Journal of Advanced Biomedical and Pharmaceutical Sciences

Journal Homepage: http://jabps.journals.ekb.eg

Biological Potential of *Moluccella laevis* L. Aerial Parts, Family Lamiaceae (Labiatae)

Nousiba A. Abdelaty, Eman Z. Attia, Ashraf N. E. Hamed*, Samar Y. Desoukey

Department of Pharmacognosy, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt.

Received: May 26, 2021; revised: September 10, 2021; accepted: September 12, 2021

Abstract

The current study aimed to determine the various *in vivo* biological activities of *Moluccella laevis L.* aerial parts. The anti-inflammatory activity demonstrated after 2 h, for both the total ethanolic extract (TEE) and the petroleum ether (Pet. ether) fraction, exhibited the same percentage of inhibition (26.24%) as that of the indomethacin, whereas the following hour showed results quite higher than that of the positive control. Additionally, the TEE showed higher antipyretic activity compared to the used standard acetylsalicylic acid with a rapid onset (30 min) and a longer duration exhibiting the maximum activity. After 4 h from the beginning of the experiment, the Pet. ether, ethyl acetate (EtOAc) and aqueous fractions displayed nearly the same effect as that of acetylsalicylic acid, which lasted up to 5 h. Moreover, the aqueous fraction exhibited the maximum analgesic activity with a rapid onset and a longer duration followed by the Pet. ether fraction, TEE and the EtOAc fraction. These activities could be explained by the presence of the highest amount of phenolic and flavonoidal contents, which are found in both EtOAc fraction and TEE. Due to the pronounced results of *M. laevis* L. aerial parts, it can be considered as a success of natural products in further drug discovery.

Key words

Analgesic; Anti-inflammatory; Antipyretic; Lamiaceae (Labiatae); *Moluccella laevis*; Phenols; flavonoids.

1. Introduction

Lamiaceae is one of the greatest important families containing essential oils. It was called formerly the Mint family or Labiatae. It included 236 genera and more than 7,000 species. They have various pharmacological activities. Additionally, many of them are edible and can be used in perfume manufacture. They are used in folkloric medicine as antispasmodic, carminative, antiemetic, choleretic, anti-inflammatory, diaphoretic, emmenagogue and antimicrobial agents [1]. *Moluccella* (syn. *Lamium*) is considered one of the most significant genera of this family. It is native to North Africa, Asia and Europe. It comprised about 40 species [2].

Moluccella laevis L. is an important research plant for chemical and biological investigation due to the presence of very few studies on it. The volatile oils and the saponifiable matters of the petroleum ether (Pet. ether) fraction of the total ethanolic extract (TEE) of *M. laevis* were analyzed by GC-MS [3,4]. Moreover, the antibacterial activity of TEE and its different fractions was investigated against Gram-positive and Gram-negative bacteria [4].

Additionally, both the aqueous and EtOAc fractions of TEE demonstrated the most potent cytotoxic effect against immortalized cell lines of human colorectal adenocarcinoma (CACO-2) and human breast cancer cell lines (MCF-7), respectively [5].

Moreover, twenty compounds were tentatively identified from LC-HR-ESI-MS metabolomic analysis. *In silico* docking study for these compounds revealed that stachydrine is more likely to account for the antiproliferative activity of both the EtOAc and aqueous fractions [5].

Furthermore, eight compounds: palmitic acid, tricosan-1-ol, pentadecan-1-ol and icosyl acetate, a mixture of *β*-sitosterol & stigmasterol, *β*-sitosterol-7-*O*-*β*-D-glucopyranoside and luteolin-7-*O*-*β*-D-glucopyranoside, were isolated [5].

According to the recent review, the most chemically investigated genus is *Leonurus*, while, *Lavandula latifolia*, *Lamium garganicum*, *Lamium purpureum* and *Melissa officinalis*. While, *M. laevis* need more phytochemical attention [6].

