International Journal on Science and Technology (IJSAT)
E-ISSN: 2229-7677 ● Website: www.ijsat.org ● Email: editor@ijsat.org

In-vitro Antioxidant and Anti-Inflammatory Potential of herbal elixir containing Crocus Sativus and Nigella Sativa

Aisha Siddiqua[1], Nahida Siddiqui[2], Bhukya Kalyan[3], Bai Raveena[4], Tadikonda Rama Rao[5]

[1,2,3,4,5] Associate Professor CMR College of Pharmacy, Kandlakoya (V), Medchal Road, Hyderabad, Telangana, India
[2]siddiquinahida92@gmail.com

Abstract

Background

Oxidative stress and chronic inflammation are interlinked and associated with several major diseases, including cancer, cardiovascular, and neurodegenerative disorders. Although modern pharmacotherapy is effective, issues such as high cost, side effects, and limited accessibility—particularly in underdeveloped regions—have driven interest in herbal alternatives. Elixirs, as sweetened hydro-alcoholic solutions, offer an effective delivery system for herbal drugs.

Objective

To formulate and evaluate a herbal elixirs containing Crocus sativus (saffron) and Nigella sativa (black seed) for its in-vitro antioxidant and anti-inflammatory activities.

Methods

A hydro-alcoholic (40%) elixirs were prepared using different ratios of coarsely powdered C. sativus stigmas and N. sativa seeds, based on clinically used doses. Formulations were filtered after 48 hours and sweetened with 0.075 g sodium saccharin. Physicochemical parameters (viscosity and pH) were measured. Antioxidant activity was evaluated via hydrogen peroxide (H_2O_2) scavenging assay; anti-inflammatory activity was assessed through inhibition of egg albumin denaturation assay.

Results

Among all formulations, F2 elixir demonstrated significant antioxidant activity with 55 ± 0.23% inhibition at a 4 ml dose, comparable to ascorbic acid (56 ± 0.31% at 20 mg/ml). It also showed >50% inhibition of protein denaturation, with effects comparable to 60 µg/ml of diclofenac sodium.

Conclusion

The F2 herbal elixir exhibits potent antioxidant and anti-inflammatory activities in-vitro. These findings

support that it could be cost-effective and accessible alternatives or adjuncts to conventional therapies for controlling oxidative stress and inflammation-related disorders.

Key words: Anti-inflammatory, Antioxidant, Crocus sativus, Elixir, Nigella sativa.

1. Introduction

Oxidative stress and chronic inflammation are two interrelated processes implicated in the pathogenesis of numerous diseases, including cancer, cardiovascular disorders, and neurodegeneration [1,2,3]. These conditions are often worsened by excessive production of reactive oxygen species (ROS), which disrupt cellular redox balance and trigger tissue damage [4,5]. Despite remarkable advancements in modern medicine, access to effective healthcare remains a challenge, particularly in developing and underdeveloped regions. Modern pharmacological therapies often face limitations such as adverse effects, microbial resistance, and high costs, prompting a growing global interest in traditional, herbal, and complementary medicines. Their lower toxicity, natural origin, and multi-targeted action make them attractive alternatives or adjuncts to conventional allopathic therapies [6,7,8,9].

Elixirs, a traditional sweetened hydro-alcoholic solutions, offer a practical and palatable medium for delivering herbal remedies. They increase solubility of phytoconstituents compared to decoctions and also improve oral palatabilty. The present in-vitro study, developed a novel herbal elixir containing stigma of Crocus sativus (saffron) belongs to Iridaceae family and seeds of Nigella sativa (black seed) from Ranunculaceae family, both of which are revered for their powerful antioxidant and anti-inflammatory properties [10,11, 12, 13,14,15,16,17,18,19,20]. Crocin from saffron, in particular, has shown potent free radical scavenging activity, making it a vital component in managing oxidative stress-related disorders [20].

Given the increasing evidence supporting the efficacy and safety of herbal therapies, our study aims to establish base for therapeutic utilization of herbal-based elixirs over conventional allopathic treatments, especially in managing inflammation and oxidative stress-associated disorders. By integrating traditional knowledge with modern pharmaceutical techniques, such formulations offer a promising pathway toward safer, more accessible, and effective healthcare solutions. Therefore present study was evaluated in-vitro antioxidant and anti-inflammatory activity of a herbal elixir containing Crocus sativus and Nigella sativa.

2. Materials and Methods

Procurement of Crocus sativus and Nigella sativa

Stigmas of Crocus sativus (Saffron) and seeds of Nigella sativa (Kalonji or black seeds) were procured and authenticated by a botanist of CMR college of pharmacy, Hyderabad, India.

