Research Article

Formulation of Herbal Emulsion based Anti-inflammatory Cream for Skin Diseases

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ABSTRACT

Ethanolic extracts of *Emblica officinalis, Eculaptus globules* and *Cymbopogon citratus* were taken for this research work to elucidate their anti-oxidant, anti inflammatory and anti bacterial activity against skin diseases and to formulate an emulsion based cream. 2, 2-diphenyl-1-picrylhydrazyl test and DNA protection test was carried out to evaluate anti oxidant activity. Four different anti inflammatory assays, namely albumin denaturation, HRBC membrane stabilization, anti-proteinase and heat induced hemolysis were performed. The anti bacterial activity was evaluated using microtitre well dilution method against *S.aureus and S.pyogenes*. Maximum anti oxidant activity was found in the case of *E.officinalis* (84.37 %) whereas *C.citratus* was good DNA protecting agent (16.98%). Anti inflammatory tests yielded maximum inhibition for *E.globulus* (91.32%). *E.officinalis* showed a promising result for anti-bacterial (MIC_{90} of *S.aureus*= 0.251mg/ml and of *S.pyogenes*=0.325mg/ml). The cream retained its original property only at the RT (pH=6.55, viscosity=12.2cps, Spreadability=14.88 g.cm/s).

Keywords: Anti-inflammatory, DNA protection, Emulsion based cream, denaturation.

INTRODUCTION

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers.[1] In the case of oxidative stress, reactive oxygen species are generated. These agents play an important role in causing various serious diseases such as ageing[2] cancer, coronary heart disease, Alzheimer's disease and inflammation. Hence, anti oxidants that can scavenge these reactive oxygen species can be beneficial in the treatment of inflammatory disorders. Synthetic antioxidants have restricted use because of their toxicity and DNA damaging properties.[3] Whereas natural products obtained from plant extracts prove to be a better alternative against such modern medicines that have numerous side effects.

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend us against attack from predators such as insects, fungi and other microrganisms. Three such medicinal plants were selected for this study. It was reported that *Emblica officinalis* has a strong antioxidant activity, which may be partially due to the existence of flavonoids and several gallic acid derivatives including epigallocatachin gallate.[4] The essential oil of the plant *Cymbopogon citrates*, Citral, has applications in food, perfumery, soap, cosmetic, pharmaceutical and insecticide industries.[5] Aqueous extracts of dried leaves of this plant are used in traditional medicine for the treatment of inflammation, digestive disorders, diabetes, nervous disorders, cancer, fever, etc. [6,7] Thirdly, the leaves of *Eucalyptus globules* have been externally used as traditional remedies for inflammation in China.[8] Essential oil from this species has a therapeutic application in treatment of pulmonary infections by inhalation[9], and the monoterpene extracted from *Eucalyptus citriodora*, *Eucalyptus globulus* and *Eucalyptus teretcorni* exhibit antibacterial activity.[10]

MATERIALS AND METHODS

Plant Material Collection and Extract Preparation

Fresh leaves of Eucalyptus globules and Cymbopogon citrates were collected from CSIR-Centre for aromatic and medicinal plants, Allalasandra, Bangalore, Karnataka. Similarly fresh *Emblica officinalis* fruits were collected from Bangalore. These samples were then washed thoroughly and cut into pieces and shade dried at room temperature for 15 days. After the samples were dried completely, they were grounded to make it into powdered form. This was further used for extract preparation.

The raw plant material was then subjected to ethanol extraction with soxhlet apparatus. Weight of plant material was taken before loading in the Soxhlet apparatus and the extracts obtained were concentrated using rotary evaporator and then dried. Weight of the dried extract was also noted.

Bacterial Strains

The two organisms (*S.aureus* and *S.pyogenes*) used for the testing anti-microbial activity were purchased from MTCC. The strains from the mother culture plate were

cultured in L.B.broth and grown for overnight before use. These were frequently subcultured every 7 days in new media for fresh use.

Anti Oxidant Test

DPPH (1, 1Diphenyl2picrylhydrazyl) radical scavenging activity:

The ability of the extract to scavenge DPPH radicals was determined using the standard protocol with minor modification.[11]

The percentage of inhibition was calculated using the following formula:

Percentage inhibition = ($Abs_{control}$ – Abs_{sample}) X 100/$Abs_{control}$)

DNA Protection Activity

To study the free radical damage, 4ul of 100ng/ul of plasmid was mixed with 2ul of TE buffer (Tris HCl, 200nM and EDTA, 5mM) and 4ul of 30% hydrogen peroxide.[12] Plant extracts of different concentrations was made and 4ul of these were added to the above mixture each. Volume was made up to 20ul with sterile distilled water. One negative control was prepared in the same way except that no plant extract was added. All these samples were placed under UV light (To generate free radicals) for 5mins. One positive control was also prepared by taking 4ul of plasmid and volume was made up to 20ul with sterile distilled water. No UV treatment was given to positive control.

