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Original Article

Comparative Study of Polyherbal Formulation for Antiarthritic Activity Having Cockle Shell, Egg Shell, Ginger and Balloon Vein in Gel Form and Oil Form: A Novel Preparation for Anti-Oxidant Activity

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ABSTRACT

Inflammatory and chronic disease of the joints and tissues surrounding them, rheumatoid arthritis is known as the most common form of arthritis. Traditional medicines plays major role because of more advantageous like lesser side effects, naturally available and cost effective. A formulation for anti arthritis activity was developed, isolated, and evaluated in this study. Based on the extensive review of the literature, we have formulated three formulation like gel, polyherbal oil formulation with extract of herbs and polyherbal formulation with powders of herbs. We have selected, traditional herbs (Cockle shell, Egg shell, Ginger and Ballon Vein) based on the literature and does a comparative study between gel and the oil formulation to check which has better anti arthritis activity. The selected herbs for formulation of gel are cockle shell and egg shell which has rich calcium content and for oil formulation herbs like ballon vein and ginger were chosen.The chemical constituent present in herbs plays a major role in curing rheumatoid arthritis.Then finally we have done a evaluation like ph measurement, spreadability, specific gravity, antioxidant study etc., between the comparison of DPPH assay of the formulation, clearly reported that the efficacy in the medicated oil in the extract and well in the macerated oil showed significant anti-oxidant activity when compared to the gel.

Key words: Herb formulation, RA, *In vitro* studies – DPPH.

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INTRODUCTION

Rheumatoid arthritis is an inflammatory disease that commonly affects the joints and peri-articular tissues. Rheumatoid arthritis is a chronic, progressive, autoimmune condition. Pathological techniques are caused by antigen antibody complexes. Joint inflammation is triggered by infection-causing mediators. Lysosomal enzymes are released by inflammatory cells, damaging bone and animal tissue, leading to disability. Those are as a result of extra variety of proinflammatory molecules loose from macrophages inclusive of reactive chemical element species and eicosanoids. Eicosanoids consisting of prostaglandins, leukotrines those are secreted by way of immune cells and macrophages.[1] Inhibiting Cox and Lox enzymes modulates arachidonic acid metabolism in persistent inflammatory conditions, so inhibiting Cox and Lox enzymes modulates arachidonic acid metabolism.[2] RA is a collection of procedure, such as proliferation of synovial mobile, fibrosis, formation of pannus and cartilage and bone destruction. The prevailing takes a look at is to formulate different forms of anti -arthritic formulation like gel and oil with its assessment parameter assessment.

MATERIALS AND METHODS

A local beach in Chennai provided us with cockle shells.

Gel formulation

Isolation of cockle shell

After cleaning the shells with a brush and rinsing them with double-distilled water (DDWD), they were scrubbing under tap water to remove dirt. A mortar and pestle are used for fine crushing of the cockle shells, which are then blended in a blender machine into fine cockle shell powder. Micron size (10-63 m) was obtained by sieving the powder using an AS 200 Control Sieve Shaker made of stainless steel. The micron-sized cockle shell powder was dissolved in 20 mL of 5 M hydrochloric acid (HCl) and the following procedures were conducted to produce calcium chloride (CaCl2). Emsure® (Merck KGaA, Darmstadt, Germany), 37%. Filter sheets (Fioroni Filter Circles 70 mm, Lab Logistic Group GmbH, Meckenheim, Germany) were used to filter the solution. To remove the contamination products from the solution, a CaCl2 and DDW combination was spun at 500 rpm at room temperature for one hour. Following the formation of the liquid, 1 L of DDW was added to make the stock solution. 10 mL of DDW and 5 g of K2CO3 ACS reagent, 99% (SigmaAldrich, Steinheim, Germany) were dissolved in 100 mL of K2CO3 ACS reagent, 99% (SigmaAldrich, Steinheim, Germany) as a stock solution. Following the addition of 10 mL of diluted CaCl2 and 10 mL of diluted K2CO3 to DDW (0, 10, 20, 50, 60, and 70 mL) at varied feeding rates (1, 1.5, 2, and 2.5 h), different quantities of DDW were added to 10 mL of diluted CaCl2. To carry out the process, many solvents were used. Methanol, Analytical Reagent Grade, Lot No. 1,352,421 from Sigma Aldrich, Steinheim, Germany; 70% ethanol, ChemPur® CAS No. 64-17-5 from Systerm, GmbH, Karlsruhe, Germany; or propanol, Analytical Reagent Grade, Lot No. 1,367,431 from Sigma

