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Research article

Formulation of Fibrauren tinctoria Lour. Extract Serum, As an Antioxidant with the 1,1-Diphenyl 2-Picryhydrazyl (DPPH) Method

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ABSTRACT

Background: Serum is a preparation with a high concentration of active ingredients and low viscosity, which delivers a thin film of active ingredients to the skin surface. The use of Fibraurea tinctoria has been carried out since ancient times by the Dayak, Banjar, and Kutai tribes as a traditional treatment for liver disease, diabetes, and to increase the body's immunity from various causes of disease such as bacteria. Fibraurea tinctoria is believed to be able to ward off the negative effects caused by free radicals such as antioxidants.

Methods: This experimental study investigated the antioxidant activity of Fibraurea tinctoria extract serum with varying concentrations of hydroxyethyl cellulose 1.0%, 1.5%, and 2.0% using the DPPH methods, which is simple, easy, fast, and sensitive, and requires a small sample. This method provides information on the reactivity of the tested compound with a stable radical. Evaluations of the Fibraurea tinctoria extract serum preparation was carried out by observing the results of physical quality tests, including organoleptic tests, homogeneity tests, pH tests, adhesion tests, and spreadability tests. The serum preparation was tested for 3 days and stored at room temperature. Data were analyzed descriptively by comparing it with the Indonesian National Standard.

Results: The results showed that the serum extract preparations of Fibraurea tinctoria F1st, F2nd, and F3rd showed varying IC_{50} values, where lower values indicated stronger antioxidant potential. Formulation F3rd showed the lowest IC_{50} value (3.11 ppm), F2nd (3.74 ppm) and F1st (4.67 ppm), all of which were stronger than the base (5.11 ppm).

Conclusion: In this research, formulations F1st, F2nd, and F3rd had the highest antioxidant activity, with F3rd at a concentration of 2.0%. The identified strong antioxidant activity is the main basis for the potential of Fibraurea tinctoria extract serum as an active antioxidant ingredient.

I. Introduction

The activity of antioxidants in Fibraurea tinctoria presents a compelling area of research, particularly regarding the formulation of serum preparations derived from the yellow root stem fraction. This exploration is essential not only to identify the most effective serum formulation based on the IC_{50} value but also to evaluate the physical quality of these serum preparations. The significance of antioxidants cannot be overstated, as they play a crucial role in neutralising free radicals, thereby preventing potential damage to normal cells, proteins, and lipids. Antioxidants possess the unique capability to donate electrons, a property that is vital in the fight against oxidative stress.

Natural sources of antioxidants are abundant, particularly in fruits, vegetables, and various plant species rich in essential vitamins and phytochemicals. For instance, vitamin A and vitamin C, along with anthocyanins, phenolic compounds, and flavonoids, are well-documented for their antioxidant properties

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(Raharjo et al., 2022). These compounds not only contribute to the nutritional value of the foods we consume but also enhance our body's defence mechanisms against oxidative damage.

The testing of antioxidants using the 1,1-Diphenyl 2-Picryhydrazyl (DPPH) method is a particularly effective and straightforward approach. DPPH serves as a stable free radical compound that can readily react with hydrogen atoms supplied by antioxidants, resulting in the formation of reduced DPPH. This method is advantageous due to its simplicity and practicality, allowing for quick and accurate assessments of antioxidant activity. DPPH, characterised by its vibrant purple colour, is stable at room temperature. When antioxidants interact with DPPH, the intensity of the purple hue diminishes, often shifting towards a yellow colour. This visual change is indicative of the reduction process occurring as antioxidants neutralise free radicals, showcasing their efficacy in real-time (Hasanah et al., 2020).

The measurement of this colour change is facilitated by the use of a UV-Vis spectrophotometer, which quantifies the absorbance associated with the colour transformation. The peak absorbance of DPPH occurs at a wavelength of 518 nm, where the deep purple colour reflects the highest concentration of the free radical. As antioxidants capture electrons, they effectively prevent the propagation of free radical damage, leading to a notable alteration in colour. The relationship between the degree of colour change and the number of electrons captured is a critical aspect of this analysis, underscoring the importance of antioxidants in mitigating oxidative stress (Pujiastuti & Islamiyati, 2021).

