

Formulation and Evaluation of Anti-Microbial Polyherbal Ointment

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Abstract

The majority of the population in developing countries uses plants or plant preparations in their basic health care. Many plant species have been proved to have antimicrobial activity. Lots of the antibiotics had been at the beginning derived from micro-organisms even as the chemotherapeutic agents are from vegetation. Together with other dosage varieties, natural drugs are additionally formulated within the type of ointment. An ointment is a viscous semisolid preparation used topically on a variety of body surfaces. Thus, the main objective of the present study is to formulate and evaluate a polyherbal ointment with antimicrobial activity. Ointments were formulated using methanolic extracts (by continuous hot percolation-soxhlation) of Shatavari, Terminalia chebula and were evaluated for its physicochemical properties. The methanolic extracts of the chosen plants were taken in specific ratio randomly and the antimicrobial tests of the combinations had been applied. Ointments were all set making use of special concentrations of the extracts with the aid of fusion procedure utilizing emulsifying ointment as a base. Formulations had been then tested for its physicochemical properties like loss on drying, pH, spread ability, extrudability 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 antimicrobial undertaking against various gram positive and gram terrible. 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 antimicrobial property. Overall result of this study reveals that this is an effective polyherbal antimicrobial ointment.

Keywords: Antimicrobial activity; Eucalyptus; Poly herbal; Shatavari; Terminalia

Introduction

Herbal medication, also called botanical treatment or phytomedicine, refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbal drug treatments are in general used to furnish first-line and common health provider, both to persons dwelling in faraway areas the place it is the only on hand wellness service, and to people residing in bad areas the place it offers the one cheap relief. Even in areas where cutting-edge treatment is to be had, the curiosity on herbal drug treatments and their utilization had been growing swiftly in up to date years. Medicinal vegetation are principal sources for pharmaceutical manufacturing. Medicinal plants and herbal drugs account for a giant percent of the pharmaceutical market [1-5].

Plant Profile

Eucalyptus

Eucalyptus oil is acquired by steam distillation and rectification from the contemporary leaves or the fresh terminal branchlets of more than a few species of Eucalyptus wealthy in 1, eight-cineole. Chemical materials of the leaves of Eucalyptus globules are The isolation of the water-distilled unstable oil from the leaves of *E. Globuluslabill*, the qualitatively examination of the chemical constituents of the most important oil and their quantitative selection by using direct comparison with results from MS databases attached to the GC-MS instruments following GC-MS evaluation. Chemical constituents in the fruits of Eucalyptus globules are 15 compounds have been obtained and recognized as beta-sitosterol, betulinic acid, stigmasterol, euscaphic acid, 2a-Hydroxybetulinic acid, macrocarpal B, macrocarpal A, oleanolic acid, ellagic acid and gallic acid [6-10].

Pharmacological activity

Myrtaceous plants are known to be rich source of biologically active terpenoids and polyphenols, including flavonoids, phloroglucinol

derivatives and tannins. Phytochemical studies on *Eucalyptus globulus labill*. One new phloroglucinol by-product named eucalypt one G, alongside 9 identified compounds. The antibacterial undertaking of the new compound has been studied [10-13].

Shatavari

Asparagus racemosus is a species of asparagus produced throughout Nepal, Sri Lanka, India and the Himalayas. *Asparagus racemosus* (Shatavari) is encouraged in Ayurvedic texts for the prevention and medication of gastric ulcers, dyspepsia and as a galactagogue. *A. racemosus* has additionally been used by some Ayurvedic practitioners for worried problems. The title "Shatavari" approach "curer of 100 ailments" (shat: "hundred"; vari: "curer"). Biologically active constituents of shatavari are aside from saponins, the material comprises alkaloids, proteins, starch, tannin, mucilage and diosgenin. The style of saponin varies with the geographical distribution of the species. Two new steroidal saponins, shatavarside A and shatavarside B at the side of a known saponin, filiasparoside C, have been isolated from the roots of *Asparagus racemosus*. 5 steroidal saponins, shatavarins VI-X, in conjunction with five recognized saponins, shatavarin I (or asparoside B), shatavarin IV (or asparinin B), shatavarin V, immunoside and schidigerasaponin D5 (or asparinin A), have been remoted from the roots of *Asparagus racemosus* [14-16].

