Journal of Natural Product Research and Applications (JNPRA)

Home page : https://journals.univ-tlemcen.dz/JNPRA/index.php/JNPRA

Research Article

OPEN ACCESS

Comparative Phytochemical Profiling and HPLC-DAD Analysis of Aqueous Infusions from *Melissa officinalis*, *Matricaria chamomilla*, and *Passiflora incarnata* as Potential Sedative Agents

Sarra KADI [1]*, Meriem BELARBI [1], Chahrazed BELARBI [1], Selma Abou-Bekr[1], Imad EL HACI[1,2]

[1] Laboratory of Natural Products, Department of Agronomy, Faculty of Natural and Life Sciences, Abou Bekr Belkaïd University, Tlemcen, Algeria, 13000 Tlemcen, Algeria

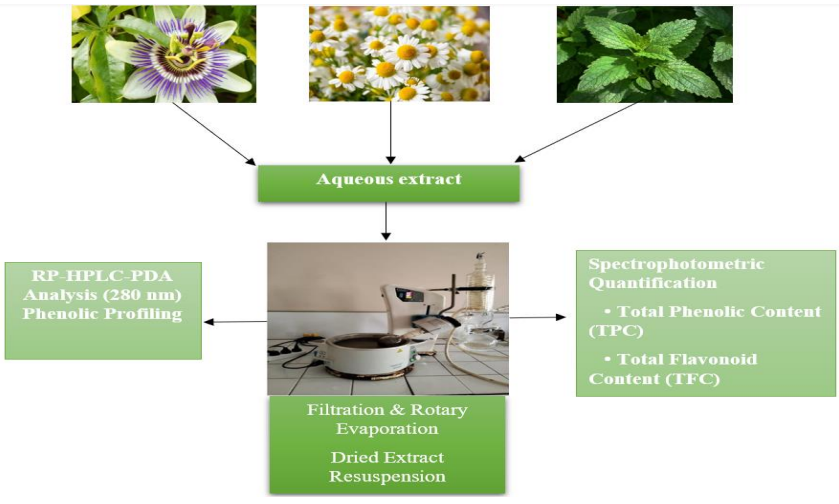
[2] Scientific and Technical Research Centre in Physico-Chemical Analysis (CRAPC), Bou-Ismaïl, 42004 Tipaza, Algeria

*Corresponding author: sarra-kadi@univ-tlemcen.dz; kadisarra36@gmail.com

Highlights

- Aqueous infusions analyzed by TPC/TFC and RP-HPLC-PDA (280 nm).
- *M. officinalis* showed highest TPC; rutin was the main peak.
- *M. chamomilla:* TFC 212.53 mg CE/g DM with several peaks.
- *P. incarnata*: TPC 93.72, TFC 49.11 mg/g; major p-coumaric acid peak.
- Phenolic profiles are complementary and may act together in infusions.

Graphical Abstract



Abstract

Sedative plants are increasingly considered natural alternatives to benzodiazepines, but variability in composition complicates standardization. We compared aqueous infusions of *Melissa officinalis* leaves, *Matricaria chamomilla* flowers, and *Passiflora incarnata* aerial parts. Total phenolics (TPC) and flavonoids (TFC) were determined by colorimetry (mg GAE/g DM; mg CE/g DM). Phenolics were profiled by RP-HPLC-PDA at 280 nm.

Melissa officinalis showed the highest TPC and TFC (372.41 ± 62.88 mg GAE/g DM; 824.72 ± 136.51 mg CE/g DM), with a chromatogram dominated by rutin. *Matricaria chamomilla* showed moderate TPC (97.81 ± 4.95 mg GAE/g DM) and higher TFC (212.53 ± 20.36 mg CE/g DM), with multiple medium peaks (gallic acid, chlorogenic acid, resorcinol, *p*-coumaric acid, rutin, naringenin, quercetin). *Passiflora incarnata* had TPC similar to chamomile (93.72 ± 6.32 mg GAE/g DM) but lower TFC (49.11 ± 0.00 mg CE/g DM), with a major p-coumaric acid peak and additional phenolics (gallic acid, chlorogenic acid, resorcinol, catechin, rutin, naringenin, quercetin).

These data show distinct yet complementary phenolic signatures and support combined use. Aqueous infusion extracts key polar phenolics relevant to sleep and anxiety with a favorable safety profile.

Keywords: *Melissa officinalis; Matricaria chamomilla; Passiflora incarnata*; Polyphenols; HPLC profiling; Sedation.

