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Research Article

Development, characterization and evaluation of poly-herbal ointment and Gel formulation containing *Nerium Indicum Mill*, *Artocarpus Heterophyllus Lam*, *Murraya Koenigii Linn*, *Punica Granatum Linn*

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ABSTRACT

Objective: The objective of present study was the development, characterization and evaluation of poly-herbal ointment and gel formulations of *Nerium Indicum Mill*, *Artocarpus Heterophyllus Lam*, *Murraya Koenigii Linn*, *Punica Granatum Linn* 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 and gel formulation of plant extract has a sound approach, The poly-herbal ointment and gel formulation were evaluated for its physicochemical parameters like color, odour, pH, spreadability, extrudability, consistency, diffusibility, solubility, washability, non-irritancy and stability. **Materials and methods:** Extract of all four plants has been separated by the soxhlet extraction by 70% ethanol. Poly-herbal ointment and gel formulation has been prepared by mixing the extract of *Nerium Indicum Mill*, *Artocarpus Heterophyllus Lam*, *Murraya Koenigii Linn*, *Punica Granatum Linn* with carbapol 940 for Gel formulation and PEG 2000 & PEG 600 for ointment formulation. **Results and conclusion:** Physicochemical evaluation shows that the poly-herbal ointment and gel formulations fulfilled the criteria as herbal formulation and irritancy test also performed to indicate the effectiveness and efficacy of prepared ointment and gel formulations. The poly-herbal ointment and gel 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: *Nerium Indicum Mill*, *Artocarpus Heterophyllus Lam*, *Murraya Koenigii Linn*, *Punica Granatum Linn*, extract, ointment, gel.

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INTRODUCTION

Artocarpus heterophyllus Lam: *Artocarpus heterophyllus Lam* is a species of tree of the mulberry family Moraceae. It is also known as jackfruit (Eng.), Kathal (Hindi), Kanthal (Beng.). It is native to Western Ghats of India, Malaysia, central and eastern Africa and south-eastern Asia¹. Jackfruit (*Artocarpus heterophyllus Lam*) was flakes of ripe fruits are high in nutritive value; every 100 gm of ripe flakes contains 287-323 mg potassium, 30.0-73.2 mg calcium and 11-19 gm carbohydrates. The *Artocarpus heterophyllus* (Figure 1) contains various chemical constituents as several flavones colouring matters, morin, dihydromorin, cynomacurin, artocarpin isoartocarpin, cycloartocarpin, artocarpesin, oxydihydro artocarpesin, artocarpetin, norartocarpetin, cycloartinone and artocarpanone².

The heart wood on analysis yields moisture 6.7%, glucosides 38.0%, lipids 0.7%, albumin 1.7% and cellulose 59.0%. The plant also contains free sugar (sucrose), fatty acids, ellagic

acid and some essential Amino acids like Arginine, cystine, histidine, leucine, lysine, methionine, theonine, tryptophan³. The leaves and stem show the presence of saponins, cycloartenone, cycloartenol, β -sitosterol and tannins, they show estrogenic activity. A root contains β -sitosterol, ursolic acid, betulinic acid and cycloartenone⁴.

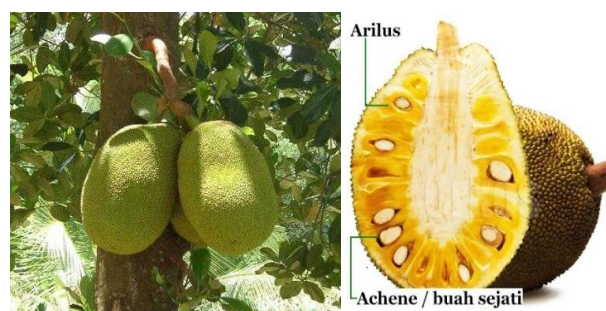


Figure 1: Fruit of *Artocarpus heterophyllus Lam* (Jackfruit, Kathal)

The wood is nervine, antidiabetic, sedative and is useful in convulsions⁵. The wood has a sedative property and its pith is said to be abortifacient. Latex is used as an anti-inflammatory agent⁶.

