Formulation and Evaluation of Lemon Balm Herbal Chocolate as Anxiolytic

Kiran Andhale*[1], Mukesh Mohite[2], Jay Ahire[3]

Dr D Y Patil College of Pharmacy, Akurdi, Pune, Maharashtra, India-411044.

ABSTRACT:

Background: Lemon balm (Melissa officinalis) have anxiolytic effects. Many studies have demonstrated lemon balm and supplements can improve measures of calmness, memory and alertness in adult experiencing mental stress. The active compounds in lemon balm, such as rosmarinic acid, appear to contribute to these calming and anti-anxiety properties.

Main Body: Formulating lemon balm into a food product like chocolate could be an effective way to deliver these beneficial effects. It has also been associated with potential health benefits such as anti-inflammatory, neuroprotective, and hepatoprotective properties. In this study, a formulation of Lemon Balm chocolate was prepared after varying the excipients concentration, while the drug extract is kept constant. HPTLC image of lemon balm extract after developed at 254 & 366 nm. The concentration of herbal extract and chocolate formulation was determined by High-Performance Thin Layer chromatography (HPLC).

Conclusion: The results showed that the Quantification of Lemon Balm extract and Chocolate Formulation by HPTLC for determination of concentrations of Rosmarinic Acid (RA). The ready chocolate definition has been evaluated for Organoleptic properties, Hardness, pH, Moisture content, Viscosity determination, Bloom test and Stability study. Total 8 formulations were prepared. The formulas were evaluated for chocolate as drug delivery system for Anxiolytic.

1.   Introduction

People may sometimes feel anxious, but the people suffering from anxiety disorders often experience intense fear and worry. Feelings of unease often come with physical tension & other cognitive & behavioral signs. Managing them can be tough, causing significant distress & potential lasting a while without treatment. Anxiety disorders disrupt everyday tasks & can hinder someone's family, social, & professional life. About 4% of the global population deals with anxiety disorder. As of 2019, an estimated 301 million individuals worldwide experienced anxiety disorder, making them the most prevalent mental health condition [1].

Even though there's highly efficient remedy for anxiety disorders, merely around one-fourth (27.6%) of individual requiring assistance actually receive it. Obstacles to accessing care encompass unawareness regarding the treatability of this health condition, insufficient funding in mental health services, shortage of adequately trained healthcare professionals & societal stigma [2].

In recent time, *M. Officinalis* bioactive characteristics, particularly its impact on central nervous system, have garnered growing interest in research. Concerning its effect on neurocognition, there was significant variability observed in cholinergic nicotinic & muscarinic receptor binding in human brain samples among different strains of *M. Officinalis* [3]. A particular extract, which showed minimal binding to cholinergic receptors, elicited behavioral outcomes in humans that align with its historical usage as a gentle anxiolytic agent [4].

On the flip side, an extract evaluated for its strong binding to nicotinic & muscarinic receptors in human

brain tissue exhibited similar calming effects, along with enhancements in memory function [5]. These results imply that the potent anxiolytic properties of *M. Officinalis* may rely on a yet-to-be-identified mechanism within the plant, distinct from cholinergic pathways [4-6].

Although binding to nicotinic & muscarinic receptors has been shown, it's uncertain whether this occurs specifically with receptor subtypes nicotinic α4β2 & α7 receptors, as well as muscarinic M1, M2 & M4 receptors, which are recognized for their cognitive effects. The anxiolytic effectiveness of *M. Officinalis* might be linked to its established interaction with GABA-A receptors. [7].

One of the medicinal herbs high in Rosmarinic Acid is lemon balm, with dry leaves with have up to 6% RA [8]. Several health advantages exhibited by RA including anxiolytic, neuroprotective [9], hepatoprotective [10], and anti-inflammatory properties [11-12].

The microbial growth and hydrolysis of water-sensitive active agent is resisted because anhydrous nature of chocolate which contains polyphenols, saturated fat, di and triterpenes, sterols, methylxanthines, alcohols and aliphatic [13]. Chocolate's versatility allows for the creation of entirely novel flavours and tactile sensations when combined with drugs [14]. Lemon balm (Melissa officinalis) has been shown to have anxiolytic effects. In 2016, research indicated that lemon balm tea enhanced anxiety symptoms and sleep quality among individuals who had suffered burns. Similarly, a double-blind study in 2019 revealed that individuals who underwent recent heart bypass surgery and consumed capsules containing 1.5 grams of dried lemon balm daily experienced reduced anxiety levels compared to those taking a placebo. Due to potential interactions with sedatives, consulting a physician before using lemon balm is recommended [15].

