Research Article

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FORMULATION DEVELOPMENT AND EVALUATION OF MICROEMULSION GEL SYSTEM OF EXTRACT OF *QUERCUS INFECTORIA* OLIV. FOR TOPICAL USE

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ABSTRACT

Quercus infectoria Olivier Family Fagaceae common name Mayphal have a great medicinal value and have different proved pharmacologic activities like astringent, anti-diabetic, anti-tremor, local anesthetic. In present work topical Microemulsion of extract of *Quercus infectoria* prepared on the basis of traditional claim. Preliminary phytochemical screening, Fingerprinting analysis UV spectroscopic analysis for the said extract along with the biomarker, gallic acid was performed for establishing the profile of extract. The extract was formulated in Microemulsion gel with different oil samples and optimized formulation was evaluated for various parameters like appearance, spreadability, pH, viscosity, *in-vitro* diffusion and antimicrobial study. The R_f value of extract was found to be 0.19. The extract exhibited good correlation with selected marker. The optimized formulation exhibited significant results for *in vitro* drug diffusion, viscosity and antimicrobial activity with acceptable spreadability and elegant appearance. Further study of formulation using some animal models will explore the use of extract in the treatment of different skin ailments.

Keywords: Antimicrobial activity, Microemulsion, *Quercus infectoria*, Skin ailments

INTRODUCTION

The Modern system of medicines possess many types of dosage form for treating dermatological diseases. The conventional dosage forms include pastes, ointments, creams, gels but due to their certain shortcomings new forms came in to the picture for topical delivery to name a few, Microemulsion, lipogels, organogels, micelle system etc. these dosage forms offer improved drug diffusion, spreadability and thermodynamic stability. Two immiscible liquids (e.g. water and oil) can be brought into a single phase with appropriate use of surfactant and co-surfactant(s). This thermodynamically stable optically clear system is called as 'Microemulsion'. In present study Microemulsion-gel system has been prepared with the help of appropriate concentration of surfactant and co-surfactant. It is considered that the envelope formed by surfactant monolayer at the oil/water interface decides phase behavior and microstructure of final formulation hence Tween 80 and Polyethylene glycol 400 selected as surfactant and co-surfactant respectively as it gives oil-in-water (O/W) microemulsions[1-3]. Ayurveda and other alternative system of medicines have effective solutions for treating skin diseases which uses natural herbs, minerals and many more natural origin materials. Different naturally occurring drugs like minerals, herbs, vitamins have been formulated in Microemulsion-gel system for their effective delivery[4-6]. In folklore galls have been used for treating inflammatory diseases. The direct application of boiled and bruised galls on skin effectively cures any swelling or inflammation. In present study an attempt has been made to formulate hydroalcoholic extract of *Q. infectorea* in to Microemulsion gel so as to improve its efficacy in terms of drug permeation, spreadability and also patient acceptance as it does not leave oil residue on skin after washing [7-10]

MATERIALS AND METHODS

Materials

Oil phase- Captex 200 which is propylene glycoldicaprylate obtained as gift sample from Abitech Corporation Surfactant- Tween 80 (Research Lab Ltd.), Co surfactant- PEG 400 (Research Lab. Ltd.) are procured from local chemical supplier.

Plant collection and extraction

The fresh fruits of *Q. infectoria* were selected for this study. Selected plant was collected from Pune region and authenticated from Department of Botany, University of Pune, Maharashtra, India (Voucher No. Bot/34/2010). Proximate analysis of powder was carried out for different physicochemical standards such as ash values, extractive values and loss on drying**.** The hydroalcoholic extracts in the proportion 60:40 for the selected herbal plant was prepared by simple maceration for about 72 h and concentrated and stored in air tight container [11-15].

Fingerprinting Analysis

The crude extract of selected plant was analyzed for HPTLC fingerprinting using Camag HPTLC system equipped with an automatic TLC sampler and TLC scanner with a UV cabinet. Planer chromatograms of samples prepared using general solvent system Toulene:Chloroform:Ethanol (8:8:2) and solution prepared as 100 mg in 5 ml of methanol.

UV-Spectroscopic Analysis

UV-Spectroscopic analysis of extract and biomarker was performed using Shimadzu 1700 UV spectrophotometer. Absorbance maxima for extract was established and UV calibration curve of extract was prepared using UV spectrophotometer.

