

Formulation and Evaluation of Antibacterial Polyherbal

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ABSTRACT

Herbal drugs predominate in traditional medicine as well as in alternative medicine practiced in the developed world. Among the various indications where traditional herbal medicines are used, skin related disorders is ranked top. The majority of the population in developing countries uses plants or plant preparations in their basic health care. In present study is to formulate and evaluate a poly herbal ointment with antibacterial activity. Ointments were formulated using methanolic extract of *M. Koenigii*(L.) spreng, essential oil from *T. erecta* (L.) flower methanolic extract of honey were evaluated for its physicochemical properties. The prepared extracts of the choose plants were taken in specific ratio randomly for antibacterial evaluation. Ointments were all set making use of special concentrations of the extracts with the aid of fusion procedure utilizing simple ointment as a base. Formulations had been then tested for its physicochemical properties like colour, odour, pH, consistency and gave satisfactory results. Probably the most powerful mixture used to be then determined via evaluating the results of the zone of inhibition given through distinct extract ratios on *Escherichia coli*, *Staphylococcus aureus* of antibacterial undertaking against various gram positive and gram negative. The entire formulations confirmed predominant recreation against selected species. The formulations are found to be very efficacious in all the parameters which has conducted and also found enhance Antibacterial property. Overall result of this study reveals that this is an effective Polyherbal Antibacterial ointment.

1. Introduction

Medicinal plants are naturally gifted with invaluable bioactive compounds which form the backbone of traditional medicines [1]. The basic herbs have the answer with no side effects and effective remedies and the golden fact is use of herbal treatment is independent of any age group. When two or more herbs are used in the formulation they are known as Polyherbal formulations [2]. Antibacterial activity is the ability of the substance to inhibit or kill bacterial cells. Microorganisms such as bacteria can cause many types of skin-related diseases such as skin rashes, acne, eczema, psoriasis, dermatitis and etc. *Staphylococcus aureus* and *Escherichia coli* are the main pathogen that causes these skin infections. Topical ointment containing extract of medicinal plant is one alternative to treat the skin infection caused by bacteria and prevent the use of oral antibiotic which then can develop bacterial-resistant.[3,4,5] Honey consists of various constituents such as water, carbohydrates, proteins, vitamins, amino acid, energy and minerals. Besides the major ones, there must also be several minor constituents in honey, which may be playing a key role in determining the antibacterial behavior of honey.[6,7] *Murraya koenigii* (Linn.)spreng, a member of the family Rutaceae, is a deciduous to semi-evergreen aromatic tree found throughout India. Curry leaf tree is commonly used as spice due to the aromatic nature of leaves. Carbazole alkaloids, the major constituents of the plant are known to possess Cytotoxic, Antioxidative, Antimutagenic and Anti- inflammatory, Antibacterial activities. *Tagetes erecta* L.(Asteraceae) is a medicinal and ornamental plant as nematocide, cosmetic and medicinal properties. It is used in olden days for the treatment of wounds, burns, and skin rashes. [8,9]

2. Materials and methods

Collection of Plants and Material

Fresh leaves of *Murraya koenigii* (L.)spreng. were collected from local area of Gondia District, Marigold flowers (*T. erecta*) and Honey were purchased from local market.

Physicochemical Analysis of Curry Leaves

The collected leaves of curry leaves were shade dried for 7 days and finally pulverized in to coarse powder. It was stored in a well closed container free from environmental climatic changes till usage.

Preparation of Herbal Extracts

Preparation of Methanolic Extract of *M Koenigii* Leaves[[7,8]

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 with methanol solvent. Methanolic extract was evaporated by heating until dried residue and concentrated methanolic extract was collected.

hytochemical Analysis of *M Koenigii* Extract [7]

The methanolic extract of *M. koenigii* leaves were subjected to the phytochemical analysis by using various chemical tests

Preparation Of Methanolic Extract Of Honey [5]

Extraction of raw honey was performed by using organic solvents, for this, 10g of honey was taken in a centrifuge tube with 25 ml of solvent and then mixed well by vortexing and shaking with hands for about 30 minutes. This was centrifuged at 3000 rpm for 10 minutes at 25°C. Supernatant was collected from each centrifuged tube in a round bottom flask by filtration. The extracts were prepared using the solvent methanol. The

methanolic Extracts dissolved in DMSO were collected in sterilized glass tube and used within 24 h for the evaluation of Bacteriostatic and Bactericidal activity. After extraction residue of each honey were also checked for their Antibacterial activity.