Chocolate, particularly with a high cocoa content, has also been associated with potential health benefits. A study in 2021 found that chocolate with a high cocoa content could help reduce stress levels and improve mood. Another study in 2022 found that dark chocolate consumption could improve cognitive function and reduce inflammation [16]. Combining lemon balm with chocolate could potentially enhance the anxiolytic effects of lemon balm while also providing the potential health benefits of chocolate. This could be achieved by creating a lemon balm herbal chocolate, where lemon balm extract is added to the chocolate during the manufacturing process.

2. Materials & Methods

2.1 Materials: Lemon Balm herbal extract received from Aushadhi Herbal, New Delhi. cocoa powder received from Arboreal Bioinnovations Private Limited, Uttar Pradesh. cocoa butter, sucralose received from Shandong Kanbo Biochemical Technology Co., LTD, vanillin, soya lecithin.

2.2 Methods:

2.2.1: Formulation Method:

1. All the components were precisely weighed.

2. Cocoa powder and sucralose were combined in one beaker and thoroughly mixed.

3. In another beaker, cocoa butter was melted, and this melted butter was blended with the powder mixture until a smooth consistency was achieved.

4. Soya lecithin, acting as an emulsifier, was then incorporated and mixed.

5. Subsequently, the herbal drug extract was accurately measured and added to the chocolate mixture.

6. Vanilla, serving as a flavor enhancer, was introduced before the mixture was poured into molds.

7. The prepared chocolate, containing the herbal drug extract, was poured into molds and placed in the freezer overnight to set.

A total of 8 formulations were prepared, varying the concentrations of excipients while keeping the herbal extract concentration constant. The compositions of all 8 formulations are detailed in Table No. 1.

Contents (in gm)	F1	F2	F3	F4	F5	F6	F7	F8
Cocoa Butter	3	3	3.4	2.4	2.6	3.4	3.5	2.6
Cocoa Powder	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Sucralose	1.3	0.5	0.6	0.9	1.2	1.2	0.9	0.6
Soya Lecithin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vanillin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Drug Extract	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

Table No.: 1: Formulation Table

2.2.2: HPTLC of Herbal Drug [17]

2.2.3: Evaluation Parameters [18-19]

I. **Organoleptic Properties**

II. **Dimension:** Vernier's calliper

III. **Hardness:** Pfizer Hardness Testers measure the force required to break a tablet. The unit of measurement is kg/cm2.

IV. **pH:** 2gm of prepared chocolate were dissolved in 100ml of phosphate buffer solution & the pH of the resulted solution was analyzed using a digital pH meter equipped with glass electrode.

V. **Moisture Content:** A desiccator was employed to assess the moisture content, aiming to ascertain the moisture level in the dry chocolate. The chocolate mixture obtained was accurately weighed & placed in a desiccator containing anhydrous silica gel. Following a 24-hr period, the formulations were taken out, reweighed & the percentage of moisture absorbed was calculated utilizing a specific formula.

$$\% \, Moisture = \frac{Intial \, Weight - Final \, Weight}{Final \, Weight}$$

VI. **Stability Study:** Store the for 1 month at room temperature.

VII. **Viscosity determination:** Brookfield to determine the viscosity of the prepared chocolate foundation, a rotating digital viscometer is utilized.

VIII. **Bloom test:** The sample was heated to 40 degrees for eight hours and then cooled to 20 degrees. After being at 20°C for 8 hours, a test sample was checked to see if bloom had occurred or not.

3. RESULTS

1. Standardization of Herbal Drug: Table No. 2

Parameters	Observed Value	Standard Value
Loss of Drying	2.68%	NMT 5%
Bulk Density (Tapped)	0.45 gm/ml	0.30-0.80 gm/ml
Particle Size	100%	100% passing through 40 mesh
Hydro Alcoholic Acid	Complies	10:1
Total Plate Count	Complies	NMT 1,000 CFU/g
Yeast & Mould	Complies	NMT 100/g
Heavy Metals	Complies	NMT 10ppm

