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Original Research Article

Formulation Development and Evaluation of Microemulsion Gel System of Extract of *Tephrosia purpurea* for Topical Use.

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Abstract

The ancient knowledge of Ayurveda and drugs of herbal origin can be replicated in to a modern science. The present work elaborates the use of microemulsion drug delivery system of extract of *Tephrosia purpurea* to treat different skin ailments in better manner. In the present work whole plant of *Tephrosia purpurea family; Fabaceae* which is also traditionally known as *Sharpunkha* selected on the basis of traditional claim. The reported constituents of plant are alkaloids, tannins and carbohydrates. These constituents are considered to be having antiinflammatory, and blood purifying activity. The proximate analysis, Preliminary Phytochemical Screening and HPTLC fingerprinting analysis of hydroalcoholic extract of plant was done to confirm the presence of alkaloids and tannins. 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 optimized formulation exhibited significant results for *in vitro* drug diffusion, viscosity and antimicrobial activity with acceptable spreadability and elegant appearance. Further study of formulation on specific skin disease using some animal models will explore the use of *Tephrosia purpurea* in cosmeceuticals.

Keywords: Microemulsion, Extract*, Tephrosia purpurea,* Skin disease, Cosmeceuticals

1. Introduction

Microemulsions due to their versatile physicochemical attributes like optically clarity, thermodynamic stability and droplet size ranging from 10-140nm stands as delivery of choice for topical dermal application. It can interact with the stratum corneum resulting into the increased permeability due to structural rearrangement lipid layers[1,2]. Skin diseases are result of internal factors like inability of the skin to retain moisture, decrease in Elastin production, less turnover of new skin cells and external factors like lifestyle and environment of the individual like smoking, exposure to sun, exposure to cold, poor diet, too much stress and lack of exercise etc.

Ayurveda has suggested many homemade treatments for skin diseases using 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. This study focus on development of Microemulsion-gel of hydroalocoholic extract of whole plant of *Tephrosia purpurea.*[3] In Ayurveda *Tephrosia purpurea* family *Fabaceae is* referred to as *Sarwa wran vishapaha* which means that it has healing power of any type of wound. Hence on the basis of this traditional claim above plant selected.[4]

2. Materials and Methods

2.1 Plant Collection and Extraction

Fresh whole plant was selected for extraction. Selected plant was collected from Pune region

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and authenticated from Department of Botany, University of Pune. 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 above selected herbal plants were prepared by simple maceration for about 72 hours and concentrated and stored.[5]

2.2 Phytochemical Analysis

The crude extract was subjected to Preliminary Phytochemical Screening for the detection of various phytoconstituents.[6]

2.3 Fingerprinting Analysis

The crude extract of selected plant was analyzed by HPTLC fingerprinting from Anchrom Enterprises, Mumbai. Camag HPTLC system equipped with an automatic TLC sampler and TLC scanner with a UV cabinet were used.[7]

2.4 Spectroscopic Analysis

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

2.5 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. They are involved in many secondary skin infections usually occurring in Psoriatic lesions. Internal standards used were Streptomycin and Bacitracin for bacteria and fungi respectively.[8,9]

2.6 Skin Irritation Test

The test was performed on albino mice weighing about 25-30g. The test animals were divided into two groups each containing seven animals. Cotton wool impregnated with Formulation and 0.8% Formalin (Positive Control) was applied on the back of two groups of animals respectively. The animals were observed for sign of edematous reaction. [10]

2.7 Formulation and Optimization of Microemulsion Gel

2.7.1Material and Method

- Aqueous Phase
- Oil- Captex 200
- Surfactant- Tween 80
- Co surfactant- PEG 400

Trial batches using different concentrations of oil, water, surfactant and co-surfactants were prepared to establish optimized concentrations 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) [11, 12,13]

2.8 Evaluation of Optimized Formulation

Optimized batch was evaluated for different physical parameters like Appearance, pH, Spreadability, Viscosity, *In-vitro* drug diffusion etc.

3. Results and Discussion

3.1 Phytochemical Analysis:

Preliminary phytochemical screening was performed for establishing the profile of extract for its chemical composition. The extracts showed the presence of Flavanoids, 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.

