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ORIGINAL ARTICLE

Phytochemical and Toxicological Analyses of Herbal Mixtures Containing *Hypericum perforatum* and *Melissa officinalis*

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ABSTRACT

Objectives: This study aimed to formulate a novel herbal mixture of *Hypericum perforatum* (H) and *Melissa officinalis* (M) and evaluate its toxicity, microbial load, and phytochemical content.

Materials and Methods: Total flavonoids were measured using the $AlCl_3/NaNO_2$ complex formation method and colorimetric assay. The quercetin content of the herbal mixture was determined by reverse-phase high-performance liquid chromatography. The *in vitro* and *in vivo* safety of the herbal formulations were analyzed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and acute oral toxicity analysis in the rat model, respectively.

Results: The formulated extract (HM), compared with the standard rutin extract, had a total flavonoid content of 15.29 ± 0.64 mg rutin per mL sample. Reverse-phase high-performance liquid chromatography revealed a quercetin content of 0.187 mg/mL. Microbial tests for *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Salmonella* spp. were negative. Colony counts for total aerobic microbial and yeast and mold counts were 10 in each case. The MTT assay (with up to about 5% *v/v* HM extract) using the NIH/3T3 cell line revealed no cell toxicity in the range of concentrations tested. Acute oral toxicity was tested in the Wistar rat model, and the LD_{50} was 695.2 ± 7.5 mg/kg. The dry weight of the HM extract was 38.1 mg/mL.

Conclusion: Preliminary results proved the safety of the HM herbal mixture, with its toxicity and microbial load within the limits of accepted guidelines allowable for use in clinical trials.

Keywords: *Melissa officinalis*, *Hypericum perforatum*, combined hydroethanolic extract, cell toxicity, animal toxicity

INTRODUCTION

In the course of history, humans have always looked toward nature for food, nutrients, and natural substances to treat various illnesses. Traditional medicine was founded on the relationship between man and nature thousands of years ago.[1] To date, only a small percentage of the total number of plant species on earth has been analyzed phytochemically, and even a smaller percentage has been screened for potential pharmacological use. Despite recent advances in our fundamental understanding of many disease mechanisms, such as cancer, chronic inflammation, diabetes, and neurodegenerative disorders, there is a great need for more effective pharmaceutical solutions. Plant extracts containing many secondary metabolites with various biological/pharmacological activities can affect many targets and potentially fill this gap. Previous studies have shown that essential oils and extracts isolated from different plant species exhibit powerful antimicrobial and antioxidant activities, as well as anti-inflammatory, anti-cancer, and

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hepatoprotective activities.[2-6] A significant proportion of all drugs produced worldwide are either plant products or derived from them and phytopharmaceutical research has played an important role in the discovery and development of many more synthetic drugs.[7] At the same time, mixed herbal extracts can be used as multi-target drugs that act synergistically, have fewer side effects, and in the process lower treatment costs.[8]

Lemon balm, with the scientific name *Melissa officinalis* (M), is a well-known perennial herbaceous plant belonging to the Lamiaceae family that has been studied extensively for its medicinal properties. It is native to Asia, North America, the Mediterranean, and Southern Europe, and is extensively cultivated in these regions.[9] The leaves of M have been used to treat a number of ailments, including digestive and inflammatory diseases and microbial infections.[10] The presence of phenolics, terpenoids, and flavonoids, such as quercetin, rutin, and quercetin, in the M extract is believed to be responsible for their ability to treat various diseases.[11] Essential oils contain important secondary metabolites, such as geranial, cineol, and caffeic acid, which are capable of reducing serum cholesterol and triacylglyceride. Flavonoids are known for their powerful antioxidant activities, and they are also present in M extract.[12] *Hypericum perforatum* (H), also known as St. John's wort, is another medicinal plant of great importance that has been used for thousands of years. This perennial herb belongs to the Hypericacea family and is native to temperate regions of the world. It has been studied extensively because of its importance as a powerful medicinal plant that can treat various illnesses.[13] Leaves of plants are known to contain active metabolites such as flavonoids, naphthodianthrone, and phloroglucinol. The antidepressant effects of H extract are comparable to those of common synthetic antidepressants in mild to moderate cases.[14] Many studies have focused on the antiviral activity of H, and the results appear promising.[15,16]

Each of these plants contains a multitude of secondary metabolites that can exert a positive influence on the treatment of various diseases. A question that comes to mind is whether a combined extract of these two plants could be formulated for clinical use and maximize their therapeutic efficacy. Previous studies have confirmed that the combination of herbal formulations is generally associated with increased functional properties and biological activities of mixed extracts.[17,18] However, scientific data on the phytochemical analysis of the combined extract of M and H, particularly in, the determination of cell and animal toxicities, are scarce. In the current study, a new formulation of the combined ethanolic herbal extracts of H and M was prepared to perform preliminary safety and phytochemical analyses in preparation for future clinical testing.