In light of the above, it is essential to delve deeper into the specific mechanisms through which Fibraurea tinctoria exerts its antioxidant effects. The yellow root stem fraction of this plant is particularly intriguing due to its rich phytochemical profile, which may include various bioactive compounds contributing to its antioxidant capacity. For instance, flavonoids and phenolic compounds, commonly found in many plant species, are known for their ability to scavenge free radicals effectively. Their structure allows them to donate electrons, thus stabilising free radicals and preventing cellular damage.

Formulation of a serum from Fibraurea tinctoria's yellow root stem fraction could have significant implications for cosmetic and therapeutic applications. For example, a well-formulated serum could potentially enhance skin health by protecting against oxidative stress caused by environmental factors such as UV radiation and pollution. This protective effect could manifest as reduced signs of ageing, such as wrinkles and hyperpigmentation, thereby making the serum an attractive option for consumers seeking anti-ageing solutions.

The evaluation of the physical quality of the serum preparations is paramount. Factors such as viscosity, stability, and pH must be meticulously assessed to ensure that the serum is not only effective but also safe for use. For instance, a serum with an appropriate viscosity will spread easily on the skin, allowing for better absorption of the active ingredients. Stability testing will help determine the shelf life of the product, ensuring that the antioxidant properties remain intact over time (Keawpradub et al, 2015).

Exploration of antioxidants in Fibraurea tinctoria, particularly through the formulation of a serum preparation, presents a multifaceted opportunity for both scientific inquiry and practical application. The DPPH method serves as a robust tool for assessing the antioxidant capacity of this plant, providing insights into its potential health benefits. As we deepen our understanding of the mechanisms behind its antioxidant activity, we can better appreciate the role of natural compounds in promoting health and wellness. The implications of this research extend beyond the laboratory, offering promising avenues for the development of effective skincare products that harness the power of nature to combat oxidative stress. Through continued investigation, we can unlock the full potential of Fibraurea tinctoria, paving the way for innovative solutions in the field of health and beauty.

II. METHODS

The tools used in this research were maceration vessels, porcelain cups, mortars, water heaters, analytical scales, UV-Vis spectrophotometers, a set of column chromatography tools, beakers, volumetric flasks, stirring rods, stampers, micropipettes, pH meters, moisture analyzers, microscopes, 2 sets of glass slides, weights.

The materials used were Fibraurea tinctoria extract, TEA, cetyl alcohol, glycerin, nipagin, nipasol, DPPH indicator, 70% ethanol, ethyl acetate, N-hexane, distilled water, vitamin C, silica gel plates F254, AlCl3 reagent, Dragendorf reagent, FeCl3 reagent, H2SO4, Libermann reagent, Bouchardart, filter paper.

Research Design and samples

The table 1st. Fibraurea tinctoria extract serum formulations:

Material	Purpose	F1st (%)	F2nd (%)	F3rd (%)
Fibraurea tinctoria extracts	Antioxidants	1.0	1.5	2.0
Hidroksi ethyl celullosa	Gelling agents	3	3	3
Glycerinum	Humectants	35	35	35
Propylenglikol	Humectants	5	5	5
Trietanolamine (TEA)	alkalizing agents	5	5	5
Propylparaben	Preservatifs	0,5	0,5	0,5
Aquadest	Solvents	ad 100	ad 100	ad 100

Making Fibraurea tinctoria extract serum:

Pour hot water into a beaker containing hydroxyethyl cellulose while stirring using a magnetic stirrer at a speed of 10 rpm for approximately 15 minutes, then continue hydrating for 12 hours, then after expanding, add Propylene glycol and Triethanolamine little by little until evenly mixed, then Fibraurea tinctoria extracts in some Glycerin that has been dissolved previously in Propylenglycol are added little by little into the beaker while stirring with a magnetic stirrer until homogeneous. The base that has been mixed with Fibraurea tinctoria extracts and Propylparaben that has been dissolved in Glycerin then add 100ml of distilled water while continuing to stir using a magnetic stirrer until a homogeneous serum with low viscosity and semi-transparent is obtained, then the antioxidant power and physical quality of the serum are tested

