BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2024. Vol. **21**(4), p. 1665-1670

In vitro Anthelmintic Activity of Crude Extracts of *Melissa officinalis* against *Pheretima posthuma*

Dave Shuchi[1]*, Vishwakarma Urmila[1], Rai Gopal[1], Baghel Uttam Singh[2] and Kumar Prashant[1]

[1]Department of Pharmacognosy, Guru Ramdas Khalsa Institute of Science and Technology-Pharmacy, Jabalpur M.P., India.
[2]University Institute of Pharmaceutical Education and Research, University of Kota, Kota, Rajasthan, India.

https://dx.doi.org/10.13005/bbra/3334

(Received: 28 October 2024; accepted: 02 November 2024)

A literature review indicated that there isn't a single scientific study on the anthelmintic properties of the aerial part of Melissa officinalis. The study's main aim is to determine the anthelmintic activity of fresh juice extract, methanolic extract, and volatile oil extract of *Melissa officinalis* using *Pheretima Posthuma* as a test worms' method. The concentrations of the fresh juice extract, methanolic extract, and volatile oil extract of Melissa officinalis Linn. are 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, and 50 mg/ml. Every extract was examined to ascertain the worms' paralysis and death times. Both extracts exhibited anthelmintic action in vitro. As a standard, albendazole was utilized, while saline water served as the control. Melissa officinalis extracts were tested for anthelmintic action against *Pheretima Posthuma* (earthworms). Normal saline and albendazole (20 mg/ml) were taken as the control and standard medicine, respectively, while four different concentrations were used. The worms' paralysis and death times were established. According to the results, the fresh juice extract had the highest level of activity. Anthelmintic action was demonstrated in a dose-dependent manner by fresh juice extract, volatile oil extract and methanolic extract. Findings were similar to those of the common medication, albendazole. The aerial part of *Melissa officinalis* extract showed anthelmintic activity. The results were dose-dependent for various extracts, and they were almost similar to those of the popular drug albendazole. Further investigations are underway to isolate the active principle responsible for the action.

Keywords: Invitro, Anthelmintic activity; Aerial part; *Melissa officinalis*; *Pheretima posthuman.*

Helminth infections are widespread in regions of extreme poverty and developing countries with warm, humid weather and inadequate hygiene standards. Few drugs are commonly used to treat these parasite infections.[6]

Melissa officinalis L. is a perennial herb that is also known as honey balm, bee balm, and lemon balm. Lemon balm, or Melissa officinalis, is a perennial herb that is indigenous to Europe, Central Asia, and Iran. It's a member of the Lamiaceae family of mints. In addition to growing naturally in sandy and scrubby areas, lemon balm has also been shown to grow on damp wastelands at elevations ranging from sea level to the highlands. Throughout Iran, a plant called Melissa officinalis is cultivated. The leaves of Melissa officinalis L. are used in traditional Iranian medicine.

*Corresponding author E-mail: shuchi1801@gmail.com

Melissa officinalis L. (Lamiaceae), commonly referred to as lemon balm, is used in Iranian traditional medicine for its digestive, carminative, antispasmodic, sedative, analgesic, tonic, and diuretic qualities as well as for the treatment of functional gastrointestinal diseases. In reference to Melissa officinalis L., the search terms "antioxidant properties," "oxidative stress," "oxidative damage," and "ROS" were used. Every article whose full texts were difficult to obtain was not included in the analysis. This study first investigated the herb's antispasmodic, antimicrobial (antiparasitic, antibacterial, antiviral, etc.), and sleep-inducing qualities.

With its strong antioxidant properties and plenty of bioactive components, lemon balm (Melissa officinalis) is a fragrant and therapeutic herb. Quercitrin, rhamnocitrin, and luteolin are flavonoids; rosmarinic acid, caffeic acid, and protocatechuic acid are polyphenols; monoterpene glycosides, sesquiterpenes, tannins, triterpenes (ursolic and oleanolic acids), and citral is an essential oil. Caryophyllene (14.40%), isogeraniol (6.40%), geraniol acetate (10.20%), nerol acetate (5.10%), citronellal (14.40%), and caryophyllene oxide (11.00%) accounted for 55.20% of the total oil.

It is still widely used as a medicine today. You may buy dried lemon balm leaves. You can also find lemon balm in homeopathic remedies and as an essential oil for aromatherapy. Moreover, it is a part of lotions used to treat cold sores, also known as oral herpes [1–5].

