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RESEARCH ARTICLE

Preparation and Evaluation of Hydrogels Containing Methanolic Extract of Brassica *Oleracea ver. Italic*

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Abstract

Objective: Herbal medicine usage is increasing nowadays because their usage is acceptable by most people to be safe and produce fewer side effects than synthetic drugs. This present study has been undertaken with the aim to formulate and evaluate herbal gel containing *Brassica oleracea ver. italica* methanolic extract. Method: The gel formulation was designed by using methanolic extract of *Brassica oleracea var. italica*, Carbopol 940, propylene glycol, methyl paraben, propyl paraben and required amount of distilled water. PH of skin was maintained by adding tri-ethanolamine drop wise. The prepared herbal gel formulation was subjected to preliminary evaluation such as pH, total phenolic content and total flavonoid content, viscosity, drug diffusion study and anti-inflammatory study. Result: The result of anti-inflammatory study showed that the gels with extract concentration of 1000 µg/ml have comparable anti-inflammatory activity with the Diclofenac sodium (100 µg/ml).Conclusion: Formulation of gel containing *B. oleracea var. italica* extract is a good attempt as herbal formulation has high demand in the market.

Keywords: *Brassica oleracea var. italica, Carbopol 940, Hydrogel.*

Introduction

Natural substances have gained major interest in design and development of drug delivery system. The use of metals, fungi, alginates, polysaccharides as formulation or formulation additives have invited a lot of research in health industry [1-9]. The advent of micro and nanotechnology has added an increased potency in application of natural substances for treatment of diseases or as pharmaceutical additives [10, 11]. Family *Brassicaceae* consists of 350 genera and 3500 species. One of the most important genus in this family is Brassica which include crops and economically important species such as *Brassica oleracea L., Brassica napus L and Brassica rapa L. Brassica oleracea* which include vegetables and forage forms is the main vegetables species. It includes vegetables such as kale, cabbage, broccoli, Brussel sprouts, cauliflower and others [12].

Broccoli is an edible green plant in the cabbage family whose large flowering head is eaten as a vegetable. The word broccoli comes from the Italian plural of broccoli, which means "the flowering crest of a cabbage" and is the diminutive form of broco, meaning "small nail" or "sprout" [13]. The sprouts refer to the branching habit of broccoli and young edible inflorescences. They are introduced from eastern Mediterranean to the Italy where diversity of broccoli-like vegetables was developed [14]. Broccoli provides many health-promoting properties which attributes to its antioxidant and anti-carcinogenic compounds. It is composed of polyphenols, glucosinolates, sulforaphane and selenium [15]. They are also known to contain a high content of flavonoids, vitamins, and mineral nutrients [16-18]. The pharmacokinetics of broccoli explains that when hydrolysis takes place, glucoraphanin produces many products that include the bioactive isothiocyanate sulforaphane. The percentage of isothiocyanate sulforaphane present in these vegetables varies depending upon the conditions of hydrolysis, food

handling, and preparation procedures. The food in broccoli family results in under dosing of metabolic drugs. The drug-food interactions of broccoli are further described under pharmacological activities [19]. Sulforaphane contribute to anti-inflammatory properties and activate phagocytosis to reduce and clear inflammatory insults. Moreover, researcher discovered that sulforaphane might be a new therapeutic agent for rheumatoid arthritis as it is a potent anti-inflammatory [14]. Analgesic effects of Broccoli extract may be attributed to Vitamin C, Quercetin and Sulforaphane [20].

Inflammation is defined as the natural process of our body in response to cells destruction, microbial infection and chemical irritation. [21]. Studies suggest that inflammatory conditions are related to consumption of western diet which primarily devoid vegetables, fruits and fish [22]. Traditionally, pain induced by rheumatoid arthritis are treated by non-steroidal anti-inflammatory drug (NSAID), such as aspirin, to relieve pain, corticosteroids or even disease-modifying anti-rheumatic drugs to reduce other symptoms of the disease [21]. However, synthetic drugs can cause negative and undesirable effects on the body [20]. Some of the major adverse effects associated with anti-inflammatory agents such as NSAIDs are gastric ulceration and, infrequently, myocardial infarction and stroke [23]. Withal, topical anti-inflammatory can cause photosensitivity and allergic reaction. A trend has witnessed among denizens of using traditional use of plant based medicines. In conjunction, studies reported the reduction in pro-inflammatory cytokines IL-1B, TNFa & IL-6 with an increased intake of cruciferous vegetables [24]. It is further envisaged that traditional and herbal medicine is cost saving and claimed to be innocuous, and it is also realized that herbal products have fewer unwanted adverse reactions that is witnessed in most of the synthetic agent. Natural constituent in herbs produce limited side effects on the human body but it enriches the body with nutrients and useful minerals. Aloe vera has been reported for its wound healing properties, *Daucus carota* has been effectively screened for its antioxidant properties and *Cucumis sativa* has been suggested to cool, heal, and sooth irritated skin due to sunburn and cutaneous eruption. *N. jatamansi* can be used for its stimulant, tonic and antiseptic action [25].

