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FORMULATION AND EVALUATION OF ANTI-INFLAMMATORY & ANALGESIC POLYVALENT HERBAL OINTMENT

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Abstract: The current study was meant to created detailing on the Anti-inflammatory, and Analgesic action of Capsicum annuum, Mentha piperita, Curcuma longa, Syzygium aromaticum . Non-steroidal anti-inflammatory drugs (NSAIDs) are related with an excess of side effects and unfavourable medication responses. Steady utilized of NSAIDs produces gastrointestinal aggravation and another incidental effects on body organs like liver and kidneys. Mitigating, and Analgesic movement of Capsicum annuum, Curcuma longa, Syzygium aromaticum, Mentha piperita extract was recently covered distinctive experimental models. Capsicum annuum (chilli pepper) contains an assortment of carotenoids, including capsanthin, capsorubin, beta-carotene, cryptoxanthin, lutein, phytofluene, and xanthophyll, and steroids, including capsicoside. One of the primary constituents is capsaicin, which creates a serious consuming sensation when it comes into contact with the skin, eyes, or mucous films and which gives peppers their consuming taste. Syzygium aromaticum (clove) is a conventional flavour that has been utilized for food conservation and has different pharmacological exercises. S. aromaticum is wealthy in numerous phytochemicals as follows: sesquiterpenes, monoterpenes, hydrocarbon, and phenolic compounds. Eugenyl acetic acid derivation, eugenol, and β-caryophyllene are the main phytochemicals in clove oil. Curcuma longa has been utilized for millennia as a cure in the conventional Indian and society medication for the fix of a huge assortment of ailments, like irritation, irresistible illnesses, and gastric, hepatic, and blood issues. Menthol from Mentha piperita (40-90 percent) is an element of numerous beauty care products and a few scents.

Ointment formulation of these herbal drug shows a good result in all the evaluation test parameters such as General appearance, Consistency, pH, Spread ability, Extrudability, Diffusion study, Non irritancy test, & Stability study etc.

Keywords: Capsicum annuum, Mentha piperita, Herbal Ointment, Inflammation, analgesic.

INTRODUCTION

In the last few years there has been rapid growth in the field of herbal medicine and these drugs gaining popularity both in developing & developed countries due to their natural origin and less side effects. Therefore used of herbal medicine is essential for to overcome the problem of adverse drug reactions.[7]

Inflammation is an essential response provided by the immune systems that ensures the survival during infection and tissue injury. When an inflammation occurs in your body, many different immune system cells may be involved. They release various substances, known as inflammatory mediators. These include the hormones bradykinin and histamine. They cause the small blood vessels in the tissue to become wider (dilate), allowing more blood to reach the injured tissue. For this reason, inflamed areas turn red and feel hot. A drug or substance that reduces inflammation (redness, swelling, and pain) in the body. Anti-inflammatory agents block certain substances in the body that cause inflammation. They are used to treat many different conditions .An effective anti-inflammatory drug should be able to inhibit the development of inflammation without interfering in normal homeostasis.[7]

Chilly pepper, with the scientific name of Capsicum annuum, belongs to the Solanaceae family. Hot Chilly peppers consist of spicy compounds called capsaicinoids which include capsaicin, dihydrocapsaicin, nordihydrocapsaicin and other compounds. Capsaicin, water-insoluble derivative of homovanillic acid and the main active ingredient in capsicum fruits, is responsible for hot sensation to the tongue and is utilized for the treatment of inflammatory disorders such as psoriasis and rheumatoid arthritis , diabetic neuropathy, postherpetic neuralgia, cluster headache, postmastectomy

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syndrome, reflex sympathetic dystrophy , dermatitis or eczema itching , postoperative nausea and vomiting, bladder hyperactivity , gallstone , anorexia, haemorrhoids, liver congestion, foodborne gastrointestinal pathogens including Listeria monocytogenes, Salmonella typhimurium and Bacillus cereus , tonsillitis and rhinitis and fibromyalgia . It is also used as pesticides analgesic, antiobesity, antihypertensive , antiarrhythmic, antiischemic , and gastroprotective agent. It can stimulate saliva and digestive enzymes of the pancreas, small intestine , and also stimulate hair growth in alopecia areata. Anticoagulant activity, prevention of aspiration pneumonia, protecting neuromuscular junctions from Clostridium botulinum neurotoxin A and improving cognitive function are also attributed to capsaicin beneficial properties.[10]