Materials

Ascorbic acid, hydrogen peroxide, diclofenac sodium, and all organic solvents were acquired from SD Fine-Chem and Research Labs in Mumbai, India. Two solutions were made: phosphate buffer solution (PBS) and phosphate buffer saline solution (PBSS). Every chemical employed in the research was of AR grade.

Preparation of Herbal Elixirs

The seeds of Nigella sativa and was stigma of Crocus sativus were grounded into coarse powder. The different ratios of both the crude drugs (on the basis of clinical trial doses) has been taken for the preparation of herbal elixir containing 40% of hydro-alcoholic solution [13, 14] (Table 1). Filtered them after 2 days and finally added 0.075g of sodium sachharin to all the elixir as sweetner. Physiochemical properties such as viscosity of all elixirs was determined by using Brookfield viscometer (Brand name and serial no.) at 30, 50, 60 rpm with spindle number 63. Additionally, pH of all elixirs was determined by using Digital pH meter (Brand name and serial no.)

In-vitro anti-oxidant activity: Hydrogen-peroxide (H_2O_2) Assay

The doses of 2, 4, and 8 ml were taken for each elixirs and combined with 0.6 ml of a 4 mM H_2O_2 solution made in PBS (0.1 Mmo_2, pH 7.4), and then incubated for 10 minutes. Ascorbic acid was used as standard (20, 40, and 60 mg/ml). At 230 nm, all the doses of elixirs absorbance was measured (n=3). Water is used as a blank. The following formula was used to determine the percentage inhibition of doses of elixirs and ascorbic acid.

% inhibition = 1- Absorbance of fractions /Absorbance of H_2O_2 X 100

The dose of elixir at which 50% of the inhibition of oxidation caused by H_2O_2 was achieved was then evaluated (IC_{50}) [21].

Anti-Inflammatory Activity: Egg Albumin denaturation Assay

The 5 ml reaction mixture was prepared by adding 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of Phosphate buffer saline solution (PBSS) (pH 6.8) and added 2, 4, and 8 ml doses of all elixirs. Diclofenac sodium (10-100 mg/ml) was used as a standard. Equal volume of double-distilled water and DMSO served as blank. These mixtures were incubated at (37° C) in a BOD (Biological oxygen demand) incubator for 30 min and then heated at 75° C for 10 min. After cooling, their absorbance was measured at 660 nm. The percentage inhibition of protein denaturation was calculated by using the following Formula [22]-

% inhibition =1- Absorbance test or std/ Absorbance control X 100

3. Results

Different ratios of both the crude drugs were used and elixirs prepared(Table 1). The physico-chemical properties were indicated that all elixirs had maple syrup like consistency due to below 100 cp of viscosity values. Moreover, values of pH indicated that all the elixirs were acidic in nature (Table 2).

Table 1. **F1, F2, F3, and F4 Elixirs preparation**

Elixirs	Crocus sativus (mg)	Nigella sativa (mg)
F1	100 mg	2000 mg
F2	50 mg	1000 mg
F3	25 mg	500 mg
F4	12.5 mg	250 mg

Table 2. Viscosity and pH values of all elixirs

Test	F1	F2	F3	F4
Viscosity	62cp	89cp	76cp	36cp
P^H	2.79	3.95	4.09	4.19

In-vitro antioxidant activity of Elixirs using H_2O_2 assay

The antioxidant activity of all doses of elixirs was assessed against H_2O_2 assay (Table 3). The results showed significant inhibitory potential (IC_{50}) was obtained with F2 elixir at 4 ml of dose (55±0.23 % inhibition) against H_2O_2 free radicals. The standard ascorbic acid showed better inhibitory activity (IC_{50}) at 20 mg/ml of concentration (56±0.31) compared to all doses of the elixirs which showed highest free radical scavenging over all the elixirs. However, study demonstrated that 4 ml of F2 formulation has free radical scavenging potential equivalent to 20 mg/ml of ascorbic acid.

Table 3. % inhibition of different formulations of Elixirs

Doses (in ml)	**% inhibition**					
	F1	**F2**	**F3**	**F4**	**Ascorbic acid**	
					Dose (mg/ml)	**% inhibition**
2 ml	21 ± 0.16	37 ± 0.36	23 ± 0.23	13.5± 0.38	20	56 ± 0.31
4 ml	34± 0.48	55 ± 0.23	29.6 ± 0.39	17.7 ± 0.51	40	65 ± 0.26
8 ml	22 ± 0.29	49 ± 0.37	37.5 ± 0.22	26.9 ± 0.38	60	83 ± 0.17
Standard deviation = ± value						

Anti-inflammatory activity using albumin denaturation assay

The in-vitro anti-inflammatory activity of all elixirs was assessed against denaturation of egg albumin (Table 4). The present findings exhibited > 50% of inhibition of protein (albumin) denaturation at the dose of 4 ml only by F2 elixir. The % inhibition (IC_{50}) 4 ml of F2 elixir was found to be equivalent of 60 µg/ml of diclofenac sodium anti-inflammatory potential.