Antimicrobial activity of Extract
The crude extract and optimized formulation was screened for antimicrobial action on selected microbes i.e. *Staphylococcus aureus* and *Candida albicans* which are normal flora of skin and also *Pseudomonas aeruginosa* using Cup Plate Method. Previous two microbes are opportunistic pathogens. The above mentioned microorganisms were selected on the basis that they are the usual pathogens which causes many secondary skin infections usually occurring in Psoriatic lesions. This study will be further explored to establish antipsoriatic activity of extract. Internal standards used were Streptomycin and Bacitracin for bacteria and fungi respectively. Based on minimum inhibitory concentration (MIC), concentration of extract in formulation was decided[16,17].

Preparation of Microemulsion Gel
Trial batches of Microemulsion gel were prepared using different concentrations of oil, water, surfactant and co-surfactants to establish optimum concentrations of ingredients which will give stable and elegant gel. Mixture of oil, surfactant and co-surfactant prepared to form Oil Phase whereas extract was dissolved in aqueous phase and then oil phase was added to aqueous phase followed by gentle stirring. It gave Microemulsion gel instantaneously. The oil and aqueous phase mixed in 1:1 ratio (Table 1)[18-20].

Evaluation of Optimized Formulation
Optimized batches of *Q. infectoria* were evaluated for different physical parameters like Appearance, pH, Spreadability, Viscosity, Skin irritation test, *In-vitro* drug diffusion etc.[21-2].

Skin Irritation Test
Skin irritation test was performed on albino mice weighing about 25-30g. The two groups of animals were prepared. Optimized formulation 0.5 g and 0.8% Formalin (Positive Control) was applied to an area of approximately 6 cm^2 of skin and covered with a gauze patch on the back of animals of respective groups. The patch was loosely held in contact with the skin by means of a
semi-occlusive dressing**.** The animals were observed for sign of edematous reaction for next seven days. The protocol of study was approved by Institutional Animal Ethical Committee and care of lab animals was taken as per the guidelines laid by CPCSEA (Reg. No. 1554/PO/a/11/CPCSEA)[24]

RESULT

Phytochemical Analysis
Preliminary phytochemical screening was performed for establishing the profile of extract for its chemical composition. The extracts showed the presence of tannins, mucilage and saponins. The results are listed in Table 2. The various physicochemical standards such as Ash values, Extractive values and loss on drying were performed. The results were reported in Table 3.

Fingerprinting analysis
HPTLC fingerprinting showed better separation of the components. Planer chromatogram generated was used to determine existence of present phytoconstituents. The end R_f values of extract were found to be in the range of 0.03 to 0.19 which can be better correlated with biomarker. (Figure 1 and 2)

Spectroscopic Analysis
The extract and biomarker exhibited 0.074, 0.0511 and0.003, 0.0429 intercept and slope values, respectively (Table 4 and 5; Figure 3 and 4). UV-Spectroscopic analysis showed good correlation between plant extract and standard (gallic acid).

Antimicrobial Activity
Different concentrations of extract were screened for antimicrobial activity, based upon zone of inhibition MIC of extract of above mentioned three microbes are reported in Table 6 and Figure 5.

Skin Irritation Test
Formulation did not show any major changes at site of application, irritation or edema formation during or after seven days of observation.

Evaluation of Microemulsion gel System

Appearance and pH: The optimized formulations were found to be stiff, homogenous and translucent in appearance. The pH of formulations was determined by using Digital pH meter, 1g gel dissolved in 100 ml distilled water and readings taken in triplicate. pH of optimized formulations was found in range of 6.8 to 7.2. (Table 6)

Spreadability
Spreadability was determined by pressing 1 g of a sample for 1 min. between two 20 x 20 cm horizontal plates, the upper of which weighed 125 g. The spread diameter (Φ) was measured and expressed as spreadability. (Table 7)

Viscosity
Viscosity determination was done using Brookfield Viscometer at a different RPM and corresponding torque**.** The viscosity observations are expressed as Mean of three readings **(**Table 7**)**

***In-vitro* drug diffusion**
In-vitro drug diffusion was carried out in Franz Diffusion Cell using prehydrated Cellophane membrane. Study performed for 06 Hours using Phosphate Buffer of pH 7.4. Percent drug diffused after 06 h across membrane and percent drug retained in membrane was determined. It was found that F4 formulation showed better drug release and retention which was 63.74 % and 18.46 % respectively. (Figure 6, Table 8 and Table 9)

DISCUSSION

The proximate analysis of extract showed acceptable range of all parameters which are evaluated. Phytochemical screening showed presence of tannins, saponins and mucilage. Analytical screening of extract with HPTLC and UV Spectroscopy has given insight about chemical composition which was correlated with biomarker, Gallic acid. The extract exhibited acceptable antimicrobial and antifungal activity against selected microbes. Optimized formulations showed acceptable pH, spreadability, *in vitro* diffusion and percent drug retention.