Hydrogels are three-dimensional, cross-linked networks of water-soluble polymer. Hydrogel is desirable in drug delivery application because of its unique physical properties [26]. The present research methanolic extracts of broccoli was incorporated in a gel to treat some inflammatory conditions. The formulated gel uses natural and semisynthetic chemicals to produce safe and stable gel. Varied concentration of gelling agent was used to prepare the gels and pharmacological effects of extracts were measured as anti-inflammatory effect by conducting human red blood cells membrane stabilization study. This topical gel formulation of broccoli extract formulated may have good utility and option for effective management of inflammatory conditions.

Materials and Methods

Materials

Methanol extract of broccoli, Carbopols 940 (Sigma Aldrich), Propylene Glycol (Qualikem), Methyl Paraben, Propyl Paraben, Methanol (Merck), Ethanol (Merck), Tri-ethanolamine (Sigma). All other reagents used were of analytical grade.

Formulation of Hydrogel

Carbopol based hydrogels were formulated according to the formula depicted in Table 1. 1 g of extract was dissolved in distilled water, the suspension was sonicated for 30 minutes. Accurately weighed carbopol was dispersed in sufficient quantity of distilled water and the dispersion was stirred with magnetic bead overnight to allow complete swelling. Weighed quantity of methyl paraben and propyl paraben were separately dissolved in ethanol and added to the overnight soaked carbopol suspension.

Triethanolamine was added dropwise until good consistency of gel was obtained. The pH was adjusted and the formulated gels were further sonicated for 6 hours to remove any air bubbles. The gels were transferred to suitable container with closure and was kept at room temperature and away from direct sunlight for further evaluation [27]

Measurement of pH

pH of the gels was measured by using pH meter.

Table 1: Formulation of gel

Formulation	F1	F2
Carbopol 940	0.5 g	1.0 g
Extract	1.0 g	1.0 g
Propylene Glycol	4.0 ml	4.0 ml
Ethanol	4.0 ml	4.0 ml
Methyl Paraben	0.2 g	0.2 g
Propyl Paraben	0.02 g	0.02 g
Tri-ethanolamine	Quantity sufficient	Quantity sufficient
Water	Up to 100 ml	Up to 100 ml

Total Phenolic Content and Total Flavonoid Content

Total phenolic and flavonoid content was determined by using method as described by Baba and Malik. Total phenolic content of extract was determined by using Folin-Ciocalteu method. 200 µL of crude extract (1mg/ml) were made up to 3 mL with distilled water, it was mixed thoroughly with 0.5mL of Folin-Ciocalteu reagent for 3 minutes, followed by the addition of 2 mL of 20% (w/v) sodium carbonate. The mixture was allowed to stand for 60 minutes in dark, and the absorbance was measured at 650 nm. The total phenolic content was calculated from calibration curve and the result were expressed as mg of gallic acid equivalent per g dry weight.

The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method. 50 µL of crude extract (1mg/ml ethanol) were made up to 1 mL with methanol, it was mixed with 4 ml of distilled water, 0.3 ml of 5% NaNO2 solution, 0.3 ml of 10% $AlCl_3$ solution. The mixture was incubated for 5 min and was allowed to stand for 6 min after incubation. To the mixture, 2 ml of 1mol/L NaOH solution was added and the final volume of the mixture was brought up to 10 ml with distilled water. The mixture was allowed to stand for 15 minutes and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve ant the result was expressed as mg rutin equivalent per g dry weight [28, 29].

HRBC Membrane Stabilization Study

The method human red blood cells membrane stabilization was used in this research to study the anti-inflammatory activity of the gel because of its simplicity and time saving. 10 ml of human blood sample was taken from a healthy volunteer who have not consume any NSAIDs and antihistamine for at least two weeks before conducting the test.