MATERIALS AND METHODS

Plant acquisition and extraction

Both H and M herbal extracts were obtained from a medicinal plant farm in Ardabil, Iran. The samples were carefully cleaned of debris and air-dried in the laboratory. Herbarium samples of the plants were sent to the Institute of Pharmacology, Tehran University of Medical Sciences for identification. After proper identification, samples were registered and code numbers were assigned (PMP-2310 and 14001 for H and M, respectively). After weighing the plants, they were ground together in a pharmaceutical grinding mill to an adequate size for extraction (coarse ground). An Accelerated Solvent Extraction system (ASE) was used to perform extraction using 70% pharmaceutical-grade ethanol in water. Preliminary test trials indicated that extraction temperatures of 10 MPa were not required and the extraction was performed. The mixed ground plant tissue was loaded into extraction vessels and allowed to fill with the extraction solvent. The system was allowed to stand for static extraction for 24 hours, after which dynamic extraction was resumed. The extraction solvent was fluxed through the ASE system twice (*i.e.*, a total of three times). The final product (HM extract) was used for subsequent analysis. To determine the dry weight, 10 mL of the HM extract was dried in triplicate at 35-40 °C in a drying oven until no further weight change was noted. The total extraction volume and final percentage of ethanol in the extract were also measured and recorded.

Phytochemical analysis

Total flavonoid content

The aluminum chloride/sodium nitrite method was used to determine the total flavonoid content of the HM extract.[19] In this method, the o-nitroso derivatives of flavonoids form a complex with Al III, which absorbs maximally at or near 510 nm. Rutin (Sigma-Aldrich, USA) was used as the standard to determine the total flavonoid content of HM as µg rutin/mL. Sodium hydroxide (4%), sodium nitrite (5%), and 10% aluminum chloride in deionized water were used for the procedure. Samples were prepared by adding 25 µL (1:10 dilution) of HM extract to 100 µL water and 7.5 µL sodium nitrite solution into a 96-well Enzyme-Linked Immunosorbent Assay (ELISA) plate in 8 replicates. After six minutes, 7.5 µL aluminum chloride, 100 µL sodium hydroxide, and 110 µL of deionized water were added to each well, and the wells were covered with aluminum foil for 15 minutes. Absorption at 510 nm was read and recorded using an ELISA reader (Synergy, BioTek Instruments Inc., Germany). The same procedure was repeated for different rutin standard concentrations. The 6 best results were selected for total flavonoid determination.

Quercetin concentrations determined by reversed-phase high-performance liquid chromatography (RP-HPLC)

Quercetin, a key marker and constituent of both H and M, was analyzed using HPLC. RP-HPLC is routinely used for the optimal separation of flavonoids due to their hydrophobicity and low solubility in aqueous solutions.[20] All HPLC-grade solutions and equipment were obtained from the Alborz Academic Institute, Iran. To prepare the standard solution (Figure 1), 10 mg of quercetin dihydrate (Sigma-Aldrich, USA) was dissolved in 20 mL methanol, 15 mL dilute hydrochloric acid, and 5 mL water, and the final volume was adjusted to 50 mL with methanol. To prepare the sample solution, 15 mL of the HM extract was first dried. The dry extract was then added to the same series

of solutions as the standard with a final volume of 50 mL, as described above. A 25 cm C18 column (Phenomenex, USA) with a 4 mm diameter and 5 µm particle size was used for HPLC on an Alliance E2695 (Waters, USA). Gradient elution was performed with a mobile phase consisting of 0.3 g/L phosphoric acid (solution A) and pure methanol (solution B). The gradient started with 60% solution A and 40% solution B and ended with 0% A and 100% B. The injection volume was 10 µL and the running time was 25 minutes at 25 °C. The detector was set at a wavelength of 370 nm with a flow rate of 1 mL/min. Quercetin was identified by comparing the retention times of the sample peaks with the quercetin peak in the standard (Figure 2).

