dose exhibited the lowest locomotor activity in animal models during the Hole Cross Test, suggesting its potential as a more potent sedative. Thus, the EEGO nasal spray formulation demonstrated both sedative and anxiolytic effects, with results close to the control drug Diazepam, highlighting its potential as an alternative option for sedation and anxiety management. Furthermore, the pharmaceutical properties of the EEGO nasal spray, including pH, clarity, and pump delivery performance, were found to meet established quality standards. The findings support the null hypothesis that the sedative and anxiolytic properties of the ethanol extract of *papait* nasal spray are not significantly different from those of Diazepam, indicating their acceptability based on the results.

Recommendations

1. A negative control is recommended for further comparison of the formulated nasal spray.
2. Increase the concentration of the crude extract to ensure the optimum dose will be delivered with fewer pumps.
3. Further stability testing for the formulated EEGO *papait* nasal spray, such as microbial stability and shelf-life stability.
4. Enhance the clarity of the nasal spray by applying other filtration methods during formulation.
5. Improve the odor of the *papait* nasal spray by using excipients that enhance fragrance.
6. Isolate flavonoids and identify secondary metabolites that are responsible for the *papait* nasal spray's sedative and anxiolytic effects.

7. The formulated *papait* nasal spray may undergo clinical trials.

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