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Comparative evaluation of the antioxidant activity of mixed extracts of Achillea filipendulina and Salvia sclarea by the DPPH radical scavenging assay

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Сравнительная оценка антиоксидантной активности смешанных экстрактов Achillea filipendulina и Salvia sclarea методом DPPH

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Abstract

Mixed extracts of Achillea filipendulina Lam. and Salvia sclarea L. prepared at ratios of 1:1, 3:1, and 1:3 were evaluated for activity using the DPPH assay. The study examined effects of extraction solvent and plant ratio on antiradical activity. The mixtures were extracted with 96 % ethanol, 70 % ethanol, and water. One gram of plant material was extracted with 25 mL of solvent in an ultrasonic bath for 20 min, filtered through a 0.45 μm syringe filter, and diluted tenfold. Activity was determined spectrophotometrically at 517 nm for 30 min. At 100 μL, the highest activity was observed for the 1:3 mixture extracted with 70 % ethanol, reaching 42.64 %. Values for the 3:1 and 1:1 mixtures were 37.92 % and 34.55 %, respectively. The calculated IC50 value of the most active sample was 119.2 μL. Results demonstrate dependence on solvent composition and plant ratio, with the 1:3 mixture in 70 % ethanol showing the effect.

Аннотация

Проведена сравнительная оценка антирадикальной активности смешанных экстрактов Achillea filipendulina Lam. и Salvia sclarea L. в соотношениях 1:1, 3:1 и 1:3 методом DPPH. Цель исследования заключалась в определении влияния экстрагента и соотношения растительных компонентов на антирадикальную активность. Для экстракции использовали 96 %-ный этанол, 70 %-ный этанол и воду. Один грамм растительного материала экстрагировали 25 мл растворителя в ультразвуковой ванне в течение 20 мин, фильтровали через шприцевой фильтр 0,45 мкм и разбавляли в десять раз. Антирадикальную активность определяли спектрофотометрически при 517 нм в течение 30 мин. При объеме образца 100 мкл максимальная активность установлена для 70 %-ного этанольного экстракта смеси 1:3 и составила 42,64 %. Для смесей 3:1 и 1:1 значения составили соответственно 37,92 и 34,55 %. Расчетное значение IC50 наиболее активного образца составило около 119,2 мкл. Результаты показывают зависимость активности от природы экстрагента и соотношения компонентов. Наиболее эффективным среди исследованных вариантов оказался 70 %-ный этанольный экстракт смеси растений в соотношении 1:3.

Keywords: Achillea filipendulina, Salvia sclarea, DPPH, antioxidant activity, antiradical activity, ethanol extraction, IC50.

Ключевые слова: Achillea filipendulina, Salvia sclarea, DPPH, антиоксидантная активность, антирадикальная активность, этанольный экстракт, IC50.

Introduction

Oxidative processes are closely associated with the formation of reactive species, and plant phenolics are widely investigated as natural radical scavengers. The DPPH assay remains one of the most frequently used spectrophotometric approaches for screening hydrogen- or electron-donating capacity because the reduction of the stable violet DPPH radical can be monitored as a decrease in absorbance [1–3]. Methodological variables, including solvent, reaction time and sample concentration, can substantially influence the measured antioxidant response [3].

Achillea filipendulina L. is a medicinal species rich in phenolic acids, flavonoids and other bioactive metabolites. Recent phytochemical studies confirm substantial variation in its antioxidant activity depending on plant part, morphotype and extraction conditions [4–8]. Salvia sclarea L. also contains antioxidant constituents; recent studies have demonstrated that its biological activity depends strongly on extraction procedure and solvent system [9–11]. These findings make solvent selection particularly important when plant mixtures rather than single species are evaluated.

The objective of the present work was to compare the DPPH radical-scavenging activity of Achillea filipendulina–Salvia sclarea mixtures at 1:1, 3:1 and 1:3 ratios after extraction with water, 70 % ethanol and 96 % ethanol, and to identify the combination showing the most favorable antiradical response under the experimental conditions.

Materials and Methods

A DPPH working solution (7.92 mM) was prepared in ethanol in a 100 mL volumetric flask, protected from light with aluminum foil and kept for 30 min at room temperature. Mixed Achillea filipendulina and Salvia sclarea samples were prepared at 1:1, 3:1 and 1:3 ratios. For each extraction, 1 g of plant sample was treated with 25 mL of 96 % ethanol, 70 % ethanol or water in an ultrasonic bath for 20 min. The extracts were passed through 0.45 μm syringe filters and diluted tenfold before analysis.

For the blank, 3.0 mL DPPH solution and 100 μL ethanol were placed in a 4 mL quartz cuvette. Absorbance was measured at 517 nm with a K7000 spectrophotometer (YOKE, China) every 5 min for 30 min. For test samples, 25, 50, 75 or 100 μL extract was mixed with 3.0 mL DPPH solution, and ethanol was added to a total volume of 3.1 mL. Antiradical activity (ARF, %) was calculated as $ARF = [(A0 - As)/A0] \times 100$, where A0 is the blank absorbance and As is the sample absorbance.