This encouraged us to carry out these extensive biological studies in addition to quantitative analyses of the total phenols and flavonoids of *M. laevis L.* aerial parts.

2. Material and methods

2.1. Plant material

The aerial parts of *M. laevis* L. were collected from the Nursery of Faculty of Agriculture, Minia University in March 2016. It was identified by Prof. Nasser Barakat, Botany Department, Faculty of Science, Minia University. A voucher specimen was kept in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University, Minia, Egypt, under registration number (Mn-ph-Cog-35).

2.2. Preparations of samples

The fresh aerial parts of *M. laevis* were air-dried in the shade then reduced to a coarse powder. The powdered plant material (5 kg) was then extracted by maceration in 95% ethanol (10 L, 3x, a week interval) and the ethanolic extract was concentrated

**** Correspondence:** **Ashraf N. E. Hamed***

***Tel.:** +201091-237-437**; Fax:** +2086-236-90-75.*

***Email Address:** ashrafnag@mu.edu.eg*

under vacuum to a syrupy consistency (TEE, 400 g). TEE was suspended in the least amount of distilled water (100 mL), then transferred to a separating funnel. It was successively fractionated with Pet. ether (one L, 3x) and ethyl acetate (one L, 3x) to afford two fractions; Pet. ether (90 g) and EtOAc (25 g). While, the remaining mother liquor was the aqueous fraction (285 g).

2.3. Estimation of total phenolic content

The content of total phenolic content for the TEE and its derived fractions of *M. laevis* was determined by the Folin-Ciocalteu method [7]. Analysis was performed by adding 3.5 mL of deionized H_2O, 50 µL of the TEE or its derived fractions, 50 µL Folin-Ciocalteu reagents (2N) and 300 µL of Na_2CO_3 (10 %). The reaction was left for 30 min and then the absorbance was measured in triplicate at 730 nm. The blank consisted of all reagents excluding the sample. A standard curve was made using gallic acid so that the total phenolic concentrations were expressed as mg of gallic acid equivalents per gram dried sample.

2.4. Estimation of total flavonoidal content

Total flavonoidal content was determined following Samy et al. method [7]. Where, 0.3 mL of the TEE or its derived fractions, 3.4 mL of 30% MeOH, 0.15 mL of $NaNO_2$ (0.5 M) and 0.15 mL of $AlCl_3$.6 H_2O (0.3 M) were mixed. After 5 min, 1 mL of NaOH (1 M) was added. The solution was mixed well and the absorbance was measured in triplicate against the reagent blank at 506 nm. A standard curve for the total flavonoids was made by using rutin standard solution at different concentrations under the same procedure. The total flavonoids were expressed as mg of rutin equivalents per gram of the dried sample.

2.5. Biological study

2.5.1. Animals

The animals used in this study were female albino rats weighing (200±20 g) for antipyretic and anti-inflammatory activities. Moreover, mice (30±5 g) were used for analgesic activity. They were obtained from the animal house of the Faculty of Medicine Assiut, Egypt, then housed and bred under standardized environmental conditions and fed with a standard diet and water. Animal experiments were conducted following the guidelines for the care and use of laboratory animals of the National Institutes of Health (NIH publication No. 85-23, revised 1985). The study protocol was approved by "The Research Ethics Committee" (# 29/2018), Faculty of Pharmacy, Minia University, Egypt. The animals were grouped into six groups (4 animals each). All samples and standard drugs were suspended in carboxymethyl cellulose (CMC) before administration. They received the following treatments orally as following:

Group **1**: Control group, received 0.5% CMC solution (negative control).
Group **2**: TEE group, received a dose of 300 mg/kg.
Group **3**: Pet. ether fraction group, received a dose of 300 mg/kg.
Group **4**: EtOAc fraction group, received a dose of 300 mg/kg.
Group **5**: Aqueous fraction group, received a dose of 300 mg/kg.
Group **6**: Standard groups (positive control), received [indomethacin 8 mg/kg (for anti-inflammatory activity) and acetylsalicylic acid 100 mg/kg, for both antipyretic and analgesic activities] [8]. All the experiments continued for 5 h.