Cell culture and toxicity

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to assess the toxicity of the HM hydroethanolic extract in the NIH/3T3 cell line based on the general protocols described in ISO-10993-5 and Danihelová et al.[21] The NIH/3T3 cell line was obtained from The Iranian National Center for Genetics and Biological Resources. The cell culture medium consisted of complete Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10 IU/mL penicillin, and 100 IU/mL streptomycin (Capricorn, Germany). Standard growing conditions included 5% CO_2 and 95% humidity at 37 °C. After three passages, cells were grown in T175 cell culture flasks until reaching confluence. The samples were then trypsinized and centrifuged at 1200 rpm for 5 min. After suspending the cells in the cell culture medium, viable cells were counted using a hemocytometer aided by trypan blue to identify non-viable cells. An average number of 2×10^4 cells were incubated (24 hours at 37 °C) in the wells of a flat-bottom ELISA plate and used to perform the MTT assay. The culture medium was replaced with various concentrations of HM extract (0.5 to 50% *v/v*), which was added to NIH/3T3 cells and incubated at 37 °C for 24 hours. The control wells received media containing complete DMEM supplemented with FBS and antibiotics, but no HM extract. To perform the MTT assay, the culture medium was removed from each well, and the cells were washed with 100 µL of fresh DMEM. To each well, 50 µL of cell culture medium and 50 µL 5 mg/mL MTT reagent (Sigma-Aldrich, USA) were added, and the flasks were incubated for 4 hours at 37 °C. MTT is a yellow solution converted to formazan crystals by mitochondrial nicotinamide adenine dinucleotide phosphate-dependent oxidoreductases in metabolically active cells. To dissolve the formazan crystals, 150 µL dimethyl sulfoxide (DMSO) was added to each well and shaken for 15 min. A blank containing MTT reagent and DMSO was used to adjust baseline absorption. Absorbance was measured at 570 nm using an ELISA reader (Synergy, BioTek Instruments Inc., Germany). Results are expressed as percentage control ± standard error of the mean (SEM).

Acute oral toxicity, LD_{50} in Wistar rats

The acute oral toxicity of HM was studied in Wistar rats obtained from the Institute of Pharmacological Sciences, Tehran University of Medical Sciences (Iran). Procedural and ethical considerations were based on OECD UDP Procedure 425 (2020). Animal procedures were approved by the Payame

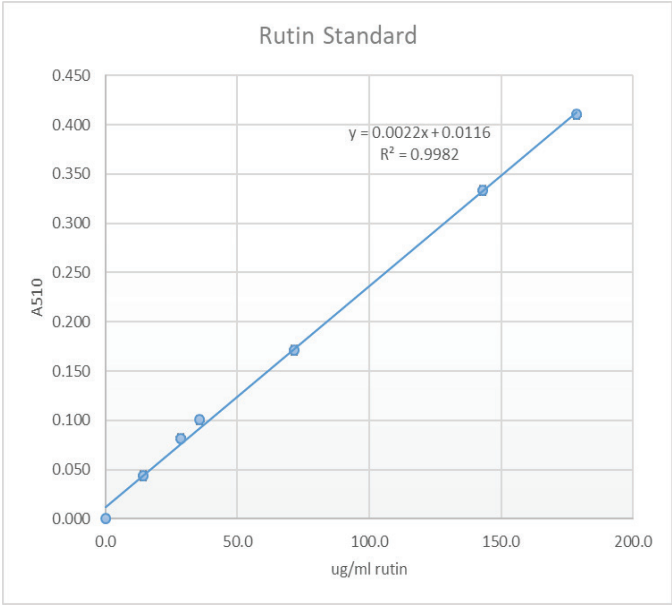

Figure 1. Standard calibration curve of rutin. The total flavonoid content of HM was determined by linear regression analysis

HM: *Melissa officinalis-Hypericum perforatum*

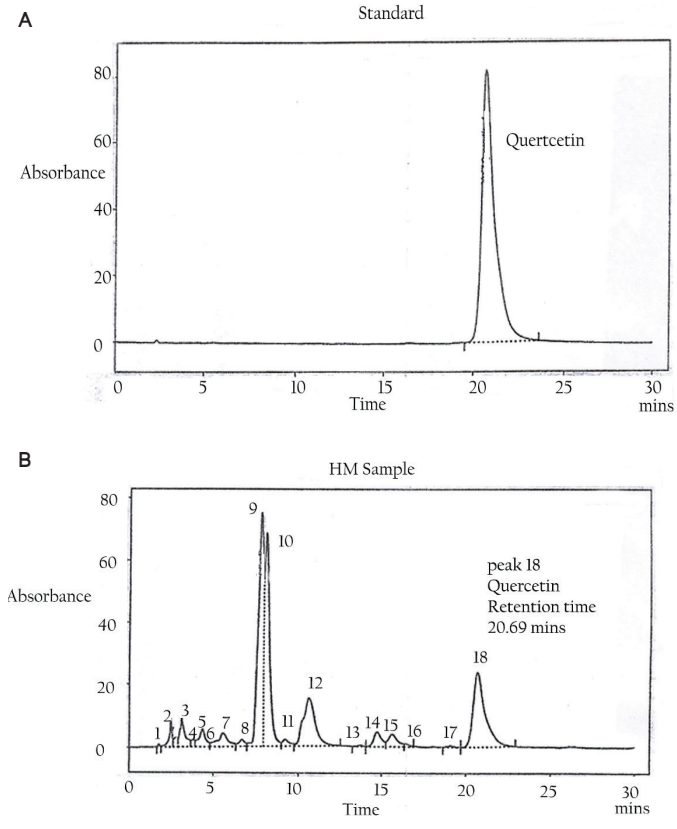

Figure 2. A) Chromatograph of standard quercetin at a concentration of 200 mg/mL, B) Chromatograph of the HM extract. An arrow indicates the Quercetin peak

