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EXPLORATION OF *TRIGONELLA FOENUM-GRAECUM* EXTRACT: CHEMICAL SCREENING, ANTI-WRINKLE ACTIVITIES, AND THERAPEUTIC POTENTIAL IN SKINCARE AND SUPPLEMENT

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ABSTRACT

This study investigates the chemical screening and anti-wrinkle activities of *Trigonella foenum-graecum* extract. Chemical screening revealed significant yields of diosgenin, supported by Thin Layer Chromatography (TLC) analysis showing aligned Rf values with a standard reference compound. The extract exhibited MMP-1 and MMP-2 IC50 values of 34.87 ± 1.29 µg/mL and 22.11 ± 2.98 µg/mL, respectively, while gallic acid demonstrated lower IC50 values, suggesting superior anti-wrinkle properties. These findings highlight *Trigonella foenum-graecum* as a potential source of bioactive compounds with therapeutic implications for skincare. Further research is warranted to explore the extract's full chemical composition and pharmacological potential, paving the way for the development of novel anti-wrinkle formulations and pharmaceutical applications.

KEYWORD: *Trigonella foenum-graecum*, Anti-collagenase, Anti-elastase, Diosgenin.

INTRODUCTION

Trigonella foenum-graecum, commonly known as fenugreek,[1,2] is a versatile herb that has been utilized for centuries in traditional medicine due to its various health benefits.[3] One of the intriguing properties of fenugreek lies in its potential as an anti-collagenase[4,6] and anti-elastase agent.[7,8] Collagenase and elastase are enzymes involved in the breakdown of collagen and elastin, respectively[9,11], which are essential components of connective tissues in the body.[12,13] Excessive activity of collagenase and elastase can lead to tissue damage, aging of the skin, and contribute to the development of various skin conditions such as wrinkles, sagging, and loss of elasticity.[14,16] Studies have indicated that *Trigonella foenum-graecum* extract possesses inhibitory effects against collagenase and elastase enzymes. This suggests its potential utility in skincare formulations aimed at preventing or mitigating the effects of skin aging and promoting skin health.[17,19]

In addition to its anti-collagenase and anti-elastase properties, fenugreek extract is rich in bioactive compounds such as flavonoids, alkaloids, and saponins[20,22], which exhibit antioxidant, anti-inflammatory, and wound-healing properties.[23,25] These additional benefits further enhance the potential of *Trigonella foenum-graecum* extract as a valuable ingredient in skincare products targeting aging and damaged skin.[26,27]

Overall, the exploration of *Trigonella foenum-graecum* extract for its anti-collagenase and anti-elastase activities opens up exciting possibilities in the development of innovative skincare formulations aimed at maintaining youthful and healthy skin. Further research and development in this area hold promise for unlocking the full potential of fenugreek extract in skincare and cosmetic applications. This research aims to study the preliminary phytochemical analysis, extraction process quality, and collagenase activities of *Trigonella foenum-graecum* extract.

Reagents

Trigonella foenum-graecum was collected from the Samunpai Thaprachan Corporation Bang Chak, Mueang phasi charoen, Bangkok, Thailand., Diosgenin was purchased from Natural Remedies, Bangalore, (India)., Methanol, Ethyl acetate, acetone, and n-hexane were purchased from Qrec, (Newzealand)., Petroleum, Ether–isopropanol was purchased from RCI Labscan, Ireland., EnzChek® collagenase was purchased from Sigma-Aldrich (USA)., 5,5′dithiobis nitro benzoic acid, Ellman reagent and acetylthiocholine iodide were purchased from Sigma-Aldrich (USA)., TLC Aluminium sheet was purchased from MACHEREY-NAGEL Corporation, (Germany)., Ultrasonic Bath Model GT SONIC-D13 Made in (China)., and Rotary Evaporator RV 10 digital V, IKA, (English), Black-Box Type UV Analyzer model BTU-6 Made in (China).

Preparation and characterization of plant extract

This process was modified by Chumchuen S et al.[28] Briefly,100 g *Trigonella foenum-graecum* showed in figure 1 were separately soaked in 500 mL of deionized water at 25°C for 1 week and then each extract was filtered. The mask of the herb was re-extracted by 500 mL of ethanol. Afterward, the water and ethanol liquid were mixed, and then evaporation by a rotary evaporator. Then, 50 mL of extract was mixed with 100 mL of methanol. The solution was evaporated for further study.