Curcuma longa L. (turmeric) of ginger family (Zingiberaceae) belongs to the group of oldest cultivated spice plants in the south-east Asian countries. Curcuma longa (turmeric) is a popular spice in India and many other Asian countries. Major active ingredients of turmeric include three curcuminoids; curcumin (diferuloylmethane, the primary constituent responsible for yellow colour of turmeric), desmethoxycurcumin, and bisdemethoxycurcumin. In addition, volatile oils (turmerone, atlantone, and zingiberene) also have pharmacological activity. In addition, sugars, proteins, and resins are also present in turmeric. Turmeric has excellent anti-inflammatory properties and is a superior antioxidant. The World Health Organization (WHO) stated the acceptable daily intake of curcuminoids as a food additive in the range of 0–3 mg/kg. Curcuminoids and turmeric products have been characterized as safe by the Food and Drug Administration. In addition, active ingredients of turmeric have neuroprotective, antitumor antiacidogenic, and excellent anti-inflammatory properties. Therefore curcuminoids have therapeutic potential in treating many chronic diseases that contain an element of inflammation, including colon cancer, lung cancer, breast cancer, and inflammatory bowel diseases.[8]

Syzygium (S.) aromaticum, also known as clove, is a dried flower bud belonging to the Myrtaceae family that is indigenous to the Maluku islands in Indonesia but has recently been farmed in different places worldwide. Clove essential oil has been used as a topical anaesthetic and flavouring for years. It is known to have antimicrobial, anti-inflammatory, and antioxidant activity, mostly related to its content of eugenol and other polyphenolic compounds. Other uses of clove have also arisen, like insect repellent or growth promoter agent. Clove oil is a popular remedy for toothache due to its potent antiseptic and analgesic activity. It also has strong antioxidant and antiviral activities.[9] Eugenol is the main component of clove oil. The lipid profiles, the contents of tocols, and total phenolics in cold pressed clove oil are well documented. Cold pressed clove oil was reported to possess stronger radical scavenging and antimicrobial activity compared to virgin oil. Similarly, the cold pressed clove oil was also reported to possess hepatoprotective activity in experimental animals along with other biological activities.[11]

Mint consists of the dried leaves and flowering tops of Mentha piperita L. (peppermint) belonging to the family Lamiaceae. Peppermint is a popular herb that can be used in numerous forms (oil, leaf, leaf extract, and leaf water). Peppermint oil has the most uses, and use data on the oil are considered relevant to the leaf extract formulations as well. This herbal preparation is used in cosmeceuticals, personal hygiene products, foods, and pharmaceutical products for both its flavouring and fragrance properties. Peppermint oil possesses a fresh sharp menthol odour and a pungent taste followed by a cooling sensation. It also has a variety of therapeutic properties and is used in aromatherapy, bath preparations, mouthwashes, toothpastes, and topical preparations. Topical preparations of peppermint oil have been used to calm pruritus and relieve irritation and inflammation.[12]

A number of topical dermatologic products, ranging from solids to liquids, are available for the treatment of skin diseases . Majority of ointments consist of a base, which mainly acts as a carrier or vehicle for the medicaments. The nature of the base also controls its performance; hence, selection of ointment base is a very important aspect of formulation. Traditional ointment bases have been oleaginous in nature, which include hydrocarbons such as petrolatum, beeswax, and vegetable oils that do not allow inclusion of any water inversely to fatty alcohols. Ointments are used topically for several purposes such as protective, antiseptic, emollient, antipruritic, keratolytic, and astringent. The base of an ointment is of prime importance if the finished product is expected to function as any of the above categories. The ointment base composition determines not only the extent of penetration but also controls the transfer of medicaments from the base to the body tissues.[7]

MATERIALS & METHODS

Materials: The plant parts were washed with tap water, rinsed well and dried at room temperature in open air. The dried material was properly ground into powder. This powder material was separated according to particle size with the help of sieves no; #44,#60,#80,#85 to obtained different batches for further Preformulation Study.[12]

Excipients

Paraffin wax, Petroleum Jelly, Cetyl alcohol, White soft paraffin etc. obtained from Merck Specialties pvt ltd & SD Fine-chem limited.

