International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Formulation of topical Herbal Gel from the extract of Purslane *(Portulaca oleracea)* leaves in two concentration and its evaluation

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Abstract

The objective of the study is to formulate oral herbal gel in two concentrations (5% & 10%) from the extract of purslane leaves. Purslane (Portulaca oleracea) is an incredible herb that is edible with medicinal properties used to treat a variety of diseases. It exhibits a myriad of pharmacological effects, including antibacterial, anti-ulcerogenic, anti-inflammatory, antioxidant, wound-healing properties, purgative, cardiac tonic, emollient, muscle relaxant, and diuretic treatment. This study is aimed to formulate topical gel from purslane extract in two different concentrations. The phytochemical analysis and the antibacterial, antioxidant, and cytoprotective properties of the gel concentrations were evaluated. The necessity to formulate this gel preparation is that it can be used to alleviate the symptoms of various oral mucosal lesions. As it is a herbal preparation it has no serious side effects.

Keywords: *Portulaca oleracea*, Purslane, gel formulation, antioxidant, cytoprotective

Full length article **Corresponding Author*, **e-mail:** aruljom*@gmail.com*

1. Introduction

Purslane (*Portulaca oleracea*) is incredible herb which is edible with medicinal properties used wide spectrum of diseases (Figure 1). [1] The name Portulaca is thought to be derived from the Latin 'porto' meaning "to carry" and 'lac' meaning milk, since the plant contains a milky juice.[2] It is known with different names such as sanhti, punarva, paruppu keerai, gangavalli or kulfa. It is commonly known as purslane (USA and Australia), rigla (Egypt), pigweed (England), pourpier (France), and Ma-Chi-Xian (China)[3]. It is listed by the World Health Organization as one of the most used medicinal plants, and it has been given the term "Global Panacea" [4]. Purslane contains various phytochemicals like flavonoids, alkaloids, coumarins, anthraquinone glycoside, cardiac glycoside, fatty acids, terpenoids, polysaccharides, vitamins, sterols, proteins, and minerals. It has myriad of pharmacological effects, including antibacterial [5], anti-ulcerogenic [6], anti-inflammatory [7], antioxidant[8], wound-healing[9] properties, purgative, cardiac tonic, emollient, muscle relaxant, diuretic treatment and in the treatment of osteoporosis and psoriasis[10]. It possesses comparatively higher β-carotene, ascorbic acid, alpha-linolenic acid (ALA) and antioxidant properties, hence can be considered as an important substitute of fish for vegetarian and vegan people [1]. *Portulaca oleracea* also provides a source of nutritional benefits owing to its rich omega-3 fatty acids and antioxidant properties [11]. The Chinese folklore described it as "vegetable for long life" and it has been used for thousands of years in traditional Chinese Medicine [12,13]. *Portulaca oleracea* is distributed all over the world and easily grows in diverse soil and climatic conditions. It has been traditionally used as a nutritious food across the globe [14,15].

To formulate oral herbal gel in two concentrations (5% & 10%) from the extract of purslane leaves and evaluate its phytochemical constituents, antibacterial, antioxidant and cytoprotective properties of the prepared gel concentrations.

2. Materials and methods

2.1. Collection and Authentication of the plant

The leaves of the Purslane plant is easily available in the local market. It is collected from the local market, Tondiarpet, Chennai, Tamil Nadu. The plant specimen is authenticated by Institute of Herbal Science (Plant Anatomy Research Centre- PARC), West Tambaram, Chennai. Reg. No. of the certificate - PARC/2021/4619.

2.2. Chemicals

Ethanol, Carbopol 940, Methyl paraben, Propyl paraben, Propylene glycol 400, Triethanolamine, distilled water.

2.3. Preparation of collected leaves & extracts

The purslane plant leaves which were freshly collected were washed with running water, shade dried and powdered to granules. They were then weighed accurately and extracted with ethanol for three consecutive days. The mixture was then separated through Whatman filter paper and concentrated using Rotary vacuum evaporator. The dried extracts were fractionated with equal volumes of EtOAc and water using a separating funnel. The Purslane leaves ethyl acetate fraction was dried under vacuum and the extract were used for further analysis. Dried, crushed Purslane leaves resulted in 380gms of coarse powder which upon extraction with 400ml of ethanol yielded a dark colored crude extract which weighed approximately 5.2 g.

