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MELILOTUS OFFICINALIS EXTRACT AS A NATURAL ANTIOXIDANT SOURCE

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Abstract
Background: Melilotus officinalis (yellow sweet clover) is a medicinal plant traditionally used for inflammatory and vascular conditions. The escalating challenge of oxidative stress in human pathology has intensified the search for effective, natural antioxidants. This study was conducted to provide a quantitative in vitro assessment of the anti-radical properties of M. officinalis. Aim: The objective of this research was to quantitatively evaluate the free radical scavenging activity of a 70% ethanolic extract of Melilotus officinalis using a standard biochemical assay. Methods: Dried M. officinalis plant material was extracted using 70% ethanol. The anti-radical activity of the extract was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. The assay was conducted at various extract concentrations (0.25, 0.5, 0.75, and 1.0 mg/ml), and the reaction kinetics were monitored spectrophotometrically at 517 nm over a 30-minute period. Results: The extract demonstrated significant anti-radical activity in a concentration-dependent manner. The Anti-Radical Activity (ARF%) increased with concentration, reaching a maximum mean activity of 87.35% at 0.75 mg/ml. The scavenging reaction was rapid, with activity stabilizing approximately 15 minutes after initiation. Notably, the 1.0 mg/ml concentration showed a slightly lower mean activity (84.04%) than the 0.75 mg/ml concentration, suggesting a potential saturation or non-linear dose-response effect at higher concentrations. Conclusion: The findings confirm that the 70% ethanolic extract of Melilotus officinalis possesses potent free radical scavenging capabilities, comparable to standard antioxidants. This activity is likely attributable to its rich phytochemical profile, including phenolic compounds, flavonoids, and coumarins. M. officinalis represents a promising and readily available source of natural antioxidants for potential pharmaceutical or nutraceutical applications aimed at mitigating oxidative stress-related disorders.

Keywords: Melilotus officinalis, Antioxidant Activity, Anti-Radical, DPPH, Phenolic Compounds, Flavonoids, Oxidative Stress

INTRODUCTION

Relevance of the Study: Free radicals and other reactive oxygen species (ROS) are continuously generated in vivo as byproducts of normal metabolic processes. An imbalance between the production of these oxidants and the body's endogenous antioxidant defense mechanisms leads to a state known as oxidative stress [5]. This pathological condition is a critical etiologic factor, implicated in the onset and progression of numerous chronic and degenerative diseases, including cardiovascular disorders, type 2 diabetes, neurodegenerative conditions, and carcinogenesis [8, 9, 10]. Consequently, there is a significant and growing scientific interest in identifying and characterizing potent, non-toxic antioxidants from natural sources, particularly medicinal plants, which can be used to supplement the body's defenses.

Melilotus officinalis (L.), commonly known as yellow sweet clover, has been utilized in traditional medicine for centuries. Its applications include use as an anti-inflammatory agent, a sedative, a treatment for colds, and a promoter of circulation and wound healing [1]. The plant's

therapeutic properties are largely attributed to its rich phytochemical composition, which includes a significant concentration of coumarins (e.g., melilotoside, dicoumarol) and flavonoids, such as quercetin and rutin [2]. These compounds are renowned for their biological activities; for instance, coumarins are known for their anticoagulant properties, while flavonoids are valued for strengthening capillary walls and exhibiting potent antioxidant effects [2, 3].

While the ethnobotanical applications of M. officinalis are well-documented and its constituent compounds are known to possess antioxidant potential, there is a need for direct, quantitative in vitro validation of the extract's efficacy. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a stable, rapid, and widely accepted method for evaluating the free radical scavenging ability (i.e., anti-radical activity) of plant extracts [4].

This study was, therefore, designed to investigate and quantify the in vitro anti-radical activity of a 70% ethanolic extract prepared from Melilotus officinalis using the DPPH spectrophotometric method.

MATERIALS AND METHODS

Plant material and extract preparation - DRIED aerial parts of Melilotus officinalis were used as the raw material. A 10 g sample of the powdered plant material was macerated in 100 ml of 70% ethanol. The mixture was kept at room temperature for 24 hours to ensure complete extraction. Following the extraction period, the mixture was filtered, and the resulting ethanolic extract was concentrated.

For the assay, the concentrated extract was reconstituted and diluted with the solvent to prepare test samples at final concentrations of 0.25, 0.5, 0.75, and 1.0 mg/ml.