Table No.: 2: Standardization of Herbal Drug

2. Design of Experiment Model Graph
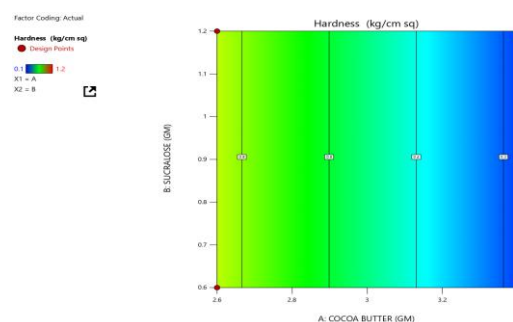
I. Contour Graph: Figure No. 1

Figure No.: 1: Contour Graph
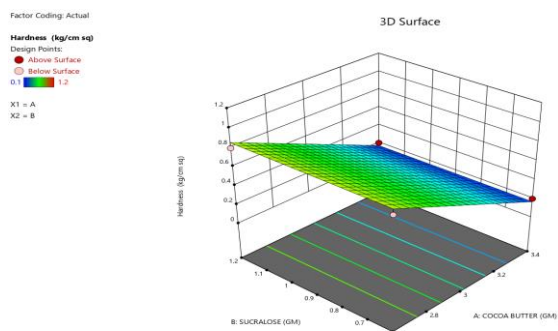
II. 3D Surface Graph: Figure No. 2

Figure No.: 2: 3D Surface Graph

3. Estimation of Herbal Drug by HPTLC

a. HPTLC image of Lemon Balm Extract after developed at 254 & 366 nm: Figure No. 3 & Figure No. 4 respectively:
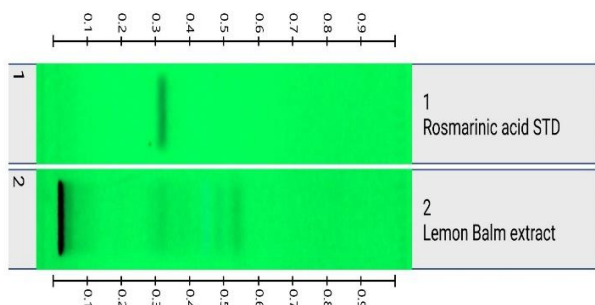

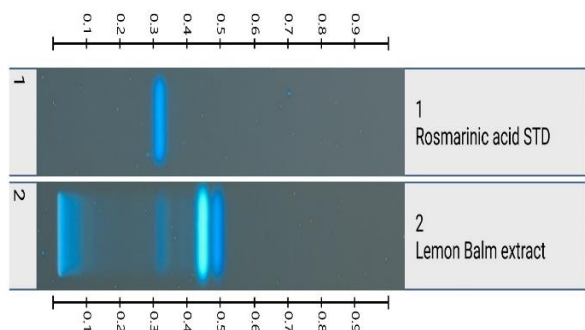**Figure No.: 3:** HPTLC image of Lemon Balm Extract after developed at 254.

Figure No.: 4: HPTLC image of Lemon Balm Extract after developed at 366 nm.

b. HPTLC image of Chocolate Formulation after developed at 254 & 366 nm: Figure No. 5 & Figure No. 6 respectively:
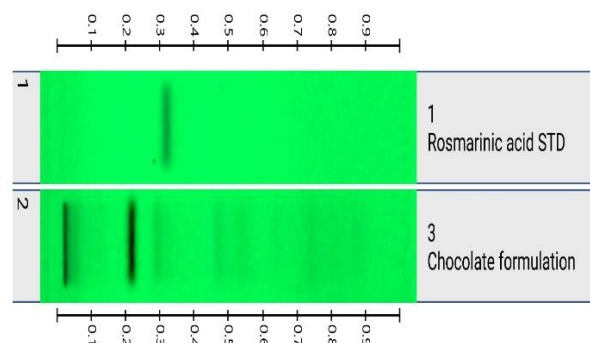

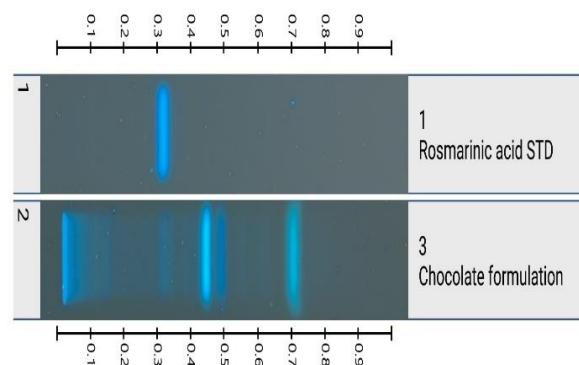**Figure No.: 5:** HPTLC image of Chocolate Formulation after developed at 254.