***Nerium indicum* Mill:** *Nerium indicum* mill, also known as Kaner (Figure 2). It is evergreen shrub or small tree that grows up to 5m in height⁷. Leaves and flowers are used to treat malaria and as traditional medicine it induces the termination of embryo. *Nerium indicum* Mill has gain many properties like bitter, acrid, astringent, anthelmintic, aphrodisiac, stomachic, febrifuge, It is also used as antimalarial, anti-diuretic, emetic, expectorant, cardio tonic, anticancer and applied in the treatment of cardiac asthma, renal and vesicle calculi, chronic stomach, snake bites, joint pains, ulcers etc⁸.



Figure 2: Leaves and flower of *Nerium indicum* Mill (Kaner)

***Murraya koenigii* Linn:** *Murraya koenigii* Linn (Figure 3), called curry leaf, is a small, tropical to sub-tropical tree or shrub that typically grows to 6-15' tall and is noted for its pungent, aromatic, curry leaves which are an important flavoring used in Indian/Asian cuisine. Aroma/flavor of the fresh leaves is enhanced when the leaves are fried in oil or butter⁹. Curry leaves are often added to vegetable dishes.



Figure 3: Leaf of *Murraya koenigii* Linn (curry leaf)

It is a warming, strongly aromatic herb that improves appetite and digestion. The leaves, roots and bark can all be used internally in the treatment of digestive problems. The oil obtained from the leaves used as antioxidant, anthelmintic¹⁰ and antibacterial properties¹¹. It has been shown that the leaves increase digestive secretions; relieve nausea, indigestion and vomiting. The leaves can be used internally in treating constipation, colic, antimicrobial¹² and diarrhea. The leaves are used in the treatment of diarrhea and dysentery. The leaves can be applied externally as a poultice to treat burns and wounds¹³. The roots and bark are harvested as required and can be used fresh or dried. A paste made from the bark is applied to the bites of poisonous insects and other animals.

***Punica granatum* Linn:** *Punica granatum* Linn (Figure 4) is a deep-rooted but slow-growing, spiny, deciduous shrub or small multipurpose tree, it is particularly valued for its edible fruit, but also has medicinal properties, is a good source of tannins and has many agro-forestry applications.

Fruit—raw, Juicy and refreshing with a sub-acid flavor¹⁴. The fruit is 5-13 cm in diameter; it has a hard, tough case that contains lots of seeds, each surrounded by a delicious juicy red flesh¹⁵. The fruit juice can be used in soups, sauces, jellies, ice cream, cakes etc. The fruit contains about 1.5% protein, 1.6% fat, 16.8% carbohydrate, 0.6% ash¹⁶.



Figure 4: Fruit of *Punica Granatum* Linn (Anardana)

It is used externally in the treatment of vaginal discharges, mouth sores and throat infections. The whole plant, but in particular the bark, is having antibacterial, antifungal¹⁷, antiviral, anticancer¹⁸ and astringent properties. The flowers are used in the treatment of dysentery, stomach ache and cough. The juice of the flowers is used to treat nose bleeds. The seeds are demulcent and stomachic. It is also used as anti-diabetic¹⁹ and having antioxidant, anti-inflammatory²⁰ and anti-proliferation properties. A decoction of the seed is used to treat syphilis. The fruit is a mild astringent and refrigerant in some fevers and especially in biliousness. It is also cardiac and stomachic. The juice of the fruit is used to treat jaundice and diarrhea. It is a specific remedy for tapeworm infestation. The rind of the fruit is ground in water and drunk every morning by diabetics.

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 odour, consistency, spreadability, extrudability and chemical parameters includes pH, diffusion study, loss on drying (LOD), solubility, washability, non irritancy and stability.

MATERIALS AND METHOD

Plant material collection and authentication

The leaves of *Nerium indicum* Mill (NI) were collected at in the month of June, 2016 from local area of Shubham Nursery Bhopal M.P., Pulp of *Artocarpus Heterophyllus* Lam (AH) and Whole plant of *Murraya Koenigii* Linn (MK) from local market of Barkheda and Bark of *Punica Granatum* Linn (PG) from Vidisha district, Madhya Pradesh. The specimens were submitted and identified as leaves of *Nerium indicum* Mill, pulp of *Artocarpus Heterophyllus* linn, bark of *Punica Granatum* Linn, Whole plant of *Murraya Koenigii* Linn and authenticated by Dr. Zia ul Hassan, Department of Botany, Saifia Science College, Bhopal. The oppression no. for the specimen is 498/BS/saifia/NI_MK_PG_AH /04/16/07 has been preserved for future identification. The samples were shade dried so as to protect its chemical constituents not to get degrade at high temp.