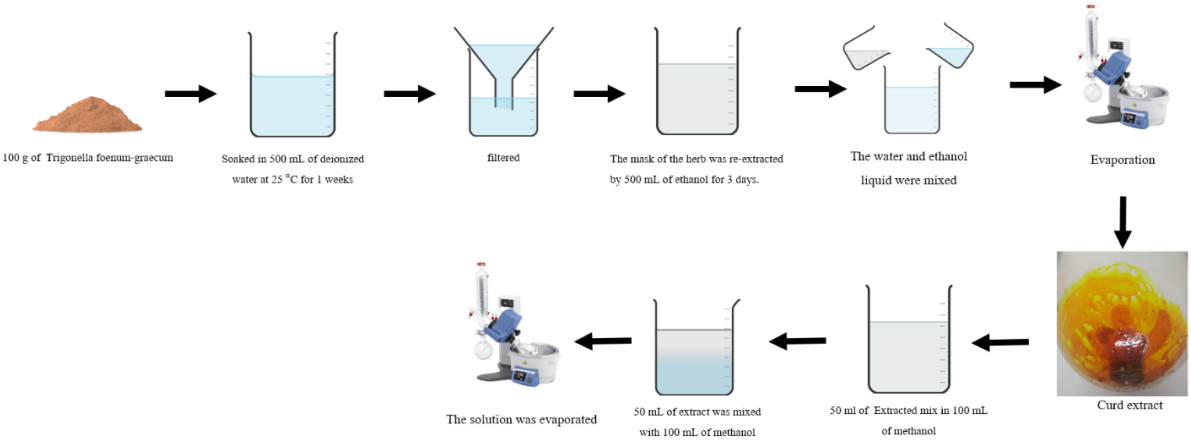

Figure 1: **Process extract of *Trigonella foenum-graecum.***

Preliminary phytochemical screening

Standard preparation

Briefly, the solution of standard, diosgenin (2 mg/mL) was prepared 5 mL in methanol and made up 15 mL with methanol. The final concentration of diosgenin (600 µg/mL) was used as the work standard for the TLC method.

Sample preparation

Accurately weight 10 mg of semisolid extraction were dissolved into methanol 10 mL and sonication for 10 minutes.

The solution was filtered through an 11 µm membrane before the next study.

The mobile phase was prepared from a combination of petroleum: Ether–isopropanol (23:2, v/v) mL, which was validated by Amir A et al.[29] The optimized chamber saturation time for the mobile phase was 50 minutes at room temperature (25 °C).

Anti-collagenase inhibitory activity

Anti-collagenase inhibitory activity which is modified from Chumchuen S.[30] The assay was followed EnzChek® collagenase/gelatinase assay kit. Briefly, the extract was calculated as minimum as 1.5 % w/v on the 96-well plate and add DQTM collagen (MMP-1) and DQTM gelatin (MMP-2), 30 µl. Continuously added Collagenase obtained from Clostridium histolyticum about 300 µl has been incubated 60 min. Fluorescence intensity was measured with the excitation and the emission wavelength at 485 nm and 538 nm, respectively, using a fluorescent microplate reader and compared with epigallocatechin gallate (EGCG).

Anti-elastase inhibitory activity

Anti-elastase inhibitory activity which is modified from Chumchuen S.[30] Briefly, the extract amount was calculated as minimum as 1.5% w/v on the 96-well plate and add porcine pancreatic elastase (PE), 20 µl was preprad by 0.2 mM Tris-HCL buffer (pH 8.0). Continuously added 1.6 mM N-Succinyl- Ala-Ala-pnitroanilide (AAAPVN) 350 µL/plate. Measured with microplate reader that wavelength at 485 nm and 538 nm, respectively. Was compared with epigallo catechin gallate (EGCG).

Statistical analysis

Statistical analysis was performed ANOVA method, confidence level 99 % of the comparison was compared by individual pair Tukey's test.

RESULTS AND DISCUSSION

Chemical screening of the extract

The extraction processes employed with *Trigonella foenum-graecum* yielded a significant amount of diosgenin, a bioactive compound of interest. This assertion was supported by preliminary scanning compared with a standard, as illustrated in Figure 1. The choice of Thin Layer Chromatography (TLC) method for this study was deliberate due to its simplicity and minimal preparation requirements. Upon analysis, the study revealed that the extraction process resulted in Rf values for both the herbal extract and the standard that were closely aligned, indicating the presence of similar chemical constituents. This observation suggests that the extraction method utilized was effective in isolating diosgenin and other chemical substances from *Trigonella foenum-graecum*. Consequently, this extraction technique holds promise for further investigations into the chemical composition and pharmacological properties of the herb. Additionally, the successful

isolation of diosgenin underscores the potential of *Trigonella foenum-graecum* as a natural source of bioactive compounds with therapeutic implications. Further studies are warranted to explore the full spectrum of compounds present in the extract and to elucidate their biological activities and potential applications in medicine and nutraceuticals. Moreover, optimizing the extraction process to enhance the yield and purity of diosgenin could lead to the development of novel pharmaceutical formulations or dietary supplements with enhanced efficacy and safety profiles.