Figure No.: 6: HPTLC image of Chocolate Formulation after developed at 366 nm.

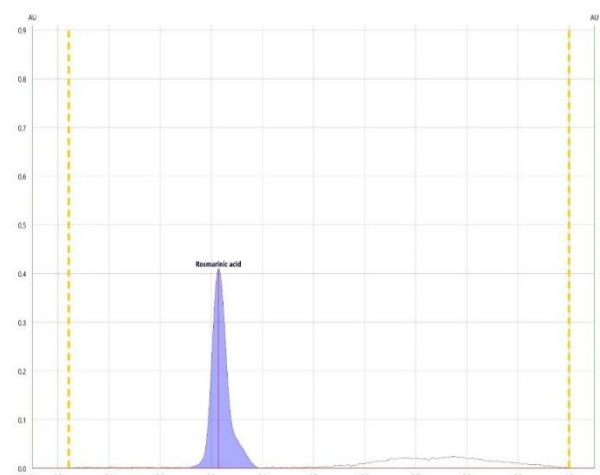**c.** HPTLC Densitogram of Rosmarinic Acid Standard at 330 nm: Figure No. 7:

Figure No.: 7: HPTLC Densitogram of Rosmarinic Acid Standard at 330 nm.

d. HPTLC Densitogram of Rosmarinic Acid in Lemon Balm Extract: Figure No. 8:
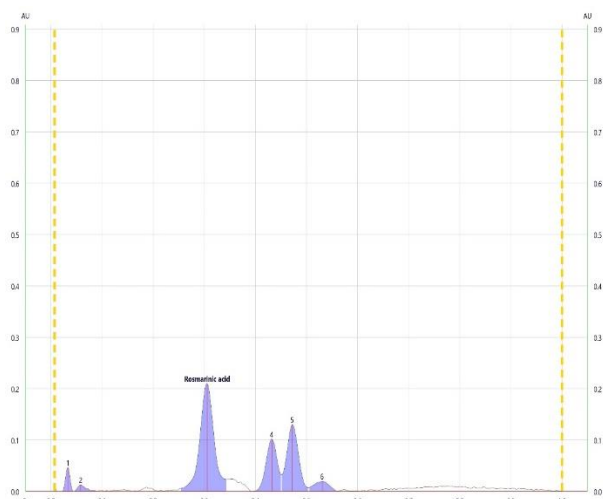

Figure No.: 8: HPTLC Densitogram of Rosmarinic Acid in Lemon Balm Extract.

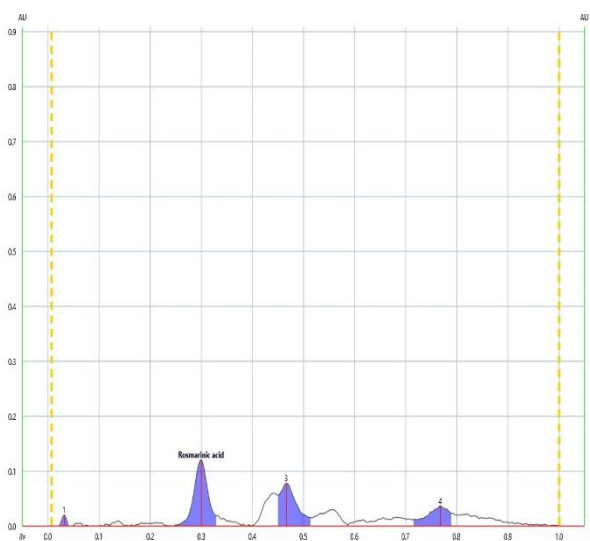**e.** HPTLC Densitogram of Rosmarinic Acid in Chocolate Formulation: Figure No. 9

Figure No.: 9: HPTLC Densitogram of Rosmarinic Acid in Chocolate Formulation.

f. Quantification of Lemon Balm extract and Chocolate Formulation by HPTLC: Table No. 3

Test	Lemon Balm Extract (%)	Chocolate Formulation (%)
Rosmarinic Acid (1000PPM)	1.73	0.10

Table No.: 3: Quantification of Lemon Balm extract and Chocolate Formulation by HPTLC.