3.2 Fingerprinting analysis

HPTLC fingerprinting showed better separation of the components. Planer chromatogram generated was used to determine existence of present phytoconstituents. The end Rf values of extract were found to be in the range of 0.02 to 0.81 (Figure 1 and 2)

3.3 Spectroscopic Analysis

UV-Spectroscopic analysis showed absorbance maxima at 311 nm and obeyed Beer Lamberts law in the range of 30-210 mcg/ml. It showed 0.997 regression coefficient with 0.008 and 0.025, intercept and slope values respectively. (Figure 3 and Table 4)

3.4 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 5 and Figure 4.

3.5 Skin Irritation Test

Formulation did not show any irritation or edematous reaction during or after seven days of observation.

3.6 Evaluation of Microemulsion gel System

3.6.1 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 6.5 to 7.2. (Table 6)

3.6.2 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 6) [14]

3.6.3 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 6)[15]

***3.6.4 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 Hrs. across membrane and percent drug retained in membrane was determined. It was found that F3 formulation showed better drug release and retention which was 62.65% and 20.47% respectively. (Figure 5, Table 7 and Table 8)[16]

Discussion

Phytochemical investigation has provided better understanding of chemical make-up of *T. purpurea.* In fingerprinting analysis end R_f value of extract is in the range of 0.02 to 0.81 which can resemble with standard values of active chemical constituents of extracts like Quercetin and Rutin. Spectroscopic analysis also shows good linearity with r^2 value 0.997. Optimized formulations were found to be significant in terms of appearance, water washability, viscosity, spreadability, % drug released and anti microbial activity when compared with synthetic drug. Drug release of formulations F1, F2, F3 and F4 was found to be 53.24%, 51.28%, 62.65% and 49.05% respectively after 06 hrs. and that of retention of drug in diffusing membrane was 25.63%, 31.65%, 20.47% and 18.75% respectively.

Conclusion

Thus in upcoming era there is need of Ayurvedic and other Alternative Systems of medicines due to shortcomings of modern medicines. Flavanoids and Tannins serves as natural defense mechanism against microbial infections, inflammatory conditions and they also serves as immunomodulators. This study reveals that F3 formulation shoed good drug release thus *T. purpurea* can serves as drug of choice for many skin diseases and disorders like eczema, psoriasis, due to presence of Flavanoids, tannins and many other constituents. Formulation retained in membrane can serves as drug depot for its topical action. This study can further be explored with some animal models to establish significance of *T. purpurea* in the treatment of skin diseases like psoriasis.

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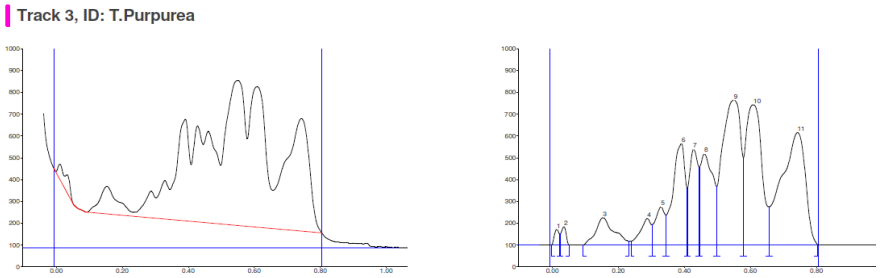

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.00	0.8	0.01	73.0	1.95	0.02	51.3	937.5	0.62	unknown *
2	0.02	51.4	0.04	85.2	2.28	0.05	0.1	1135.4	0.75	unknown *
3	0.09	0.2	0.15	126.5	3.39	0.23	18.4	6279.9	4.12	unknown *
4	0.24	18.8	0.29	122.5	3.28	0.30	93.2	3613.5	2.37	unknown *
5	0.30	93.9	0.33	176.4	4.72	0.34	138.0	4548.9	2.99	unknown *
6	0.35	138.1	0.39	466.6	12.49	0.41	259.7	15703.3	10.31	unknown *
7	0.41	262.0	0.43	440.8	11.80	0.45	357.9	10587.1	6.95	unknown *
8	0.45	358.0	0.46	419.3	11.22	0.50	268.6	14659.1	9.63	unknown *
9	0.50	269.9	0.55	665.3	17.80	0.58	401.0	33243.1	21.83	unknown *
10	0.58	401.6	0.61	644.4	17.25	0.66	175.4	28189.4	18.51	unknown *
11	0.66	175.6	0.74	516.6	13.83	0.81	2.2	33357.0	21.91	unknown *