The blood was kept into heparinized tubes to prevent from coagulation. 2 ml of blood was mixed with 2 ml of Alsever's and centrifuged at 3000 rpm for 30 minutes. The packed cells obtained was used to make 10% of iso-saline suspension. Varied concentrations of extract (250, 500 and 1000 µg/ml in phosphate buffer pH 7.4), 2 ml of hyposaline (0.45% w/v NaCl) and 0.5 ml of HRBC suspension were mixed in a test tube. The test tubes were incubated at 37º C for 30 minutes and later centrifuged at 3000 rpm for 20 minutes. The supernatant solution was extracted to estimate hemoglobin content using UV visible spectrophotometer (Shimadzu, 1900) at a wavelength of 560 nm. Diclofenac sodium (100 µg/ml) was used as the reference standard while control was prepared without the extract. Percentage HRBC membrane stabilization was calculated using the following formula 1

% Protection= 100 – (OD of drug treated sample/OD of control) x 100 ……..1

In-vitro Diffusion Study

In vitro diffusion study of the prepared gels was carried out in Franz diffusion cell (Electrolab, India) for studying the rate of drug release from the formulated gels. 1.0 g of the gel was taken on a dialysis membrane and the diffusion studies were carried out at 37±1 ^{o}C in 100 ml phosphate buffer pH 7.4. The release was studied for 120 minutes. 10 ml of each sample was withdrawn periodically at 10, 20, 30, 40, 50, 60, 120 and each sample was replaced with equal volume of fresh diffusion medium. Sample were analyzed for the drug release as gallic acid equivalent.

Viscosity

Viscosity of both gels was determined by using Anton Paar rheometer.

Results and Discussions

Measurement of PH

Table 2: pH of gels

Formulation	F1	F2
pH	6.9	7.1

The pH of both gels was found to be 6.9 and 7.1 for F1 and F2 respectively. The pH was suitable for topical application. The percentage of tri-ethanolamine was good enough to maintain the pH compatible with skin [30].

Total Phenolic Content

The calculation of total phenolic content of plant extract was carried out using the standard curve of gallic acid and presented as gallic acid equivalents (GAE) per gram.

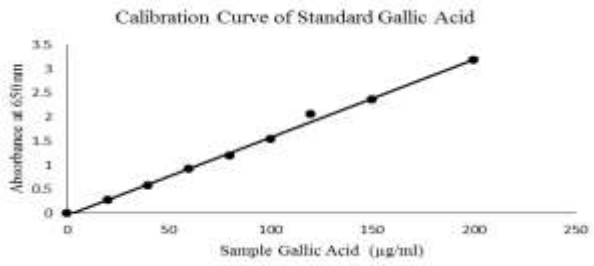

Figure 1: Calibration Curve of standard gallic acid

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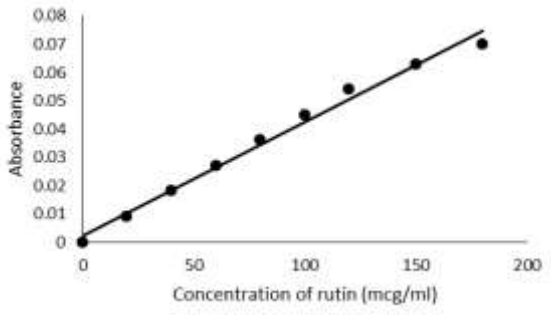

Figure 2: Calibration curve of rutin

The total phenolic content of *Brassica oleracea var. italica* was calculated using the calibration curve of standard gallic acid (Figure 2). The extract was found to have 68.907 µgGAE/g of phenolic content. The calculation of total flavonoid content of plant extract was carried using the standard curve of rutin and presented as rutin equivalents per gram.

The total flavonoid content of *Brassica oleracea var. italica* was calculated using the calibration curve of standard rutin (Figure 1. The extract was found to have 680.907 µg rutin equivalent/g.

The phytochemical screening test has confirmed that *B. oleracea var. italica extract* contained phenolic and flavonoid compound. The total phenolic content of methanolic *B. oleracea var. italica* extract, calculated from the calibration curve, was 68.907 µg gallic acid equivalents/g and the total flavonoid content was 680.907 µg rutin equivalents/g. Studies have suggested the presence of phenolic compound in cruciferous vegetables[31]. The flavonoid content was high for *B. oleracea var. italica* which may be responsible for the anti-inflammatory activity of the extract. [19]

HRBC Membrane Stabilization Study

Diclofenac sodium (100µg/ml) was used as the reference standard while control was prepared by excluding the extract. As for calculation for percentage hemolysis, the hemolysis by control assumed to be 100%. Percentage HRBC membrane stabilization was calculated using the following formula:

% Protection= 100 – ((OD of drug treated sample/OD of control) x 100)