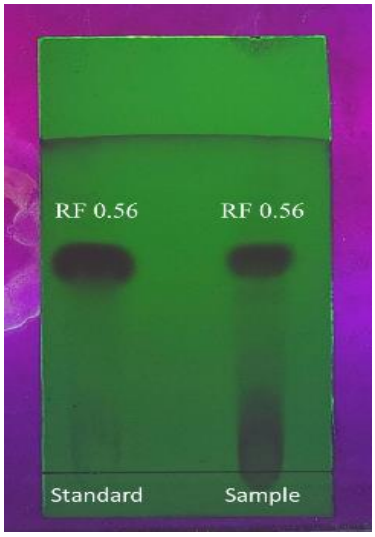

Figure 2: Characterization of *Trigonella foenum-graecum* extract compared with standards diosgenin.

Anti-wrinkle Activities

For the table 1, The anti-collagenase and anti-elastase activities of the extract, Diosgenin purified to 98%, and Gallic acid were evaluated using MMP-1 and MMP-2 IC_{50} values, respectively. The results indicated that the extract exhibited an MMP-1 IC50 value of 34.87 ± 1.29 µg/mL and an MMP-2 IC_{50} value of 22.11 ± 2.98 µg/mL. Upon purification to 98%, Diosgenin demonstrated slightly lower IC_{50} values for both MMP-1 (31.01 ± 9.29 µg/mL) and MMP-2 (20.87 ± 18.99 µg/mL) compared to the crude extract, although the differences were not statistically significant.

Gallic acid, on the other hand, exhibited significantly lower IC_{50} values for both MMP-1 (11.56 ± 0.33 µg/mL) and MMP-2 (19.11 ± 7.35 µg/mL) compared to both the extract and Diosgenin. These findings suggest that while both the extract and Diosgenin possess anti-collagenase and anti-elastase activities, Gallic acid demonstrates superior inhibitory effects against both MMP-1 and MMP-2. The significant difference in IC_{50} values indicates the potent anti-wrinkle properties of Gallic acid compared to the other compounds tested. It's worth noting that the variability in IC_{50} values, particularly for the extract, could be attributed to the complexity of its chemical composition, which may contain a mixture of compounds with varying bioactivities.

Further studies are warranted to elucidate the precise mechanisms underlying the observed effects and to identify additional bioactive constituents present in the extract. In conclusion, while both the extract and Diosgenin show promising anti-wrinkle activities, Gallic acid emerges as a potent inhibitor of collagenase and elastase, highlighting its potential for further investigation and development as an anti-aging agent. Further research into the synergistic effects of compounds within the extract and their potential applications in skincare formulations is warranted.

Table 1: The results extracted for anti-collagenase and anti-elastase activities.

Chemicals	Anti-collagenase inhibitor		Anti-elastase inhibitor
	MMP-1 IC_{50} (ug/mL)	MMP-2 IC_{50} (ug/mL)	IC_{50} (ug/mL)
Extract	34.87 ± 1.29	22.11 ± 2.98	18.99 ± 21.09
Diosgenin purify 98%	31.01 ± 9.29	20.87 ± 18.99	18.11 ± 91.08
Gallic acid	11.56 ± 0.33	19.11 ± 7.35	8.98 ± 12.87

[a] Paired sample T-test; significant difference $p < 0.05$

CONCLUSION

The chemical screening of the extract derived from *Trigonella foenum-graecum* has revealed promising insights into its potential as a source of bioactive compounds, particularly diosgenin. Through meticulous extraction processes, a significant quantity of diosgenin was obtained, as evidenced by preliminary scanning compared with a standard reference compound (Figure 1). The deliberate selection of the Thin Layer Chromatography (TLC) method proved instrumental in validating the successful extraction of diosgenin, with the observed Rf values for both the herbal extract and the standard closely aligned. This alignment suggests the presence of similar chemical constituents in the extract, affirming the efficacy of the extraction method in isolating diosgenin and other relevant substances from *Trigonella foenum-graecum.*

The successful isolation of diosgenin underscores the potential of *Trigonella foenum-graecum* as a natural reservoir of bioactive compounds with promising pharmacological properties. Further investigations into the chemical composition and therapeutic potential of the herb are warranted to unlock its full spectrum of benefits. Additionally, optimizing the extraction process to enhance the yield and purity of diosgenin could pave the way for the development of novel pharmaceutical formulations or dietary supplements with improved efficacy and safety profiles.