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Aldrich, Steinheim, Germany. After that, precipitation was permitted to fall for 24 hours. The resulting product was centrifuged at 7000 rpm for 7 minutes in an Eppendorf Centrifuge 5804 (Eppendorf North America Inc., Enfield, CT, USA) after the supernatant solution was removed. The acidity of the resulting solution was neutralised by washing it three times with DDW. To freezedry the finished product, it was first placed in the Ilshin Europe Biobase DF8517 freezer at 80 degrees C for 24 hours and then in the freeze-drying machine at 108 degrees C for 48 hours.[3-5]

Isolation of egg shell[6]

EGG SHELL
↓ Grounded, blended and seived
EGG SHELL POWDER
↓ Dissolved in Hcl
$CaCl_2$
↓ Titration of K_2CO_2
$CaCO_3$
↓ Freeze dry
CaO Powder

Preparation of gel base

To avoid agglomeration, 60 mL of demineralized water was heated, and carbopol 934 was slowly dissolved, while stirring constantly, for 1 hour to avoid agglomeration. Disodium edetate and triethanolamine were then dissolved separately in 10 mL of demineralized water, and stirred continuously for 10 minutes. After mixing 4.83 mL of propylene glycol with 12 mL of demineralized water for 10 minutes, the solution should be clear. Stirring the solution for 10 minutes adjusted the pH of the carbopol solution to 7.4 after adding sodium edetate and triethanolamine solution. A clear consistency gel base was obtained by stirring propylene glycol solution for 10 minutes and adding methyl salicylate until a clear consistency gel base was obtained by stirring.[7-10]

Preparation of gel formulation

In order to mask unpleasant odors, menthol crystals were added to the gel base created with isolated calcium oxide from cockle shell and eggs shell.[11-13]

Preparation of poly herbal oil using extract

Preparation of methanolic extract of balloon vein: A thorough cleaning and drying of the collected leaves was carried out under shade at room temperature, followed by powdering. It was defatted with n.-hexane (25 g) after the powder had been ground. Defatted materials were then repeatedly extracted with methanol at room temperature until the extraction solvent became colorless[14,15] through repeated extractions with methanol at room temperature. A rotary evaporator is used to remove methanol from the dried methanol extract with a rotary filter paper No. 1 followed by a rotary evaporator at 50°C to give a dried methanol extract after the filtered specimen has been collected.[16,17]

Preparation of ginger extract: Dried ginger was used to make ground ginger powder. A Soxhlet extractor was used to purify the methanolic extract. An extraction was performed by loading 30 grams of ginger powder into the cartridge, adding 300 mL of methanol, and running the extraction. Condenser and vacuum pump purification were used to purify the extract.[18,19]

Preparation of polyherbal sesame oil: The prepared methanolic extract of ballon vein and ginger were directly added to the sesame oil.[20]

Preparation of polyherbal oil using powder of herbs:[21] It was collected, cleaned, dried and powdered under shade at room temperature and then added directly to sesame oil to test for its activity after that. Ballon vein and ginger were collected, cleaned, dried, and powdered.

Physical evaluation

The above formulation were preliminary evaluvated for its pH measurement, Physical evaluvation (Colour, Odour, Consistency…), Washability, Viscosity, Spreadability and specific gravity.

Evaluation of anti oxidant activity by using *in-vitro* dpph assay:[21]

In the bleaching of purple colored methanolic solutions of DPPH, hydrogen atoms or electrons were measured in the corresponding extracts and pure compounds. An analysis of DPPH radicals was conducted using methanolic extracts and essential oils. Each extract in methanol sample of 4 mL was added separately to a DPPH radical solution in methanol of 0.2 mM concentration (final concentration of DPPH). Shaking vigorously for 30 minutes and then allowing the solution to stand for 30 minutes was followed by measuring the absorbance at 517 nm with a spectrophotometer (Shimadzu UV-1240, Kyoto, Japan). As a percentage [I (%)], the inhibition of free radical DPPH was calculated as follows:

$$I\ (\%) = 100 \times (A_{blank} - A_{sample}) / A_{blank}$$

A sample represents the test compound's absorbance and A blank represents the absorbance of the control (containing no test compound). A DPPH radical is classified as being scavenged by 50% when a DPPH concentration of 50 grams per milliliter is applied to the radical.