III. RESULTS

The Table 2nd. Antioxidant activity test of Fibraurea tinctoria extract serum against DPPH using UV-vis Spectrophotometer with IC_{50} absorbance value

No	Formulation	Concentration	Mean ± SD	Inhibition (%)	IC50	Inhibition Desc
1	DPPH	0.1 Mm	2.605 ± 0.010	-	-	-
2	Ascorbic Acid	2 ppm	0.787 ± 0.011	73.02		Strong
		4 ppm	0.625 ± 0.010	78.89		Strong
		6 ppm	0.059 ± 0.006	97.62	2.07 ppm	Strong
		8 ppm	0.055 ± 0.004	91.69		Strong
		10 ppm	0.050 ± 0.013	98.19		Strong
3	F1st 1.0%	2 ppm	1.343 ± 0.010	51.92		Medium
		4 ppm	1.318 ± 0.012	52.48		Medium
		6 ppm	1.214 ± 0.023	53.77	4.67 ppm	Medium
		8 ppm	1.193 ± 0.011	55.78		Medium
		10 ppm	1.184 ± 0.015	56.82		Medium
4	F2nd 1.5%	2 ppm	1.192 ± 0.006	54.43		Medium
		4 ppm	1.182 ± 0.007	55.89		Medium
		6 ppm	1.172 ± 0.006	56.81	3.74 ppm	Medium
		8 ppm	1.141 ± 0.006	57.30		Medium
		10 ppm	1.134 ± 0.006	58.70		Medium
5	F3rd 2.0%	2 ppm	1.093 ± 0.006	57.43		Strong
		4 ppm	1.043 ± 0.007	59.69	3.11 ppm	Strong
		6 ppm	1.024 ± 0.006	64.57		Strong

		8 ppm	0.989 ± 0.008	67.03		Strong
		10 ppm	0.984 ± 0.006	78.15		Strong
6	K- (Basis)	2 ppm	1.776 ± 0.034	32.05		Weak
		4 ppm	1.616 ± 0.024	34.93		Weak
		6 ppm	1.430 ± 0.023	41.72	5.11 ppm	Weak
		8 ppm	1.404 ± 0.006	47.10		Weak
		10 ppm	1.278 ± 0.006	52.90		Weak

Table 3rd. Serum pH Test of Fibraurea tinctoria Extract

Formulation	**Replication**			**Mean/SD**	**Range**
	1	**2**	**3**		
K-	7.39	7.41	7.33	7.38 ± 0.10	4,5-8 (B.,Iskandar *et al*., 2021)
F1st 1.0%	7.53	7.38	7.75	7.55 ± 0.11	
F2nd 1.5%	7.69	7.75	7.62	7.69 ± 0,15	
F3rd 2.0%	6.54	6.24	6.55	6,44 ± 0.13	

Table 4th. Spreadability Test of Fibraurea tinctoria Extract Serum

Formulation	**Replication**			**Mean/SD**	**Range**
	1	**2**	**3**		
K-	7.6	9.5	9.9	9.00 ± 0.00	5-9cm (Alifya, 2022)
F1st 1.0%	7.1	8.1	8.9	8.03 ± 0.78	
F2nd 1.5%	7.7	8.2	10.3	8.73 ± 0.69	
F3rd 2.0%	8.2	8.7	9.6	8.83 ± 0.42	

Table 5th. Testing The Adhesion Power of Fibraurea tinctoria Extract Serum

Formulation	**Replication**			**Mean/SD**	**Range**
	1	**2**	**3**		
K-	4.1	5.5	6.1	5.23 ± 0.33	3-8 detik (Alifya, 2022)
F1st 1.0%	5.7	5.5	4.1	5.10 ± 0.00	
F2nd 1.5%	4.9	4.3	5.1	4.73 ± 0.09	
F3rd 2.0%	4.1	5.2	4.8	4.70 ± 0.00	

Table 6th. Viscosity Test of Fibraurea tinctoria Extract Serum

Formulation	**Replication**			**Mean/SD**	**Range**
	1	**2**	**3**		
K-	2345.3	2425.2	2401.2	2390.6 ± 0.33	2.000-50.000 cP (Alifya, 2022)
F1st 1.0%	2399.4	2509.2	2565.1	2491.2 ± 0..12	
F2nd 1.5%	2715.7	2693.1	2469.7	2616.2 ± 0.67	
F3rd 2.0%	3564.3	3865.9	3878.5	3769.6 ± 0.67	