2.4. Formulation Table

With the method described above, the formulae were tabulated. Along with control sample, gel were prepared by addition of required quantity of Portulaca oleracea leaves extracts to prepare 5% & 10% respectively **(Table 1).**

2.5. Preparation of Gel

Different combinations of Portulaca oleracea leaves ethanol extract (5% & 10%) were tried with the polymers of Carbopol 940 using above mentioned formulae (Table 1). The following combination with Carbopol 940 resulted in the best gel formulation, which was smooth and stable. Control sample also was prepared for testing to check the activity of control ingredients. Method for Preparation of Gel Containing Extract 1 g of Carbopol 940 was dispersed in 10 ml of distilled water kept the beaker aside to swell the Carbopol 940 for 30 minutes and stirred with Carbopol 940 to form gel. Take 5 ml of distilled water and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath. Solution was brought to room temperature and Propylene glycol 400 was added. Further required quantity of portulaca oleracea leaves extract was mixed to the above mixture. Finally, all the ingredients were mixed properly to the Carbopol 940 gel with continuously stirred and triethanolamine was added in drops to adjust the pH (6.8-7) and to obtain the gel at required consistency (Fig. 2: 5 & 10% gel preparation of P. oleracea)

2.6. Phytochemical analysis of the prepared gel concentration

The phytochemical studies done with purslane provides evidence that it is one of the most abundant terrestrial sources of ω-3 and ω-6 fatty acids, ascorbic acid, tocopherols, glutathione and β-carotene advocating its prospects pertaining to nutrition [16]. Phytochemical analysis of the prepared formulations was done and the results are tabulated (Table 2).

3.7. .Evaluation of Antimicrobial, Antioxidant and Cytoprotective properties of extracts

3.7.1. The antimicrobial activity and its Minimal Inhibitory Concentration

The antimicrobial activity of the prepared formulation is investigated to provide evidence to the ethnopharmacology usage of the plants. The property is evaluated using microdilution method to determine its minimum inhibitory concentration against bacterial strains as described by Elshikh et al (2016) using Resazurin indicator [17]. The antibacterial activity of extract and its formulation against the bacterial strains, *Staphylococcus aureus and Escherichia coli* is determined using Resazurin as indicator. This method is simple, sensitive, rapid, and reliable, and could be used successfully to assess antibacterial properties of compounds. Resazurin is a cell permeable redox indicator that can be used to monitor viable cell number with protocols similar to those utilizing the tetrazolium compounds in extremely small volumes of solution in microliter plates without the use of a spectrophotometer. *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-Negative) was used for determining the MIC in the current study. Bacterial cultures were grown in discrete colonies and inoculated in the nutrient broth after 48 hrs. Both the cultures were monitored for growth using a Fluorescence plate reader after 24 hrs at a wavelength of 600 nm to obtain a final OD 1.0. Resazurin solution was prepared by dissolving a 13.5mg of Resarzurin in 2.0 mL of sterile distilled water. A solution was vortexed for 1min to get a well-dissolved and homogenous solution. A sterile 96-well plate was inoculated with bacterial culture along with, either antibiotic (Chloramphenicol 1µg/10µl) or Compounds and indicator. The layout is given below in results (Fig. 3).

3.7.2. Determination the antioxidant potential of leaf extract at different concentration (5% and 10%)

The antioxidant activity of given formulation is determined using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical gives colorless ethanol solution on reduction in presence of an antioxidant molecule at room temperature. DPPH was used to determine the free radical scavenging activity of methanolic extracts using colorimetric method as

described by Sannasimuthu A et al (2018) with minor modifications.[18]
The radical scavenging efficacy of the isolated compounds was determined using the formula

Radical scavenging activity (%) = $[(A_{control}-A_{sample})/A_{control}]\times 100\%$

On reduction, stable nitrogen centered free radical DPPH changes from violet to yellow color. The change in the color depends upon the scavenging abilities of antioxidant extracts or pure compounds as it reduces the DPPH radical by donating hydrogen. In all essays, Ascorbic acid is positive control. All the 3 samples showed radical scavenging activities.