CONCLUSION

The above study indicates that hydroalcoholic extract of *Q. infectoria* Oliv. can be presented to human body in more effective manner when formulated in Microemulsion-gel system. Tannins which are prime constituent of galls can effectively treat different skin conditions. This work will be further extended to establish antipsoriatic activity of extract.

Table 1: Formulation of Microemulsion-gel System

Ingredients	F1	F2	F3	F4
Q. infectorea (% w/v)	1	2	3	4
Distilled Water	49	48	47	46
Captex 200 (0il) %	35	35	35	35
Tween 80 (Surfactants) %	10	10	10	10
PEG 400 (Co-surfactants) %	5	5	5	5

Table 2: Preliminary Phytochemical Screening of *Quercus infectoria*

Sr. No	Nature of Constituent	Sample (*Q. infectoria*)
1.	Alkaloids	-
2.	Carbohydrates	+
3.	Flavonoids	-
4.	Glycosides	-
5.	Lipids	+
6.	Mucilage	+
7.	Phytosterols	-
8.	Proteins and Amino acids	-
9.	Saponins	+
10.	Tannins	+
11.	Volatile oils	-

Table 3: Proximate analysis of *Quercus infectoria*

Sr. No.	Proximate analysis	Observed Value
1.	Total Ash (%)	11.4
2.	Acid Insoluble Ash (%)	7.1
3.	Sulphated Ash (%)	8.43
4.	Alcohol Soluble Extractive Value (%)	16.38
5.	Water Soluble Extractive Value (%)	34.16
6.	% LOD	18.5

Table 4: Calibration of *Quercus infectoria* at 275 nm

Concentrations (mcg/ml)	Absorbance
0	0
15	0.407
30	0.798
60	1.568
90	2.161
120	2.763

Table 5: Calibration Curve of Gallic acid at 260 nm

Concentrations (mcg/ml)	Absorbance
0	0
2	0.101
4	0.215
6	0.313
8	0.399
10	0.51

Table 6: Antimicrobial Activity of *Quercus infectoria*

Ps. aeruginosa	*Candida albicans*	*Staph. aureus*
7 mg/ml	3 mg/ml	2 mg/ml

Table 7: Physical parameters of Optimized Formulation

Sr. No.	Formulation Code	pH	Viscosity (cps)	Spreadability Spread Diameter (Φ) mm
1.	F1	6.80 ± 0.12	532± 0.27	48 ± 0.41
2.	F2	6.93 ± 0.27	523± 0.58	45 ± 0.25
3.	F3	7.11 ± 0.14	485± 0.41	52 ± 0.14
4.	F4	7.18 ± 0.35	436 ± 0.25	58 ± 0.18

Table 8: Percent Drug Release of Optimized Formulations of *Quercus infectoria*

Sampling Time	Cumulative % Drug Release			
	F1	F2	F3	F4
15 min.	3.81±0.25	2.30 ± 0.19	2.85±0.42	1.52±0.65
30 min.	4.56±0.35	2.94 ±0.65	5.28±0.56	4.54±0.31
1 h	6.38±0.41	4.18±0.10	9.11±0.52	8.06±0.44
2 h	10.36±0.22	8.56±0.14	16.52±0.17	14.67±0.74
3 h	21.28±0.45	18.65±0.27	28.65±0.65	21.07±0.36
4 h	35.20±0.26	29.26±0.24	41.85±0.86	38.81±0.20
5h	48.65±0.12	41.38±0.28	54.47±0.	52.45±0.05
6 h	58.48±0.16	55.32±0.18	60.31±0.14	63.74±0.30

* Readings are in triplicate (± SD)

Table 9: Percent Drug retained in Cellophane membrane after 06 h

Sr. No.	Formulation	% Drug Retention
1.	F1	22.32±0.32
2.	F2	28.36±0.15
3.	F3	16.85±0.85
4.	F4	18.46±0.52

* Readings are in triplicate (± SD)