HM: *Melissa officinalis-Hypericum perforatum*

Noor University Research Ethics Committee (approval number: IR.PNU.REC.1401.082, date: 15.05.2022). Animals were housed under standard conditions of a 12-hours/12-hours light/dark cycle with no food or water restrictions at 25 °C. All animals were acclimated to standard conditions for 10 days prior to dosing. The average weight of the animals was 200-250 grams, and all doses were calculated according to body weight. Animals were singly dosed in the test stage using the default doses recommended by the OECD guidelines (50, 100, 500, 1000 mg/kg) to find a suitable range of doses/response for the main stage of the experiment. After the initial dosing, the animals were monitored for 72 hours for signs of toxicity. The HM doses selected for the main stage of the experiment were 381.0 mg/kg, 571.5 mg/kg, 685.8 mg/kg, and 762.0 mg/kg administered by oral gavage. The main stage consisted of 1 control and 4 treatment groups with 10 rats in each group (n= 10). Clinical manifestations of toxicity as well as mortality were monitored and recorded for 14 days. LD_{50} was calculated.

Microbial content analysis

In order to analyze the microbial load of the HM extract, the USP-40 general protocol for the analysis of non-sterile products was used. Serial dilutions of 1:10, 1:100, and 1:1000 HM extract were prepared in peptone water buffer, and the total aerobic microbial count (TAMC) was performed by adding 1 mL of each dilution to 15 mL plate count agar in 9 cm Petri dishes in duplicates. The incubation period was 5 days at 30 °C. The same procedure was used to obtain total yeast and mold counts (TYMCs) on casein-soybean digest agar. The presence of the four main groups of food pathogens, *i.e.*, *Escherichia coli, Salmonella* spp., *Staphylococcus aureus,* and *Pseudomonas aeruginosa*) in HM extract was explored on MacConkey, bismuth sulfite, mannitol salt, and cetrimide agars. Uninoculated negative control plates were run in parallel, and the results were recorded as colony-forming units per mL (colony-forming units/mL).

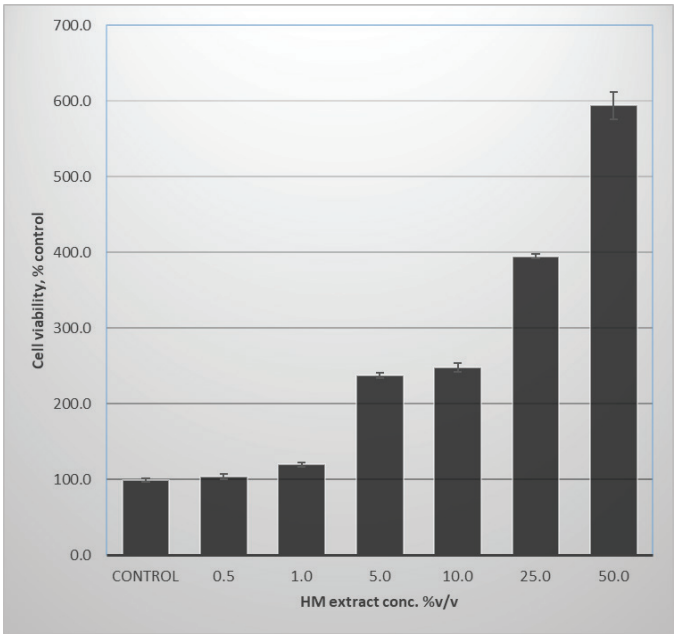

Figure 3. Graphical results of the MTT viability test of the NIH/3T3 cell line in response to various concentrations of the HM extract, expressed as a percentage of the control

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, HM: *Melissa officinalis-Hypericum perforatum*

Statistical analysis

One-Way analysis of variance and Probit analysis of LD_{50} were performed using SPSS 26 software. The Dunnett test was performed as a post hoc analysis method where $p < 0.05$ was statistically significant. The final results are presented as mean ± standard error of the mean (SEM).