1. Introduction

Sleep quality is important for public health. Individuals suffering from chronic insomnia have a twofold higher risk of developing major depression compared to healthy subjects (Baglioni et al., 2011). In the short term, sleep disturbances impair stress reactivity, mood stability, and cognitive performance. Furthermore, long-term sleep deprivation and chronic activation of the sympathetic nervous system lead to major cardiovascular consequences, notably an increased prevalence of arterial hypertension (Medic et al., 2017).

Contemporary clinical practice limits the prolonged use of benzodiazepines due to their well-known side effects, which are particularly severe in older adults, including cognitive impairment, daytime somnolence, and an increased risk of falls. Clinical data indicate that herbal alternatives can be effective, making the chronic prescription of these synthetic molecules often avoidable (Markota et al., 2016).

Among these natural alternatives, Passionflower (*Passiflora incarnata* L.), Lemon balm (*Melissa officinalis* L.), and Chamomile (*Matricaria chamomilla* L.) are commonly used for sedation. While their anxiolytic and hypnotic effects have been validated by clinical trials(Zick et al., 2011; Miroddi et al., 2013; Soltanpour et al., 2019), the inherent variability in the concentration of their active constituents complicates the standardization of clinical extracts.

The primary objective of this study is to establish a comparative phytochemical characterization of these sedative plants. We quantified total phenolic and flavonoid fractions and described their RP-HPLC-PDA profiles. These analyses allow the identification of key biomarkers and help elucidate the biochemical complementarity that supports the synergistic use of this traditional triad for sleep improvement.

2. Materials and Methods

2.1. Plant material

The study was carried out at the Laboratory of Natural Products (LAPRONA). The investigation focused on the aerial parts of *Passiflora incarnata* L., the leaves of *Melissa officinalis* L. (sourced from a specialized supplier, imported), and the flowers of *Matricaria chamomilla* L. (from a local herbalist). Botanical identification was confirmed according to reference floras.

2.2. Preparation of aqueous extracts

Dried plant materials were ground into a fine powder. Extraction involved infusing 5 g of the plant powder in 50 mL of boiling distilled water for 10 minutes with stirring. After filtering through Whatman paper No. 5895 to remove solid residues, the filtrate was concentrated using a rotary evaporator. The resulting dry extracts were resuspended in distilled water to maintain metabolite stability during subsequent process assays.

2.3. Spectrophotometric assays

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965) and expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM). Total flavonoid content (TFC) was quantified using the aluminum chloride method(Zhishen et al., 1999) and expressed as milligrams of catechin equivalents per gram of dry matter (mg CE/g DM). All measurements were performed in triplicate to ensure analytical reproducibility.

2.4. HPLC analyses (High Performance Liquid Chromatography)

2.4.1. HPLC–PDA analysis

The reversed-phase High-Performance Liquid Chromatography with Photodiode Array Detection (RP-HPLC-PDA) analysis of phenolic compounds was performed on a Perkin Elmar Flexar system. The system was equipped with a binary pump delivery system and a C18 column (150 mm × 4.6 mm, 5 µm).

The mobile phase consisted of solvent A (2% Acetic acid in water) and solvent B (Acetonitrile). The chromatographic separation was achieved using the following gradient elution system:

1. 5 min at a constant concentration of 90% A;
2. 25 min at 10% A;
3. 15 min linear gradient from 90% to 100% B;
4. 20 min for column re-equilibration to initial conditions.

The flow rate was maintained at 1 mL/min. The chromatograms were monitored at 280 nm (Imad et al., 2020). Compound identification and peak assignments were accomplished by comparing their retention times, UV-Vis spectra, and co-elution with commercial standards under identical chromatographic conditions.

3. Results

3.1. Total Phenolic and Flavonoid Contents

The total phenolic content (TPC) and total flavonoid content (TFC) of the three plant species are presented in Table 1. The aqueous extract of M. officinalis leaves was characterized by the highest TPC (372.41 ± 62.88 mg GAE/g DM) but a moderate TFC (824.72 ± 136.51 mg CE/g DM). The *M. chamomilla* flower extract displayed a substantial TFC (212.53 ± 20.36 mg CE/g DM) while maintaining moderate phenolic levels (97.81 ± 4.95 mg GAE/g DM). The aerial parts of *P. incarnata* exhibited a TPC comparable to chamomile (93.72 ± 6.32 mg GAE/g DM), but a significantly lower TFC (49.11 ± 0.00 mg CE/g DM).

Table 1. Total phenolic and flavonoid contents of aqueous extracts.

