ABSTRACT

Even though aging is a natural part of life, anti-aging cream masks or combats with visible signs of aging through natural anti-oxidants which hydrates, firms, and rejuvenate the skin. Quercetin, a flavonoid exhibit potent anti-oxidant and anti-inflammatory properties.This study aimed to extract analyse, and utilize the bio-active compounds such as quercetin from *Tabernaemontana divaricata* leaves and *Clitoria ternatea* flowers for the formulation of an anti-aging cream. The extracts underwent phytochemical screening and chromatographic profiling via TLC and HPTLC, with quercetin as the reference standard. HPTLC analysis revealed that the ethyl acetate extract of *T. divaricata* and aqueous extract *C. ternatea* had more concentration of quercetin than other extract.

Key words: Quercetin, HPTLC, Anti-aging, *Tabernaemontana divaricata, Clitoria ternatea*

INTRODUCTION

In recent years, the global cosmetic industry has experienced a significant shift toward natural and plant-based skincare solutions, driven by increasing consumer awareness of product safety, environmental sustainability, and long-term skin health.Among these, anti-aging cosmetics remain at the forefront, as individuals seek effective ways to maintain youthful skin without the adverse effects associated with synthetic ingredients.

Skin aging is a multi-factorial process characterized by wrinkles, hyper-pigmentation, loss of elasticity, and dryness, driven by intrinsic factors such as cellular senescence and oxidative stress, as well as extrinsic influences like ultraviolet (UV) radiation and pollution. These factors contribute to DNA damage, epigenetic modifications, and the degradation of structural skin proteins like collagen. Recent trends in cosmetic science emphasize the shift from synthetic chemicals to bioactive compounds derived from natural sources, particularly those with antioxidant, anti-inflammatory, and skin-rejuvenating properties.

ROLE OF QUERCETIN IN SKIN AGING

Quercetin (Que), 3,3′,4′,5,7-pentahydroxyflavone, is an organic compound in the group of flavonols which are a class of flavonoids. In nature, quercetin occurs as the aglycone of flavonoid glycosides forming isoquercetin with glucose, rutin with rutinose, hyperoside with galactose, and quercetrin with rhamnose. The compound is sparingly soluble in water[1], and soluble in Alcohol[2], lipids[3]and organic solvents.[4-5]

Quercetin, a naturally occurring flavonol found in various fruits, vegetables, and medicinal plants, has emerged as a promising candidate in this regard due to its potent

biological activities and safety profile[5-12].Quercetin demonstrates strong anti-aging potential by inhibiting matrix metalloproteinase-1 (MMP-1) and cyclooxygenase-2 (COX-2), key enzymes in collagen degradation and inflammation. It also modulates key signaling pathways including AP-1, NF-κB, ERK, JNK, AKT, and STAT3—factors closely linked to skin aging, UV damage[13], and carcinogenesis. Additionally, it activates proteasome function and supports fibroblast regeneration, contributing to skin repair and resilience.Topical formulations of quercetin have shown favorable skin penetration and efficacy in reducing oxidative damage and pigmentation by inhibiting tyrosinase, while also suppressing pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6[11.] Given these properties, quercetin is increasingly recognized as a valuable alternative to synthetic anti-aging ingredients in modern dermatological and cosmetic formulations.

Tabernaemontana divaricata and *Clitoria ternatea*, two botanicals traditionally used in herbal medicine, are rich sources of flavonoids and other bio-active compounds, including quercetin. Despite their traditional uses, scientific research exploring their combined potential in cosmetic formulations remains limited.

This study explores the isolation of quercetin from *Tabernaemontana divaricata* and *Clitoria ternatea* and its application in a novel anti-aging poly-herbal cream, aligning with the demand for safer, plant-based skincare solutions

AIM: The main aim of the research work is to standardize and formulate an anti-aging poly-herbal cream.

OBJECTIVE

- To extract the bioactive compounds from *Tabernaemontana divaricata* leaves and flowers of *Clitoria ternatea* using successive solvent extraction and maceration method respectively.
- To standardize the extract through organoleptic evaluation, phytochemical analysis, determination of weight and chromatographic techniques (TLC and HPTLC) using quercetin as a marker.
- To formulate an anti-aging cream using the extract containing more quercetin content.
- To carry out the evaluation of the formulated cream.

MATERIALS AND METHODS

- Collection and identification of plant materials are as follows,
- Leaves of *Tabernaemontana divaricata* and flowers of *Clitoria ternatea* were collected from Gandhinagar, Kottayam district of Kerala.