Phytochemical Analysis of Honey

Tests for carbohydrates

A. Fehling's test: Take 1 ml of extract in the test tube and to this added the equal quantities of Fehling's solution A and B, a brick precipitate indicates the presence of carbohydrate.

B. Fehling's test: Take 1 ml of extract in the test tube and to this added the equal quantities of Fehling's solution A and B, a brick precipitate indicates the presence of

carbohydrate.

Extraction of *T. erecta* flowers [8]

The fresh flowers of *T. erecta* were shade dried at room temperature for 10 days and ground to a fine powder using electronic blender. Herb samples (100g) were hydro-distilled in Clevenger type apparatus. The essential oils were collected and dried over anhydrous sodium sulphate. The essential oil sample were stored in the dark at 4°C.

Formulation of Polyherbal Ointment

The base and extracts of the mentioned three plants were added and stirred well until a homogeneous mass were obtained.

Table no. 1: Formulation of Polyherbal Ointment

Sr. no.	Ingredients	F1 (2%)	F2 (4%)	F3 (6%)
1.	Methanolic <i>M.koenigii</i> extract	2 gm	4 gm	6 gm
2.	<i>T. erecta</i> essential oil	2 gm	4 gm	6 gm
3.	Methanolic honey extract	2 gm	4 gm	6 gm
4.	Ointment base (q.s.)	100 gm	100 gm	100 gm

Evaluation of Antibacterial Activity Of Ointments

Antibacterial Activity Procedure

Anti-microbial activity is a process of killing or inhibiting the growth of microbes. Antimicrobial agent either kills (bactericidal) the microbes or inhibits the growth (Bacteriostatic) of microbes. The standard bacterial test organisms were sub cultured freshly prepared nutrient agar and the extracted samples were inoculated into the culture using paper cup plate method.

Test Microorganisms

The test organisms include Gram negative bacteria - *Escherichia coli* and one Gram positive bacteria *Staphylococcus aureus*.

Methodology

The methanolic extracts of following plants were taken in different ratios were carried out for anti-microbial activity using cup plate method. Nutrient agar medium was prepared, sterilized and used as growth medium for bacterial culture. 25 ml of sterilized medium was poured into each petri plates, covered semi half and allowed it to solidify. Then the test micro organisms like *Escherichia coli*, *staphylococcus aureus* were inoculated into the petri plates. Then different formulations was poured inside the plates were incubated at 37°C overnight for observation. The presence of zone of inhibition was noted after 24 hrs. The susceptibility of the test to the tested plant extracts

was determined by observing the zone of inhibition around each well.

3. Result and discussion

The zone of inhibition (mm) measured in different extraction ratios on *Escherichia coli*, *Staphylococcus aureus* were fig.1.and 2 The agar well diffusion was employed to evaluate the antibacterial efficacy of the extract combination.[4] The diameter of the borer used was 6 millimeter. The combinations having the biggest zone of inhibition were shown in Table-2.

Formulation F3 (6%) showed greater activity against *Staphylococcus aureus* and *Escherichia coli*. *Murraya koenigii*(L.)spreng, *T. Erecta* (L), Honey have Antibacterial activity. Extraction and phytochemical screening were done. Phytochemical screening confirmed the presence of various phytoconstituent like carbohydrate, glycosides, flavanoids and tannins. The anti-bacterial activity of prepared ointments were compared with 5%w/w Mupinase ointment using selected species of microorganism such as *Staphylococcus aureus* and *E.coli*. It observed that formulation F3 showed greater activity *Staphylococcus aureus* and *E coli* compared to 2% Mupinase ointment. So, the prepared ointments has better activity against *Staphylococcus aureus* and *E.coli* compared to standard 2% w/w Mupinase ointment.