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Medicinal uses

Asparagus racemosus is a principal medicinal plant of tropical and subtropical India. Its medicinal utilization has been pronounced in the Indian and British pharmacopeia it's quite often identified for its phytoestrogenic residences. In Ayurveda, *Asparagus racemosus* has been described as a rasayana herb and has been used commonly as an adaptogen to expand the non-distinctive resistance of organisms against a variety of stresses. Apart from use within the healing of diarrhea and dysentery, the plant also has antioxidant, immunostimulant, anti-dyspepsia and antitussive results. The roots are utilized in Ayurvedic medicine, following a regimen of processing and drying. It's normally used as a uterine tonic, as agalactagogue (to give a boost to breast milk), in hyperacidity, and as a best common wellness tonic.

Pharmacological activity

A. racemosus is a traditional galactagogue both in animals and human's therapy of duodenal ulcer with *A. racemosus* is mentioned. Antioxitocin action of this plant has been documented and immunomodulatory effects are ascribed to the basis.

Terminalia chebula

It's a deciduous tree developing to 30 metre (98 ft) tall, with a trunk as much as 1 metre (3 ft 3in) in diameter. The leaves are alternate to sub opposite in association, oval, 7–eight-centimetre (2.8–3.1 in) long and 4.5–10 cm (1.8–3.9 in) wide with a 1–3 cm (0.39–1.18 in) petiole. The fruit is drupe-like, 2–4.5 cm (0.79–1.77 in) lengthy and 1.2–2.5 cm (0.47–0.98 in) huge, blackish, with five longitudinal ridges. *Terminalia chebula* is an important medicinal plant in Indian typical medicine and it's most on the whole used herb in Ayurveda. *Terminalia chebula* is a medium- to significant-sized tree expended in the course of tropical and subtropical Asia, together with China and Tibet. This tree is found in the forests of northern India, Uttar Pradesh and Bengal, and is normal in Tamil Nadu, Karnataka and southern Maharashtra. The *Terminalia* consists of 250 species and extensively distributed in tropical areas of the arena. The fruit of *Terminalia chebula* is keep in mind as the "king of drug treatments" with the aid of Tibetans and 2nd-to- none with the aid of ayurvedic apothecaries, and in addition held in high regard by way of other folks medicinal practitioners. *T. Chebula* is an important ingredient of the well identified natural preparation, Triphala, which is used for the therapy of enlarged liver, stomach problems and soreness in eyes. *Terminalia chebula* (*T. Chebula*) is a flowering evergreen tree of the family Combretaceae. Haritaki is broadly used medicinal plant in Ayurveda. Haritaki is used as a natural cleaner of the digestive procedure. It improves the functioning of the liver, spleen and the colon and thus it is largely used as a digestive tonic. The extract of Haritaki fruit is also used widely in many Ayurvedic formulations. Haritaki is also used in the combination with two more herbs to prepare a formulation know as Triphala, This medicine is widely used for Anti-aging activity. It is also used for increasing the immunity of the body [17,18].

Pharmacological properties

Antibacterial activity: Two antibacterial compounds, gallic acid and ethyl ester towards methicillin-resistant *Staphylococcus*, have been isolated from ethyl alcohol extract of fruits of *T. Chebula*. Various extracts of *T. Chebula* exhibit antibacterial responsibility in opposition to a number of bacterial species 21. The ethanolic extract of *T. Chebula* fruit was once observed strong in opposition to both gram-optimistic and gram-negative micro-organism corresponding to *Salmonella typhi* SSFP 4S, *Staphylococcus epidermidis* MTCC

3615, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* MTCC 441 and *Pseudomonas aeruginosa* ATCC 27853 suggesting its extensive spectrum antimicrobial activity [19-21].

Antianaphylactic and adaptogenic activities: *T. chebula* along with several other medicinal plants helps to resist against a number of stressors in different ways. *T. chebula*, when given following anaphylactic shock, reduces the serum histamine level showing a strong antianaphylactic activity.

