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FORMULATION AND EVALUATION OF HERBAL OINTMENT OF *MURRAYA KOENIGII*

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Abstract: The leaves of *Murraya koenigii* (L) spreng (rutaceae) are reported to have great medicinal value such as antibacterial, anti inflammatory, antidiabetic, etc. Pharmacognostic evaluation including foreign matter, moisture content, ash value and extractive values were carried out. Phytochemical screening including qualitative chemical examination was performed. Phytochemicals such as carbohydrates, alkaloids, sterols, tannins, volatile oils, saponins, Anthraquinone glycosides, and flavanoids are reported. Carbazole alkaloids which are abundantly present in the leaves, fruits, roots and bark of this plant have been reported for their anti diabetic, antioxidant, wound healing activity, anticancer. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Wounds are inescapable events in life. Wounds may arise due to physical, chemical or microbial agents. Healing is survival mechanism and represents an attempt to maintain normal anatomical structure and function. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. Literature reports elsewhere indicate that *Murraya koenigii* possess wound healing property. Therefore this work is aimed at investigating the wound healing effect of herbal ointment formulated with *Murraya koenigii*.

Keywords: *Murraya koenigii*, Physiochemical studies, pharmacognostic, Phytoconstituents, etc.

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INTRODUCTION

Nature has provided a complete store-house of remedies to cure all aliments of mankind. In addition to being a good source of anti-infective agents, they are also cost-effective and have fewer side effects. The presence of various life sustaining constituents in plants has always encouraged scientists to carry out investigations for finding new therapeutic agents for the various fields of biomedicine. WHO estimates indicate that 80% of the population, mostly in developing countries still relies on plant-based medicines for primary care.. The different systems of medicinal usage practiced in India, Ayurveda, Unani, Siddha, and Homeopathy, utilize a large number of plants for treatment of human and animal diseases. Thus, India has rich plant diversity and houses about 47,000 plant species, out of these 7,500 have medicinal value; but only 800 plant species are used in the preparation of herbal drugs. A large number of plants still remain unexplored with regard to their medicinal properties and they can be sources of potential bioactive compounds for the development of new "leads" to combat various diseases.

Murraya koenigii L. (family: *Rutaceae*) with very pungent aromatic leaves is a plant which has various important uses in traditional system of medicine. Based on ethnomedicine, *Murraya koenigii* is used as a stimulant, antidysentric, antiemetic, flavoring agent and for management of diabetes mellitus[1]. Carbazole alkaloids which are abundantly present in the leaves, fruits, roots and bark of this plant have been reported for their anti diabetic, antioxidant, wound healing activity, anticancer. Diabetes mellitus is metabolic disorders of multiple actiology characterize by chronic hyperglycaemia with disturbance of carbohydrates, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. Management of this disease may include life time modifications, diet, and exercise long term use of oral hypoglycaemic agents or insulin therapy. Since ancient times plant have been plant have been and exemplary source of medicine. The search for plants with hypoglycaemic even though, literature reports elsewhere indicates that *Murraya koenigii* possess hypoglycaemic property. Therefore this work is aimed at investigating the wound healing effect of herbal ointment formulated with *Murraya koenigii*.

MATERIALS AND METHODS:

Collection of plant: The fresh leaves of *M. koenigii* were collected from Mansarovar, Jaipur , Rajasthan, in the month of December,2014 and were identified by the taxonomist, Mr. Vinod Kumar, Department of Botany, Rajasthan University, Jaipur, Reg. No. RUBL211486**.**

Chemicals and Reagents: Emulsifying Wax, Liquid Paraffin, White Soft Paraffin, Tween 80, Methanol, chloroform, Ethyl acetate, Petroleum ether, Dragendoff's reagent, Hager's Reagent, Wagner's Reagent, Mayer's Reagent, Tannic acid, Vanilline HCl, Bromine water, Conc. HCl, Dil.

HCl, Conc. H_2SO_4, Gelatin, Magnesium turnings, ammonia solution, CCl_4, Antimony Trichloride, Acetic anhydride.

Equipments: Soxhlet apparatus, digital balance, bunsen burner, pH meter, glass wares, UV spectrophotometer, Magnetic stirrer, hot air oven.

i. Physiochemical analysis:-

- **Determination of Loss on Drying:** 5 g of the sample (without preliminary drying) was weighed and placed in a tarred evaporating dish. It was dried at 105˚ C for 5 hours and at 1 hour interval until difference two successive weighing corresponded to not more than 0.25%.

- **Foreign Organic Matter:** 100 g of drug sample to be examined was spread on thin layer. Foreign matter was inspected with lens. Foreign matter was separated and drug sample was weighed again.

- **Determination of Total Ash:** About 2 g of sample was accurately weighed in a tarred silica dish at a temperature not exceeding 450^o C until it was free from carbon. Then it was cooled in desiccator and weighed. The percentage of total ash was calculated with reference to the air dried drug.