Anti Inflammatory Activity

Inhibition of albumin denaturation[13], anti-proteinase activity[14], human red blood cell membrane stabilization method[15] and heat induced hemolysis assay[16] were done according to the standard protocols, with minor modifications.

Anti Microbial

Minimum Inhibitory Concentration Assay

MIC test was done to find the minimum concentration of extract required to inhibit the visible growth of microorganism after overnight incubation.

The test was carried out on an elisa plate. A extract stock of 1ml of concentration 10mg/ml was taken in the first well. It was then serially diluted to half the concentration in every other well.

Later to every well, 0.5ml of an overnight culture of the organism in LB broth was added and kept for overnight incubation for 24 hrs at 37 °C. OD reading was then taken in 550nm against LB broth as blank.[17]

Combinatorial Approach

After the individual activities of the extracts were observed, the extracts were then mixed in different proportion and anti inflammatory activity through HRBC membrane stabilization method was carried out to find out the best combination.

Absorption based Cream Formulation

Hot Infusion Oil Extract

1g of the extract (the combination that gave best result) was mixed in 10ml of olive oil and boiled in water bath for 60-70°C.[18] 10ml of this mixture was then cooled and filtered using cotton cloth. The filtrate was collected and heated again in water bath. For this mixture, 2.8g of Beeswax was added when the solution was hot. Wait till the solution melts. This is the oil phase of the cream.

Water Extract

1g of extract (the combination that gave best result) was mixed in 10 ml of water. This mixture was then boiled in water bath up to 60-70°C. It was then cooled down to room temperature and filtered using cotton cloth. The filterate collected is the water extract.

Mixing

Mortar and pestle was taken for mixing the two phases. The oil phase was poured into the mortar before solidification. 5 drops of mineral oil was added to this oil phase. The water extract was added drop by drop and the mixture was being mixed continuously. This was done until the consistency of a cream was obtained.

Physical Evaluation of Cream

pH

pH was determined by using Digital pH meter. One gram of cream was dissolved in 100ml of distilled water and stored for two hours.

The measurement of pH of the cream formulation was done in triplicate and average values were taken.[19]

Viscosity

The viscosity of the prepared cream was measured with the help of viscometer. The values were measured in triplicate and average value was considered.[20]

Spreadability

A special apparatus was designed to study the spreadability of cream formulation.[21] The spreadability is expressed in terms of times in seconds taken by two slides to slip off from cream and placed in between the slides, under the direction of certain load. Lesser the time taken taken for separation of two slides, resultant the better spreadability. Spreadability was calculated by using the formula.

S = M.L/T

S = Spreadability

M = Weight tied on upper slide

L = Length of glass slides

T = Time taken to separate the slides completely from each other.

Organoleptic and Stability Test

Changes in organoleptic properties of the creams were evaluated by visual inspection and the properties evaluated included the colour of the creams, texture and consistency.[22]

Phase separation was another test carried out using centrifuge.

These were evaluated over a period of 45 days at specific time intervals of 15 days each using environment chamber to test its stability at different temperatures (4 °C, 37 °C and 50 °C).

RESULTS

Statistical Tool

All the statistical work for this research was done by using graphpad prism, version 5.1. All the assays were done three times and the results were expressed in mean ± SEM.

Yield

The maximum yield was found in *Eucalyptus globules* with 18.4 (w/w) when compared with *Emblica officinalis* and *Cymbopogan citrates* (Table 1).

Table 1: Extract Yield for the Selected Medicinal Plants

Plant used	Amount of Powder Taken (g)	Volume of Ethanol Taken (ml)	Volume of Extract Obtained in Liquid Form (ml)	Amount of Extract Obtained After Evaporation (g)	% Yield (w/w)
Emblica officinalis	5	150	100	0.57	11.4
Eucalyptus globules	5	150	120	0.92	18.4
Cymbopogan citratus	5	150	80	0.72	14.4

Table 2: % of Intensity of Plasmid DNA Bands

Lane	Contents	% Intensity
1	Plasmid	17.054
2	Plasmid + H_2O_2	11.81
3	Plasmid + H_2O_2 + UV treatment	8.981
4	Plasmid + H_2O_2+ UV treatment + *E.officinalis*	16.53
5	Plasmid + H_2O_2+ UV treatment + *E.globules*	16.12
6	Plasmid + H_2O_2+ UV treatment + *C.citrates*	16.981
7	Plasmid + H_2O_2+ UV treatment + Taurine	16.58