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PREFORMULATION STUDY

a) General Appearance
Physical examination like Colour, Odour, Taste is done by visual Inspection.

b) Bulk Density: It refer to packing of particles in powder sample. Bulk density is used to determine the amount of powder sample that occupies the volume in g/ml. Weighed quantity of powder sample was transferred into 100ml measuring cylinder. The volume occupied by powder material was measured. Bulk density was calculated by using formula;
Bulk density= mass of powder/bulk volume of powder

c) Tapped density: Weighed accurate quantity of powder sample was transfer into a graduated measuring cylinder. Volume occupied by the powder was noted down. Then cylinder was subjected to 100-300 taps in tap density apparatus. Tapped density was calculated by using formula;
Tapped density= mass of powder/tapped volume

d) Carr's Index (Compressibility): The compressibility index and Hausner's ratio was measures the property of powder to be compressed. The packing ability of powder material was evaluated from change in volume, which is due to rearrangement of packing occurring during tapping. It was indicated as Carr's compressibility index was calculated by following formula;
Carr's index= [Tapped density-bulk density]/tapped density x 100

e) Hausner s' Ratio: It is measurement of frictional resistance of powder. The ideal range should be 1.2-1.5. It was determined by the ratio of tapped density and bulk density.
Hausner's = Tapped density/bulk density

f) Angle of Repose (θ): It is defined as the maximum angle that can be obtained between the free standing of powder heap and horizontal plane, which is determined by the equation;
angle of repose (θ)= $\tan^{-1}$(h/r)
Where, θ = Angle of repose.
h = Height of powder heap.
r = Radius of the powder cone.

g) Flow Rate: Weighed accurate quantity of powder sample . Place a cotton plug at the neck of a clean and dry funnel of stem diameter 1-2.5cm. Place powder sample in the funnel. Remove plug from the neck & Record the total time required for all the powder to flow. Calculate flow rate by using formula.[13]
Flow rate= weight of powder/ time required to flow.

h) Water Soluble Extractive: Useful for the evaluation of a crude drug. Give idea about the nature of the chemical constituents present in a crude drug. Weigh about 5gm of the coarsely powdered drug and transfer it to a dry 250ml conical flask. Fill a 100ml graduated flask with water and transfer into conical flask. Cork the flask and set aside for 24 hours, shaking frequently. Filter into a 50ml cylinder. When sufficient filtrate has collected, transfer 25ml of the filtrate to a weigh thin porcelain dish. Evaporate to dryness on a water- bath and complete the drying in an oven at 105 0 C for 6 hours. Cool and weigh immediately. Calculate the percentage w/w of extractive with reference to the air-dried drug.[2]
a) Weight of empty porcelain dish =.....(X).....gm
b) Weight of porcelain dish with residue =.....(Y).....gm
c) Weight of residue = ...(X –Y).....gm
W.S.E.(%) = weight of residue x 100 x 100/ weight of drug taken x volume of filtrate (25ml)

i) Alcohol Soluble Extractive: Useful for the evaluation of a crude drug. Give idea about the nature of the chemical constituents present in a crude drug. Weigh about 5gm of the coarsely powdered drug and transfer it to a dry 250ml conical flask. Fill a 100ml graduated flask with ethanol and transfer into conical flask. Cork the flask and set aside for 24 hours, shaking frequently. Filter into a 50ml cylinder. When sufficient filtrate has collected, transfer 25ml of the filtrate to a weigh thin porcelain dish. Evaporate to dryness on a water- bath and complete the drying in an oven at 105ºC for 6 hours. Cool and weigh immediately. Calculate the percentage w/w of extractive with reference to the air-dried drug.[2]
a) Weight of empty porcelain dish =.....(X).....gm
b) Weight of porcelain dish with residue =.....(Y).....gm
c) Weight of residue = ...(X –Y).....gm

