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Development, characterization and evaluation of herbal ointment containing *momordica charantia, pongamia glabra and piper nigrum*

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ABSTRACT

Objective: The objective of present study was the development, characterization and evaluation of poly-herbal ointment formulation of *pongamia glabra, piper nigrum and momordica charantia* extract. Plant derived substances and herbal medicines have recently attracted the great interest towards their versatile application. Medicinal plants are the richest source of bioactive compounds used in traditional and modern medicine. The ointment formulation of plant extract has a sound approach to develop a poly-herbal ointment. The poly-herbal ointment formulation was evaluated for its physicochemical parameters like color, odor, pH, spreadability, extrudability, consistency, diffusibility, solubility, washability and stability. **Materials and methods:** Extract of all three plants has been separated by the soxhlet extraction. Poly-herbal ointment has been prepared by mixing the extract of *momordica charantia, pongamia glabra and piper nigrum* with wool fat, Cetostearyl alcohol and paraffin. **Results and conclusion:** Physicochemical evaluation shows that the poly-herbal ointment fulfilled the criteria as herbal formulation and irritancy test also performed indicate the effectiveness and efficacy of prepared ointment formulations. The poly-herbal ointment formulations were also evaluated for its stability at various temperature conditions which shows no change in the irritancy, spreadability and diffusion study. Thus it could become a medium to use the medicinal properties of extracts effectively and easily as a simple dosage form.

Keywords: *Momordica charantia, pongamia glabra, piper nigrum,* ointment, Cetostearyl alcohol.

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INTRODUCTION

Pongamia glabra (Figure 1) belong to *Fabaceae* family [1] (Sharma et al., 2004). Fruit contains furano-flavonoids, coumestan and pongacoumestan. *P. glabra* has been reported to contain a large number of furano flavonoids e.g. karanjin, pongapin, kanjone, pongamol, and pongaglabrone, along with a number of simple flavonoids and lipid like arachidonic acid [2] (Bandivdekar et al., 2002). It used as bacteriocidal activity against V. cholerae and E. coli, as well as anti-inflammatory and antipyretic properties [3] (Krishnan et al., 2013).

Piper nigrum (Figure 2) belonging to family piperaceae [4] **(**Sunila et al., 2004**)**. The fruits have small globose drupe and was known as a peppercorn when dried [5] (Sudjarwo et al., 2005). Antioxidant active chemicals isolated from black pepper includes camphene, carvacrol, eugenol, myrcene myristic-acid, myristicin, palmitic-acid and ubiquinone were the major chemical compounds responsible for the aroma, pungency and medicinal property of the black pepper. Black pepper is used to improve digestion, stimulate appetite, and treat gastrointestinal problems, including diarrhea, dyspepsia and flatulence. It is also used to treat colds, coughs and sore throats.

Momordica charantia belong to Cucurbitaceae family [6] (Jayasooriya et al., 2000). The fruit has a distinct warty looking exterior and an oblong shape (Figure 3: Fruit of *M. Charantia*). *M. Charantia* consists the following chemical constituents charantin, diosgenin, gentisic acid, momorcharasides, momorcharins, momordenol, multiflorenol, myristic acid and nerolidol. *Momordica charantia* is used as anthelmentic, anti-mycobacterial, antioxidant, antitumor, wound healing, antiulcer, antiviral, hypoglycemic and immune-stimulant [7, 8, 9, 10] (Chia et al., 2011; Leelaprakash et al., 2011; Rajaram et al., 2012; Rawat et al., 2012).

Figure 1: Seeds of *Pongamia glabra*

Figure 2: Unripe and ripe seeds of *Piper nigrum*

Figure 3: Fruit of *M. Charantia*

The object of the present paper was to develop poly-herbal ointment formulation of three plants include *momordica charantia, pongamia glabra and piper nigrum.* The poly-herbal ointment was characterized and evaluated by following physicochemical properties includes physical parameters color and odor, consistency, spreadability, extrudability and chemical parameters includes pH, diffusion study, loss on drying (LOD), solubility, washability, non irritancy and stability.