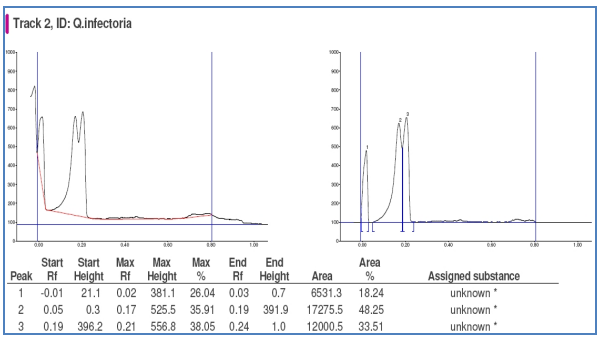


Figure 1: Planer Chromatogram of *Quercus infectoria*

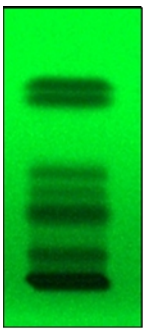


Figure 2: HPTLC band of *Quercus infectoria*

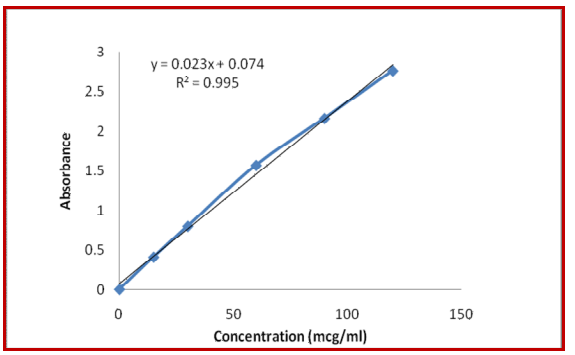


Figure 3: Calibration Curve of *Quercus infectoria* at 275 nm

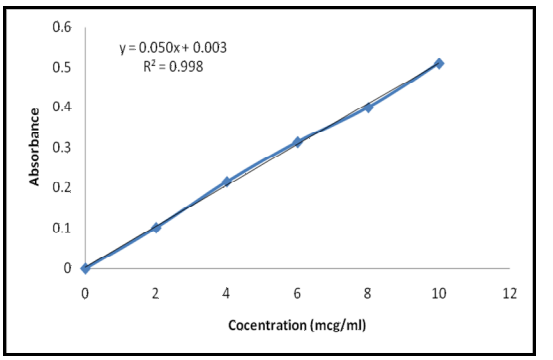
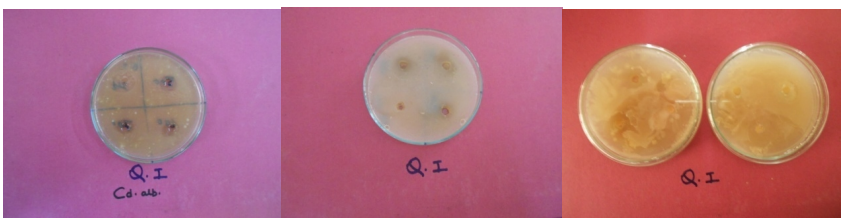


Figure 4: Calibration Curve of Gallic acid at 260 nm



A B C

Figure 5: A. Antimicrobial activity of extract and Std. on (Bacitracin) *Candida albicans*, B. Antimicrobial activity of extract and Std. (*Streptomycin*) on *Ps.aeruginosa* C. Antimicrobial activity of extract and Std. (Streptomycin) on *Staph. aureus*