2.5.2. Evaluation of anti-inflammatory activity

The TEE and its different fractions of *M. laevis* L. aerial parts were evaluated for their anti-inflammatory activity using the carrageenan-induced paw edema method [9]. The tested TEE, the fractions in addition to the reference drug were administrated orally 1 h before carrageenan injection (0.01 mL, 1% w/v in normal saline, subcutaneous injection) as a phologistic for the inflammation and were injected into the sub-plantar tissue of the right hind paw of the animals under study.
The paw thickness (mm) was measured by a Vernier caliper immediately at 0, 0.5, 1, 2, 3, 4 and 5 h after carrageenan injection. The percentage of inhibition of rat paw edema was calculated as follows:

$$\% \text{ Inhibition} = \frac{(\text{Control mean} - \text{treated mean})}{\text{Control mean}} \times 100$$

2.5.3. Evaluation of the antipyretic activity

The TEE and its different fractions of *M. laevis* L. aerial parts were evaluated for their antipyretic activity using yeast-induced pyrexia method [10].
The test was performed on female albino rats by subcutaneous injection (in the back, below the nape of the neck) of 20% aqueous suspension of yeast in a dose of 10 mL/kg to induce pyrexia. TEE and its different fractions were administered orally after 2 h from yeast injection. The rectal temperature of each animal was recorded by inserting a thermometer 2 cm into the rectum at 0, 0.5, 1, 2, 3, 4 and 5 h following the administration of the tested TEE, fractions as well as the reference drug.

2.5.4. Evaluation of the analgesic activity

The TEE and different fractions of *M. laevis* L. aerial parts were evaluated for their analgesic activity using the hot plate method described by Hosseinzadeh et al. [11]. The animals were placed on a hot plate and the temperature of the metal surface was maintained at 54 °C. The time (sec) of the response produced by the animal as tail withdrawn, licking paws or jumping due to radiant heat is noted and recorded at 0, 1, 2, 3, 4 and 5 h after the administration of the tested TEE, fractions and the standard drug.

2.6. Statistical analysis

Data were expressed as mean±standard error. The student's T-test followed by Dunnett's test was applied. Graph Pad Prism 5 was used for statistical calculations. Results were considered significant at p values < 0.05, 0.01 and 0.001.

3. Results and discussion

3.1. Estimation of the total phenolic contents

Results varied from 0.304±0.00057 to 6.7±0.058 mg gallic acid equivalent/g dried sample. The highest amount of phenols was found in the EtOAc fraction (6.7±0.058), followed by the TEE (4.58±0.0058), the aqueous fraction (3.58±0.0058) and lastly the Pet. ether fraction (0.304±0.00057). The results were demonstrated in Table 1.

Table 1: The total phenolic and flavonoidal contents of the TEE and its different fractions of *M. laevis* aerial parts.

Extract or fraction	Total phenolic contents (mg gallic acid equivalent/g dried sample)	Total flavonoidal contents (mg rutin acid equivalent/g dried sample)
TEE	4.58±0.0058	49.52±0.0.01
Pet. ether fraction	0.304±0.00057	7.6±0.01
EtOAc fraction	6.7±0.058	86.66±0.01
Aqueous fraction	3.58±0.0058	31.9±0.1

Table 2: The anti-inflammatory activity of the TEE and its different fractions of *M. laevis* aerial parts using carrageenan-induced paw edema method.