MATERIALS AND METHOD

Drugs are collected from wild plants or cultivated plants. The season in which the drug is collected plays an important role in determining the quality of drug. Organoleptic, morphological and microscopic examination would help in identifying crude drug. Generally, three methods were employed in the extraction of plant materials including maceration, percolation and soxhlet extraction. Maceration and percolation may be employed for the extraction of thermo-labile constituents. Soxhlet extraction is rapid, continuous and may be employed in the extraction of sparingly soluble constituents due to repeated extraction, which cannot be done by either maceration or percolation methods. Plant collection and authentication has the prime source and criteria to select the exact plant material. The fruits of *Momordica charantia* were collected in the month of July, 2016 from local field areas of Bhopal region, Madhya Pradesh. Leaves of *Pongamia glabra* obtained from botanical garden and fruits of *Piper nigrum* collected from local market of Bhopal, Madhya Pradesh. The specimens were submitted and identified as fruits of *Momordica charantia* (MC) belong to family-Cucurbitaceae, Leaves of *Pongamia glabra* (PG) belong to family-*Fabaceae* and fruits of *Piper nigrum* (PN) belong to family-Pipereacea. All three plants and plants parts has authenticated by Prof. Zia ul Hassan, Department of Botany, Saifia Science College, Bhopal. The appession number for the specimen is 490/BS/saifia/16 has been preserved for future identification. The samples were shade dried to protect its chemical constituents and not to get degrade at high temp.

Soxhlet extraction

Soxhlet extraction has been performed by using different solvents system and selected on the basis of polarity of the solvent system after defatting of the crude drug by petroleum ether. Solvents used were petroleum ether, ethyl acetate, ethanol and distilled water. The moderately coarse powder of the drugs e.g. *Momordica charantia* (MC), *Pongamia glabra* (PG) and *Piper nigrum* (PN) were subjected to soxhlet extraction with different solvents in increasing order of polarity from non-polar to polar.

The 80 gm of dried coarsely powdered drug was packed in soxhlet apparatus and defatted with 1000 ml of petroleum ether at 40-60^{0}C temperature, until the complete defatted. Complete defatting ensured by placing a drop by thimble on the filter paper which did not exhibited any oily spot. The defatted material was removed from the soxhlet apparatus and air dried to remove the last traces of petroleum ether. The defatted material was subjected to extraction by ethyl acetate and then with ethanol as solvent by soxhlet apparatus and finally with distilled water by maceration process. The completion of extract was confirmed by evaporating a few drops of the extract on the watch glass and ensuring that no residue remained after evaporating the solvent.The marc was air dried before extracted with the next solvent. Dried marc was macerated with water for 24 h. The extracts were evaporated under reduced pressure at low temperature (30^{o}C) to dryness to yield different extracts, stored in an airtight container in refrigerator for further experimental studies. They were weighed to a constant weight and percentage w/w basis was calculated **.**

Preparation of poly-herbal formulations

Phytochemical screening has performed prior to the development of poly-herbal ointment formulation to determine the active constituent [11] (Shukla et al., 2018). An ointment with water soluble base was of first choice by their ease of preparation and cleaning after application. The alcoholic and aqueous extracts of all three plants were selected for ointment formulation as they have the higher content of flavanoid and phenol compounds.

Formulation of Ointment (British Pharmacopoeia, 1996)

Preparation of Simple Ointment (B.P.) Base

The simple ointment base was prepared by mixing the wool fat, hard paraffin, Cetostearyl alcohol and white soft paraffin with gentle heating with stirring. The obtained ointment base is then cooled and stored **(Table 1)**. Two formulations were prepared by fusion method e.g. one containing all three extracts of above mentioned plants parts in equal ratios of

alcoholic and aqueous extracts i.e. 3.33% w/w of each extract for the preparation of 10 % w/w ointment in ointment base (Treated as polyherbal-I or F-1) and the other one containing all three extracts of above mentioned plants parts in equal ratios of alcoholic and aqueous extracts i.e. containing 5.0% w/w of each extract, equal to total 15% w/w in ointment base (Treated as polyherbal-II or F2) **(Table 2)**.