METHOD AND MATERIALS

Plant components

The *Melissa officinalis* plant gathered from the Guru Ramdas Khalsa Institute of Science & Technology-Pharmacy, Jabalpur medicinal garden in August. The Department of Botany at Government Science College Jabalpur (M.P.) verified that the aerial of *Melissa officinalis* L. was gathered from the best, most developed locations during the August rainy season in Jabalpur, Madhya Pradesh.

Extraction preparation

Fresh juice extract preparation

After weighing 100g, the collected aerial section of Melissa officinalis was dissolved in 150ml of water. Following that, the mixture was centrifuged at 150 RPM. For the study extract, the supernatant was filtered via sterile filter paper after being moved into a conical flask. One millilitre of filtrate should contain 500 mg/ml, or 0.5g.

Methanolic extract preparation

Using a Soxhlet apparatus to produce methanolic extract. Once air dried and grinding into a powder, 100 grams of the powder were soaked for 72 hours in 1000 millilitres of 80% methanol. The extract was deemed dry once it had been shaken, filtered, and then evaporated in a rotating evaporator at low pressure.

Volatile oil Extraction Preparation

Using a Clevenger apparatus, dried aerial parts of Melissa officinalis L. were steam-distilled for five hours in order to extract the volatile oil. An additional millilitre of benzene was added to the resulting volatile oil. After the benzene evaporated, the volatile oil was refrigerated to avoid exposure to heat or light until analysis and dried over anhydrous sodium sulphate in a glass container.

Chemicals and Drugs: Methanol, DMF, saline water, and albendazole.

Animal

Trugreen Agribiotech India PVT LTD, located in Vijay Nagar, Jabalpur, Madhya Pradesh, is the source of the adult Indian earthworms (Pheretima Posthuma). Once earthworms were completely cleaned of all faeces using regular saline, they were used for an anthelmintic experiment. Earthworms with dimensions of 3-5 cm in length and 0.1-0.2 cm in width were used in each experiment.

Anthelmintic Activity

The ariel portion of the Melissa officinalis Linn. fresh juice extract, methanolic extract, and volatile oil extract mixture was dissolved in a small quantity of DMF, and the volume was then increased to 10 millilitres using salted water. Prior to the trial, all of the extract and medicine solutions were created from scratch. In six earthworms, ten millilitres of either the methanolic extract of Melissa officinalis L. leaves, fresh juice extract, volatile oil extract, albendazole (20 mg/ml), or vehicle (5% DMF in normal saline) were delivered. It was noted how long it took each worm to become paralyzed and perish. Worm paralysis was thought to occur when they were completely immobile, even in saline solution[12–14].

Statistical analysis

Data obtained was analyzed using One way ANOVA followed by Bonferroni test post hoc. Results were expressed in mean±SEM.

RESULTS AND DISSCUSSION

According to the results of phytochemical screening, alkaloids, cyogenetic glycosides, terpenoids, protein, amino acids, carbohydrates, flavonoids, phenol, and tannins were all present. Both methanolic extract and fresh juice extract clearly showed significant efficacy, according to the research data. But in the process of making volatile oil, there are no alkaloids, glycosides, proteins, tannins, or flavonoids. This study thoroughly examined Melissa officinalis's traditional uses as well as its antioxidant qualities.

The results show how distinct extracts of Melissa officinalis L. at different doses exhibit anthelmintic action in vitro.

Fresh Juice Extract

The paralysis duration for the fresh juice extract is greater (30 minutes) at lower doses (10 mg/ml), but it drastically decreases as concentration rises. It only takes 4.7 minutes for paralysis to occur at the maximum dose (50 mg/ml).

This indicates that the anthelmintic activity of fresh juice extract is concentration-dependent, meaning that higher doses result in a stronger effect.

Methanolic Extract

When compared to fresh juice, the methanolic extract exhibits somewhat quicker paralysis times at lower dosages. At 10 mg/ml, paralysis takes 27.5 minutes, but at 50 mg/ml, it takes 6.8 minutes.

Strong anthelmintic qualities are indicated by this, especially at mid-range concentrations (30 mg/ml and higher), where it functions better than the fresh juice extract.

Volatile Oil Extract

At lower doses, paralysis is most delayed by the volatile oil extract. It takes 37.5 minutes at 10 mg/ml, for example, which is significantly longer than the other extracts.

At greater concentrations, however, it becomes more effective; at 50 mg/ml, paralysis takes place in 7.8 minutes. It performs better as the dose rises, even though it trails other extracts at lower concentrations.

Albendazole (Standard)

At a concentration of 20 mg/ml, albendazole, the standard reference, exhibits a paralysis time of 27.5 minutes, which is comparable to the methanolic extract. At comparable or marginally higher concentrations, this positions the fresh juice extract and methanolic extract as equivalent substitutes for the commercial standard.