Table 3: MIC Test for Selected Medicinal Plants Against Pathogens

Concentration (mg/ml)	% inhibition for S.*aureus*			% inhibition of s.*pyogenes*		
	E.officinalis	*E.globules*	*C.citratus*	*E.officinalis*	*E.globules*	*C.citratus*
0	0	0	0	0	0	0
0.0195	2.540541	5.702703	3.567568	5.647321	4.098214	7.241071
0.039	6.081081	8.810811	6.675676	12.125	10.41964	10.69643
0.078	14.91892	18.40541	13.45946	24.01786	20.24107	22.61161
0.156	27.45946	32.91892	23.45946	41.62946	36.49554	41.62946
0.31254	46.05405	36.43243	27.13514	47.65625	39.28571	53.90625
0.625	55.24324	53.40541	54.37838	65.51339	65.40179	60.26786
1.25	61.40541	66.27027	57.62162	73.66071	76.00446	65.40179
2.5	72.32432	70.91892	64.54054	79.01786	79.01786	73.99554
5	86.91892	83.24324	67.67568	80.73214	85.9375	76.11607
10	96.10811	96.97297	87.13514	82.09375	87.61161	83.14732
MIC_{90} values (mg/ml)	0.251	0.414	0.577	0.325	0.375	0.330

Table 4: Combinatorial Results for Anti-inflammatory Activity

Combination	*E.officinalis*	*E.globulus*	*C.citratus*	Anti Inflammatory (% inhibition in 1mg/ml conc.)
Combination 1	33%	33%	33%	52.26%
Combination 2	50%	25%	25%	76.58%
Combination 3	25%	50%	25%	83.1%
Combination 4	25%	25%	50%	79.64%

Table 5: Evaluation of Physical Parameters for Prepared Cream

Physical Parameters as on 45th Day	4 °C	37 °C	50 °C
Color	Light green	Green	Light green
Texture	Semi hard	Soft	Soft
Consistency	Hard consistency	Cream like	Cream like

Anti Oxidant Test

The results show that *E.officinalis* and *C.citratus* have radical scavenging activity higher than eucalyptus with 84.53% and 79.83% respectively at higher concentrations (Fig. 1). At higher concentrations, all the selected medicinal plant was found to inhibit the free radicals and even equal to their respective standard concentrations (Fig. 1).

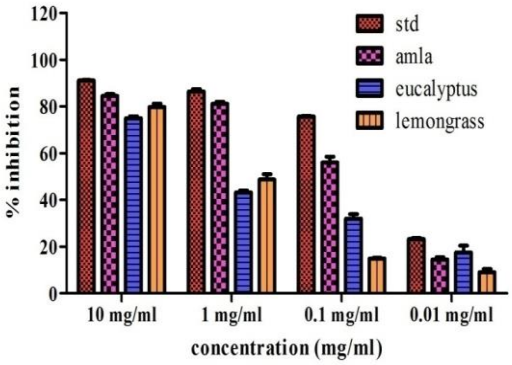

Figure 1: Comparison of DPPH assay for various medicinal plant extracts at different concentration

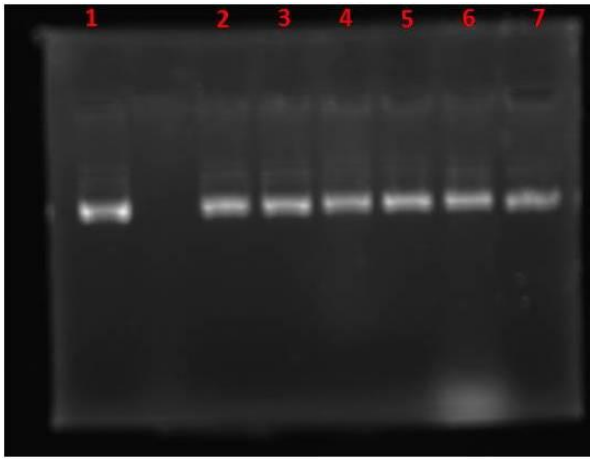

Figure 2: Gel Picture represents DNA Protection Activity

DNA Protection Activity

As the extracts showed excellent radical scavenging properties, their radioprotective activity was also studied. It was found that exposure of plasmid pBR322 to stress converted it to linear and circular forms that are visible in the smear form as shown in the figure.

Further, addition of plant extracts protected the Plasmid from getting damaged and the band was intact. The maximum protection was found in the case of *C.Citratus* extract with 16.98% as shown in Table 2, Figure 2.