Plant Species	Plant Organ	Total Phenolic Content (mg GAE/g DM)	Flavonoids (mg CE/g DM)
M. officinalis	Leaves	372.41 ± 62.88	824.72 ± 136.51
M. chamomilla	Flowers	97.81 ± 4.95	212.53 ± 20.36
P. incarnata	Aerial parts	93.72 ± 6.32	49.11 ± 0.00

Values represent the mean of three replicates ± standard deviation; DM: dry matter; GAE: gallic acid equivalents; CE: catechin equivalents.

3.2. Identification and Quantification of Phenolic Compounds by HPLC

HPLC–PDA analysis was performed (Fig. 1). Compounds were identified by comparing their retention times and spectral data at 280 nm with those of eight reference standards.

The chromatogram of the lemon balm extract (Fig. 1A) showed a profile dominated by a single major peak at approximately 13.5 min, identified as rutin, with an absorbance of about 350 mAU. Minor phenolic compounds were also detected, including gallic acid, chlorogenic acid, resorcinol, *p*-coumaric acid, and quercetin, eluting at approximately 28.5 min.

The chromatographic profile of the chamomile extract (Fig. 1B) was characterized by a distinct pattern with several moderate peaks. The identified compounds included gallic acid, chlorogenic acid, resorcinol, *p*-coumaric acid, rutin (≈ 13.5 min), naringenin (≈ 17.5 min), and quercetin. The most intense absorption was observed at around 25 min, with an absorbance of approximately 95 mAU.

The passionflower extract chromatogram (Fig. 1C) exhibited a profile dominated by a peak at approximately 12.5 min, corresponding to *p*-coumaric acid, with an absorbance of about 70 mAU. Minor phenolic compounds were also detected, including gallic acid, chlorogenic acid, resorcinol, catechin, rutin, naringenin, and quercetin, eluting at approximately 28.5 min.