3.7.3. Cytoprotective effect of extract in fibroblast cell line using MTT assay

The cytoprotective effect of the exract was evaluated against fibroblast cell line using MTT assay based on literature Ghazali et al. 2022 [19]. The viability of THP-1 cells against 5% and 10% formulation along with the plant extracts was checked. 20000 cells seeded in 96 well plates and incubated for 1 hr. Cells treated with 10mg/ml concentrations of formulation and extracts. Followed by 24 hrs incubation 10 µl of MTT solution along with 5% FBS added. Incubated for 3 hrs, finally 100 µl of DMSO is added and quantified at 570 nm.

3. Results and Discussions

3.1. Antimicrobial activity

Of the formulations (10% and 5%), Extract 10% and the material inhibited the growth of both gram +ve and gram -ve bacteria at all tested concentrations (5 - 0.132 mg/ml). Whereas, 5% extract showed activity against Staphylococcus aureus at all the tested concentrations but against Escherichia coli the extract did not show inhibitory activity at the lowest concentration of 0.132mg/ml. Blue color indicates No growth; Pink color indicates Growth. (Figure 3).

3.2. Antioxidant property

The base material extracts showed very low scavenging activity of ~17% with significant variation due to concentration. The 10% formulation showed the highest radical scavenging activity of ~25% - 78% (Table 3 & Figure 4). The 5% formulation showed ~24% - 44% (Table 4 & Figure 5). The compounds showed significant ($p < 0.01$) concentration dependent antioxidant activities, the higher concentration exhibited highest radical scavenging activity. This was on par with the positive control ascorbic acid which possessed 91% - 95% antioxidant activity. Both 5% and 10% formulation have significant antioxidant activity.

3.3. Cytoprotective effect of extract in fibroblast cell line using MTT assay

The plant extracts and the formulations did not show cytotoxicity against the human monocyte cell lines (THP-1) (Table 5 & Figure 6). From the above results it is clearly shows that all the prepared gel formulations was green in color without any smell or odour and has good homogeneity and gelling property.The phytochemical analysis of the prepared gel has the following constituents (Steroids, Flavonoids, Terpenoids, Proteins, Coumarins, Glycosides, Phenols & Tannins, Quinones, Alkaloids, Diterpenes). Thus overall, the gel formulations comply with all parameters of an ideal gel. The studies showed the gel formulation has desired antimicrobial, antioxidant and cytoprotective effects which makes the gel formulations suitable for the use in the oral cavity [15]. Hosseini et al., (2009), in their study, has previously formulated the systemic dosage of Purslane in tablet form(235mg) from the ethanolic extract of Portulaca oleracea form the treatment of oral lichen planus (OLP). The results showed satisfactory improvement in alleviating the symptoms of OLP with no side effects [1]. Our study is first in literature to use extracts of Purslane for formulating gel preparation indicated for topical oral use.

Table 1: Formulation proposed for gel preparation

S.NO	Ingredients	Control	PO1	PO2
1.	Carbopol 940	1gm	1gm	1gm
2.	Methyl paraben	0.4 ml	0.2ml	0.2ml
3.	Propylene glycol 400	5ml	5ml	5ml
4.	Triethanolamine(q.s)	1.2ml	1.2ml	1.2ml
5.	Distilled Water	Required volume	Required volume	Required volume
6.	P.O extract(5%)	-	0.5g	-
7.	P.O extract(10%)	-	-	10.0g