Figure 1: Planer Chromatogram of *T. purpurea*

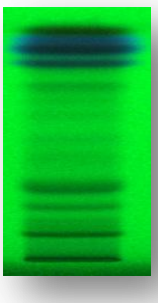

Figure 2: HPTLC Band of *T. purpurea*

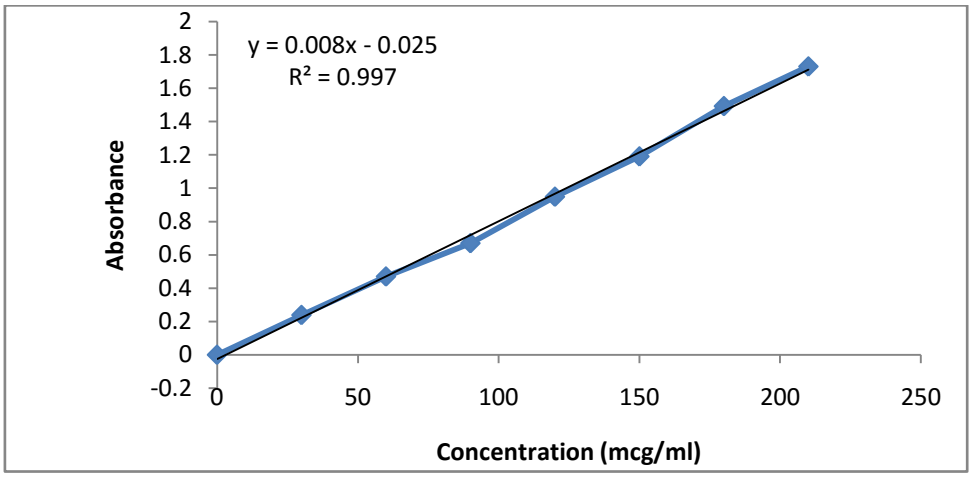

Figure 3: UV Calibration Curve of *Tephrosia purpurea* at 311 nm

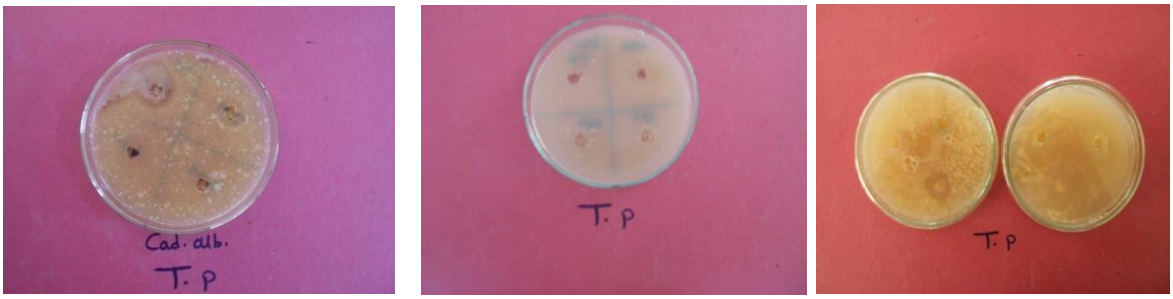

Fig. 4.1: Antimicrobial activity of extract and Std. on (Bacitracin) *Candida albicans*
Fig. 4.2: Antimicrobial activity of extract and Std. (Streptomycin) on *Ps. aeruginosa*
Fig. 4.3: Antimicrobial activity of extract and Std. (Streptomycin) on *Staph. aureus*