4. Evaluation

a. Organoleptic Properties: Table No. 4

Parameters	F1	F2	F3	F4	F5	F6	F7	F8
Colour	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown
Odour	Chocolaty	Chocolaty	Chocolaty	Chocolaty	Chocolaty	Chocolaty	Chocolaty	Chocolaty
Taste	Sweet	Slightly Bitter	Slightly Bitter	Slightly Bitter	Sweet	Sweet	Slightly Bitter	Slightly Bitter
Mouth Feel	Smooth & Pleasant	Smooth & Pleasant	Smooth & Pleasant	Smooth & Pleasant	Smooth & Pleasant	Smooth & Pleasant	Smooth & Pleasant	Smooth & Pleasant
Appearance	Glossy	Glossy	Glossy	Dull	Dull	Glossy	Glossy	Dull

Table No.: 4: Organoleptic Properties

b. Dimension
- Length: 32.62 mm
- Width: 32.62 mm
- Thickness: 5.83 mm

c. Hardness: Table No. 5

Formulation Code	F1	F2	F3	F4	F5	F6	F7	F8
Hardness (kg/cm sq)	0.4	0.4	0.2	1.2	0.8	0.2	0.1	0.8

Table No.: 5: Hardness

d. pH: Table No. 6

Formulation Code	F1	F2	F3	F4	F5	F6	F7	F8
pH	6.6	6.5	6.6	6.4	6.5	6.7	6.3	6.5

Table No.: 6: pH

e. Moisture Content: Table No. 7

Formulation Code	Initial Weight	Final Weight	% Moisture
F1	6.93	6.21	0.11%
F2	6.88	6.01	0.14%
F3	7.10	6.51	0.09%
F4	6.81	5.82	0.17%
F5	6.85	5.69	0.20%
F6	7.12	6.75	0.05%
F7	7.15	6.28	0.13%
F8	6.80	5.53	0.22%

Table No.: 7: Moisture Content

f. Stability Study: Table No. 8

Parameters	Storage Condition	At the time of Preparation	After 1 Month
Colour, Odour, Taste, Mouth Feel, Appearance	At room temperature	Brown, Chocolaty, Smooth, Glossy, Sweet	No Change

Table No.: 8: Stability Study

g. Viscosity determination: Table No. 9

Formulation Code	Viscosity (mPa.s)
F1	1650.5
F2	1738.0
F3	1420.6
F4	2186.3
F5	1856.4
F6	1439.8
F7	1295.9
F8	1756.1

Table No.: 9: Viscosity determination

h. Bloom test: No blooming.

4. Discussion

Total 8 batches formulated and F6 is the optimized formulation according to hardness, colour, taste & appearance. The process of formulate chocolate with lemon balm extract involves adding the extract in powdered form to the chocolate. The formulation and evaluation of lemon balm herbal chocolate involve chocolate with lemon balm extract to enhance its properties. Lemon balm extract is rich in rosmarinic acid which shows the Anxiolytic effect. HPTLC report shows the presence of rosmarinic acid in the chocolate. This study carried out evaluation of the chocolate.

5. Conclusion

The finding suggests that the F6 batch, being optimized, offers improved sweetening properties compared to others, with satisfactory appearance and hardness. There is potential for further investigation using the Elevated Plus Maze Model for in vivo studies. Lemon Balm herbal extract was effectively incorporated into chocolate formulation, containing active constituents like rosmarinic acid for its anxiolytic effects. The sensory properties of chocolate are great for masking unpleasant tastes of active ingredients and giving a smooth texture for oral delivery. This chocolate formulation offers a pleasant way to administer medicines orally, as the dosage range of the drug extract used is safe for consumption and can be ingested without causing systemic side effects.

6. Acknowledgement

7. Conflict Of Interest

The authors have no conflicts of interest.

Refrences

1. GBD Results Tool. In: Global Health Data Exchange [website]. Seattle: Institute for Health Metrics and Evaluation; 2019 (https://vizhub.healthdata.org/gbd-results?params=gbd-api-2019-permalink/716f37e05d94046d6a06c1194a8eb0c9, accessed 5 September 2023).

2. Alonso J, Liu Z, Evans-Lacko S, et al. Treatment gap for anxiety disorders is global: results of the World Mental Health Surveys in 21 countries. Depress Anxiety. 2018;35(3):195–208. doi:10.1002/da.22711.

3. Wake, G.; Court, J.; Pickering, A.; Lewis, R.; Wilkins, R.; Perry, E. CNS acetylcholine receptor activity in European medicinal plants traditionally used to improve failing memory. *J. Ethnopharmacol.* **2000**, *69*, 105–114.