Phyto constituents	Experiment	Observation
Steroids	Extract + 2ml $CHCL_3$, then add 2ml of con.H_2SO_4& acetic acid	Greenish coloration - Presence of steroids confirmed.
Flavonoids	2ml of extract + 2ml of 2% NaOH Add few drops of dil. acid	Yellow color formation- Turns colorless - Presence of Flavonoids confirmed.
Terpenoids	200µl of extract+1ml of $CHCl_3$+1ml of conc.H_2SO_4	Presence of deep red color. - Presence of terpenoids confirmed.
Proteins	1ml of extract + 1ml H_2SO_4(Conc)	Absence of white precipitate Presence of Proteins confirmed.
Coumarins	2ml of extract + 2ml of NaOH	Absence of yellow color Absence of coumarins confirmed.
Glycosides	2ml of extract+2ml of $CHCl_3$ + 2ml of CH_3COOH	Presence of Green color Presence of Glycosides confirmed.
Phenols & Tannins	Extract with 2ml of 2% $FeCl_3$	Black coloration Presence of phenols and tannins confirmed.
Quinones	1ml of extract + 1ml conc.H_2SO_4	Formation of red color Presence of quinones confirmed.
Alkaloids	200µl extract + few drops Wagners reagent	Presence of reddish-brown color Presence of alkaloids confirmed.
Diterpenes	Extract dissolved in water + 3,4 drops of copper acetate	Formation of emerald green color. Presence of diterpenes confirmed.

Table 2: Phytochemical analysis of the prepared gel concentration

Concentration	10% formulation	10% formulation	10% formulation	Average %	SD
5	78.333	80.417	77.917	78.889	1.093612
2.5	48.333	47.083	49.167	48.194	0.856169
1.25	35.833	33.750	35.833	35.139	0.982093
0.625	30.417	33.333	31.250	31.667	1.226633
0.132	25.833	26.250	25.833	25.972	0.196419

Table 3: Antioxidant activity of the 10% formulation

Concentration	5% formulation	5% formulation	5% formulation	Average %	SD
5	44.167	45.417	45.000	44.861	0.519675
2.5	36.250	35.000	34.583	35.278	0.708197
1.25	31.250	30.833	31.667	31.250	0.340207
0.625	29.167	27.917	28.750	28.611	0.519675
0.132	25.000	23.333	24.167	24.167	0.680414

Table 4: Antioxidant activity of the 5% formulation

Sample	% Viability	STD.Dev
CONTROL	100.00	0.1642
MATERIAL	98.19	0.6568
10% FORMULATION	99.01	0.4926
5% FORMULATION	98.52	0.3284
10% EXTRACT	99.34	0.4926
5% EXTRACT	98.69	0.8210