RESULTS

Phytochemical analysis

The total extraction volume, percent ethanol, and dry mass of the HM extract are presented in Table 1. Total flavonoid content was determined using the aluminum chloride/sodium nitrite method by measuring A_{510}. Comparing the A_{510} of the HM sample (Table 2) with the rutin standard curve revealed a total flavonoid content of 15.29 ± 0.64 mg rutin/mL (Table 3) HM extract (*$p < 0.05$).

Eighteen peaks were detected in the reverse-phase HPLC chromatogram of HM. The quercetin peak in the HM sample (peak number 18, chromatograph b) was identified by comparing peak retention times with those of the quercetin standard (chromatograph a). The areas under the peaks were compared, and quercetin concentration per mL of HM extract was calculated relative to the standard. These numbers were corrected for dilutions to determine quercetin concentrations in the HM samples. The quercetin concentration was 0.187 mg/mL.

Microbial content

The presence of four main pathogenic contaminants in food and non-sterile products, *i.e. E. coli*, *P. aeruginosa*, *Salmonella* spp.,

Table 1. Percent alcohol content, volume, and mass of dried extract per mL HM extract

Total volume extracted	Alcohol (%)	Dried extract
1320 mL	58	38.1 mg/mL

HM: *Melissa officinalis-Hypericum perforatum*

Table 2. The absorbance of different rutin concentrations (at 510 nm) used to construct the standard curve

Rutin standard (µg/mL)	A510 ± SEM
0.0	0.000 ± 0.001
14.2	0.044 ± 0.001
28.6	0.082 ± 0.001
35.7	0.100 ± 0.001
71.4	0.171 ± 0.001
142.9	0.333 ± 0.001
178.6	0.410 ± 0.001

SEM: Standard error of mean

and *S. aureus,* was tested, and growth was negative in all cases. The TAMC and TYMC yielded negative results. The results of the microbial load tests are summarized in Table 4. According to USP-40 guidelines for microbial testing of non-sterile pharmaceutical products, HM extract meets the requirements for human consumption.

Toxicity assays

Cellular toxicity: The MTT assay was used to determine HM toxicity in the NIH/3T3 cell line. Results are summarized in Figure 2 and Table 5. Cellular toxicity was measured by colorimetric assay of formazan crystals produced by viable cells. No toxicity was noted in the range of tested concentrations. However, a significant concentration-dependent increase in apparent cell viability was observed above 5% v/v for HM, which may indicate interference with the assay. Such effects are commonly observed with electron transport chain uncouplers or molecules that directly reduce the MTT reagent to form formazan crystals independent of mitochondrial oxidoreductases. Examples include NADH, ascorbic acid, glutathione, and flavonoids, which are powerful antioxidants present at significant levels in HM extract. This would explain the dose-dependent increase in absorption at HM concentrations above 5% *v/v*.

Animal toxicity: The results for acute oral toxicity (LD_{50}) in Wistar rats are summarized in Table 6. Animals were monitored for 14 days for the visual clinical manifestations of toxicity (Table 7) following the administration of HM extract by gavage. The data indicate an LD_{50} of 685.8 mg/Kg. SPSS software was used to perform non-linear regression analysis (PROBIT analysis) of the LD_{50}. The software calculated an LD_{50} of 695.2 ± 7.5* mg/kg ($p < 0.05$) with a 95% confidence interval of 599.4-913.3 mg/kg. The only clinical signs noted were abdominal distension and diarrhea in the dose range of 571.5-685.8 mg/kg, as shown in Table 6. Therefore, the maximum dose without signs of toxicity was 381.0 mg/kg of the HM extract.

DISCUSSION

Previous studies have reported that the extracts obtained from several natural sources, such as *Zataria multiflora*[22], *Carum carvi*[23], *Rosa damascenes*[24], and *Mentha piperita*[25] possessed

Table 3. Total flavonoid expressed as mg standard rutin (*$p < 0.05$)

Total flavonoids in HM, corrected for 1:140 dilution SEM ± mg/mL	Total flavonoids as mg standard rutin SEM ± µg/mL	A510 ± SEM
15.29 ± 0.64*	109.2 ± 4.6*	0.259 ± 0.001

Significant differences were assessed by *$p < 0.05$. SEM: Standard error of mean, HM: *Melissa officinalis-Hypericum perforatum*

Table 4. Summarized results of microbiological tests

Test	Results	Acceptable range	Standard	unit
Pseudomonas aeroginosa	-	-	USP 40	CFU/mL
Staphylococcus aureus	-	-	USP 40	CFU/mL
Salmonella spp.	-	-	USP 40	CFU/mL
Escherichia coli	-	-	USP 40	CFU/mL
TYMC	*10›	Max 10^2	USP 40	CFU/mL
TAMC	*10›	Max 10^3	USP 40	CFU/mL