Extraction

The leaves of NI, pulp of AH, bark of PG and leaves of MK were separated from the fresh and dried on filter paper

sheets under shade at room temperature until with changing of color of filter papers. The shade-dried, coarsely powdered materials (500g) were defatted by petroleum ether (45°C). The defatted marc was then subjected to Soxhlet extraction with 70% ethanol to obtain hydro-alcoholic extract. The hydro-alcoholic extracts were evaporated under reduced pressure at low temperature (30°C) to dryness to yield different extracts, stored in an airtight container in refrigerator for further experimental studies.

Preparation of ointment and gel formulations

Poly-herbal formulation of all four plants has been prepared after completion of extraction and phyto-chemical studies. In this regards water soluble base is first choice for the formulation development of poly-herbal ointment and gel formulations. Water soluble base was used by their ease of preparation and cleaning after application.

Formulation of ointment

Poly-herbal ointment formulation was prepared by the fusion method²¹. Polyethylene glycol (PEG) Ointment base, a mixture of PEG 4000 and PEG 600²² found to have sufficient consistency in ratio 3:7 respectively, thus suitable for ointment preparation with concentration of 10 % & 15% w/w of all plants extracts. Two formulations F1 and F2 were prepared by Fusion method e.g. one containing all four extracts of above mentioned plants parts in equal ratio i.e. 2.5% w/w of each extract for the preparation of 10 % w/w ointment in PEG ointment base (treated as poly-herbal-I or test-I) and the other one containing all four extracts of above mentioned plants parts in equal ratios i.e. containing 3.75% w/w of each extract, equal to total 15% w/w in PEG ointment base (treated as poly-herbal-II or test-II). The prepared formulations was then evaluated by various parameters e.g. consistency, stability²³. The Quantity used for the ointment formulation was depicted in **Table-1**.

Table 1: Formulation of Ointment

S. No.	Content	Quantity (%)	
		F1	F2
1.	<i>Nerium indicum</i> Linn. (Leaves extract)	2.5	3.75
2.	<i>Murraya koenigii</i> (whole plant extract)	2.5	3.75
3.	<i>Punica granatum</i> (bark extract)	2.5	3.75
4.	<i>Artocarpus heterophyllus</i> (Pulp extract)	2.5	3.75
5.	Ointment base	Q.S.	Q.S.

Formulation of topical Gel

Topical gel formulations of different concentration using carbopol base were formulated. The various ingredients used for the two different formulations are mentioned in

Table 2. All formulations were having carbopol gel base. Formulations I was 2.5% (w/w) of each extract for the preparation of 10% (w/w) gel and formulation II was 3.75% concentration of each extract for the preparation of 15% (w/w) gel, mixed properly with stirrer²⁴⁻²⁶.

Table 2: Formulation of Topical Gel

S. No.	Content	Quantity (For 100 ml)	
		F3 (10%(w/w))	F4 (15%w/w)
1	<i>Nerium indicum</i> Linn. (Leaves extract)	2.5	3.75
2	<i>Murraya koenigii</i> (Whole plant extract)	2.5	3.75
3	<i>Punica granatum</i> (Bark extract)	2.5	3.75
4	<i>Artocarpus heterophyllus</i> (Pulp extract)	2.5	3.75
5	Carbopol 940	1	1
6	Glycerin	5 ml	5 ml
7	Tri-ethanolamine	Q.S.	Q.S.
8	Propyl paraben	Q.S.	Q.S.
9	Distilled water	Q.S.	Q.S.

Evaluation of formulation

Colour and odour: Physical parameters like colour and odour 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 hrs. pH was determined in triplicate for the solution 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. Lesser the time

taken for separation of two slides result shown better spreadability. Spreadability was calculated by following formula: $S = M \times L / T$; 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 board at the center of medium and ointment was by placed in it. The time taken by ointment to get diffused through was noted. (after 60 minutes)

Loss on Drying (LOD): LOD was determined by placing the formulation in petri-dish on water bath and dried for the temperature 105°C.