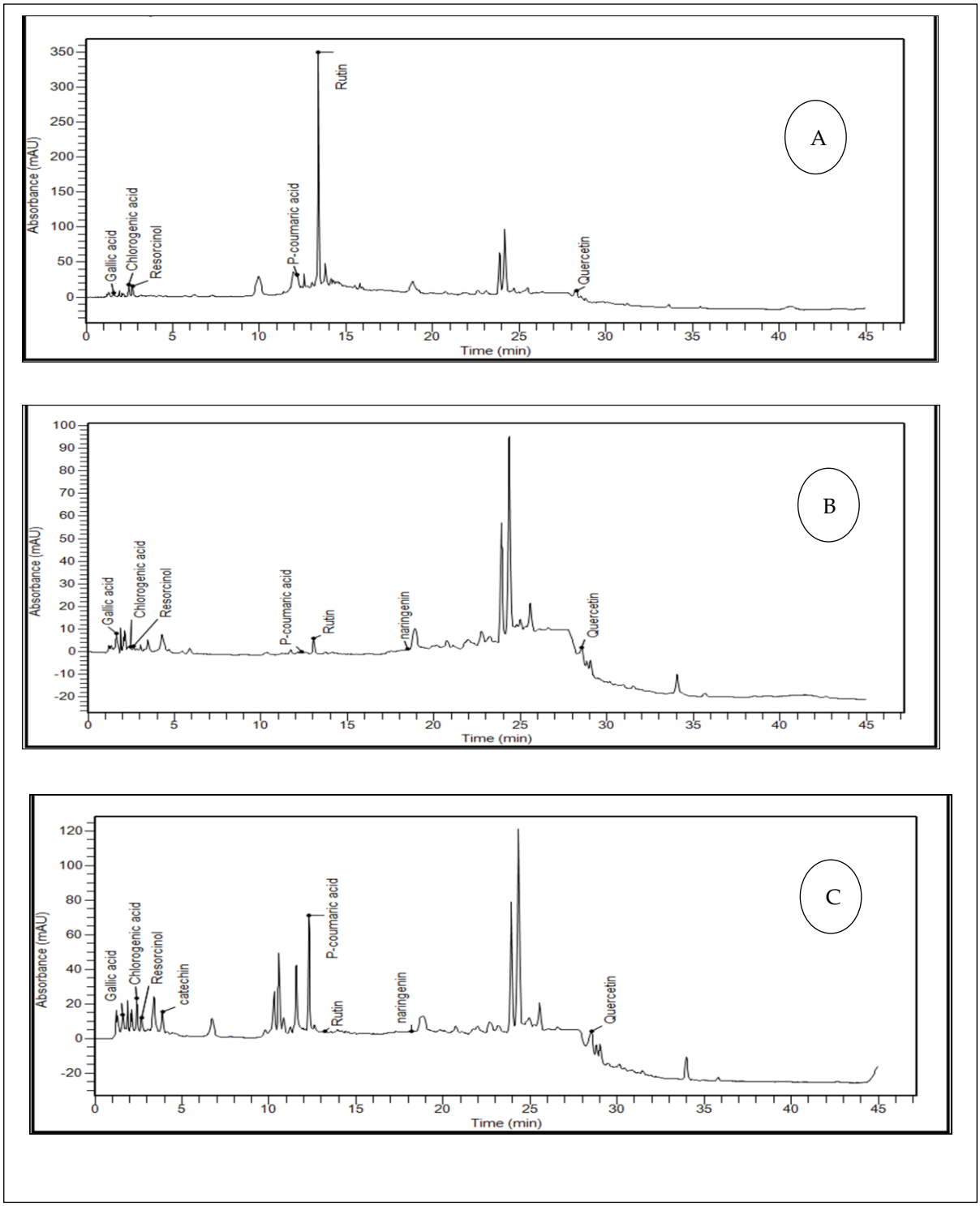


Figure 1. RP-HPLC-PDA chromatograms of aqueous extracts from the three sedative medicinal plants monitored at 280 nm. **A**: Profile of M. officinalis leaves, **B**: Profile of M. chamomilla flowers, **C**: Profile of P. incarnata aerial parts.

4. Discussion

4.1. Correlation Between Phytochemical Composition and Chromatographic Profiles

The integration of spectrophotometric assays and HPLC-DAD profiles reveals distinct phytochemical fingerprints for *Melissa officinalis*, *Matricaria chamomilla*, and *Passiflora incarnata*, underscoring their complementary roles in managing insomnia and anxiety.

4.1.1. Melissa officinalis: Phenolic Dominance and GABAergic Synergy

M. officinalis exhibited the most significant phenolic and flavonoid density, with values reaching 372.41 mg GAE/g DM and 824.72 mg CE/g DM, respectively. These results confirm its status as a superior source of bioactive polyphenols (Stini et al., 2024). Although the HPLC profile at 280 nm (Fig. 1(A)) is dominated by rutin (≈ 350 mAU), the presence of rosmarinic acid—a hallmark of the Lamiaceae family— remains a key therapeutic contributor. The underrepresentation of rosmarinic acid in this specific chromatogram is likely due to its absorption maxima (320–330 nm), which fall outside the 280 nm detection window. From a pharmacological perspective, rosmarinic acid offers promising sedative potential (Priya et al., 2025), while rutin has been shown to exert anxiolytic effects comparable to diazepam via systemic or intra-amygdala pathways (Hernandez-Leon et al., 2017). This synergy between GABAergic modulation and neuroprotection validates the traditional use of lemon balm as a potent sedative.

4.1.2. Matricaria chamomilla: Targeted Action on Sleep Homeostasis

Chamomile displayed a substantial flavonoid concentration (212.53 mg CE/g DM) alongside moderate phenolic levels (97.81 mg GAE/g DM). These values exceed those previously reported for decoctions (Bekhti et al., 2022), suggesting that a 100°C infusion protocol optimizes the extraction of heat-stable polyphenols. The efficacy of *M. chamomilla* in promoting sleep onset is primarily driven by apigenin. This flavonoid acts as a high-affinity ligand for GABA-A receptors, directly modulating sleep homeostasis (Kramer & Johnson, 2024). Additionally, the presence of chlorogenic acid (Fig. 1(B)) provides an indirect sedative benefit. By mitigating oxidative stress and anxiety-driven hyperarousal, chlorogenic acid helps lower the physiological barriers to sleep (Valcheva-Kuzmanova et al., 2015), creating a balanced sedative effect.

4.1.3. Passiflora incarnata: Phytochemical Diversity and Synergistic Balance

Despite exhibiting the lowest quantitative phenolic (93.72 mg GAE/g DM) and flavonoid (49.11 mg CE/g DM) levels, *P. incarnata* presented the most diversified chromatographic profile (Fig. 1(C)). The HPLC analysis identified a complex array of compounds, including gallic, chlorogenic, and p-coumaric acids, as well as catechin, rutin, naringenin, and quercetin. This structural variety is consistent with the findings of Appel et al. (2011), who emphasized that passionflower's sedative efficacy stems from a multi-target "entourage effect" rather than a single molecule. Specifically, p-coumaric acid reduces neuronal oxidative stress and modulates the GABAergic system (Pei et al., 2016), while catechin further bolsters neuroprotective defenses (Godos et al., 2020). This synergistic mechanism provides a gentle therapeutic approach, making *Passiflora* particularly effective for long-term stress management and sleep quality improvement.