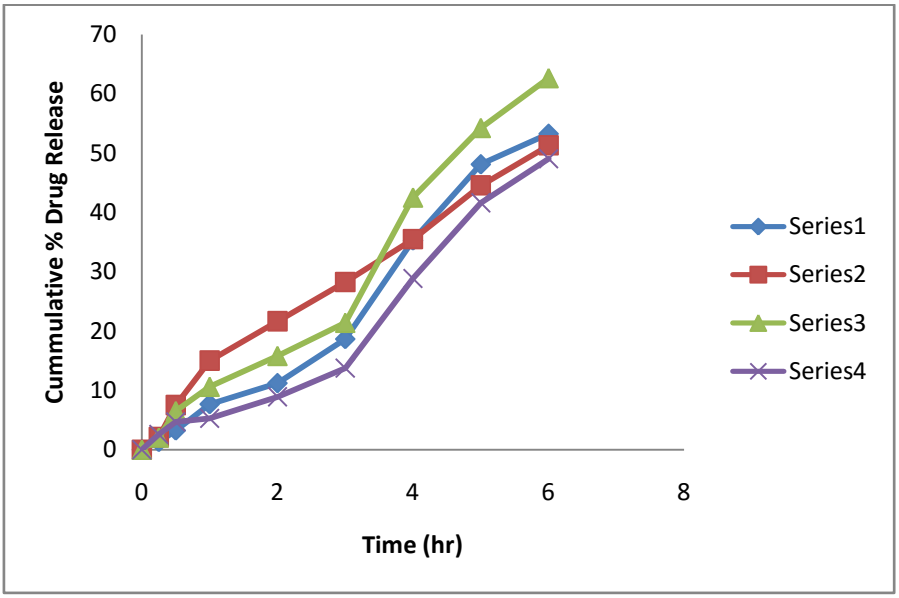

Figure 5: *In vitro* Drug Diffusio

Table1: Formulation of Microemulsion-gel System

Ingredients	F1	F2	F3	F4
T. purpurea (% w/v)	2	3	4	5
Distilled Water	48	47	46	45
Captex 200 (0il) %	30	30	30	30
Tween 80 (Surfactants) %	15	15	15	15
PEG 400 (Co-surfactants) %	5	5	5	5

Table 2: Phytochemical Screening of *T. purpurea*

Sr. No	Nature of Constituent	Hydroalcoholic Extract of *T. purpurea*
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 *T. purpurea*

Sr. No.	Proximate analysis	Observed Value
1.	Total Ash (%)	7.3
2.	Acid Insoluble Ash (%)	4.8
3.	Sulphated Ash (%)	4.14
4.	Alcohol Soluble Extractive Value (%)	7.10
5.	Water Soluble Extractive Value (%)	32.26
6.	% LOD	5.8

Table 4: UV Calibration of *T. purpurea*

Concentration (mcg/ml)	Absorbance
0	0
30	0.239
60	0.47
90	0.67
120	0.949
150	1.191
180	1.493
210	1.731

Table5: Antimicrobial Activity of *T. purpurea*

Ps. aeruginosa	***Candida albicans***	***Staph. aureus***
6 mg/ml	4 mg/ml	3 mg/ml

Table 6: Physical parameters of Optimized Formulation

Sr. No.	**Formulation Code**	**pH**	**Viscosity (cps)**	**Spreadability Spread Diameter (ϕ) mm**
1.	F1	6.85 ± 0.12	403 ± 0.47	53 ± 0.41
2.	F2	6.70 ± 0.27	412 ± 0.32	50 ± 0.25
3.	F3	7.13 ± 0.14	382 ± 0.15	54 ± 0.34
4.	F4	7.22 ± 0.35	370 ± 0.62	56 ± 0.28

**Readings are in triplicate (*± SD*)*

Table 7: Percent Drug Release of Optimized Formulations of *T. purpurea*

Sampling Time	**Cumulative % drug release**			
	F1	**F2**	**F3**	**F4**
15 min.	1.30 ± 0.52	2.12±0.25	2.05±0.42	2.58±0.21
30 min.	3.24 ±0.40	7.52±0.31	6.52±0.56	4.70±0.15
1 hr.	7.68±0.16	15.02±0.14	10.61±0.52	5.28±0.67
2 hr.	11.21±0.11	21.64±0.22	15.81±0.17	8.93±0.83
3 hr.	18.65±0.47	28.26±0.07	21.40±0.65	13.77±0.37
4 hr.	35.26±0.26	35.47±0.62	42.52±0.86	28.87±0.14
5hr.	48.10±0.78	44.55±0.51	54.25±0.08	41.63±0.63
6 hr.	53.24±0.33	51.28±0.26	62.65±0.35	49.05±0.45

** Readings are in triplicate (*± SD*)*

Table 8: Percent Drug retained in Cellophane membrane after 06 hrs.

Sr. No.	**Formulation**	**% Drug Retention**
1.	F1	25.63±0.46
2.	F2	31.65±0.58
3.	F3	20.47±0.16
4.	F4	18.75±0.34

** Readings are in triplicate (*± SD*)*

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Conflict of Interest: None declared

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