RESULT

Results shown in Table 2 and Figure 11.

Table 1: List of materials and their uses in formulation.

NAME OF MATERIAL	CATEGORY
Cockle Shell	Active Ingredient
Egg Shell	Active Ingredient
Ginger	Active Ingredient
Ballon Vein	Active Ingredient
Sesame Oil	Active Ingredient
Carbopol	Polymer
Triethanolamine	P^H Adjustor
Propylene Glycol	Humectant
Methyl Salicylate	Cooling Agent
Menthol Crystal	Volatile Oil

Figure 1: Isolated CaO powder

Table 2: List of materials and their uses in formulation.

FORMULATION	PH	COLOUR	CONSISTENCY	GREASINESS	ODOUR	WASH ABILITY	SPREAD ABILITY	VISCOCITY	SPECIFIC GRAVITY
GEL	7.4	CREAMY WHITE	SOFT AND SMOOTH	GREASY	MENTHOL	WASHABLE	5	1.70poise	0.9118
Poly herbal oil with extract	6.6	YELLOW	SOFT AND HOMOGENOUS	GREASY	CHARACTERISTIC	WASHABLE	5	0.9123	0.9731
Poly herbal oil with herbal powder	6.4	GRENISH BROWN	SOFT AND HOMOGENOUS	GREASY	CHARACTERISTIC	WASHABLE	5	0.91	0.97

Figure 2: Magnetic stirrer with CaCo3

Figure 4: Electric stirrer

Figure 3: Isolation of cockle shell

Figure 5: Gel formulation

Figure 6: Methanol extraction of balloon vein

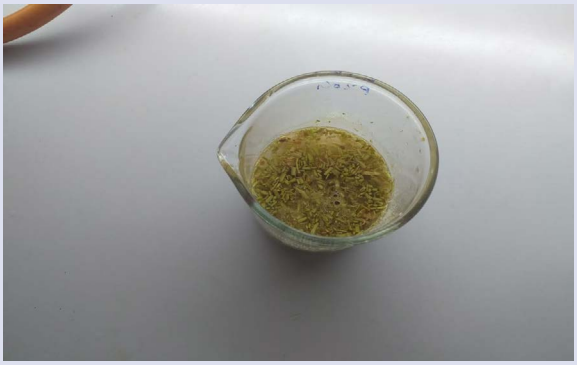

Figure 7: Methanol extract of ginger

Figure 8: Polyherbal oil

Figure 9: Photo colorimeter

Figure 10: DPPH assay

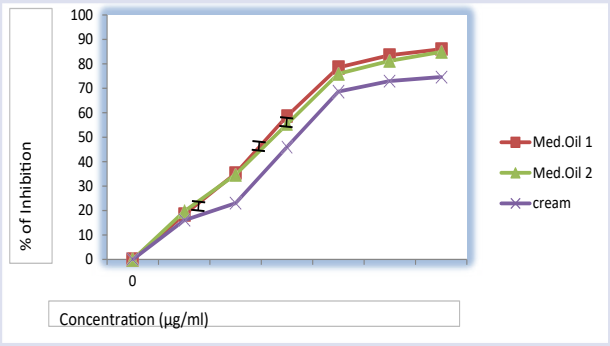

Figure 11: Results and comparison of *In-vitro* DPPH assay

DISCUSSION

Based on the extensive review of the literature, we have formulated three formulation like gel , polyherbal oil formulation with extract of herbs and polyherbal formulation with powders of herbs.We have selected this herbs based on the literature and does a comparative study between gel and the oil formulation to check which has better anti arthritis activity.In the medicated oil shown the pH with the range of 6.4 to 6.6, as per literature review the pH will be suitable for the highl penetration of formulation to the skin epidermal layer. The viscosity of the medicated oil is in the range of 0.9 which is useful of the stability maintainance during the winter season also. The formulation of gel are cockle shell and egg shell which has rich calcium content supported with pH 7.4 and viscosity 1.7. The comparison of DPPH assay of the two formulation, clearly reported that the efficacy in the medicated oil in the extract and well in the macerated oil showed significant anti-oxidant activity when compared to the gel. From this we have concluded that poly herbal oil has better anti oxidant activity then gel further study requires to be carried out for its arthritis activity.

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