Apparent IC50 values were estimated from the linear relationship between extract volume and ARF measured at 30 min. For a regression $y = mx + b$, the extract volume corresponding to 50 % inhibition was calculated as $IC50 = (50 - b)/m$. Because the tested range did not reach 50 % inhibition, these IC50 values are extrapolated estimates and should be interpreted as comparative indicators rather than directly observed endpoints.

Results and Discussion

All tested extracts reduced DPPH absorbance over the 30 min reaction period, and the magnitude of the response generally increased with extract volume. This concentration- and time-dependent behavior is consistent with the established DPPH mechanism, in which antioxidant molecules donate hydrogen atoms or electrons to the stable radical [1–3]. The clearest separation among the samples was observed at 100 µL after 30 min.

Table 1. DPPH antiradical activity and apparent IC50 of the mixed extracts

Solvent	A. filipendulina:S. sclarea	ARF at 100 µL, 30 min (%)	Apparent IC50 (µL)
96% EtOH	1:1	28.01	174.7
96% EtOH	3:1	20.50	244.1
96% EtOH	1:3	26.47	188.5
70% EtOH	1:1	34.55	143.8
70% EtOH	3:1	37.92	127.6
70% EtOH	1:3	42.64	119.2
Water	1:1	26.56	185.0
Water	3:1	24.06	206.5
Water	1:3	33.21	147.5

The 70 % ethanolic series was the most active group. At 100 µL, ARF reached 34.55 % for the 1:1 mixture, 37.92 % for 3:1 and 42.64 % for 1:3. In comparison, the corresponding 96 % ethanolic extracts produced 28.01, 20.50 and 26.47 %, while the aqueous extracts produced 26.56, 24.06 and 33.21 %, respectively. Thus, the 1:3 mixture in 70 % ethanol exceeded the corresponding 96 % ethanol and water extracts by 16.17 and 9.43 percentage points, respectively.

The regression-derived apparent IC50 values supported the same ranking. The lowest value was obtained for the 1:3 mixture in 70 % ethanol (119.2 µL), followed by the 3:1 70 % ethanolic extract (127.6 µL) and the 1:1 70 % ethanolic extract (143.8 µL). The 3:1 mixture extracted with 96 % ethanol showed the highest estimated IC50 (244.1 µL), indicating the weakest response in this comparison. Importantly, none of the measured samples reached 50 % inhibition within the tested 25–100 µL range; consequently, the IC50 values are extrapolations from highly linear regressions and should be verified experimentally at higher sample volumes or concentrations.

The superior performance of 70 % ethanol is chemically plausible because aqueous ethanol provides intermediate polarity and can extract a broader spectrum of phenolic constituents than highly concentrated ethanol or water alone. A recent solvent-comparison study reported maximal flavonoid recovery and strong DPPH activity around 70 % ethanol in plant matrices [12]. Studies on A. filipendulina likewise show that antioxidant performance varies with solvent and phenolic composition [4–8]. For S. sclarea, different extraction techniques and solvent systems also produce marked differences in DPPH activity and phenolic yield [9–11]. Therefore, the present solvent-dependent pattern is consistent with current international evidence, although direct chemical profiling of the mixed extracts would be required to identify the compounds responsible.

The influence of plant ratio was also solvent-dependent. Within the 70 % ethanol and water series, enrichment with S. sclarea (1:3) produced the highest ARF. However, the 96 % ethanol series did not show the same monotonic ratio effect, indicating that mixture composition and solvent polarity interact. The data therefore do not by themselves prove synergism between the two plants; demonstration of synergy would require comparison with extracts of each individual species at matched concentrations and an appropriate combination-effect model. The present results instead support a narrower conclusion: among the tested mixed systems, the 1:3 mixture extracted with 70 % ethanol showed the strongest DPPH-scavenging response.

Conclusion

Mixed extracts of Achillea filipendulina and Salvia sclarea displayed measurable DPPH radical-scavenging activity that depended on both extraction solvent and plant ratio. The best result within the tested conditions was obtained with the 1:3 mixture extracted with 70 % ethanol, which reached 42.64 % ARF at 100 μL after 30 min and gave the lowest extrapolated IC50 (approximately 119.2 μL). The 70 % ethanolic extracts were consistently more active than the corresponding 96 % ethanolic extracts and, for the most active 1:3 ratio, also outperformed the aqueous extract. These findings identify 70 % ethanol as the most promising of the tested solvents for this herbal combination. Future work should directly measure IC50 within an expanded concentration range and determine total phenolic/flavonoid contents and chromatographic profiles to clarify the chemical basis of the observed activity.

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