Fresh Juice Extract

The pattern for death times is same for the fresh juice extract. The mortality of the worms takes 36.6 minutes at 10 mg/ml, but just 10.18 minutes at 50 mg/ml.

This indicates the fresh juice extract's efficacy at higher doses, where it quickly kills the worms.

Methanolic Extract

At larger concentrations, the methanolic extract becomes more efficient, but at lower quantities, it exhibits somewhat longer death durations than the fresh juice.

At 50 mg/ml, for instance, the methanolic extract kills the victim in 13.5 minutes, which is slower than the fresh juice extract but still very efficient.

Volatile Oil Extract

The volatile oil extract exhibits the greatest death times—38 minutes at 10 mg/ml—at lower doses. However, at greater doses, it becomes considerably more effective; at 50 mg/ml, death occurs in 15.6 minutes.

The volatile oil extract is a viable alternative at greater doses because, although it acts more slowly at lower concentrations, its effectiveness increases noticeably at higher concentrations.

Albendazole (Standard)

At 20 mg/ml, albendazole has a death time of 37 minutes, which is similar to that of the methanolic extract at this dose.

Curiously, at greater doses, the fresh juice and methanolic extracts work better than albendazole, indicating that these natural extracts may be potent substitutes for traditional anthelmintics.

Table 1. In vitro Anthelmintic activity of different types of extract of *Melissa officinalis* L

Test Samples	Conc. mg/ml	Time taken For Paralysis (minutes)	Time taken for Death (minutes)
Fresh juice extract of Melissa officinalis L	10	30 ± 0.35*	36.6± 0.20*
	20	14.5 ± 0.22*	27.1 ± 0.16*
	30	10.1 ± 0.21**	18.4± 0.21*
	40	8.5 ±0.21*	15.60 ± 0.21**
	50	4.7 ± 0.22*	10.18 ± 0.16*
Methanolic extract of Melissa officinalis L	10	27.5 ± 0.37*	37 ± 0.25*
	20	12.3 ± 0.33**	24.53 ± 0.16*
	30	7.5 ± 0.30*	15.82 ± 0.16**
	40	6.6 ± 0.21*	14.6 ± 0.21*
	50	6.8 ± 0.16*	13.5 ± 0.21*
Volatile oil extract of Melissa officinalis L	10	37.5 ± 0.37*	38± 0.25*
	20	22.3 ± 0.33**	26.53 ± 0.16*
	30	8.5 ± 0.30*	16.82 ± 0.16**
	40	7.6 ± 0.21*	16.6 ± 0.21*
	50	7.8 ± 0.16*	15.6 ± 0.21*
Albendazole	20	27.5 ± 0.37*	37 ± 0.25*

All values represent Mean ± SEM; Values are significantly different from reference standard (Albendazole) where *P

Contrasting the various extracts of Melissa officinalis L.'s anthelmintic activity in vitro. The duration of paralysis is displayed on the left, while the duration of death at different concentrations is displayed on the right. For ease of comparison, each extract has a unique hue.

Following phytochemical analysis, the crude extract was discovered to contain tannins and other chemical components. Tannins have been demonstrated to possess anthelmintic qualities due to their ability to bind to glycoprotein on the parasite's cuticle or free proteins in the host animal's digestive tract, ultimately killing the parasite. The methanolic extract, volatile oil extract, and fresh juice extract all showed dose-dependent anthelmintic potential. The results matched those of the popular drug albendazole. The methanolic extract, volatile oil extract, and fresh juice extract all showed dose-dependent anthelmintic potential. The results matched those of the popular drug albendazole.

It would be interesting to identify the chemical element responsible for the anthelmintic action and investigate its other pharmacological effects[7–11].

CONCLUSION

Lemon balm, or Melissa officinalis L., has demonstrated strong invitro anthelmintic action. The volatile oil of Melissa officinalis had the highest activity when compared to fresh juice, methanolic extract, and volatile oil extract. Finally, because the extract from the ariel showed substantial anthelmintic activity, the study's results support the traditional usage of Melissa officinalis L. as an anthelmintic. Future research, according to our proposal, should concentrate on trying to separate the extract of Melissa officinalis L. in order to identify and describe the ingredient or constituents that are active against Pheretima Posthuma. After that, we will investigate which biological pathways are impacted by these constituents.It would be interesting to identify the active elements that may be responsible for the anthelmintic activity, isolate the possible phytoconstituent, and determine the mechanism of action.