During an inflammation, lysosomal enzyme is released. Inhibition of this enzyme or stabilization of its membrane by NSAIDs leads to inflammation suppression. The enzyme membrane is similar to HRBC membrane which was used to study *in vitro* anti-inflammatory activity by checking HRBC membrane stability produced by extract. This *in vitro* method was time saving, flexible and convenient than other method to study anti-inflammatory activity. Methanolic extract of *Brassica oleracea var. italica* shows the ability to resist cell lysis in low concentration compared to Diclofenac sodium at 100 µg/mL. The highest concentration of extract, 1000 µg/mL shows comparable prevention of lysis to the standard diclofenac sodium (100 µg/mL). It was subsumed 1 gm of methanolic extracts of *Brassica oleracea var. italica* should be used in formulating gel for pronounced anti-inflammatory effect (Figure 3) [32]

Table 3: HRBC membrane stabilization study

Sample	Absorbance at 560 nm	% Protection	% Haemolysis
Control	0.262	0	100
Diclofenac sodium	0.148	43.5	56.5
250 µg/ml	0.169	35.5	64.5
500 µg/ml	0.163	37.8	62.2
1000 µg/ml	0.155	40.8	59.2

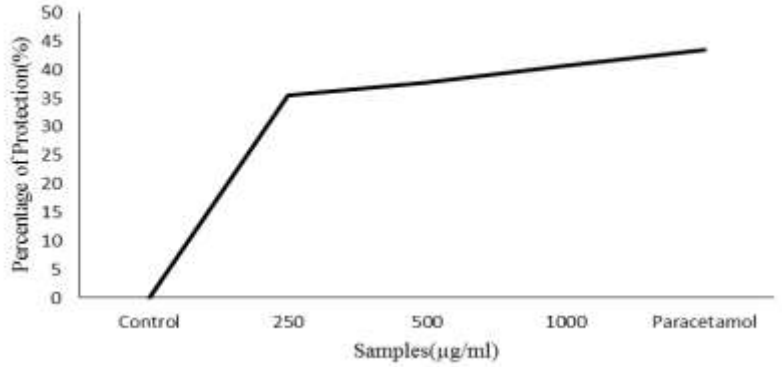

Figure 3: Graph of percentage protection against samples

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Franz Cell Diffusion Study

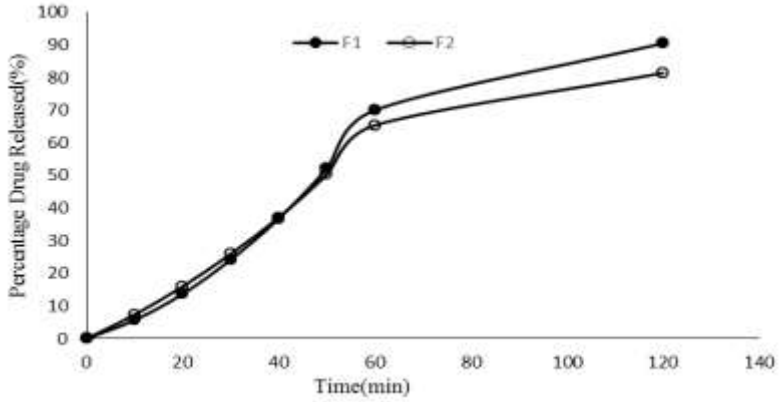

Figure 4: Comparison percentage drug release (%) of F1 and F2 against time (min)

The diffusion release study of the formulated gels is shown in Figure 4. F1 and F2 were able to release 80% rutin equivalent drug in Rapid drug release over few hours to a few days resulted from high water content and large pore sizes of hydrogel. However, the rate of release can be controlled by adjusting the amount of propylene glycol in the gels [26]

Viscosity

The viscosity at room temperature for F1 was 1010 pascal and F2 was 2110 pascal. Both the gels exhibit pseudoplastic behavior. The gels exhibited optimum viscosity which decreases as the shear rate decrease. This allows the gels to be packed easily in a collapsible tube.

Conclusion

Usage of medicinal plants in treating a disease shows an increasing trend nowadays. Their usage is acceptable by most peoples to be safe with fewer side effects than synthetic drugs. Formulation of gel containing *B. oleracea var. italica* extract is good attempts as herbal formulations have high demand in the market. From this research, it was

concluded that the methanolic extract of *Brassica oleracea var italica* have comparable anti-inflammatory activity with the Diclofenac sodium (1000 µg/mL). Therefore, gel containing methanolic *B. oleracea var. italica* extract can be use as anti-inflammatory gel.

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