- **Determination of Acid Insoluble Ash:** The total ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid; the insoluble matter obtained was collected on an ash less filter paper, washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

- **Water-soluble Ash:** The ash obtained in the determination of total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a tarred silica crucible and ignited for 15 minutes at a temperature not exceeding 450^o C. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as the water- soluble ash was calculated with reference to the air dried drug.

- **Determination of Water-Soluble Extractive:** 5 g of test sample was weighed and macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. It was filtered rapidly, taking precautions against the loss of solvent. 25 ml of the filtrate was taken and evaporated to dryness in a tarred flat bottomed shallow dish at 105^o C, to constant weight and weighed

the percentage of water soluble extractive was calculated with reference to the air dried sample.

- **Determination of Alcohol-Soluble Extractive:** 5 g of test sample was weighed and macerated with 100 ml of ethanol (95%) in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. It was filtered rapidly, taking precautions against the loss of solvent. 25 ml of the filtrate was taken and evaporated to dryness in a tarred flat bottomed shallow dish at 105^{o} C, to constant weight and weighed the percentage of water soluble extractive was calculated with reference to the air dried sample.

ii. Preparation of different extracts of *Murraya koenigii* leaves:-

The leaves of *Murraya koenigii* were dried in shade under normal environmental condition and homogenized to coarse powder and stored in opaque screw tight jars until use. Powdered drug was charged into soxhlet apparatus and extraction was carried out with following solvents succesively.1) Petroleum ether (40-60^{o}C), 2) Chloroform, 3) Ethyl acetate, 4) Methanol, 5) Water. Each time before employing the solvent of higher polarity marc was dried. Each extract was then concentrated using rotary vacuum evaporator at 40-50^{o}C under vacuum and dried residue was collected in an opaque glass bottles for further studies.

iii. Phytochemical Analysis: The methanolic extract obtained after soxhletation was subjected to various phytochemical screening to reveal the presence of various active phytoconstituents.

iv. Formulation of Ointment:

S.NO.	INGREDIENT	QUANTITY(g)
1.	Methanolic Extract	1
2.	Emulsifying Wax	3
3.	White Soft Paraffin	5
4.	Liquid Paraffin	2
5.	Tween 80	q.s.

Required quantities of ointment bases were weighed and heated up to 70-75^{o}C and stirred until a uniform mass was obtained. To this adequate amount of methanolic extract of Murraya koenigii and Tween 80 were added and stirred well until a homogenous mass was obtained.

v. Evaluation:

- **Color and Odour:**

Colour and odour was examined by visual examination.

- **Loss on drying:**

Loss on drying was determined by placing ointment in petridish on water bath and dried at 105^{o}C in hot air oven.

- **pH:**

The pH of ointment was determined by using digital pH meter, which was calibrated before each use with standard buffer solutions at pH 4, 7. 1 gm of ointment was dissolved in 100 ml of distilled water and stored for 2 hours. The electrodes were inserted into the sample to take reading at room temperature.

- **Spreadability:**

Spreadability is expressed in terms of time in sec.'s taken by two slides to slip off from the ointment when placed in between the slides under the direction of a certain load. The excess amount of sample was placed between the two glass slides and a definite amount of weight was placed on these glass slides to compress the glass slides of uniform thickness. A weight of 70 g was added and the time required to separate the two slides was noted. Spreadability was calculated by using the formula:

S=M.L/T

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

- **Viscosity:**

Viscosity of the formulation was checked using a Brookfield viscometer. The ointment was rotated at 0.3, 0.6, 1.5 rotations per min. The viscosity of the ointment was obtained by multiplying the corresponding dial reading with the factor given in the Brookfield viscometer catalog.

- **Extrudability:**

The formulation was filled in collapsible tubes after the ointment was set in the container. Extrudability of the ointment formulation was determined in terms of weight in grams required to extrude a 0.5 cm ribbon of ointment in 10 sec.

- **Diffusion study:**

The diffusion study was carried out by preparing agar nutrient medium. It was poured into petridish. A hole bored at the centre and ointment was placed in it. The time taken for the ointment to get diffused was noted.

- ***In vitro* drug release characteristics:**

The Drug release from the herbal ointment was assessed by dissolution test using egg membrane model at 37^{o}C ±0.5^{o}C with an rpm of 50. The test was performed using 500 ml of distilled water (for 2hr), as dissolution media. Dissolution studies were carried out in triplicate, maintaining the sink conditions for all the formulations. A 5 ml aliquot of samples were withdrawn at regular time intervals, filtered and assayed spectrophotometrically at 203.8 nm.

vi. Results and Discussion:

Literatures revealed that the selected plant have wound healing activity. Hence an attempt was made to formulate a herbal ointment, and to evaluate for its physical parameter, in vitro drug release. Extraction and the phytochemical screening were done using different solvent. Phytochemical screening confirmed the presence of various phytoconstituents like carbohydrate, glycosides, alkaloids, flavanoids and tannins. In the present study, herbal ointment was prepared by fusion method using emulsifying ointment as the base.