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A.S.E.(%) = weight of residue x 100 x 100/ weight of drug taken x volume of filtrate (25ml)

j) Moisture contents: Weigh 1.5g of sample in a porcelain dish containing 6-8cm diameter and 2-4cm depth in it. Dry the sample in an oven at 105ºC. cool & weigh. Calculate the moisture contents by using formula.
Moisture content(%) = final weight-initial weight x 100

k) Total Ash Value: Used to determine quality and purity of crude drug and to establish the identity of it. Weigh 2gm of powder drug into the crucible. Ignite sample on burner (flame) until all the carbon is burned off. Cool it and weigh the ash. Calculate the percentage of total ash with references to the air-dried sample of crude drug.[2]
a) Weight of the empty dish = x
b) Weight of the drug taken = y
c) Weight of the dish with ash = z
d) Weight of the ash = (z - x)
total ash = 100(Z-X)/Y

l) Antimicrobial test: Antimicrobial test Perform against Escherichia coli & Staphylococcus aureus culture medium. Weigh accurately all the ingredients & prepared nutrient broth and agar medium. Used nutrient broth for sub-culturing of pathogen (freshly prepared bacterial culture).Take Petri dish and test tube wash it properly with tap water & autoclave it (121 ºC 15lb pressure for 15-30minute). Prepared aseptic area in aseptic room. Dilute the testing sample in test tube in a range of 10^{-1} , 10^{-2} , & 10^{-3} respectively. Transfer the agar medium in Petri dish in aseptic condition allowed it cool & solidify. Then transfer the microbial culture which is required (E.coli & S.aureus) with the help of sterile disposable syringe. Shake it properly 2-3 times for proper mixing. Then transfer the sample which is diluted with the help of disc or boher plate technique. Then incubate the plate for 24-48hours in Incubator. Calculate the zone of inhibition by comparing with standard.[10]

Preparation of ethanol extract of plant drug
The collected plant parts were washed with tap water. They were prepared in to small pieces and air-dried thoroughly under shade for 15 days. The shade dried materials were converted into moderately coarse powder. Powdered material of Capsicum annuum, Mentha piperita, Curcuma longa, Syzygium aromaticum was taken in beaker and 20 ml of ethanol was added, soaked for 72 h with occasional shaking and stirring. The soaked material of plant was filtered through several layers of muslin cloth one by one for coarse filtration. The filtered extracts were concentrated under reduced pressure. Obtained semi-solid mass was stored in a desiccator.[12]

Preparation of Ointment: Preparation of 20g of Ointment Base: All the Ingredients was
mixed and heated gently with stirring then cooled. Then extract of Capsicum annuum, Mentha piperita, Curcuma longa, Syzygium aromaticum was added respectively in 20gm of Base. Then Eucalyptus oil is added as a penetration enhancer in 20gm of Base. Mixed it properly by using ointment slab. Then transfer it into suitable container.[5]

Formulation Designing.
Table No. 1: Formulation of Herbal Ointment.

S.No.	**Ingredients**		**Batch**		
	(In gm)	**F1**	**F2**	**F3**	**F4**
	Concentration	**25%**	**30%**	**35%**	**40%**
1.	Plant extract	3	4	5	6
2.	Eucalyptus oil (ml)	2	2	2	2
3.	Base material	q.s	q.s	q.s	q.s
	Total	25gm	25gm	25gm	25gm

Table No. 2: Formulation of Ointment Base.

S.No.	**Ingredients (gm)**	**Quantity taken**
1.	Paraffin wax	2gm
2.	Petroleum jelly	1gm
3.	Cetyl alcohol	1gm
4.	White soft paraffin	16gm
	total	20gm

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EVALUATION OF FORMULATION

Prepared polyvalent herbal Ointment were evaluated for the following evaluation parameters.

a) Colour & Odour: Colour and odour were examined by Visual Inspection.

b) Consistency: Smooth and no greetiness is observed.

c) pH: pH of Herbal ointment was determined by using a 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.

d) Spread ability: The spread ability was determined by placing sample between two glass slides which was compressed to uniform thickness by applying definite weight for definite time period . The time required to separate the two slides was measured as spread ability. Less time taken for separation of two slides shows better spread ability calculated by using formula.[10]