Significant differences were assessed by *$p < 0.05$. TYMC: Total yeast and mold, TAMC: Total aerobic microbial count, CFU: Colony-forming units

Table 5. MTT assay results for cell toxicity in NIH/3T3 cell line

Concentration (v/v %)	0	0.5	1	5	10	25	50
Mean (control %) ± SEM	99.20 ± 2.11	103.55 ± 3.17	119.52 ± 3.31	237.10 ± 3.67*	248.07 ± 5.65*	395.00 ± 2.69*	594.20 ± 18.4*

Significant differences were assessed by *$p < 0.05$. Results are expressed as % control. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, SEM: Standard error of mean

Table 6. Raw data for acute oral toxicity (mortality for 14 days) with single dose of HM extract in Wistar rats

Dose (mg/kg)	0	381.0	571.5	685.8	762.0
Mortality	0	0	3	5	6
Mortality (%)	0	0	30	50	60

HM: *Melissa officinalis-Hypericum perforatum*

Table 7. Visible clinical manifestations of toxicity with HM treatment in Wistar rats

Visible clinical manifestations	0 mg/kg (control)	381.0 mg/kg	571.5 mg/kg	685.8 mg/kg	762.0 mg/kg
Skin	-	-	-	-	-
Eyes	-	-	-	-	-
Abdominal distention	-	-	+	-	-
Diarrhea	-	-	+	+	-
Respiratory	-	-	-	-	-
Arrhythmias	-	-	-	-	-
Hair	-	-	-	-	-
Mobility	-	-	-	-	-
Paralysis	-	-	-	-	-
Pain	-	-	-	-	-

Animals were monitored for 14 days and results were recorded. HM: *Melissa officinalis-Hypericum perforatum*

considerable functional properties, including chemopreventive, antioxidant, and anti-inflammatory potential. These beneficial effects may be associated with the antioxidant compounds, particularly flavonoids, present in the tested plant species. Therefore, in the current study, the flavonoid content of the herbal mixtures (HM) has been measured spectrophotometrically, and the results revealed that these herbal mixtures contained a significant amount of flavonoid (40.1% of total dry weight), which is comparable with the findings reported by Arceusz et al.[26] and Germ et al.[27]. However, multiple factors, such as habitat and environmental conditions during plant growth, sample preparation, extraction methods, and analytical techniques, can affect secondary metabolite concentrations.[28] The HPLC analysis confirmed that the major flavonoid present in HM extracts was Quercetin (Figure 2). These findings are in line with Aghakarim et al.[29] and Mohagheghzadeh et al.[30].

Moreover, microbiological testing is typically used to assess the presence of harmful microbes in herbal medication products before commercialization. This testing involves measuring the total number of microorganisms, checking for the presence of specific pathogenic species, and verifying the absence of certain indicator organisms. Common test methods include microbial plate counting, as well as tests for *E. coli, Salmonella* spp., and *S. aureus*[31]. The present study confirmed the absence of harmful microorganisms such as *E. coli* and *S. aureus* in the herbal mixture (Table 4), thereby ensuring the quality and safety of the studied herbal products.

Furthermore, although there has been a growing interest in using plant-based medicines, it is necessary to assure consumers about the safety of herbal treatments. Diverse essential oils have been recognized by the United States Food and Drug Administration as Generally Recognized as Safe, underscoring their potential safety and eco-friendly nature. However, before use, their toxicity should be thoroughly tested. Hence, the current was checked for the possible toxicity of the herbal mixture using both *in vitro* and *in vivo* assays. The results showed that the toxicity level of the HM extract placed it in category 4 (medium toxicity, between 500 and 2000 mg/kg), which is less toxic than commonly used substances like caffeine, aspirin, or ibuprofen (Tables 6 and 7). Lemon balm and St. John's wort are antispasmodic and antidiarrheal and are used to alleviate abdominal discomfort.[32,33] Therefore, it is possible that at higher HM doses, abdominal distension and diarrhea are absent because of these effects.