Table 1: Formulation of Ointment Base

S. No.	Ingredients	Quantity (g)	Percentage
1.	Wool Fat	1	5
2.	Cetostearyl alcohol	1	5
3.	Hard Paraffin	1	5
4.	White soft paraffin	17	85
5.	Total	20	100

Table 2: Formulation of Ointment

S. No.	Content	Quantity (%)	
		F1	F2
1.	MCAQ (1:1)	3.33	5.0
2.	PGAQ (1:1)	3.33	5.0
3.	PNAQ (1:1)	3.33	5.0
4.	Ointment base	Q.S.	Q.S.

Where MCAQ stand for alcoholic and aqueous extracts of *momordica charantia*, PCAQ Stands for -alcoholic and aqueous extracts of *pongamia glabra*, PNAQ Stands for alcoholic and aqueous extracts of *piper nigrum*

Evaluation of formulation

The prepared poly-herbal ointment formulations were evaluated by various physico- chemical parameters**.**

Color and Odor

Physical parameters like color and odor were examined by visual examination.

Consistency

Smooth and no grittiness were observed.

pH

pH of prepared herbal ointment was measured by using digital pH meter. The solution of ointment was prepared by using 100 ml of distilled water and set aside for 2 h. pH was determined in triplicate and average value was calculated.

Spreadability

The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as Spreadability [12] **(Sawant *et al.*, 2016)**. Less the time taken for separation of two slides results in better spreadability. Spreadability was calculated by following formula: **S=M×L/T**

Where, S= spreadability M= weight tide to the upper slide L= length of glass slide T= time taken to separate the slides.

Extrudability

The formulation was filled in collapsible tube container. The extrudability was determined in terms of weight of ointment required to extrude 0.5 cm of ribbon of ointment in 10 seconds.

Diffusion study

The diffusion study was carried out by preparing agar nutrient medium. A hole has made on a board at the center and poly-herbal ointment was by placed in it. The time taken by ointment to get diffused through was noted after 1h.

Loss on drying (LOD)

LOD was determined by placing the poly-herbal ointment formulation in petridish on water bath and dried at 105^{o}C temperature.

Solubility

Solubility is main criteria for the ointment formulation to check the cleansing properties solubility has determined in boiling water, distilled water and other solvent including alcohol, ether and chloroform.

Washability

Formulation was applied on the skin and then ease extend of washing with water was checked.

Non irritancy Test

Herbal ointment prepared was applied to the skin of human being and observed for the effect.

Stability study

Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2^{o}C, 25^{o}C and 37^{o}C. The herbal ointment was found to be physically stable at different temperature i.e. 2^{o}C, 25^{o}C and 37^{o}C within four weeks.

RESULT AND DISCUSSION

Collection and authentication

The collected and authenticated *Momordica charantia* fruits, *Pongamia glabra* leaves, and *Piper nigrum* fruits were studied and confirmed organoleptically. The crude drugs were dried and powdered in shade to stop the enzymatic metabolic process within them and to prevent them from degradation of metabolic products. The extraction was done by successive solvent extraction, to increase the extraction, to achieve separation of compounds in different extracts and decrease the time taken by extraction process the flask and soxhlet apparatus was covered by cotton to increase the insulation. The drying of extract containing solvent (Petroleum ether, ethyl acetate, ethanol and distilled water) was done by vacuum distillation process. Percent yield of different extract has tabulated in Table 3.

Table 3: Percentage yield of different extracts

Parts	Solvents	Extract color	Yield (gm)	% Yield (w/w)
FMC (Fruits of *Momordica charantia*)	PFMC	Yellowish green	05.54	06.93
	EFMC	Brown	04.72	05.90
	AFMC	Dark Brown	16.05	20.00
	QFMC	Greenish brown	13.08	16.30
LPG (Leaves *of Pongamia glabra*)	PLPG	Greenish brown	01.62	02.03
	ELPG	Brown	02.94	03.67
	ALPG	Dark Brown	06.26	07.83
	QLPG	Brownish Black	05.02	06.28
FPN (Fruits of *Piper nigrum*)	PFPN	Yellowish green	02.30	02.88
	EFPN	Brown	01.66	02.08
	AFPN	Dark Brown	10.15	12.69
	QFPN	Greenish brown	08.90	11.13