4.2. Therapeutic Implications and Complementarity of the Three Species

The three plants investigated differ markedly in their phytochemical composition, and this variation helps explain their complementary therapeutic effects. *Melissa officinalis* contains high levels of rosmarinic acid and rutin, compounds associated with neuroprotective activity and non-benzodiazepine GABAergic modulation (Hernandez-Leon et al., 2017; Priya et al., 2025). *Matricaria chamomilla* acts more directly on sleep regulation due to its apigenin content, which has documented affinity for GABA-A receptors and has been linked to reduced sleep latency and improved sleep continuity (Zick et al., 2011; Kramer & Johnson, 2024). *Passiflora incarnata* relies on a broader phenolic profile; rather than acting on a single receptor type, its combined phenolic constituents contribute to reducing the state of anxiety-related hyperarousal often associated with sleep disturbances (Appel et al., 2011; Miroddi et al., 2013).

When prepared as an aqueous infusion, the three plants act through distinct but complementary pathways: lemon balm provides antioxidant and GABAergic support, chamomile promotes sleep onset through GABA-A modulation, and passionflower contributes to reducing the underlying anxious state (Markota et al., 2016; Soltanpour et al., 2019). These combined effects are consistent with the use of polyherbal preparations in traditional practice, and the present chemical data offer a rationale for this association.

5. Conclusions

This study establishes distinct yet complementary phytochemical signatures in aqueous infusions of *Melissa officinalis*, *Matricaria chamomilla*, and *Passiflora incarnata*. *Melissa officinalis* combines the highest totals of phenolic and flavonoid compounds with a rutin-dominant chromatogram at 280 nm. *Matricaria chamomilla* exhibits elevated flavonoids and several medium-intensity phenolic peaks, while *Passiflora incarnata* shows lower totals but a profile characterized by a major p-coumaric acid peak accompanied by additional phenolics. Taken together, these data support a multi-compound synergistic model, where phenolic acids, flavonols, and flavones provide complementary contributions relevant to sedative and anxiolytic potential. Aqueous infusion emerges as a selective process for recovering polar phenolics of interest and offers a practical foundation for standardized, synergy-driven formulations without reliance on a single dominant biomarker.

6. Patents

The authors declare that no patents have been filed, are pending, or have been granted that arise directly from the results reported in this manuscript.

Acknowledgement

The authors gratefully acknowledge the Directorate General for Scientific Research and Technological Development (DGRSTDT).

Author Contribution Statement

Conceptualization: Meriem BELARBI, Sarra KADI, Selma Abou-Bekr

Methodology: Meriem BELARBI, Sarra KADI, Selma Abou-Bekr

Validation: Meriem BELARBI

Formal analysis: Chahrazed BELARBI, Sarra KADI, Selma Abou-Bekr

Investigation: Sarra KADI, Selma Abou-Bekr, Chahrazed BELARBI

Resources: Meriem BELARBI, Imad El HACI
Data curation: Sarra KADI
Writing – original draft: Sarra KADI
Writing – review & editing: Meriem BELARBI
Visualization: Sarra KADI

Conflict of interest

The authors declare no competing financial or non-financial interests relevant to the content of this article.

ORCID

Sarra KADI https://orcid.org/0009-0004-3465-2960
Meriem BELARBI: https://orcid.org/0000-0002-1804-7603

References

Appel, K., Rose, T., Fiebich, B., Kammler, T., Hoffmann, C., & Weiss, G. (2011). Modulation of the γ-aminobutyric acid (GABA) system by Passiflora incarnata L. *Phytotherapy Research: PTR*, 25(6), 838-843. https://doi.org/10.1002/ptr.3352

Baglioni, C., Battagliese, G., Feige, B., Spiegelhalder, K., Nissen, C., Voderholzer, U., Lombardo, C., & Riemann, D. (2011). Insomnia as a predictor of depression : A meta analytic evaluation of longitudinal epidemiological studies. *Journal of Affective Disorders*, 135(1), 10-19. https://doi.org/10.1016/j.jad.2011.01.011