Table no. 2: Antibacterial activity of ointments

Sr. no	Ointment	Diameter of zone of inhibition (mm)	
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
1	F 1(2%)	9.87	9.61
2	F 2 (4%)	10.05	9.83
3	F 3 (6%)	12.45	13.87
4	Standard	11.88	11.65

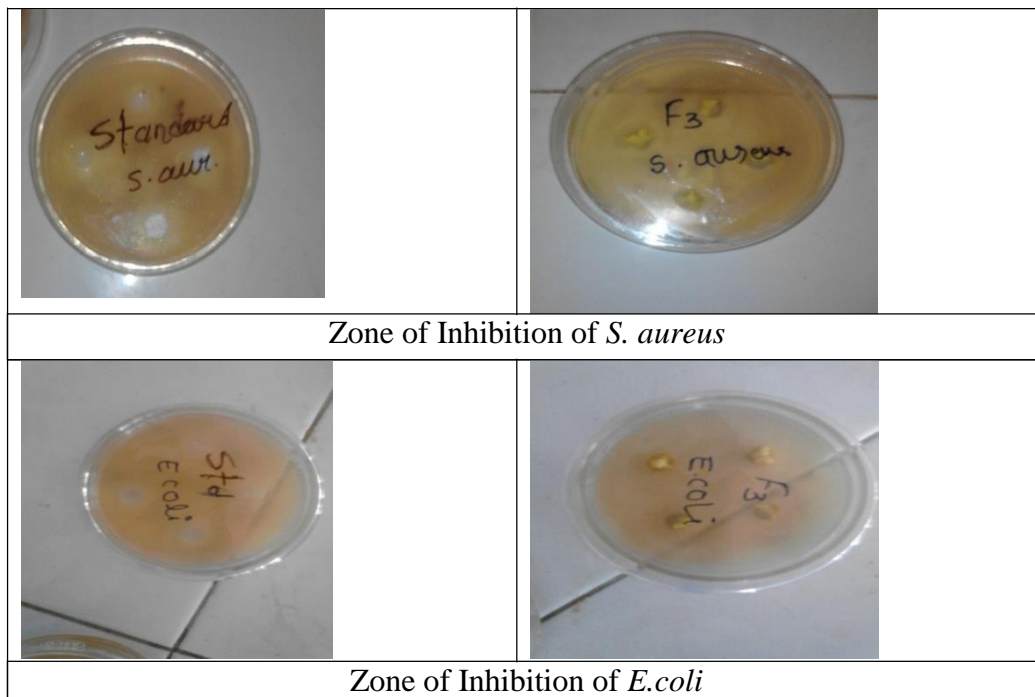


Fig-1: Zone of Inhibition.

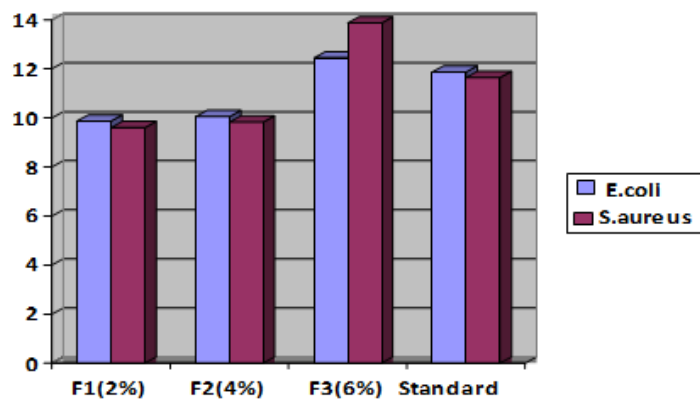


Fig.2: Various formulation Antibacterial activity of Ointments

4. Conclusion

Hence the study concludes that an efficient antibacterial ointment with antibacterial activity can be formulated

from the methanolic plant extract *M koenigii*, *T. erecta* essential oil, methanolic extract of honey which can also be used for wound healing and various skin infections.

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