Group	Thickness of the paw (mm)						
	0 h	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Negative control	7.37±0.12	7.37±0.12	7.5±0.0	7.62±0.12	7.62±0.12	7.87±0.23	7.87±0.23
TEE	7.25±0.14	6.87±0.12	6.25±0.14***	5.62±0.23***	4.62±0.23***	4.0±0.20***	3.87±0.12***
Pet. Ether fraction	7.25±0.14	6.62±0.12*	6.37±0.14***	5.62±0.23***	4.87±0.12***	4.0±0.31***	3.87±0.12***
EtOAc fraction	7.25±0.14	7.0±0.0	6.75±0.14*	6.5±0.2**	5.87±0.12***	5.37±0.37***	5.37±0.37***
Aqueous fraction	7.12±0.12	7.12±0.12	6.75±0.14*	6.5±0.2**	6.0±0.20***	4.87±0.12***	4.75±0.14***
Indomethacin (Positive control)	7.25±0.14	6.5±0.2**	6.0±0.2***	5.62±0.23***	5.37±0.23***	5.37±0.23***	3.75±0.14***

The results are presented as the mean±S.E.M. (Standard error of the mean). Differences with respect to the control group were calculated using the student's T-test ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

Table 3: The percentage of edema inhibition of the TEE and its different fractions of *M. laevis* aerial parts using carrageenan-induced paw edema method.

Group	Dose mg/kg	Percentage inhibition (%)					
		0.5 h	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Negative control	-	-	-	-	-	-	-
TEE	300	6.78	16.66	26.24	39.37	49.17	50.82
Pet. ether fraction	300	10.17	15.06	26.24	36.08	45.72	50.82
EtOAc fraction	300	5.02	10	14.69	22.96	31.76	31.76
Aqueous fraction	300	3.39	10	14.69	21.25	38.11	39.64

Table 4: The antipyretic activity of TEE and its different fractions of *M. laevis* aerial parts using yeast-induced pyrexia method.

Group	Rectal temperature (C) (Mean±S.E.M)						
	0 h	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Negative control	38.74±0.08	38.86±0.02	38.85±0.05	38.89±0.02	39.56±0.13	39.9±0.04	39.99±0.08
TEE	38.64±0.10	38.20±0.07***	37.31±0.02***	36.35±0.06***	36.24±0.04***	36.14±0.04***	36.06±0.02***
Pet. ether fraction	38.69±0.09	38.24±0.04***	38.16±0.02***	37.34±0.10***	37.41±0.17***	37.14±0.07***	37.11±0.04***
EtOAc fraction	38.76±0.09	38.51±0.04*	38.21±0.07***	37.64±0.06***	37.56±0.06***	37.21±0.04***	37.06±0.02***
Aqueous fraction	38.74±0.06	38.43±0.08**	38.01±0.13***	37.49±0.02***	37.44±0.04***	37.26±0.11***	37.14±0.07***
Acetylsalicylic acid (Positive control)	38.79±0.07	38.49±0.06**	37.71±0.16***	37.44±0.04***	37.21±0.04***	37.21±0.07***	37.16±0.02***

The results are presented as the mean±S.E.M. (Standard error of the mean). Differences with respect to the control group were calculated using the student's T-test ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

Table 5: The analgesic activity of the TEE and its different fractions of *M. laevis* aerial parts using hot plate method.

Group	Time (sec) (Mean±S.E.M)						
	0 h	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Negative control	58.01±2.51	60.67±0.88	62.01±2.30	61.34±1.85	59.34±2.02	59.5±1.55	59±1.47
TEE	60.25±1.88	57.51±1.32	94.26±4.42	94±5***	152.71±3.28***	159.8±4.19***	167±3.34***
Pet. ether fraction	59±2.48	60.76±1.93	72.26±3.98	97±1.68***	138.3±7.51***	167.3±2.72***	171±1***
EtOAc fraction	52.25±3.19	55.01±0.57	67.26±1.75	77.27±3.5**	100±2.5***	125.3±1.93***	165.3±2.95***
Aqueous fraction	62.5±2.32	75.34±2.84**	86.01±4.91**	94.76±2.72***	108±6.24***	155.3±3.70***	186.8±5.51***
Acetylsalicylic acid (Positive control)	56.25±1.70	67.51±3.61	71.51±3.66	79±4.37**	93.67±4.37	131.7±5.20***	169±7.37***