Solubility: Soluble in boiling water; miscible with alcohol, ether, chloroform.

Washability: Formulation was applied on the skin and then ease extends of washing with water was checked.

Non irritancy Test: Herbal ointment and gel formulation was evaluated for the non-irritancy test. Observation of the sites was done for 24 hrs²⁸

Stability study: The prepared herbal ointment and gel formulation were packed in aluminium collapsible tubes (5g) and subjected to stability study at 5°C, 25°C/60% RH,

30°C/65% RH, and 40°C/75% RH for a period of 3 months²⁹. The sample were withdrawn at 15 day time intervals and evaluated for physical appearance, pH, and rheological properties.

RESULT AND DISCUSSION

Extraction

The extraction was done by continuous hot percolation method i.e Soxhlet apparatus. The dried and pulverized drug was defatted with petroleum ether. The obtained marc was then extracted with 70% Hydroalcoholic solution. The drying of extract containing solvent (70% hydro-alcoholic solution) was done by rota vacuum evaporator. The percentage yield of the hydro-alcoholic extract from different drugs was tabulated in Table 3.

Table 3: Percentage yield of hydro-alcoholic extracts from different drugs

S. No.	Parts	Extract colour	Yield (in g)	% Yield w/w
1.	NIL	Greenish brown	20.43	26.28
2.	AHP	Yellowish	8.24	10.05
3.	PGB	Dark brown	8.97	11.34
4.	MKL	Greenish brown	20.76	26.11

Initially 80g of crude drug was taken. **Where NIL-** Hydroalcoholic Extract of *Neium indicum* leaves, **AHP-**Hydroalcoholic Extract of *Artrocarpus heterophyllus* pulp, **PGB-** Hydroalcoholic Extract of *Punica granatum* Linn. Bark and **MKL-** Hydroalcoholic Extract of *Murraya Koenigi* Linn. whole plant

Formulation and Evaluation

Ointment formulation

The present study was done to prepare and evaluate the herbal ointment formulation. The herbal extracts were obtained from continuous hot percolation extraction method in good yield and there was no any harm to the chemical constituents and their activity. The fusion 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 (Table 4). This polyherbal ointment formulation was also placed for a stability study at different temperature conditions range includes 5°C, 25°C/ 60% RH, 30°C/65% RH, and 40°C/75% RH for a period of 3 months and result indicated that there were no changes observed in physical appearance, pH, and rheological properties as well as formulation also tested for the irritant effect and concluded that these formulation has free from irritation. The ointment formulations have been shown the absence of redness, no sign of inflammation, and absence of edema.

Table 4: Evaluation of Ointment formulations

S. No.	Parameters	Formulations	
		F1	F2
1.	Colour	Yellowish brown	Dark Yellowish brown
2.	Odour	Characteristic	Characteristic
3.	Consistency	Smooth	Smooth
4.	Ph	5.9±0.1	6.4±0.1
5.	Spreadability (sec.)	7.2±0.38	8.6±0.25
6.	Extrudability (g)	0.41±0.06	0.47±0.11
7.	Diffusion study (after 60 min.)	0.70±0.04	0.65±0.05
8.	Loss on drying (%)	30.24±1.20	30.73±1.31
9.	Solubility	Soluble in B.W., miscible with ET, E, CL	Soluble in B.W., miscible with ET, E, CL
10.	Washability	Good	Good
11.	Non irritancy	Non irritant (Absence of redness, rashes, edema)	Non irritant (Absence of redness, rashes, edema)
12.	Stability studies 5°C, 25°C/ 60% RH, 30°C/65% RH, and 40°C/75% RH	Stable	Stable

Where, B.W.- Boiling Distilled Water, ET- Ethanol, E- Ether and CL- Chloroform

Gel formulation:

Poly-herbal gel formulation has been prepared by the addition of Indian plant extracts including *Nerium Indicum Mill*, *Artocarpus Heterophyllus Lam*, *Murraya Koenigii Linn*, *Punica Granatum Linn*. Two formulations has developed and evaluated for their physicochemical properties as well as for the stability of the formulation. The herbal extracts were extracted out by continuous hot percolation extraction process to obtain a good yield and there was no any harm to the chemical constituents and their activity. The constituents are mixed properly to prepare gel so that uniform mixing of the herbal extract with the gel 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 at 5°C, 25°C/60 % RH, 30°C/65% RH, and 40°C/75% RH for a period of 3 months and No changes were shown in physical appearance, pH, and rheological properties and gel formulation also tested for the irritant effect and concluded that these formulation has free from irritation. The gel formulation has been shown the absence of redness, rashes, and edema. The result obtained from all the studied concluded in the table 5.

Table 5: Evaluation of Gel Formulations

S. No.	Parameters	Formulations	
		F3	F4
1.	Colour	Yellowish brown	Dark Yellowish brown
2.	Odour	Characteristic	Characteristic
3.	Consistency	Smooth	Smooth
4.	pH	6.1±0.1	6.3±0.1
5.	Spreadability (sec.)	6.5±0.24	6.9±0.32
6.	Extrudability (g)	0.39±0.04	0.44±0.13
7.	Diffusion study (after 60 min)	0.65±0.02	0.60±0.02
8.	Loss on drying (%)	29.14±1.11	29.32±1.55
9.	Solubility	Soluble in B.W., miscible with ET, E, CL	Soluble in B.W., miscible with ET, E, CL
10.	Washability	Good	Good
11.	Non irritancy	Non irritant (Absence of redness, rashes, edema)	Non irritant (Absence of redness, rashes, edema)
12.	Stability studies (5°C, 25°C/ 60% RH, 30°C/65% RH, and 40°C/75% RH)	Stable	Stable

Where, B.W.- Boiling Distilled Water, ET- Ethanol, E- Ether and CL- Chloroform

CONCLUSION

The present study was carried to prepare and evaluate the poly-herbal ointment and gel formulation. The herbal extracts were prepared by soxhlet extraction using simple maceration process to obtain a good yield of extract and there was no harm to the chemical constituents and their activity. Three herbal extract was separated by the soxhlet extraction method. Poly-herbal ointment and gel formulation was prepared by mixing the extract of *Nerium Indicum Mill*, *Artocarpus Heterophyllus Lam*, *Murraya Koenigii Linn*, *Punica Granatum Linn* with the carbapol 940 and PEG 2000 & PEG 600 for gel and ointment formulation. The poly-herbal ointment and gel formulation was evaluated for its physicochemical parameters like color, odor, pH, spreadability, extrudability, consistency, diffusibility, solubility, washability. The poly-herbal ointment and gel formulations were also evaluated for its stability at various temperature conditions including at 5°C, 25°C/60% RH, 30°C/65% RH, and 40°C/75% RH for a period of 3 months which shows were shown in 5°C, 25°C/60% RH, 30°C/65% RH, and 40°C/75% RH for physical appearance, pH, and rheological properties. All the prepared gel and ointment formulations were found to be stable upon storage for 3 months, no change was observed in their physical appearance, pH, rheological properties and drug content. These gel and ointment formulation has evaluated for irritancy test. The allergy to a substance is a state of hypersensitivity of the skin, immune response to antigen that appears so excessive or in appropriate, and is also

manifested as erythema and edema. The absence of these reactions including absence of redness, rashes and edema reflects the non-irritant status of *Nerium Indicum Mill*, *Artocarpus Heterophyllus Lam*, *Murraya Koenigii Linn*, *Punica Granatum Linn*. Ointment and gel formulation was tested, found to non-irritate the skin, indicating that ointment and gel formulation may be useful in the cosmetic or cosmaceutical industry. Thus it concluded that ointment and gel formulations promote a better platform or medium to use the medicinal properties of extracts effectively and easily as a simple dosage form. The poly-herbal ointment and gel prepared from extract of *Nerium Indicum Mill*, *Artocarpus Heterophyllus Lam*, *Murraya Koenigii Linn*, *Punica Granatum Linn* showed marked reduction in wound area in comparison to control group when examined for wound healing activity by topical application in albino rats³⁰. The formulation will be helpful in wound healing with no side effects and will be beneficial for society and industry with standardization approaches.

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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