Table 4. % inhibition of protein denaturation by different formulations of Elixirs

Doses (in ml)	**% inhibition**					
	F1	**F2**	**F3**	**F4**	**Diclofenac sodium**	
					Dose (µg/ml)	**% inhibition**
2 ml	11.08± 0.49	33.8 ± 0.59	22.96 ± 0.33	19.14 ± 0.81	20	52 ± 0.31
4 ml	21.94± 0.38	65.81 ± 0.13	31.6 ± 0.29	28.7 ± 0.41	40	67 ± 0.26
8 ml	30.61± 0.19	49.21 ± 0.47	38.5 ± 0.12	35.9 ± 0.28	80	87 ± 0.17
Standard deviation = ± value						

4. Discussion

The present study has shown antioxidant and anti-inflammatory potential of the herbal elixir containing Crocus sativus and Nigella sativa. Among all the elixirs (F1, F2, F3, and F4), F2 elixir was found to be shown potent antioxidant (IC_{50} 55 ± 0.23) and anti-inflammatory activity (IC_{50} 65.81 ± 0.13) at 4 ml of its dose and it was comparable to ascorbic acid (at 20 mg/ml) and diclofenac sodium (at 40 µg/ml) standards. Pre-clinical evidences has shown that stigma of Crocus sativus and seeds of Nigella sativa have significant antioxidant and anti-inflammatory activity which may be attributed to the presence of bioactive phytoconstituents [3,13,14,24]. Elixirs may enhance pharmacological activity along with shelf life because of hydroalcoholic in nature and also improve palatability compared to the decoctions (without sweeteners, concentrated, and aqueous preparation) [23]. The present study was restricted to the preliminary biological in-vitro screening of antioxidant and anti-inflammatory activities of the developed elixirs. These findings promote further in-vivo study to find out the role of antioxidant and anti-inflammatory effect in particularly F2 elixir to control oxidative stress associated disorder including anti-inflammatory action. Furthermore, structure elucidation and characterization of antioxidant candidate with anti-inflammatory potential have to be carried out from this herbal elixir.

5. Conclusions

The F2 herbal elixir (50 mg stigma of Saffron and 1000 mg Nigella seeds) demonstrated the superior antioxidant and anti-inflammatory activities compared to other developed elixirs. These findings provide a foundation for further research, including in-vivo investigations and standardization of the elixir to improve its physicochemical properties.

6. Acknowledgements

We would like to acknowledge the CMR College of Pharmacy, Hyderabad, India for their

support.

7. Conflict of interest

There is no conflict of interest exists.

References

1. S. Furukawa, T. Fujita, M. Shimabukuro, M. Iwaki, Y. Yamada, Y. Nakajima, et al.
2. Increased oxidative stress in obesity and its impact on metabolic syndrome. J. Clin. Investig., 114 (12) (2017), pp. 1752-1761
3. C.R. Bosoi, C.F. Rose. Oxidative stress: a systemic factor implicated in the pathogenesis of hepatic encephalopathy Metab. brain Dis., 28 (2) (2013), pp. 175-178
   Yaribeygi H, Mohammadi MT, Sahebkar A. Crocin potentiates antioxidant defense system and improves oxidative damage in liver tissue in diabetic rats. Biomedicine & Pharmacotherapy. 2018 Feb 1;98:333-7.
4. J. Shiralizadeh, H. Barmaki, S. Haiaty, Y. Faridvand, M. Mostafazadeh, N. Mokarizadeh, et al. The effects of high and low doses of folic acid on oxidation of protein levels during pregnancy: a randomized double-blind clinical trial Horm. Mol. Biol. Clin. Investig., 33 (3) (2018)