Anti Inflammatory Tests

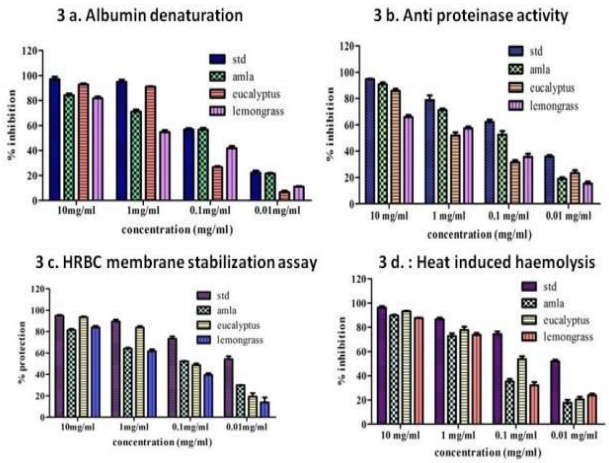

Figure 3: Anti inflammatory Assays for Various Medicinal Plants

From the four anti inflammatory tests, it was found that *E.globulus* showed the maximum activity with an average of 91.32% inhibition at its highest concentration.(Fig. 3a, b, c and d) They hence help in preventing the denaturation of protein which otherwise leads to inflammation.

The extract was also found in stabilizing the HRBC membrane which is analogous to the lysosomal membrane. Stabilization of lysosomal membrane is

important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil.

Anti Bacterial Test

MIC_{90} value indicates the concentration of the extracts required to inhibit 90% of the microorganism.

The least value was found to be that of *E.officinalis* with 0.251mg/ml and 0.325mg/ml against *S.aureus* and *S.pyogenes* respectively. Hence this plant gives a high anti bacterial activity (Table 3).

Combinatorial Approach

The best result from the above plant extracts combination was the combination 3 (25% *E.officinalis,* 50% *E.globulus* and 25% *C.citratus*) with 83.1% inhibition through HRBC cell membrane stabilization method (Table 4).

Physical Evaluation of Cream

The pH and viscosity of the formulated cream was found to be 6.55 and 12.2cps respectively. The spreadability of the cream was calculated as 14.88 g.cm/s (M=31gms, L=7.2cm and S=15sec). Further, It was observed that there were no significant difference in the organoleptic properties with the number of days whereas, the cream retained its original property only at the room temperature (37 °C) as shown in Table 5.

DISCUSSION

The extract demonstrated varied DPPH radical scavenging effect. *E.officinalis* and *C.citratus* have radical scavenging activity higher than *E.globulus.* Further, *C.citratus* showed the highest DNA protection activity owing to the fact that it has good anti oxidant properties. Among the four test carried out to evaluate the anti-inflammatory property, *E.globulus* showed the highest activity. Its membrane stabilization property was comparable to that of the standard drug, diclofenac sodium. In contrary to these findings, highest anti bacterial activity was found in that of *E.officinalis*. The pH of the prepared cream was found to be neutral with good spreadability.

The cream showed stable organoleptic properties under varied temperatures for a period of 45 days.

The conservation of supercoiled form of the DNA after irradiation by γ rays in previous study shows that the plasmid DNA was protected from radiation induced damages due to the extract of *C.citratus*.

Studies using methanolic extracts of *E.officinalis* for testing radioprotective activity were carried out using FeSo4 and H_2O_2 as free radicals.[23] It was observed that the intensity of the band decreased with time indicating damage for all the lanes except the one with extract. Further, previous studies have been performed to evaluate antibacterial activity of *E.officinalis* using MIC and agar well diffusion assay using different concentration of plant extract.[24]

The antioxidant activities of polyphenols present in the extract of the plants can be attributed to the presence of hydroxyl group (–OH) in aromatic ring, which helps in mediating redox reaction, and thereby scavenges free radicals.[25-27] The antibacterial properties of plants can be present due to the occurrence of flavonoids, alkaloids, tannins, saponins and tri-terpenoids etc.

The medicinal values of plants are numerous and proper identification and usage of these bioactive compounds can be very useful to humans.

CONCLUSION

From the present study, it can be concluded that the extracts of plants, *E.globulus, E.officinalis* and *C.citratus* have very good anti oxidation, anti inflammatory and anti bacterial properties and the emulsion based cream has good spreadability and stability.

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Competing Interest

Research can further be carried out to evaluate the biological activities of these plants by extracting certain bioactive compounds from them. Other plants with similar activities can also be used to formulate the cream which proves to have nil side effects. The cream can further be developed and used for commercial purpose. These findings can be of interest to both pharmaceutical companies and research institutes.

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