The formulation was then evaluated for their physical parameters, in vitro drug release study. These physical parameters were within the acceptable range. The stability studies were carried out and inferred that the formulation showed no signs of in stability. Hence the study concludes that an efficient herbal ointment can be formulated from the methanolic plant extract of Murraya koenigii.

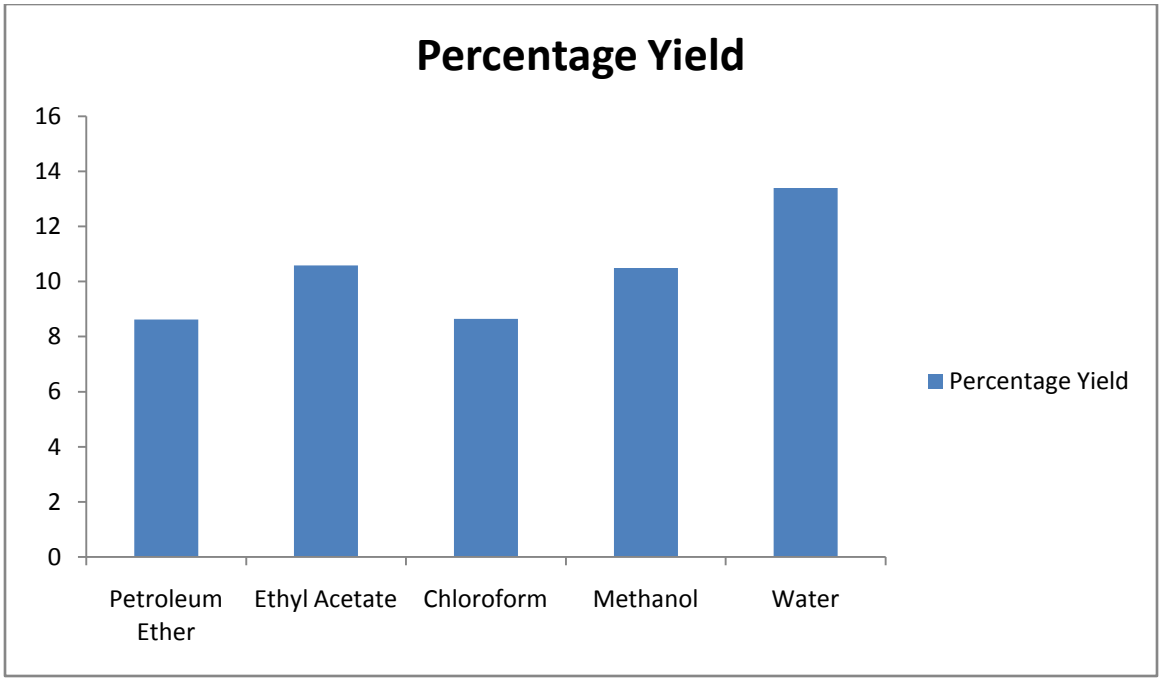
I. Macroscopic Characters:-

Sr. No	Parameters	Characters
1.	Colour and Appearance	Pale Green
2.	Taste	Bitter
3.	Odour	Pungent type

II. Physiochemical Analysis:-

Sr. No	Parameters	Physicochemical values
1.	Loss on drying	9.4% w/w
2.	Total ash	13.8% w/w
3.	Acid insoluble ash	5.0% w/w
4.	Water-soluble Ash	6.5% w/w
5.	Water-soluble extractive	3.5% w/w
6.	Alcohol-soluble extractive	7.55%w/w
7.	Foreign matter	1.0% w/w

III. Extractive value:



IV. Phytochemical examination:

Sr. NO	QUALITATIVE TESTS	RESULTS
1.	carbohydrates	++++
2.	proteins	+
3.	steroids	+
4.	tannins	+
5.	flavonoids	++
6.	Volatile oil	+
7.	Anthraquinone glycosides	++
8.	alkaloids	++++
9.	saponins	++
10.	Cardiac glycosides	+

V. Physiochemical Evaluation:

Sr. NO.	PHYSIOCHEMICAL PARAMETER	RESULT
1.	Colour	Pale green
2.	Odour	Characteristic
3.	Loss on drying	30%
4.	pH	6.91
5.	Spreadability	11
6.	Viscosity	5763 cps
7.	Diffusion Study	1.2 cm
8.	Extrudablity	5g
9.	Storage	Stable

VII. CONCLUSION:

The presences of phytochemicals make the plant useful to prevent different ailments and have a potential to provide useful drugs to mankind for various use. The quantitative determination of pharmacognostic parameters will help for setting standards for crude drugs. The total ash value is important in evaluating the purity of drug and further work aiming towards carrying out animal studies are in progress.

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