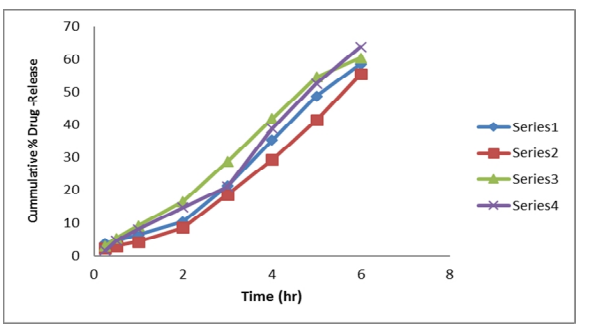


Figure 6: Cumulative Percent Drug Release

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REFERENCES

1. Paul BK and Moulik SP. Uses and applications of microemulsions. Current Science. 2001; 80(8): 990-1001.
2. Oricha BS. Cosmeceuticals: A review. African Journal of Pharmacy and Pharmacology. 2010; 4(4): 127-129.
3. Mandal S, Das S. Design and Development of Carbamazepine Mucoadhesive Microemulsion for Intranasal Delivery: AN Ex-vivo Study. International Journal of Pharmaceutical Sciences Review and Research. 2010; 3*(1):* 56-60.
4. Kapoor VP. Herbal Cosmetics for Skin and Hair. Natural Product Radiance. 2005; 4*(4):* 306-3014.
5. Rozman B, Zvonar A, Falson F, and Gasperlin M. Temperature-Sensitive Microemulsion Gel: An Effective Topical Delivery System for Simultaneous Delivery of Vitamins C and E. AAPS PharmSciTech. 2009; 10 (1): 1-9. http://dx.doi.org/10.1208/s12249-008-9172-3
6. Mishra U, Murthy PN, Pasa G and Kumar s. Formulation development and Standardization of Herbal Gel containing Methanolic Extract of *Butea Frondosa.* International Research journal of Pharmacy. 2011; 2(11): 126-129.
7. Aroonrerk N, Kamkaen. Anti-inflammatory activity of *Quercus infectoria*, *Glycyrrhiza uralensis, Kaempferia galanga* and *Coptis chinensis*, the main components of Thai herbal Remedies for aphthous ulcer. J. Health Res. 2009; 23(1):17-22.
8. Abdul-Aziz JRM. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agent. Indian J. Pharmacol. 2005; 37(1):26-29. http://dx.doi.org /10.4103/0253-7613.13851
9. Kingston C, Jeeva s, Kiruba S, Mishra BP and Kanan D. Indigenous knowledge of using medicinal plants in treating skin diseases in Kanyakumari district, Southern India. Indian Journal of Traditional Knowledge. 2009; 8 (2): 196-200.
10. Kaur G, Athar M, Alam MS. *Quercus infectoria* galls possess antioxidant activity and abrogates oxidative stress-induced functional alterations in murine macrophages. Chem. Biol. Interact. 2008; 171(3):272–282. http://dx.doi.org /10.1016/j.cbi.2007.10.002
11. Wallis TE (1985). In: Text Book of Pharmacognosy. CBS Publishers and Distributors. Delhi; 1985. p. 101-102.
12. Mukherjee PK (2002). In: Quality Control on Herbal Drugs. Eastern Publishers (Business Horizons Ltd.) New Delhi; 2002. p. 212.
13. Al-Quran S. Taxonomical and Pharmacological Survey of Therapeutic Plants in Jordan. J. Nat. Prod. 2008; 1:10-26.
14. Ghafour N, Hoshyar AA, Raad MA. Determination of Some Chemical Constitutes of Oak Plants *(Quercus spp)* in the Mountain Oak Forest of Sulaimani Governorate. J. Zankoy Sulaimani. 2010; 13(1):129-142.
15. Vermani A, Navneet P, Chauhan A. Physico-Chemical Analysis of Ash of Some Medicinal Plants Growing in Uttarakhand, India. Nat. Sci. 2010; 8(6):88-91.
16. Chusri S, Voravuthikunchai SP. Detailed studies on *Quercus infectoria* Olivier (nutgalls) as an alternative treatment for methicillinresistant *Staphylococcus aureus* infections. J. Appl. Microbiol. 2009; 106:89-96. http://dx.doi.org/ 10.1111/j.1365-2672.2008.03979.x
17. Basri DF, Fan SH. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. Indian J. Pharmacol. 2004; 37(1):26-29. http://dx.doi.org/10.4103/0253-7613.13851
18. Bergamante V, Ceschel GC Marazzita S, Ronchi C and Fini A. 2007. Effect of Vehicles on Topical Application of *Aloe vera* and Arnica Montana Components. Drug Delivery. 14(7): 427-432. http://dx.doi.org/10.1080/10717 540701202960
19. Nasseri AA, Aboofazeli R, Zia H , Thomas NE. Lecithin – Stabilized Microemulsion – Based Organogels for Topical Application of Ketorolac Tromethamine. II. *In vitro* Release Study. Iranian Journal of Pharmaceutical Research. 2003; 2: 117-123.
20. Vorarat S, Managit C, Iamthanakul L, Soparat W and Kamkaen N. Examination of Antioxidant activity and Development of Rice Bran oil and Gamma- oryzanol Microemulsion. J Health Res. 2010; 24*(2):* 67-72.
21. El laithy HM and El-Shaboury KMF. The Development of Cutina Lipogels and Gel Microemulsion for Topical Administration of Fluconazole. AAPS PharmSciTech. 2002; 3(4): 1-9.
22. Singh MK, Chandel V, Gupta V and Ramteke S. Formulation development and characterization of Microemulsion for topical delivery of Glipizide. Der Pharmacia Letter. 2010; 2(3): 33-42.
23. Mahapatra AP, Mishra DK and Panda P. Formulation and Evaluation of Cream Prepared from *Croton Spasiflorus* Morong and their Wound Healing Activity. Int. J. Res. Ayurveda Pharm. 2012; 3(6): 803-807. http://dx.doi.org/10.7897/2277-4343.03623
24. Garg A, Aggarwal D, Garg S and Singla AK. Spreading of Semisolid Formulations an update. Pharmaceutical Technology. 2002; 84-105.

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