Table 5: Cytoprotective effect of 5% and 10% formulation

Figure 1: Leaves of *Portulaca oleracea*

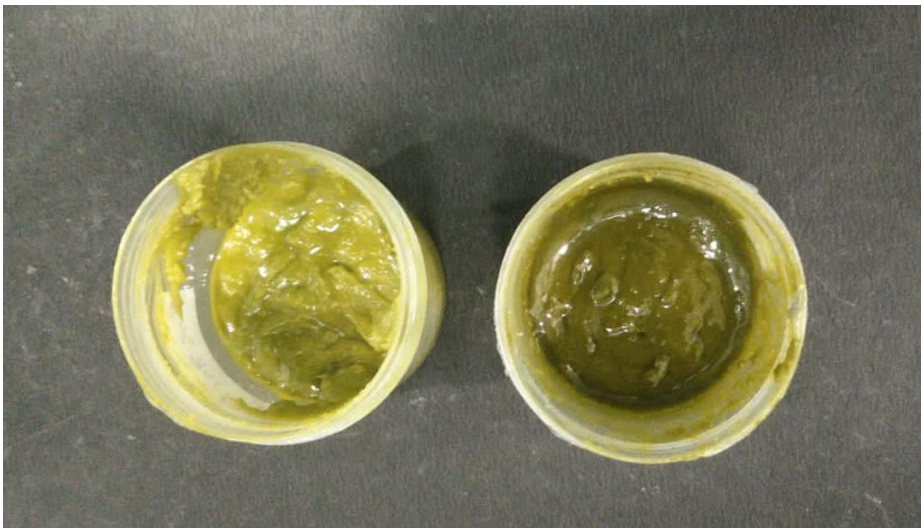


Figure 2: 5 & 10% gel preparation of *P. oleracea*

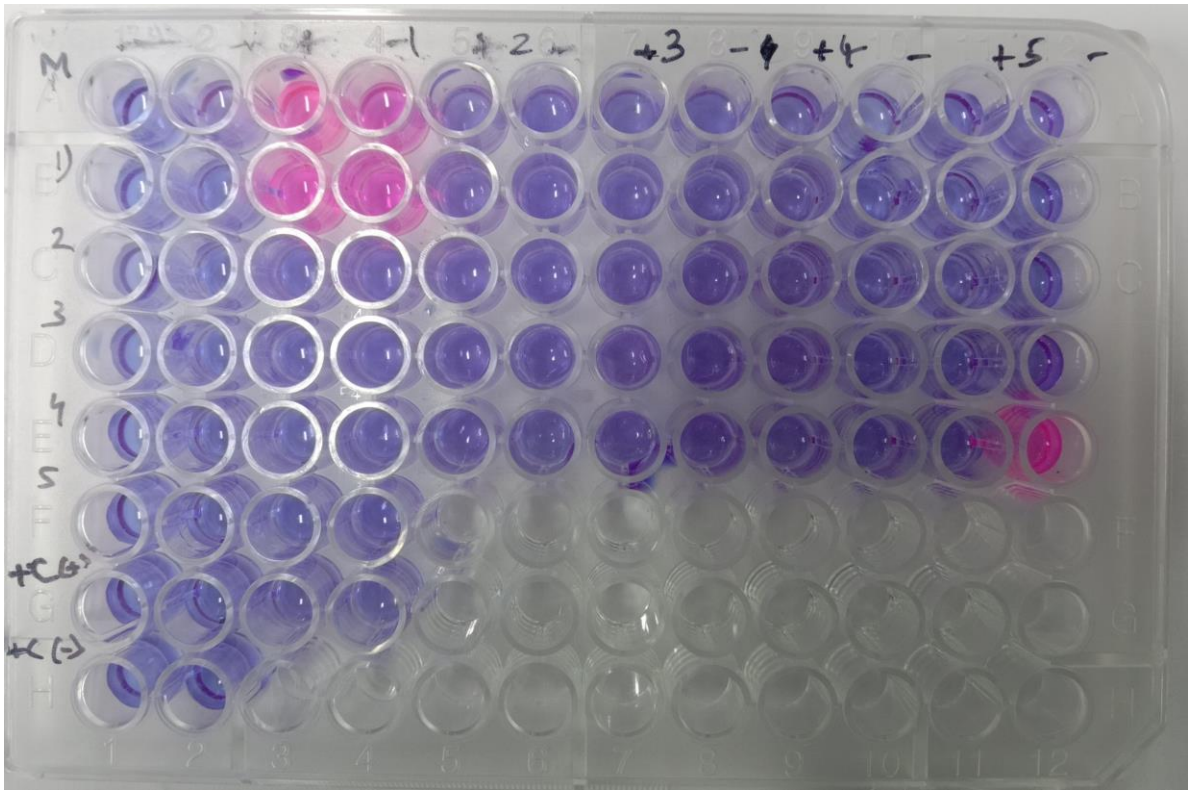


Figure 3: Antimicrobial activity of 5% & 10% Purslane gel

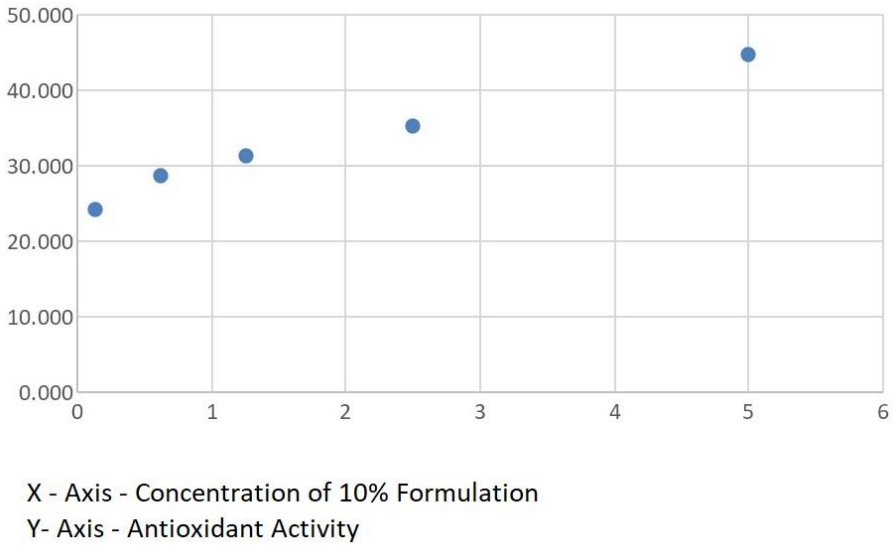
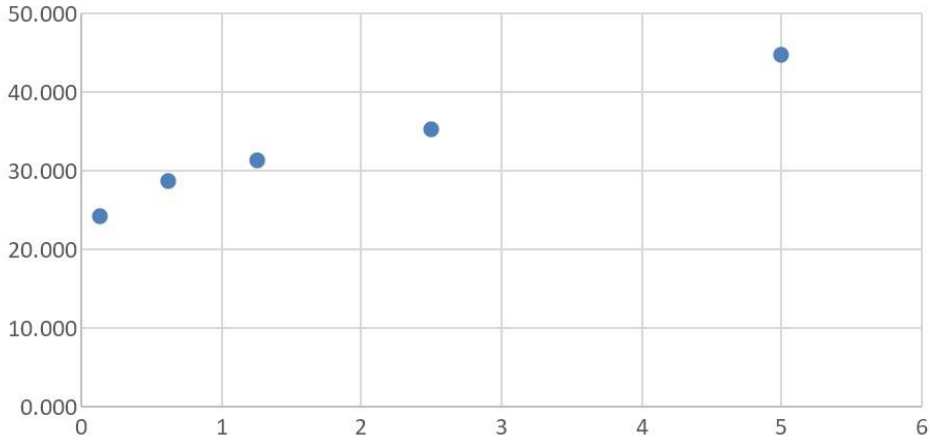
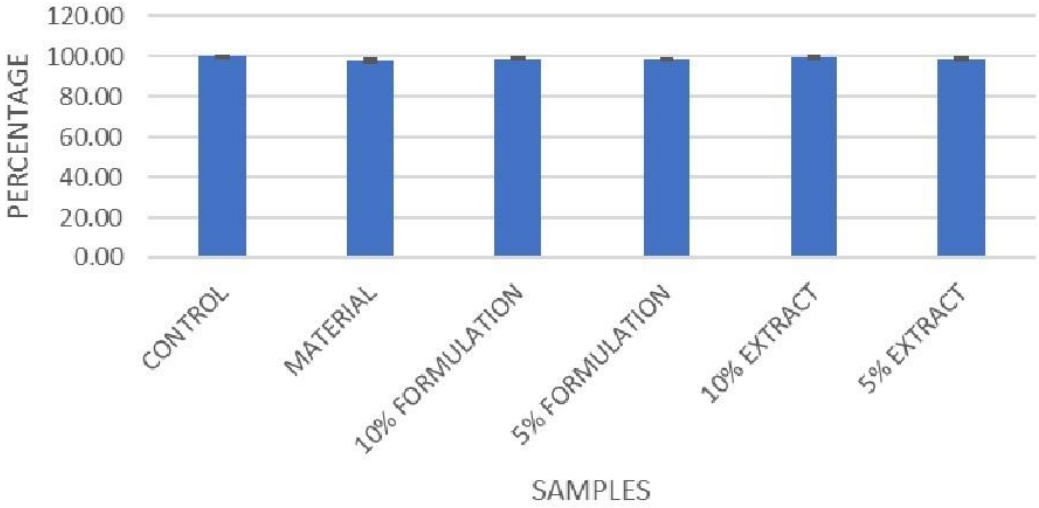


Figure 4: Antioxidant activity of the 10% formulation



X- Axis - Concentration of 5% formulation
Y- Axis - Antioxidant Activity

Figure 5: Antioxidant activity of the 5% formulation



X - Axis - Concentration of the samples
Y - Axis - % viability

Figure 6: Cytoprotective effect of 5% and 10% formulation

4. Conclusions

In recent times, the use natural medicines have become more acceptable as they are more safe and has lesser side effects compared to synthetic medicines. The preferability to use herbal products has increased in demand. The gel formulated in this study, possess all desirable properties and phytoconstituents making it suitable for its oral use without causing any undesirable effects.

Conflicts of Interest: The authors have no conflicts of interest regarding this investigation**.**

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