- The Herbarium were prepared. The plant parts were then dried in shade for almost 2 weeks in a clean area to remove moisture and to prevent degradation and it finely chopped and grounded to get the fine powder.
- The dried powdered plant material of *Tabernaemontana divaricata* was extracted using Soxhlet extraction method by soxhlet assembly successively with petroleum ether (40/60), chloroform, ethyl acetate and methanol. Finally, the drug was macerated with distilled water.Each time before extracting with the next solvent, the powdered material was dried in hot-air oven below 50℃.Each extract was concentrated by distilling off the solvent and evaporating to dryness on water bath. The extract obtained with each solvent was weighed accurately.
- The dried grounded flowers of *Clitoria ternatea* was extracted by maceration in clean beaker and about 200ml of distilled water was added into it. The mixture was allowed to stand for 7 days at room temperature with occasional stirring and then filtered.The extract was then concentrated in a heating mantle at 75℃.
- The obtained plant extracts were subjected to various standardization techniques as organoleptic evaluation and phytochemical screening.
- Colour and consistency of the extracts was evaluated by visual inspection and touch.
- Various phytochemical tests were done to the extracts to identify the bio-active compounds such as Alkaloids, Saponins, Tannins, Flavanoids, Carbohydrates, Steroids, Terpenoids and Glycosides.

CHROMATOGRAPHIC ANALYSIS

TLC and HPTLC were performed to the extracts to analyse the presence of quercetin in extracts.

TLC

- TLC was performed in pre-coated silica gel and quercetin was used as the standard.
- The solvent system used was n-hexane: ethyl acetate: acetic acid in the ratio 10:8:2.
- The sample spots were detected using UV light at 365nm.

HPTLC

- HPTLC is a sophisticated form of TLC. The component with more affinity towards the stationary phase travels slower and those with lesser affinity travels faster. Thus, the components are separated on a chromatographic plates**.**
- The stationary phase used was silica gel (60-120 mesh)**,** Column size: 2.5 cm×50 cm. Samples applied were chloroform extract, methanol extract, aqueous extract, ethyl acetate extract of *Tabernaemontana divaricata* and aqueous extract of *Clitoria ternatea.*
- Quercetin standard was dissolved in methanol to make a stock solution.
- Mobile phase used here was: n-hexane: ethyl acetate: acetic acid in the ratio 15.5:7:2.5.
- Detection wavelength: 254nm (UV detection)Results were interpreted based on Rf values, peak intensities and densitometric scanning using a CAMAG TLC Scanner. The extracts having more Quercetin content were determined from the results.

FORMULATION OF POLY HERBAL CREAM

A poly herbal cream (O/W) using the plant extracts was formulated.

Table:1 Formulation of cream base (For 10gm)

Ingredients	Quantity	Use
Shea butter	1 g	Emollient, oil phase
Almond oil	1g	Carrier oil, oil phase
Distilled water	6 g	Aqueous phase
Glycerine	0.5 g	Humectant
Phenoxyethanol	0.05 g	Preservative
Lavender oil	0.05 g	Fragrance

Table: 2 Herbal extracts

Herbal extract	Quantity
Ethyl acetate extract of *Tabernaemontana divaricata*	0.5g
Aqueous extract of *Clitoria ternatea*	0.5g

PROCEDURPE [34-36]

Weigh shea butter, almond oil, and Tween 80 accurately in a heat-resistant beaker. Melt shea butter, almond oil and Tween 80 in a beaker using a water bath (oil phase) at 70°C.Mix gently until the ingredients melt completely. In another beaker dissolve glycerine in distilled water (aqueous phase) and heat to 70°C.After heating, add oil phase slowly into aqueous phase with continuous stirring.After mixing both phases add the herbal extracts (Table:2) and continue stirring until we get a smooth cream.Allow the cream to cool at room temperature and add lavender oil as fragrance. Store the cream in a clean suitable container. and it is evaluated for various characteristics like physical appearance,wash ability, irritancy test etc.

RESULTS AND DISCUSSION

TABLE 3: ORGANOLEPTIC EVALUATION OF EXTRACTS &WEIGHT OF THE EXTRACTS

EXTRACT	COLOUR	CONSISTENCY	WEIGHT(%w/w)
ETHER EXTRACT	Blackish green	Solid	11.32
CHLOROFORM EXTRACT	Blackish green	Solid	7.46
ETHYL ACETATE EXTRACT	Yellowish brown	Solid	5.12
METHANOL EXTRACT	Brownish green	Semi – solid	23.71
AQUEOUS EXTRACT	Dark brown	Semi – solid	21.64
AQUEOUS EXTRACT(C)	DARK BROWN	Semi- solid	21.54

TABLE 4:PHYTOCHEMICAL SCREENING OF *TABERNAEMONTANA DIVARICATA & CLITORIA TERNATEA*

EXTRACT	ETHER	CHLORO FORM	ETHYL ACETATE	METHANOL	AQUEOUS(T)	AQUEOUS(C)
ALKALOIDS	+	+	+	+	-	-
SAPONINS	-	-	-	-	+	+
CARBOHYDRATE	-	-	-	+	+	+