Hepatoprotective activity: The 95% ethanolic extract of *T. chebula* fruit showed hepatoprotective activity against anti-tuberculosis (anti-TB) drug-induced toxicity which could be attributed to its prominent anti-oxidative and membrane stabilizing activities.

Ethanobotanical importance: It is commonly used in the form of Triphala. According to Ayurveda, proper digestion is the base of health. If the digestive system functions properly then it helps the other systems of the body to perform well. Triphala has Tridosahara property; it can be mixed with other herbs in compound formulations. Triphala has the quality to nourish the skin both directly and indirectly [22,23].

Reason for Taking Up the Present Work

Research literatures have revealed that *Terminalia chebula*, Shatavari and Eucalyptus oil are medicinally important. These plant materials having many research reports on (different proportions) anti-septic, anti-bacterial, anti-oxidant, anti-microbial properties etc. In the above claims and facts, the present work was undertaken to formulate and evaluate the polyherbal ointment and to find out the most effective combination having anti-microbial activity.

Plan of Work

1. Collection of crude drugs
2. Processing of crude drugs
3. Extraction of plant materials
 - Soxhalation
4. Antimicrobial activity of different formulations
 - Cup plate method

Materials and Methods

Collection of plant materials

The plant materials was collected in the month of February 2015 and authenticated by botanist in the department of Agricultural University, V. N. Pally, India. The plant material was then identified by their vernacular names and later it was compared with the herbarium of the department by the botanist. The plant materials were collected and separated and are then dried under shade drying for 4-5 days. Then the dried plant materials were grinded, sieved to get nearly fine amorphous powder.

Extraction

The plant materials were collected and separated and are then dried under shade drying for 4-5 days. Then the dried plant materials were grinded, sieved to get nearly fine amorphous powder. Extraction is the process of obtaining the constituents by separating them from crude drug by the use of solvents. Powdered material was extracted with suitable solvent or mixture of solvents for extracting the various

phytoconstituents present in the crude drug.

Soxhlation

100 g of each powdered materials (Shatavari and *Terminalia chebula*) were used for carrying out soxhlation extraction with 360 ml of Methanol for 6 hrs at room temperature. All the extracts were collected and evaporated at room temperature. The dried residues were weighed and % yield of each extract was calculated (Figure 1).

Antimicrobial activity procedure

Anti-microbial activity is a process of killing or inhibiting the growth of microbes. Antimicrobial agent either kills (bactericidal) three 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.

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, sterilised and used as growth medium for bacterial culture. 25 ml of *sterilised 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 using sterile cotton swabs. 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.

Results

The antimicrobial activity of different polyherbal formulations can be formulated from the methanolic extracts of *Eucalyptus oil*, *Terminalia chebula*, Shatavari of different proportions as shown above in Table. The formulations were then evaluated for their physical parameters (Table 1).

Anti-microbial activity

The zone of inhibition (mm) measured in different extraction ratios on *Escherichia coli*, *Staphylococcus aureus* were noted. This tests were carried out by cup plate method. Cup plate method was employed to evaluate the antibacterial efficacy of the extract combination. The diameter of the borer used was 6 millimeter. The combination having



Figure 1: Soxhlation apparatus.

Physicochemical parameters	F1	F2	F3	F4
Colour	Darkbrown	Darkbrown	Ligtbrown	Lightbrown
Odour	Characteristics	Characteristics	Characteristics	Characteristics
Loss on drying	0.95	0.91	0.91	0.86
P ^H	7.8	7.7	7.9	7.8
Spreadability (g.cm/sec)	4.66	8.60	8.60	8.86
Extrudability	120 g	115 g	114 g	130 g
Storage (4°C,24°C,37°C)	Stable	Stable	Stable	Stable

Table 1: Physicochemical Evaluation of formulated formulations.

Ointments	Diameter of zone of inhibition (mm)	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
F1	13.835	13.815
F2	12.61	12.32
F3	11.87	14.045
F4	12.315	12.35
Control	Nil	Nil

Table 2: Antimicrobial activity of polyherbal formulated ointment.