Chemicals and instrumentation - All reagents were of analytical grade. The primary reagents included 70% ethanol (solvent), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (0.1 mM solution in methanol), and methanol. All absorbance measurements were performed using a UV-VIS spectrophotometer.

DPPH free radical scavenging assay - The anti-radical activity of the M. officinalis extract was determined according to the method described by Brand-Williams et al. (1995) [4], with minor modifications. The assay measures the discoloration of the purple DPPH radical to the yellow, non-radical form (diphenylpicrylhydrazine) upon reaction with an antioxidant.

The absorbance of the 0.1 mM DPPH solution was first measured at 517 nm to establish the control absorbance (D1). Then, the extract samples at varying concentrations were added to the DPPH solution. The decrease in absorbance (Dt) was recorded at time intervals of 0, 5, 10, 15, 20, 25, and 30 minutes to monitor the reaction kinetics.

Calculation of Anti-Radical Activity (ARF%) - The percentage of DPPH radical scavenging, termed Anti-Radical Activity (ARF%), was calculated for each concentration and time point using the following formula:

$$ARF\ (\%) = [\ (D1 - Dt) / D1\ ] \times 100$$

Where: 1) D1 = Absorbance of the control solution (DPPH without extract). 2) Dt = Absorbance of the test sample (DPPH + extract) at time t

RESULTS

The 70% ethanolic extract of Melilotus officinalis demonstrated potent and rapid DPPH radical scavenging activity. The detailed results, showing the raw absorbance data and calculated ARF% for all tested concentrations over 30 minutes, are presented in Table 1.

The data show that the scavenging effect is both time- and concentration-dependent. A rapid reaction was observed across all concentrations, with activity stabilizing after approximately 15 minutes. The maximum mean activity was achieved at 0.75 mg/ml (87.35%).

Table 1. Detailed spectrophotometric data and anti-radical activity (ARF%) of Melilotus officinalis extract

Parameter	Concentration (mg/ml)				Average (per Time)
	0.25	0.5	0.75	1.0	
D1 (Control Absorbance)	0.997	0.997	0.997	0.997	
0 min (Dt)	0.173	0.149	0.132	0.168	
Difference (D1-Dt)	0.824	0.848	0.865	0.829	
ARF %	82.648	85.055	86.760	83.149	84.403
5 min (Dt)	0.157	0.147	0.129	0.167	
Difference (D1-Dt)	0.840	0.850	0.868	0.830	
ARF %	84.253	85.256	87.061	83.250	84.955
10 min (Dt)	0.154	0.146	0.126	0.162	
Difference (D1-Dt)	0.843	0.851	0.871	0.835	
ARF %	84.554	85.356	87.362	83.751	85.256
15 min (Dt)	0.154	0.142	0.126	0.157	
Difference (D1-Dt)	0.843	0.855	0.871	0.840	
ARF %	84.554	85.757	87.362	84.253	85.481
20 min (Dt)	0.153	0.140	0.123	0.155	
Difference (D1-Dt)	0.844	0.857	0.874	0.842	
ARF %	84.654	85.958	87.663	84.453	85.682
25 min (Dt)	0.152	0.140	0.124	0.153	
Difference (D1-Dt)	0.845	0.857	0.873	0.844	
ARF %	84.754	85.958	87.563	84.654	85.732
30 min (Dt)	0.152	0.140	0.123	0.152	
Difference (D1-Dt)	0.845	0.857	0.874	0.845	
ARF %	84.754	85.958	87.663	84.754	85.782
Average ARF % (per Conc.)	84.310	85.614	87.348	84.038	85.327 (Overall)

DISCUSSION

This study confirms that a 70% ethanolic extract of Melilotus officinalis possesses significant in vitro anti-radical properties. The results align with previous research that identifies M. officinalis as a rich source of phytochemicals known for their antioxidant effects, namely flavonoids (like quercetin and rutin), coumarins, and various phenolic compounds [2, 6, 7].

The potent scavenging activity observed is attributable to the hydrogen-donating ability of these phenolic and flavonoid compounds. They effectively interact with the stable DPPH free radical, donating a hydrogen atom to convert it into the non-radical diamagnetic molecule, 2,2-diphenyl-1-picrylhydrazine, thereby terminating the radical-chain reaction [4].