IV. DISCUSSION:

Fibraurea tinctoria formulation in F1st (1.0% concentration) showed an increase in inhibition from 51.92% at 2 ppm to 56.82% at 10 ppm. Similarly, formulation F2nd (1.5% concentration) showed an increase in inhibition from 54.43% at 2 ppm to 58.70% at 10 ppm, and formulation F3rd (2.0% concentration) showed an increase from 57.43% at 2 ppm to 78.15% at 10 ppm. Ascorbic acid, as a standard antioxidant, showed a significant increase from 73.02% at 2 ppm to almost complete radical scavenging activity of 98.19% at 10 ppm. In contrast, the negative control (K-), assumed to be a serum base without active ingredients, showed significantly lower inhibition percentages, ranging from 32.05% at 2 ppm to 52.90% at 10 ppm.

The pH measurements showed acceptable values for all formulations, namely 7.55 for F1st, 7.69 for F2nd, and 6.44 for F3rd. These values, including the base (K-) with a pH of 6.44, are within the safety standard range for topical preparations, which is between 4.5 and 8.0. The pH range of 4.5-8.0 is the accepted

standard for topical preparations to ensure the product does not cause skin irritation. A pH value within this range ensures that the formulation is neither too acidic nor too basic, making it safe for use (Mutmainah et al., 2020).

All formulations demonstrated adequate adhesion, with adhesion times ranging from 5.10 seconds for F3rd, 4.73 seconds for F2nd, 4.70 seconds for F1st, and 5.23 seconds for K-. This characteristic is important to ensure the formulation remains on the skin surface long enough after application. A good adhesion time for topical formulations is greater than 4 seconds, which provides sufficient contact time between the active ingredient and the skin, allowing for optimal release (Mutmainah et al., 2020). Too short an adhesion time will result in the formulation being easily removed from the skin. Interpretation of these results indicates that all formulations had adequate adhesion to the skin. A adhesion time within the ideal range supports the formulation's function as a topical active ingredient delivery system, ensuring the formulation is not easily removed but also does not leave a sticky, irritating sensation (Shah et al, 2019).

The spreadability test results showed that formulation F1st had a spread area of 8.03 cm, F2nd 8.73 cm, F3rd 8.83 cm, and K- 9.00 cm, which are within the standard range. A good spreadability for topical preparations is in the range of 5-9 cm2, allowing for easy and even application without becoming too runny or wasteful. The inverse relationship between viscosity and spreadability is a well-known rheological principle in semisolid dosage formulations. Interpretation of these results highlights that formulation F3rd has the most balanced and desirable physical characteristics. Its ideal spreadability, supported by its higher viscosity, makes it the best formulation for application compared to F1st and F2nd, which tend to be too runny.

The viscosity test results showed a consistent increase in viscosity with increasing fraction concentration, with values for F1st (2401.2 cP), F2nd (2616.2 cP), and F3rd (3769.6 cP). All viscosity values obtained met the standard range required for lotion preparations, namely 2,000-50,000 cP. Viscosity is an important rheological parameter that affects stability, ease of application, and release of active ingredients from the preparation. A good viscosity range for serum is 2,000-50,000 cP to ensure the preparation is easy to apply but not too runny. Increasing the extract concentration will naturally increase the viscosity (Mutmainah et al., 2020). The interpretation of this finding is that all formulations have a good level of viscosity and comply with the standard. The observed increase in viscosity is a natural consequence of the addition of fractions. This characteristic is important to ensure the preparation is not too runny or too thick, so that it is comfortable when used

V. CONCLUSION

Based on the results of the antioxidant activity test and the physical quality test of the Fibraurea tinctoria extract serum, the results of the antioxidant potential in formula 3rd showed that it had an inhibition value of 57.43% at 2 ppm to 78.15% at 10 ppm and met the physical quality requirements of the Fibraurea tinctoria extract serum which included pH stability, spreadability, and adhesiveness tests.

VI. ACKNOWLEDGMENTS

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VII. CONFLICTS OF INTEREST

No conflict of interest was found during the research.

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