Furthermore, the anti-wrinkle activities of the extract and gallic acid were evaluated through MMP-1 and MMP-2 IC_{50} values, respectively. While both the extract and gallic acid exhibited anti-collagenase and anti-elastase activities, gallic acid demonstrated superior inhibitory effects against both MMP-1 and MMP-2, as indicated by significantly lower IC_{50} values compared to the extract. This highlights the potential of gallic acid as a promising candidate for further exploration and development of anti-wrinkle agents.

However, it's imperative to acknowledge the complexity of the extract, which may contain a multitude of compounds that could interact synergistically or antagonistically, influencing its overall activity. Further research endeavors should aim to elucidate the precise mechanisms underlying these observed effects and identify additional bioactive constituents present in the extract. Moreover, in vivo studies are crucial to comprehensively evaluate the therapeutic potential and safety profile of these compounds for potential pharmaceutical applications.

In summary, the findings from this study contribute to our understanding of *Trigonella foenum-graecum* as a valuable source of bioactive compounds and underscore its potential applications in medicine and nutraceuticals. Continued exploration of this herbal extract holds promise for the development of novel therapeutic agents targeting various health conditions, including anti-wrinkle formulations, with significant clinical implications.

REFERENCES[30]

1. Club, Elites, and Sirjan Branch. "Fenugreek (*Trigonella foenum-graecum L.*) as a valuable medicinal plant." *International Journal of Advanced Biological and Biomedical Research*, 2013; 1.8: 922-931.
2. Patil, Sangita P., Pramod V. Niphadkar, and Mrinal M. Bapat. "Allergy to fenugreek (*Trigonella foenum graecum*)." *Annals of Allergy, Asthma & Immunology*, 1997; 78.3: 297-300.
3. Kakani, R. K., and M. M. Anwer. "Fenugreek." *Handbook of herbs and spices*. Woodhead Publishing, 2012; 286-298.
4. Yao, Y., and B. Xu. "Skin Health Promoting Effects of Natural Polysaccharides and Their Potential Application in Cosmetic Industry. *Polysaccharides.*, 2022; 3: 818–830.
5. Mersing, Johor, and Nur Amalina Binti Ismail. "documentation, anti-aging activities and phytochemical screening of selected medicinal plants used by jakun women in, 2017.
6. Napagoda, Mayuri, and Sanjeeva Witharana. "Exploring the Plant Kingdom for Sources of Skincare Cosmeceuticals: From Indigenous Knowledge to the Nanotechnology Era." *Wild Plants*. CRC Press, 2020; 426-443.
7. Figueiredo, Célia Cristina Malaguti, et al. "Biosynthesis of silver nanoparticles of Tribulus terrestris food supplement and evaluated antioxidant activity and collagenase, elastase and tyrosinase enzyme inhibition: In vitro and in silico approaches." *Food and Bioproducts Processing.*, 2023; 138: 150-161.
8. Poomanee, Worrapan, et al. "Unlocking the anti-aging ingredients of Sacha inchi husk through ultrasound-assisted extraction: Response surface methodology and comprehensive analytical approach." *Journal of Agriculture and Food Research*, 2024; 15: 101016.
9. Murphy, Gillian, et al. "Matrix metalloproteinase degradation of elastin, type IV collagen and proteoglycan. A quantitative comparison of the activities of 95 kDa and 72 kDa gelatinases, stromelysins-1 and-2 and punctuated metalloproteinase (PUMP)." *Biochemical Journal*, 1991; 277.1: 277-279.
10. Daboor, Said M., et al. "Extraction and purification of collagenase enzymes: a critical review." *Am. J. Biochem. Biotechnol*, 2010; 6.4: 239-263.
11. Alipour, Hamzeh, et al. "Therapeutic applications of collagenase (metalloproteases): A review." *Asian Pacific Journal of Tropical Biomedicine*, 2016; 6.11: 975-981.
12. Yuan, Huichin, et al. "Effects of collagenase and elastase on the mechanical properties of lung tissue strips." *Journal of applied physiology*, 2000; 89.1: 3-14.