5. N. Seyfizadeh, M. Tazehkand, A. Palideh, N. Maroufi, D. Hassanzadeh, M. Rahmati-Yamchi, et al. Is arbutin an effective antioxidant for the discount of oxidative and nitrosative stress in Hep-G2 cells exposed to tert-butyl hydroperoxide? Bratisl. Lek. Listy, 120 (8) (2019), 569-575
6. Saggar S, Mir PA, Kumar N, Chawla A, Uppal J, Kaur A. Traditional and herbal medicines: opportunities and challenges. Pharmacognosy Research, 2022; 14(2).
7. Kumari P, Luqman S, Meena A. Application of the combinatorial approaches of medicinal and aromatic plants with nanotechnology and its impacts on healthcare. Daru, 2019; 27(1): 475-89.
8. Palhares RM, Gonçalves Drummond M, Dos Santos Alves Figueiredo Brasil B, Pereira Cosenza G, das Graças Lins Brandão M, Oliveira G. Medicinal plants recommended by the world health organization: DNA barcode identification associated with chemical analyses guarantees their quality, 2015; 10(5).
9. Sen S, Chakraborty R. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. J Tradit Complement Med, 2016; 7(2): 234-44.
10. C.V.S. Subrahmanyam, J. Thimmasetty, G.L.Prabhushankar, et al. Laboratory manual of pharmaceutics including dispensing pharmacy.
11. Remington – The science and practice of pharmacy, 21 edition
12. Khare CP. Encyclopedia of Indian medicinal plants. NewYork: Springes-Verlag Berlin Heidelberg; 2004.
13. Pour FK, Aryaeian N, Mokhtare M, Mirnasrollahi Parsa RS, Jannani L, Agah S, Fallah S, Moradi N. The effect of saffron supplementation on some inflammatory and oxidative markers, leptin, adiponectin, and body composition in patients with nonalcoholic fatty liver disease: A double-blind randomized clinical trial. Phytother Res. 2020 Dec;34(12):3367-3378. doi: 10.1002/ptr.6791. Epub 2020 Aug 15. PMID: 32798261. [3:48 pm, 17/06/2025]
14. Bamosa AO, Kaatabi H, Lebdaa FM, Elq AM, Al-Sultanb A. Effect of Nigella sativa seeds on the glycemic control of patients with type 2 diabetes mellitus. Indian J Physiol Pharmacol. 2010 Oct-Dec;54(4):344-54. PMID: 21675032.
15. Bhat JV, Broker R. Riboflavine and thiamine contents of saffron, Crocus sativus linn. Nature. 1953 Sep 19;172(4377):544.
16. Moallem SA, Hariri AT, Mahmoudi M, Hosseinzadeh H. Effect of aqueous extract of Crocus sativus L.(saffron) stigma against subacute effect of diazinon on specific biomarkers in rats. Toxicology and industrial health. 2014 Mar;30(2):141-6.
17. Gismondi A, Serio M, Canuti L, Canini A. Biochemical, antioxidant and antineoplastic properties of Italian saffron (Crocus sativus L.). American Journal of Plant Sciences. 2012 Nov 27;3(11):1573.
18. Srivastava R, Ahmed H, Dixit RK, Saraf SA. Crocus sativus L.: a comprehensive review. Pharmacognosy reviews. 2010 Jul;4(8):200.
19. Alonso Gl, Salinas Mr, Garijo J, Sánchez-Fernández Ma. Composition of crocins and picrocrocin from Spanish saffron (Crocus sativus L.). Journal of Food Quality. 2001 Jul;24(3):219-33.
20. Hoshyar R, Mollaei H. A comprehensive review on anticancer mechanisms of the main carotenoid of saffron, crocin. Journal of Pharmacy and Pharmacology. 2017 Nov;69(11):1419-27.
21. Ambe DA, Odiete CE, Buba JD, Odion EE. Antioxidant Effects of Cocos nucifera L. (Asteraceae) Stem Bark Extracts using Diphenyl-I-picrylhydrazyl; Hydrogen Peroxide and Nitric Oxide

Scavenging Assay. Journal of Applied Sciences and Environmental Management. 2023; 30, 27(10): 2291-5.

22. Vinchurkar A, Valsange A, Dama L, Sonawane S, Gaikwad N, Mane P, Dama SB. Evaluation of in-vitro anti-inflammatory activity of crude Lawsonia inermis leaf extract using egg albumin denaturation assay. Trends Biotechnol Res. 2014; 8(33): 44.
23. Wax PM. Elixirs, diluents, and the passage of the 1938 Federal Food, Drug and Cosmetic Act. Annals of internal medicine. 1995;122(6):456-61.
24. V. Vahedian, A. Asadi, P. Esmaeili, S. Zamani, R. Zamani, S. Hajazimian, et al. Anti-inflammatory activity of emu oil-based nanofibrous scaffold through downregulation of IL-1, IL-6, and TNF-α pro-inflammatory cytokines Horm. Mol. Biol. Clin. Investig. 2020; 41 (2).