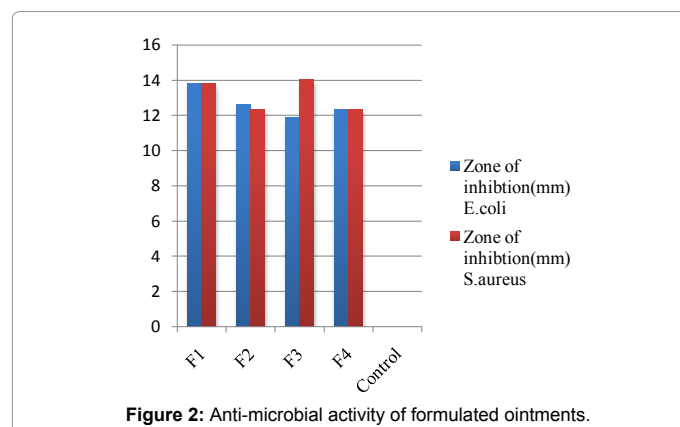


Figure 2: Anti-microbial activity of formulated ointments.

the biggest zone of inhibition were noted and the result obtained as follows (Table 2). From the above (Table 2). The results were compared with those of different formulations. Compared to all four formulations F1 formulation showed greater activity against *Escherichia coli* and F3 formulation showed greater activity against *Staphylococcus aureus*.

Graph

(Figures 2 and 3).

Zone of inhibition

(Figures 4 and 5).

Discussion

Literatures revealed that the selected 3 herbs (*Eucalyptus*, *Shatavari*, *Terminalia chebula*) have antibacterial activity. Hence an attempt was made to formulate a polyherbal ointment as in different proportions were prepared by fusion method using emulsifying ointment as the base. The formulations were then evaluate for its physicochemical parameters and to compare its antibacterial activity. Extraction was done using methanol as the solvent by successive soxhlation method. The anti-bacterial activity of prepared polyherbal ointments were used selected species of microorganism such as *Escherichia coli* and



Figure 3: Incubation of petri plates in BOD incubator.

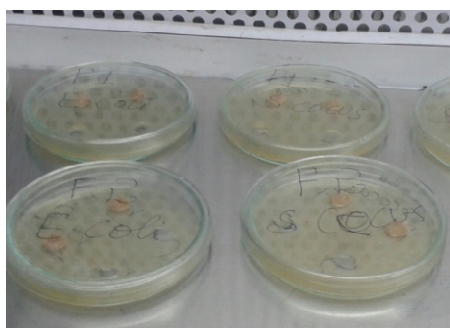


Figure 4: F1 and F2 formulations of *E.coli* and *S.aureus*.

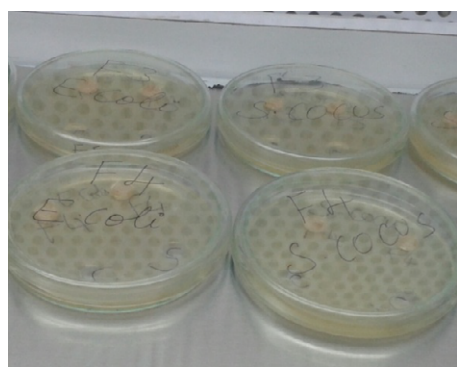


Figure 5: F3 and F4 formulations of *E.coli* and *S.aureus*.

Staphylococcus aureus and it showed the results of the formulations in different proportions. So, antimicrobial study shows that the results were compared with those of different formulations. Compared to all four formulations F1 formulation shows greater activity against *E. coli* and F3 formulation showed greater activity against *S.aureus*.

Conclusion

In the present experimental study showed that it is possible to develop and evaluate the anti-microbial polyherbal ointment with methanolic extract of plant materials of different proportions (*Eucalyptus*, *Shatavari*, *Terminalia chebula*) which is useful in the treatment of skin diseases caused by *E.coli* and *S.aureus*. The results were compared with those of different formulations. Compared to all four formulations F1 formulation shows greater activity against *E.coli* and F3 formulation shows greater activity against *S.aureus*. Hence, the

study concludes that an efficient antiseptic ointment with antimicrobial activity can be formulated from the methanolic plant extracts of *Eucalyptus*, *Shatavari* and *Terminalia chebula* which can also be used for various skin infections.

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