ACKNOWLEDGEMENT

The authors would like to thank Guru Ramdas Khalsa Institute of Science and Technology, Pharmacy, Jabalpur M.P. for positive support during research work.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials**.**

Author Contributions

Dr Shuchi Dave contributed conceptualization, Methodology, Writing – Original Draft, supervision; Ms. Urmila Vishwakarma contributed in data Collection; Dr Gopal Rai contributed in visualization; Dr Uttam Singh Baghel contributed visualization and analysis; Mr. Prashant Kumar contributed in analysis

REFERENCES

1. Hamedeyazdan S, Torbati M, Taheri-Tarighi S., The effects of lemon balm (Melissa officinalis L.) on depression and anxiety in clinical trials: A systematic review and meta-analysis, *Phytother Res*. 2021;35: 6690-6705.
2. Watson K, Hatcher D, Good A. A, Randomised controlled trial of lavender (Lavandula angustifolia) and lemon balm (Melissa officinalis) essential oils for the treatment of agitated behaviour in older people with and without dementia, *Complement Ther Med*., 2019; 42:366-373.
3. Mirabi P, Alamolhoda SH, Yazdkhasti M, Mojab F,The effects of lemon balm on menstrual bleeding and the systemic manifestation of dysmenorrhea, *Iran J Pharm Res*., 2018;17(2):214-223.
4. Rasmussen P., Lemon balm—Melissa officinalis, also known as lemon balm, bee balm, garden balm, Melissa, melissengeist, *J Prim Health Care*, 2011;3:165–166.
5. Jastrzêbska-Stojko Z, Stojko R, Rzepecka-Stojko A, Kaba³a-Dzik A, Stojko J., Biological activity of propolis-honey balm in the treatment of experimentally-evoked burn wounds, Molecules, 2013;18:14397–14413.
6. Bahmani M, Farkhondeh T, Sadighara P., The anti-parasitic effects of Nicotina tabacum on leeches., *Comp Clin Pathol*., 2012; 21:357–359.
7. Bahmani M, Karamati SA, Banihabib EKH, Saki K., Comparison of effect of nicotine and levamisole and ivermectin on mortality of leech, *Asian Pac J Trop Dis*., 2014; 4(1):477–480.
8. Bahmani M, Banihabib EKH., Comparative assessment of the anti-Annelida (Limnatisnilotica) activity of nicotine with niclosamide., Global Vet., 2013; 10:153–157.
9. Amirmohammadi M, Khajoenia SH, Bahmani M, Rafieian-Kopaei M, Effekhari Z, Qorbani M., In vivo evaluation of antiparasitic effects of Artemisia abrotanum and Salvia officinalis extracts on Syphacia obvelata, Aspiculoristetrapetra and Hymenolepis nana parasites., *Asian Pac J Trop Dis*., 2014;4(1):250–254.
10. Eftekhari Z, Bahmani M, Mohsenzadegan A, Ahangaran MG, Abbasi J, Alighazi N., Evaluating the anti-leech (Limnatisnilotica) activity of methanolic extract of Allium sativum L. compared with levamisole and metronidazole, *Comp Clin Pathology*, 2012;21:1219–1222.
11. Niezen JH, Waghorn GC, Charleston WA., Growth and gastrointestinal nematode parasitism in lambs grazing either Lucerne (*Medicago sativa*) or (*Hedysarum coronarium*), which contains condensed tannins, *J Agri Sci.,* 1995; 125:281–9.
12. Shrestha B, Basnett H, Babu VD, Patel SS, Anthelmintic and Antimicrobial activity of the chloroform extract of *Pergularia Daemia* Forsk Leaves, *Adv Pharmacol Toxicol.,* 2009; 10:13–6.
13. Athanasiadou S, Kyriazakis I, Jackson F, Coop RL, Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies, *Vet Parasitol.,* 2001;99 :205–19.
14. Thompson DP, Geary TG, The structure and function of helminth surfaces. In: Marr JJ, editor, *Biochemistry and Molecular Biology of Parasites.* 1st ed. New York: Academic Press,

1995; I: 203–32.

15. Pavicic A., Zajickova, M., Sadibolova, M., Anthelmintic activity of European fern extracts against *Haemonchus contortus*., *Veterinary Research,* 2023; 54:59.

16. Belga FN., Waindok, P, Raulf, MK, Phytochemical analysis and anthelmintic activity of *Combretum mucronatum* leaf extract against infective larvae of soil-transmitted helminths including ruminant gastrointestinal nematodes, *Parasites Vectors*, 2024;17:99.