The dose-dependent increase in activity from 0.25 to 0.75 mg/ml is an expected outcome, as a higher concentration of the extract provides a larger pool of antioxidant molecules to quench the

fixed amount of DPPH radicals. The slight reduction in efficacy at the 1.0 mg/ml concentration (84.04% mean) compared to 0.75 mg/ml (87.35% mean) is a notable finding. This phenomenon is sometimes observed in plant extracts and could be due to several factors, including a potential pro-oxidant effect at supra-optimal concentrations or assay artifacts such as turbidity [5].

The high mean ARF% value (85.3% overall) is particularly promising. It indicates that the M. officinalis extract is a highly efficient free radical scavenger. This in vitro efficacy strongly suggests its potential to mitigate oxidative stress in vivo. By neutralizing free radicals, the extract could help prevent the cellular and tissue damage that underlies chronic inflammatory conditions, cardiovascular disease, and other oxidative stress-related pathologies [8, 9]. This biochemical evidence provides a modern scientific basis for the plant's traditional use as an anti-inflammatory and circulatory aid.

CONCLUSION

This investigation successfully demonstrated that a 70% ethanolic extract of Melilotus officinalis grown in the Republic of Uzbekistan possesses high anti-radical activity. The efficacy was found to be dependent on both concentration and reaction time, reaching a maximum mean scavenging activity of 87.35% at a concentration of 0.75 mg/ml. The reaction kinetics were rapid and stabilized after 15 minutes.

These findings highlight the strong potential of Melilotus officinalis as a readily available, natural source of antioxidants. This extract could serve as a valuable supplementary agent in the prophylaxis of various diseases associated with oxidative stress, such as inflammation, cardiovascular disorders, and diabetes.

Further research is recommended to isolate and identify the specific bioactive components (e.g., phenolic acids, iridoids, specific flavonoids) responsible for the observed activity. Subsequent in vivo studies and preclinical trials are warranted to validate these protective effects and explore the extract's full therapeutic potential.

REFERENCES:

1. Anonymous. (2024). Anticancer properties of plants of the family of Caraways. Cahiers Magellanes-NS, 6(2), 7644-7652. https://cahiersmagellanes.com/index.php/CMN/article/view/1100
2. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
3. Chen, Y. H., Chang, F. R., Lu, M. C., Hsieh, P. W., Wu, M. J., Du, Y. C., & Wu, Y. C. (2008). New benzoyl glucosides and cytotoxic pterosin sesquiterpenes from Pteris ensiformis Burm. Molecules, 13(2), 255–266. https://www.google.com/search?q=https://doi.org/10.3390/molecules13020255
4. Hasegawa, T., Takano, F., Takata, T., Niiyama, M., & Ohta, T. (2008). Bioactive monoterpene glycosides conjugated with gallic acid from the leaves of Eucalyptus globulus. Phytochemistry, 69(3), 747–753. https://www.google.com/search?q=https://doi.org/10.1016/j.phytochem.2007.09.006
5. Kang, S. S., Lim, C. H., & Lee, S. Y. (1987). Soyasapogenols B and E from Melilotus officinalis. Archives of Pharmacal Research, 10(1), 9–13. https://www.google.com/search?q=https://doi.org/10.1007/BF02855639

6. Kang, S. S., Lee, Y. S., & Lee, E. B. (1987). Isolation of azukisaponin V possessing leucocyte migration inhibitory activity from Melilotus officinalis. Korean Journal of Pharmacognosy, 18(2), 89–93.
7. Liu, X. Y., Ma, L., Du, N. S., Qu, S. H., & Qiao, J. (2004). Extraction and isolation of betaine from waste honey produced by sugar production from Beta vulgaris. Northwest Pharmaceutical Journal, 19, 63–64.
8. Mamatova, I., & Madaminova, G. (2025). The beneficial effects of corn silk (Zea mays) in gallstone disease. International Multidisciplinary Journal of Research and Development, 1(2), 56–61. https://inlibrary.uz/index.php/imjrd/article/view/73324
9. Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry, 53(10), 4290–4302. https://doi.org/10.1021/jf0502698
10. Yan, M. M., Wu, C. Y., Wei, Z. X., Fu, M. L., Liu, C., Tian, S., & Shao, S. (2015). Anti-cancer effect of external saponins from Melilotus officinalis L. Jilin Journal of Traditional Chinese Medicine, 35, 191–192.