Where PFMC- Petroleum ether Extract of *Momordica charantia* fruits**, EFMC-**Ethyl acetateExtract of *Momordica charantia* fruits**, AFMC-** Etahnolic Extract of *Momordica charantia* fruits**, QFMC-** Aqueous Extract of *Momordica charantia* fruits,**PLPG-** Petroleum Ether Extract of *Pongamia glabra* leaves**, ELPG-**Ethyl acetateExtract of *Pongamia glabra* leaves**, ALPG-** Etahnolic Extract of *Pongamia glabra* leaves**, QLPG-** Aqueous Extract of *Pongamia glabra* leaves, **PFPN-** Petroleum ether Extract of *Piper nigrum* fruits**, EFPN-**Ethyl acetateExtract of *Piper nigrum* fruits**, AFPN-** Etahnolic Extract of *Piper nigrum* fruits**, QFPN-** Aqueous Extract of *Piper nigrum* fruits.

Evaluation of poly-herbal ointment

The levigation method was used to prepare ointment so that uniform mixing of the herbal extract with the ointment base was occurred which was stable during the storage. The physicochemical properties were studied which shows satisfactory results for spreadability, extrudability, washability, solubility, loss on drying and others. Also the formulation was placed for a stability study at different temperature conditions like 2°C, 25°C and 37°C within four weeks. There were no changes observed in spreading ability, diffusion study as well as irritant effect. The Evaluation of poly-herbal ointment for various physicochemical properties and stability study was tabulated in Table 4.

Table 4: Evaluation of poly-herbal ointment formulation

S. No.	Parameters	Formulations	
		F1	F2
1.	Color	Greenish brown	Dark Greenish brown
2.	Odor	Characteristic	Characteristic
3.	Consistency	Smooth	Smooth
4.	pH	6.0±0.1	6.5±0.1
5.	Spreadability (sec.)	7.3±0.29	8.7±0.42
6.	Extrudability (g)	0.42±0.03	0.48±0.09
7.	Diffusion study (after 60 min.)	0.71±0.08	0.67±0.06
8.	Loss on drying (%)	28.24±1.33	29.36±1.42
9.	Solubility	Soluble in boiled distilled water, miscible with ethanol and chloroform	Soluble in boiled distilled water, miscible with ethanol and chloroform
10.	ashability	Good	Good
11.	on irritancy	Non irritant	Non irritant
12.	Stability studies (2°C, 25°C and 37°C)	Stable	Stable

CONCLUSION

The present study was carried to prepare and evaluate the poly-herbal ointment formulation. The herbal extracts were prepared by soxhlet apparatus using simple maceration process to obtain a good yield of extract and there was no any harm to the chemical constituents and their activity. Three herbal extract was separated by the soxhlet extraction method. Poly-herbal ointment was prepared by mixing the extract of *momordica charantia, pongamia glabra and piper nigrum* with the wool fat, Cetostearyl alcohol and paraffin wax. The poly-herbal ointment was evaluated for its physicochemical parameters like color, odor, pH, spreadability, extrudability, consistency, diffusibility, solubility, washability. The poly-herbal ointment formulations were also evaluated for its stability at various temperature conditions which shows no change in the irritancy, spreadability and diffusion study. Thus it concluded that ointment formulations promote a better platform or medium to use the medicinal properties of extracts effectively and easily as a simple dosage form.

In addition our two published research paper [13, 14] (Shukla et al., 2018; Shukla et al., 2018) indicate the efficacy and effectiveness of poly-herbal ointment F2 (formulation code) in enhancing wound healing activities. In that cases when momordica and pongamia when administered orally and topically with piper they had shown significant synergistic activity. The poly-herbal ointment prepared from extract of *Momordica charantia* fruits, *Pongamia glabra* leaves and *Piper nigrum fruits* showed marked reduction in wound area in comparison to control group when examined for wound healing activity by topical application in albino rats. The formulation will be helpful in anemic and diabetic wound healing with no side effects and will be beneficial for society and industry with standardization approaches.

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