S						
PHENOLS	-	-	+	+	+	+
TANNINS	-	-	+	+	+	+
FLAVANOIDS	-	+	+	+	+	+
GLYCOSIDES	-	-	+	+	+	+
STEROIDS	+	+	+	+	-	-
TERPENOIDS	+	+	+	+	-	-

Report : The results show that:

- Ether extract contain alkaloids, steroids and terpenoids.
- Chloroform extract contain alkaloids, flavonoids, steroids and terpenoids.
- Ethyl acetate extract contain alkaloids, phenols, tannins, flavonoids, glycosides, steroids and terpenoids.
- Methanol extract contain alkaloids, carbohydrates, phenols, tannins, flavonoids, glycosides, steroids and terpenoids.
- Aqueous extract(T) contains saponins, carbohydrates, phenols, tannins, flavonoids and glycosides.
- Aqueous extract of *Clitoria ternatea* contains saponins, carbohydrates, phenols, tannins, flavonoids and glycosides.

TLC REPORT

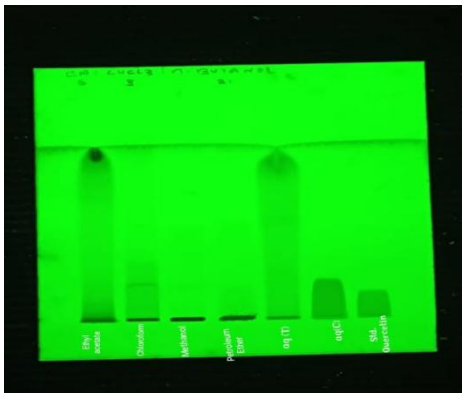


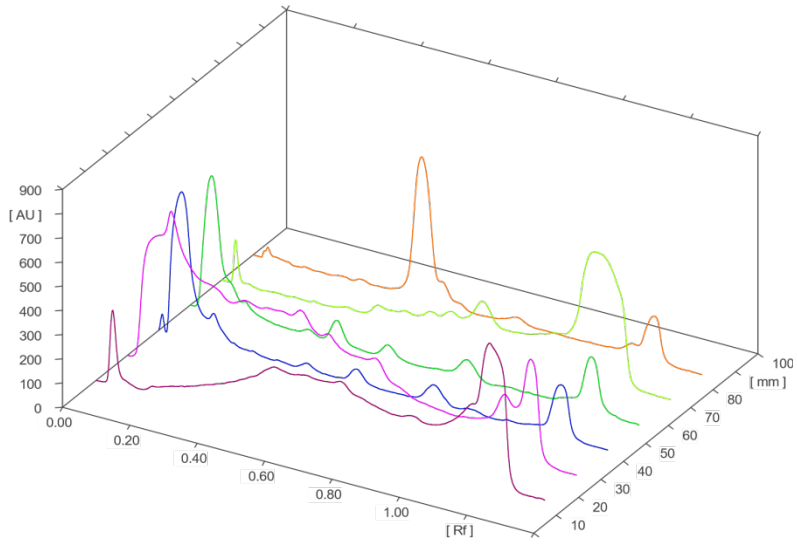
Figure 11: TLC of the samples

TLC was conducted on the herbal extracts and the results shows that ethyl acetate extract contains the highest quercetin content. Methanolic and aqueous extracts had moderate quercetin content.

HPTLC REPORT

X-axis: R_f Value

Y-axis: Absorbance units



GRAPH 1:
HPTLC PLOT OF EXTRACTS

- Track 1: Chloroform extract
- Track 2: Methanol extract
- Track 3: Aqueous extract *(T.divericata)*
- Track 4: Aqueous extract (*C.ternatea*)
- Track 5: Ethyl acetate extract
- Track 6: Standard Quercetin

RESULT: HPTLC was conducted for the herbal extracts and the results shows that ethyl acetate extract (Track 5) had the highest quercetin content (based on peak height and area at Rf -0.50). Methanol extract (Track 2) and aqueous extract of *CLITORIA TERNATEA* had moderate quercetin content. Aqueous extract and chloroform extract of *TABERNAEMONTANA DIVARICATA* had negligible quercetin content.

TABLE 8: PHYSICOCHEMICAL SCREENING OF HERBAL CREAM

PHYSICOCHEMICAL PARAMETERS	OBSERVATION
Colour	Cream to light yellow
Odour	Pleasant
Consistency	Smooth
State	Semisolid
Ph	5.8 -6
Phase seperation	No phase separation
Irritancy	Non – irritant
Wash ability	Good
Greasiness	Non – greasy

CONCLUSION

The present study successfully extracted and analyzed quercetin, a potent flavonoid known for its anti-aging and antioxidant properties, from *Tabernaemontana divaricata* and *Clitoria ternatea*. Quercetin was selected due to its well-established efficacy in protecting the skin from oxidative stress and delaying signs of aging. The extracts were characterized and confirmed for the presence of quercetin using suitable analytical techniques. A topical cream formulation was then developed incorporating the plant-derived quercetin.The final formulation exhibited satisfactory physicochemical characteristics, indicating its potential as a natural, plant-based skincare product. This study highlights the relevance of traditional medicinal plants in modern dermaceutical applications and lays the groundwork for further pharmacological and clinical evaluation.