CONCLUSION

The diverse array of secondary metabolites found in plant extracts has unlimited potential and possibilities. In the last few decades, using plant extracts and products to treat various diseases has been successful in many cases, and the results in this area appear promising. Therapeutic strategies using phytopharmaceuticals provide a new perspective to address the void that chemical drug design and manufacturing worldwide have been unable to fill. HM is a new formulation of the combined hydroethanolic extracts of Lemon balm and St. John's wort, which was analyzed for its phytochemical properties and cell and animal toxicities in the present study. HM contains flavonoids at high concentrations. No microbial growth was observed in the microbiological tests performed on the HM. At concentrations of up to 1% *v/v*, HM did not cause toxicity in the NIH/3T3 cell line, and it did not interfere with the MTT assay. Higher concentrations of HM seem to have interfered with the MTT assay and resulted in greater cell viability than the control. PROBIT analysis of the acute oral toxicity of HM extract in Wistar rats indicated an LD_{50} of 695.2 mg/kg body weight, which places HM in toxicity category 4 (moderate toxicity). Considering all the results, a range of 1-5% of the maximum dose of HM without any indications of toxicity in the animal model (*i.e.*, 3.81-19.1 mg/kg/day) is suggested for pharmacological testing in clinical trials.

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Ethics

Ethics Committee Approval: Animal procedures were approved by the Payame Noor University Research Ethics Committee (approval number: IR.PNU.REC.1401.082, date: 15.05.2022).

Informed Consent: Not required.

Authorship Contributions

Concept: F.F., S.F., S.D., Design: F.F., S.F., Data Collection or Processing: S.F., Analysis or Interpretation: S.F., Literature Search: M.Z., Writing: M.Z., S.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016;21:559.
2. Dini S, Chen Q, Fatemi F, Asri Y. Phytochemical and biological activities of some Iranian medicinal plants. Pharm Biol. 2022;60:664-689.
3. Fatemi F, Golbodagh A, Hojihosseini R, Dadkhah A, Akbarzadeh K, Dini S, Malayeri MRM. Anti-inflammatory Effects of deuterium-depleted water plus rosa damascena mill. Essential oil *via* cyclooxygenase-2 pathway in rats. Turk J Pharm Sci. 2020;17:99-107.
4. Malayeri MR, Dadkhah A, Fatemi F, Dini S, Torabi F, Tavajjoh MM, Rabiei J. Chemotherapeutic effect of Berberis integerrima hydroalcoholic extract on colon cancer development in the 1,2-dimethyl hydrazine rat model. Z Naturforsch C J Biosci. 2016;71:225-232.
5. Torabi F, Dadkhah A, Fatemi F, Dini S, Taghizadeh M, Malayeri MR. Prevention and therapy of 1, 2-dimethyl hydrazine induced colon carcinogenesis by Ferula assafoetida hydroalcoholic extract. Turkish J Biochem. 2015;40:390-400.
6. Rasooli A, Fatemi F, Hajihosseini R, Vaziri A, Akbarzadeh K, Mohammadi Malayeri MR, Dini S, Foroutanrad M. Synergistic effects of deuterium depleted water and *Mentha longifolia* L. essential oils on sepsis-induced liver injuries through regulation of cyclooxygenase-2. Pharm Biol. 2019;57:125-132.
7. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Rollinger JM, Schuster D, Breuss JM, Bochkov V, Mihovilovic MD, Kopp B, Bauer R, Dirsch VM, Stuppner H. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 2015;33:1582-1614.
8. Fatemi F, Dadkhah A, Rezaei MB, Dini S. Effect of γ-irradiation on the chemical composition and antioxidant properties of cumin extracts. J Food Biochem. 2013;37:432-439.
9. Miraj S, Rafieian-Kopaei, Kiani S. Melissa officinalis L: A review study with an antioxidant prospective. J Evid Based Complementary Altern Med. 2017;22:385-394.
10. Longevity OMAC. Retracted: Phytochemical constituents, biological activities, and health-promoting effects of the *Melissa officinalis*. Oxid Med Cell Longev. 2024;2024:9842453.
11. Petrisor G, Motelica L, Craciun LN, Oprea OC, Ficai D, Ficai A. *Melissa officinalis*: Composition, Pharmacological effects and derived release systems-a review. Int J Mol Sci. 2022;23:3591.
12. Zarei A, Changizi-Ashtiyani S, Taheri S, Hosseini N. A brief overview of the effects of *Melissa officinalis* L. extract on the function of various body organs. J Res Med Sci. 2015;17.
13. Caldeira GI, Gouveia LP, Serrano R, Silva OD. Hypericum genus as a natural source for biologically active compounds. Plants. 2022;11:2509.
14. Szewczyk B, Pochwat B, Muszyńska B, Opoka W, Krakowska A, Rafało-Ulińska A, Friedland K, Nowak G. Antidepressant-like activity of hyperforin and changes in BDNF and zinc levels in mice exposed to chronic unpredictable mild stress. Behav Brain Res. 2019;372:112045.
15. Chen H, Muhammad I, Zhang Y, Ren Y, Zhang R, Huang X, Diao L, Liu H, Li X, Sun X, Abbas G, Li G. Antiviral activity against infectious bronchitis virus and bioactive components of *Hypericum perforatum* L. Front Pharmacol. 2019;10:1272.
16. Mohamed FF, Anhlan D, Schöfbänker M, Schreiber A, Classen N, Hensel A, Hempel G, Scholz W, Kühn J, Hrincius ER, Ludwig S. Hypericum perforatum and its ingredients hypericin and pseudohypericin demonstrate an antiviral activity against SARS-CoV-2. Pharmaceuticals (Basel). 2022;15:530.
17. Javid A, Motevalli Haghi N, Emami SA, Ansari A, Zojaji SA, Khoshkhui M, Ahanchian H. Short-course administration of a traditional herbal mixture ameliorates asthma symptoms of the common cold in children. Avicenna J Phytomed. 2019;9:126-133.
18. Martinelli M, Ummarino D, Giugliano FP, Sciorio E, Tortora C, Bruzzese D, De Giovanni D, Rutigliano I, Valenti S, Romano C, Campanozzi A, Miele E, Staiano A. Efficacy of a standardized extract of *Matricariae chamomilla* L., *Melissa officinalis* L. and tyndallized *Lactobacillus acidophilus* (HA122) in infantile colic: An open randomized controlled trial. Neurogastroenterol Motil. 2017;29.
19. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10.
20. Zeliou K, Kontaxis NI, Margianni E, Petrou C, Lamari FN. Optimized and validated HPLC analysis of St. John's wort extract and final products by simultaneous determination of major ingredients. J Chromatogr Sci. 2017;55:805-812.
21. Danihelová M, Veverka M, Sturdík E, Jantová S. Antioxidant action and cytotoxicity on HeLa and NIH-3T3 cells of new quercetin derivatives. Interdiscip Toxicol. 2013;6:209-216.
22. Attaran HR, Fatemi F, Rasooli A, Dadkhah A, Mohammadi Malayeri MR, Dini S. *Zataria multiflora* essential oil prevent iron oxide nanoparticles-induced liver toxicity in rat model. Journal of Medicinal plants and By-Products, 7, 15-24.
23. Dadkhah A, Fatemi F, Mohammadi Malayeri MR, Torabi F, Sarbazi M, Dini S. The hepatoprotective activity of caraway essential oil (*Carum carvi* L.) against iron oxide nanoparticles induced toxicity in Wistar rats. J Anim Res (Iranian Journal of Biology). 32, 188-197.
24. Fatemi F, Dini S, Rezaei MB, Dadkhah A, Dabbagh R, Naij S. The effect of γ-irradiation on the chemical composition and antioxidant activities of peppermint essential oil and extract. J Essent Oil Res. 26, 97-104.
25. Irandoust F, Dini S. A new perspective of aroma face mask on COVID-19 pandemic. J Med Eng Technol. 2022;46:198-208
26. Arceusz A, Wesolowski M, Ulewicz-Magulska B. Flavonoids and phenolic acids in methanolic extracts, infusions and tinctures from commercial samples of lemon balm. Nat Prod Commun. 2015;10:977-981.