The results are presented as the mean±S.E.M. (Standard error of the mean). Differences with respect to the control group were calculated using the student's T-test ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

3.2. Estimation of the total flavonoidal contents

Results varied from 7.6±0.01 to 86.66±0.01 mg rutin equivalent/g dried sample. The highest amount of flavonoids was found in the EtOAc fraction (86.66±0.01) followed by the TEE (49.52±0.0.01), the aqueous fraction (31.9±0.1) and the Pet. ether fraction (7.6±0.01). The highest value of the total phenolic and total flavonoidal contents of the EtOAc and aqueous fractions may be attributed to its content of the phenylpropanoid derivatives. The results were shown in Table 1.

3.3. Anti-inflammatory activity

It is obvious from Tables 2 and 3 that all the tested fractions and TEE exhibited a significant (**p<0.01, ***p<0.001) decrease in the paw edema induced by carrageenan especially after 2 h from the beginning of the experiment. After 2 h, both the TEE and the Pet. ether fraction exhibited the same percentage of inhibition (26.24%) as that of the indomethacin, whereas an additional 1 h (3 h) showed results quite higher than that of the control. Similarly, after 5 h both the TEE and the Pet. ether fraction exhibited the highest anti-inflammatory activity with a percentage of inhibition (50.82%), which is close to that of indomethacin (52.35%). The aqueous fraction showed a mild inhibition of the inflammation that was increased by time to be (39.64%) after 5 h. On the other hand, the least activity was shown by the EtOAc fraction through the 5 h of the experiment (31.76%). This effect is probably attributed to their content of sterols, which were reported to have anti-inflammatory activity [12]. Also, many mechanisms proved the anti-inflammatory activity of flavonoids; inhibition of cyclooxygenase and 5-lipoxygenase pathway, inhibition of eicosanoid biosynthesis, besides its ability to inhibit neutrophil degranulation [13].

3.4. Antipyretic activity

The results of the antipyretic activity of the TEE and its different fractions of *M. laevis* aerial parts revealed that they almost exhibited a significant (***p<0.001) antipyretic activity throughout the 5 h. The TEE showed higher activity compared to the used standard acetylsalicylic acid with a rapid onset (0.5 h) and a longer duration exhibiting the maximum activity.
After 4 h from the beginning of the experiment the Pet. ether, EtOAc and aqueous fractions showed nearly the same effect as that of acetylsalicylic acid that lasted up to 5 h (Table 4). The potent antipyretic activity of the TEE of *M. laevis* may be attributed to its high content of flavonoids and sterols [14].

3.5. Analgesic activity

The TEE and its different fractions of *M. laevis* aerial parts demonstrated that they exhibited potent analgesic activity. The TEE and aqueous fraction significantly increased the reaction time (94.25±4.42 and 86±4.9, respectively) in comparison with acetylsalicylic acid (71.5±3.66) after one h from the beginning of the experiment. Furthermore, after 2 h the other fractions (except the EtOAc fraction) exhibited higher effects than acetylsalicylic acid, which lasted up to 5 h throughout the experiment (Table 5). The aqueous fraction exhibited the maximum analgesic activity with a rapid onset and a longer duration followed by the Pet. ether fraction, TEE and the EtOAc fraction. The potent analgesic activity of the TEE and the other fractions of *M. laevis* is in accordance with reported literature of *Lamium* species [15] and may be attributed to their high content of flavonoidal glycosides, sterols and iridoid glycosides, which were reported to have analgesic activity [16-18].

4. Conclusion

The high phenolic and flavonoidal contents of *M. laevis* L. aerial parts might be responsible for the outstanding results of their anti-inflammatory, antipyretic and analgesic activities. Therefore, such plant could have a supportive role in the pharmaceutical field towards the development of new drugs.

Conflict of interests

Authors declare no actual or potential conflict of interests.

Orcid

Ashraf N. E. Hamed https://orcid.org/0000-0003-2230-9909
Samar Y. Desoukey https://orcid.org/0000-0002-4462-9674

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