Bekhti, N., Fedoul, F., Fouzia, M., Merazi, Y., Piras, A., & Wafa, A. (2022). Phenolic profile and biological activities of extracts of *Matricaria chamomilla L*. from the Western Algeria. *Acta Periodica Technologica*, 48-62. https://doi.org/10.2298/APT2253048B

Godos, J., Ferri, R., Castellano, S., Angelino, D., Mena, P., Del Rio, D., Caraci, F., Galvano, F., & Grosso, G. (2020). Specific Dietary (Poly)phenols Are Associated with Sleep Quality in a Cohort of Italian Adults. *Nutrients*, 12(5), 1226. https://doi.org/10.3390/nu12051226

Hernandez-Leon, A., González-Trujano, M. E., & Fernández-Guasti, A. (2017). The anxiolytic-like effect of rutin in rats involves GABAA receptors in the basolateral amygdala. *Behavioural Pharmacology*, 28(4), 303-312. https://doi.org/10.1097/FBP.0000000000000290

Imad, E. H., Mazari, W., Bekkara, F., Mouttas-Bendimerad, F., & Hassani, F. (2020). Bioactive Compounds from the Flower Part of Polygonum maritimum L. Collected from Algerian Coast. *Current Bioactive Compounds*, 16(4), 543–545. https://doi.org/10.2174/1573407214666181116120901

Kramer, D. J., & Johnson, A. A. (2024). Apigenin : A natural molecule at the intersection of sleep and aging. *Frontiers in Nutrition*, 11, 1359176. https://doi.org/10.3389/fnut.2024.1359176

Markota, M., Rummans, T. A., Bostwick, J. M., & Lapid, M. I. (2016). Benzodiazepine Use in Older Adults : Dangers, Management, and Alternative Therapies. *Mayo Clinic Proceedings*, 91(11), 1632-1639. https://doi.org/10.1016/j.mayocp.2016.07.024

Medic, G., Wille, M., & Hemels, M. (2017). Short- and long-term health consequences of sleep disruption. *Nature and Science of Sleep*, 9, 151–161. https://doi.org/10.2147/NSS.S134864

Miroddi, M., Calapai, G., Navarra, M., Minciullo, P. L., & Gangemi, S. (2013). *Passiflora incarnata* L. : Ethnopharmacology, clinical application, safety and evaluation of clinical trials. *Journal of Ethnopharmacology*, 150(3), 791-804. https://doi.org/10.1016/j.jep.2013.09.047

Pei, K., Ou, J., Huang, J., & Ou, S. (2016). p-Coumaric acid and its conjugates : Dietary sources, pharmacokinetic properties and biological activities. *Journal of the Science of Food and Agriculture*, 96(9), 2952-2962. https://doi.org/10.1002/jsfa.7578

Priya, V., Srinivasan, D., Priyadarsini, S., Dabaghzadeh, F., Rana, S. S., Chengaiyan, J. G., Sudesh, R., & Ahmad, F. (2025). Anxiolytic, Antidepressant and Healthy Sleep-Promoting Potential of Rosmarinic Acid : Mechanisms and Molecular Targets. *Neuropsychiatric Disease and Treatment*, 21, 641-661. https://doi.org/10.2147/NDT.S501597

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16(3), 144-158. https://doi.org/10.5344/ajev.1965.16.3.144

Soltanpour, A., Alijaniha, F., Naseri, M., Kazemnejad, A., & Heidari, M. R. (2019). Effects of *Melissa officinalis* on anxiety and sleep quality in patients undergoing coronary artery bypass surgery : A double-blind randomized placebo controlled trial. *European Journal of Integrative Medicine*, 28, 27-32. https://doi.org/10.1016/j.eujim.2019.01.010

Stini, E., Tsimogiannis, D., & Oreopoulou, V. (2024). The Valorisation of Melissa officinalis Distillation By-Products for the Production of Polyphenol-Rich Formulations. *Molecules*, 29(2), 377. https://doi.org/10.3390/molecules29020377

Valcheva-Kuzmanova, S., Georgieva, A., Belcheva, I., & Tashev, R. (2015). Anxiolytic-like effect of chlorogenic acid administered subchronically to rats. *Comptes rendus de l'Académie bulgare des sciences: sciences mathématiques et naturelles*, 68, 1463-1470.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2

Zick, S. M., Wright, B. D., Sen, A., & Arnedt, J. T. (2011). Preliminary examination of the efficacy and safety of a standardized chamomile extract for chronic primary insomnia : A randomized placebo-controlled pilot study. *BMC Complementary and Alternative Medicine*, 11, 78. https://doi.org/10.1186/1472-6882-11-78