27. Germ M, Stibilj V, Kreft S, Gaberščik A, Kreft I. Flavonoid, tannin and hypericin concentrations in the leaves of St. John's wort (*Hypericum perforatum* L.) are affected by UV-B radiation levels. Food Chem. 2010;122:471-474.

28. Qaderi MM, Martel AB, Strugnell CA. Environmental factors regulate plant secondary metabolites. Plants (Basel). 2023;12:447.

29. Aghakarim, Fahimeh, Hassan Sarikhani, and Ali Azizi. Effects of supplemental light quality at the end of day on herb production and some phytochemical properties of lemon balm (*Melissa officinalis* L.). Int J Hortic Sci. 2023;66-88.

30. Mohagheghzadeh A, Badr P, Mohagheghzadeh A, Hemmati S. *Hypericum perforatum* L. and the underlying molecular mechanisms for its choleretic, cholagogue, and regenerative properties. Pharmaceuticals. 2023;16,887.

31. Stojanović NM, Randjelović PJ, Mladenović MZ, Ilić IR, Petrović V, Stojiljković N, Ilić S, Radulović NS. Toxic essential oils, part VI: Acute oral toxicity of lemon balm (*Melissa officinalis* L.) essential oil in BALB/c mice. Food Chem Toxicol. 2019;133:110794.

32. Wang H, Chen Y, Wang L, Liu Q, Yang S, Wang C. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Front Pharmacol. 2023;14:1265178.

33. Xiuying P, Jianping L, Ruofeng S, Liye Z, Xuehong W, Yan L. Therapeutic efficacy of *Hypericum perforatum* L. extract for mice infected with an influenzaa virus. Can J Physiol Pharmacol. 2012;90:123-130.