Further investigations can focus on the quantitative estimation of quercetin in the plant extracts using advanced analytical techniques such as HPLC, LC-MS, or UV-spectrophotometry to ensure consistency, standardization, and reproducibility of the formulation. Quantification will also help in optimizing the dose-dependent efficacy of the cream, ensuring maximum therapeutic benefits while maintaining safety. Additionally, stability studies, in vitro skin permeation, and in vivo efficacy testing can further validate the potential of this herbal formulation as a reliable and effective anti-aging and antioxidant topical products.

REFERENCES

1. Salehi B, Machin L, Monzote L, Sharifi-Rad J, Ezzat SM, Salem MA, Merghany RM, El Mahdy NM, Kılıç CS, Sytar O, Sharifi-Rad M. Therapeutic potential of

quercetin: new insights and perspectives for human health. ACS omega. 2020 May 14;5(20):11849-72.

2. Abraham MH, Acree Jr WE. On the solubility of quercetin. Journal of Molecular Liquids. 2014 Sep 1;197:157-9.

3. Rich GT, Buchweitz M, Winterbone MS, Kroon PA, Wilde PJ. Towards an understanding of the low bioavailability of quercetin: a study of its interaction with intestinal lipids. Nutrients. 2017 Feb 5;9(2):111.

4. Lakhanpal P, Rai DK. Quercetin: a versatile flavonoid. Internet Journal of Medical Update. 2007 Jul 1;2(2):22-37.

5. Oku S, Aoki K, Honjo M, Muro T, Tsukazaki H. Temporal changes in quercetin accumulation and composition in onion (Allium cepa L.) bulbs and leaf blades. The Horticulture Journal. 2021;90(3):326-33.

6. Mogren LM, Olsson ME, Gertsson UE. Quercetin content in field-cured onions (Allium cepa L.): effects of cultivar, lifting time, and nitrogen fertilizer level. Journal of agricultural and food chemistry. 2006 Aug 23;54(17):6185-91.

7. Heimler D, Romani A, Ieri F. Plant polyphenol content, soil fertilization and agricultural management: A review. European Food Research and Technology. 2017 Jul;243:1107-15.

8. David AV, Arulmoli R, Parasuraman S. Overviews of biological importance of quercetin: A bioactive flavonoid. Pharmacognosy reviews. 2016 Jul;10(20):84.

9. Xu D, Hu MJ, Wang YQ, Cui YL. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules. 2019 Mar 21;24(6):1123.

10. Lim HJ, Kang SH, Song YJ, Jeon YD, Jin JS. Inhibitory effect of quercetin on propionibacterium acnes-induced skin inflammation. International immunopharmacology. 2021 Jul 1;96:107557.

11. Shin EJ, Lee JS, Hong S, Lim TG, Byun S. Quercetin directly targets JAK2 and PKCδ and prevents UV-induced photo-aging in human skin. International journal of molecular sciences. 2019 Oct 23;20(21):5262.

12. Nebus J, Vassilatou K, Philippou L, Wallo W. Clinical improvements in facial photo aged skin using a novel oak quercetin topical preparation. In Journal of the American Academy of Dermatology 2011 Feb 1 (Vol. 64, No. 2, pp. AB73-AB73).

13. Casagrande R, Georgetti SR, Verri Jr WA, Dorta DJ, dos Santos AC, Fonseca MJ. Protective effect of topical formulations containing quercetin against UVB-induced

oxidative stress in hairless mice. Journal of Photochemistry and Photobiology B: Biology. 2006 Jul 3;84(1):21-7.

14. Dhase AS, Khadbadi SS, Saboo SS. Formulation and evaluation of vanishing herbal cream of crude drugs. Am J Ethnomed. 2014;1:313-8. 66. Oyedele Fluid emulsion base potential of shea butter. Journal of Pharmacy & Bioresources. 2016 Nov 29;13(2):103-13.

15. Matangi SP, Mamidi SA, Raghavamma ST, Nadendla RR. Formulation and evaluation of anti aging poly herbal cream. Skin. 2014;5(6).

16. Grace XF, Vijetha RJ, Shanmuganathan S, Chamundeeswari D. Formulation and evaluation of polyherbal cosmetic cream. Adv J Pharm Life sci Res. 2014;2(3):14-7