13. Miranda-Nieves, David, and Elliot L. Chaikof. "Collagen and elastin biomaterials for the fabrication of engineered living tissues." *ACS Biomaterials Science & Engineering*, 2017; 3.5: 694-711.
14. Imokawa, Genji, and Koichi Ishida. "Biological mechanisms underlying the ultraviolet radiation-induced formation of skin wrinkling and sagging I: Reduced skin elasticity, highly associated with enhanced dermal elastase activity, triggers wrinkling and sagging." *International journal of molecular sciences*, 2015; 16.4: 7753-7775.
15. Ghimeray, Amal Kumar, et al. "In vitro antioxidant, collagenase inhibition, and in vivo anti-wrinkle effects of combined formulation containing Punica granatum, Ginkgo biloba, Ficus carica, and Morus alba fruits extract." *Clinical, cosmetic and investigational dermatology*, 2015; 389-396.
16. Lansing, Albert I. "Ageing of elastic tissue and the systemic effects of elastase." Ciba Foundation Symposium-General Aspects (Colloquia on Ageing). Chichester, UK: John Wiley & Sons, Ltd., 1955; 1.
17. Eaknai, Waleewan, et al. "Ethanolic fenugreek extract: its molecular mechanisms against skin aging and the enhanced functions by nanoencapsulation." *Pharmaceuticals*, 2022; 15.2: 254.
18. Kaviarasan, S., P. Viswanathan, and C. V. Anuradha. "Fenugreek seed (*Trigonella foenum graecum*) polyphenols inhibit ethanol-induced collagen and lipid accumulation in rat liver." *Cell biology and toxicology*, 2007; 23: 373-383.
19. Nathiya, S., M. Durga, and T. Devasena. "Therapeutic role of *Trigonella foenum-graecum* [fenugreek]–a review." *Int. J. Pharm. Sci. Rev. Res.*, 2014; 27.2: 74-80.
20. Mahmood, N. M., and K. I. Yahya. "Nutrient and phytochemical of fenugreek (*Trigonella foenum graecum)* seeds." *International Journal of Sciences: Basic and Applied Research*, 2017; 36.3: 203-213.
21. Ali, Ammar MA, and Mawahib EM ElNour. "Antioxidant activity, total phenolic, flavonoid and tannin contents of callus and seeds extracts of fenugreek (*Trigonella foenum-graecum L.*)." *Int J Sci Res*, 2014; 3.10: 1268-72.
22. Mandegary, Ali, et al. "Alkaloid and flavonoid rich fractions of fenugreek seeds (*Trigonella foenum-graecum L.)* with antinociceptive and anti-inflammatory effects." *Food and Chemical Toxicology*, 2012; 50.7: 2503-2507.
23. Adedapo, Adeolu Alex, Sunday Oluwaseun Ofuegbe, and Kehinde Olugboyega Soetan. "Pharmacologic and medicinal properties of fenugreek (Trigonellafoenum-graecum L)." *American Journal of Social Issues and Humanities (Special Issue)*, 2014; 13-20.
24. Subhashini, N. A. T. L., A. Thangathirupathi, and N. Lavanya. "Antioxidant activity of *Trigonella foenum graecum* using various in vitro and ex vivo models." *Int J pharm pharm Sci*, 2011; 3.2: 96-102.
25. Ahmadiani, A., et al. "Anti-inflammatory and antipyretic effects of *Trigonella foenum-graecum* leaves extract in the rat." *Journal of ethnopharmacology*, 2001; 75(2-3): 283-286.
26. Yusharyahya, Shannaz Nadia, et al. "The effect of *Trigonella foenum-graceum L.*(Fenugreek) towards collagen type I alpha 1 (COL1A1) and collagen type III alpha 1 (COL3A1) on postmenopausal woman's fibroblast." *Natural Product Sciences*, 2019; 25.3: 208-214.
27. Yusharyahya, Shannaz Nadia. "Potential role of fenugreek (*Trigonella foenumgraecum*) in the prevention of skin aging." *Journal of the Medical Sciences (Berkala Ilmu Kedokteran)*, 2020; 53.1.
28. Chumchuen, Supachai., et al. "In vitro study of *Bacopa monnieri* and *Tagetes erecta* extract-loaded double emulsion for inhibitory alzheimer's disease and anti-cataractogenic" *European journal of pharmaceutical and medical research*, 2023; 10.11: 21-26.
29. Amir, M., et al. "Development and validation of HPTLC method for the estimation of diosgenin in in vitro culture and rhizome of Dioscorea deltoidea." *Acta Chromatographica*, 2012; 24.1: 111-121.
30. Chumchuen, Supachai. "Evaluation and formulation of gel form anitdesma velutinum leaves extract for antioxidant and anti-wrinkle activies." *European journal of pharmaceutical and medical research*, 2020; 7.5: 707-711.