4. Kennedy, D.O.; Scholey, A.B.; Tildesley, N.T.J.; Perry, E.K.; Wesnes, K.A. Modulation of mood and

cognitive performance following acute administration of *Melissa officinalis* (lemon balm). *Pharmacol. Biochem. Behav.* **2002**, *72*, 953–964.

5. Kennedy, D.; Wake, G.; Savelev, S.; Tildesley, N.; Perry, E.; Wesnes, K.; Scholey, A. Modulation of mood and cognitive performance following acute administration of single doses of *Melissa officinalis* (lemon balm) with human CNS nicotinic and muscarinic receptor-binding properties. *Neuropsychopharmacology* **2003**, *28*, 1871–1881.

6. Kennedy, D.O.; Little, W.; Scholey, A.B. Attenuation of laboratory-induced stress in humans after acute administration of *Melissa officinalis* (lemon Balm). *Psychosom. Med.* **2004**, *66*, 607–613.

7. Awad, R.; Muhammad, A.; Durst, T.; Trudeau, V.; Arnason, J. Bioassay-guided fractionation of lemon balm (*Melissa officinalis* L.) using an *in vitro* measure of GABA transaminase activity. *Phytother. Res.* **2009**, *23*, 1075–1081.

8. Verlag, G.T. The Scientific Foundation for Herbal Medicinal Products, 2nd ed.; ESCOP Monographs; ESCOP: Stuttgart, Germany; Thieme: New York, NY, USA, 2003; Volume 1.

9. Costa, P.; Sarmento, B.; Gonçalves, S.; Romano, A. Protective effects of *Lavandula viridis* L'her extracts and rosmarinic acid against H2O2-induced oxidative damage in A172 human astrocyte cell line. *Ind. Crops Prod.* **2013**, 50, 361–365.

10. Wang, S.J.; Chen, Q.; Liu, M.Y.; Yu, H.Y.; Xu, J.Q.; Wu, J.Q.; Zhang, Y.; Wang, T. Regulation effects of rosemary (*Rosmarinus officinalis* Linn.) on hepatic lipid metabolism in OA induced NAFLD rats. *Food Funct.* **2019**, 10, 7356–7365.

11. Georgiev, M.; Pastore, S.; Lulli, D.; Alipieva, K.; Kostyuk, V. Verbascum xanthophoeniceum-derived phenylethanoid glycosides are potent inhibitors of inflammatory chemokines in dormant and interferon-gamma-stimulated human keratinocytes. *J.Ethnopharmacol.* **2012**, 144, 754–760.

12. Zhao, L.; Zhang, Y.; Liu, G.; Hao, S.;Wang, C.; Wang, Y. Black rice anthocyanin-rich extract and rosmarinic acid, alone and in combination, protect against DSS-induced colitis in mice. *Food Funct.* **2018**, 9, 2796–2808.

13. Devare, R. R., Chaudhari, A. S., Dewang, S. P., Patil, B. V., Patil, M. A., & Pawar, P. S. (2019). Chocolate Formulation as Drug Delivery System for Paediatrics. *Indian Journal of Drugs*, *7*(4), 133-139.

14. Kolekar, S. Y., Mulani, A. S., Tamboli, A. F., More, N. H., & Misal, A. A. (2021). Formulation and Evaluation of Paediatric Herbal Chocolate. *European Journal of Biomedical and Pharmaceutical sciences*, *8*(6), 458-462.

15. Medical News Today; https://www.medicalnewstoday.com/articles/325823.

16. Weeks B. S. (2009). Formulations of dietary supplements and herbal extracts for relaxation and anxiolytic action: Relarian. *Medical science monitor: international medical journal of experimental and clinical research*, *15*(11), RA256–RA262.

17. Pawar, P. D., Bakliwal, A. A., Talele, S. G., & Jadhav, A. G. (2019). Formulation and evaluation of herbal chocolate as nervine tonic. *Journal of Pharmaceutical Sciences and Research*, *11*(5), 1808-1813.

18. Paul, M. P., Ranabhat, P. R., Khatiwara, D. K., & Bagchi, A. (2021). Review on medicated chocolate takes a patient-centered approach to drug delivery. *Journal of Applied Pharmaceutical Research*, *9*(4), 16-22.

19. Dwivedi, M., Jha, K. K., Pandey, S., Sachan, A., Sharma, H., & Dwivedi, S. K. Formulation and Evaluation of Herbal Medicated Chocolate in Treatment of Intestinal Worms and Related Problems.