S= M x L/T

S = Spread ability
M = Weight applied to slides
L = Length of glass slides
T = Time taken to separate the slides

e) Extrudability: The ointment was filled in collapsible tube. The extrudability was determined in terms of weight of ointment required to extrude 0.5 cm ribbon of ointment in 10 second.

f) Diffusion Study: The diffusion study was carried by preparing agar nutrient medium by using boher method. The hole is created on agar medium by using open mouth ampule and ointment place in it. The time taken by ointment to get diffused through was noted.(after 60min).[5]

g) L.O.D: LOD was determined by placing the formulation in china dish and dried for the temperature 105 ◦ C in hot air oven.

h) Solubility: Soluble in boiling water, miscible with alcohol & ether.

i) Washability: Ointment was applied to the skin then washability with water was checked.

j) Non-Irritancy: Prepared formulation was applied to the skin of human being and observed the effect.

k) Stability study: Physical stability of the prepared herbal ointment was carried out for 3 months at various temperature conditions like 2 ˚ C, 25 ˚ C, 37 ˚ C.[13]

RESULT AND DISCUSSION

Preformulation Study of Powder Sample.

Table No. 3: Preformulation Study of Powder sample.

S.No.	Parameters	Sieve no.#44	Sieve no. #60	Sieve no.#80	Sieve no.#85
1.	colour	Yellowish brown	Yellowish brown	**Yellowish brown**	Yellowish brown
2.	Bulk density (gm/ml)	0.585	0.547	**0.497**	0.465
3.	Tapped density(gm/ml)	0.697	0.668	**0.594**	0.572
4.	Carr's index (%)	16.06	18.11	**16.32**	18.7
5.	Hausner's ratio	1.191	1.221	**1.195**	1.23
6.	Porosity (%)	23	18.86	**17.07**	20.18

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7.	Angle of repose (θ)	32°58”	27° 64”	**30° 78”**	31°33”
8.	Moisture contents (%)	12	10	**8**	14
9.	Flow rate (gm/sec)	0.77	0.65	**0.57**	0.42
10.	Ash value	0.12	0.11	**0.12**	0.11
11.	Water soluble extractive	10	10	**10**	10
12.	Alcohol soluble extractive	11	11	**11**	11
13.	Antimicrobial test	+ve	+ve	**+ve**	+ve

From above preformulation data powder from Sieve no:#80 shows acceptable angle of repose, Bulk density, Tapped density, Carr’s index and Hausner’s ratio, Flow rate, Moisture contents. The batch shows good data as compared with other batches. Therefore it was concluded that the Powder from Sieve no:#80 consider as an optimized batch.

Evaluation of Formulation

Table No. 4: Evaluation of Formulation.

S.No.	**Parameters**	**F1**	**F2**	**F3**	**F4**
1.	Colour	brown	brown	**brown**	brown
2.	Odour	characteristic	characteristic	**characteristic**	characteristic
3.	Consistency	smooth	smooth	**smooth**	smooth
4.	pH	6.6	6.2	**5.7**	5.4
5.	Spread ability (seconds)	8	7	**8**	7
6.	Extrudability(gm)	0.5	0.5	**0.8**	0.6
7.	Diffusion study (after 60 min)	0.7	0.9	**0.5**	0.8
8.	Loss on drying	25%	30%	**25%**	20%
9.	Solubility				
	Boiling water	Freely soluble	Freely soluble	**Freely soluble**	Freely soluble
	Alcohol	miscible	miscible	**miscible**	miscible
	ether	miscible	miscible	**Miscible**	miscible
10.	washability	good	good	**good**	good
11.	Non irritancy	Non irritant	Non irritant	**Non irritant**	Non irritant
12.	Stability study (2°C,25°C,37°C)	stable	stable	**stable**	stable

From the above evaluation parameters it can be concluded that overall batches the F3 batch show all parameter in acceptable limit. Therefore it is considered as a good formulation.

Figures:

Fig.1

Fig.2

Fig.3

Fig.4

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Fig.5

Fig.6

CONCLUSION

The Polyvalent herbal powder was used to formulate anti-inflammatory and analgesic herbal ointment & evaluated for physical parameters. Preformulation study and Physical Parameter exposed that all the values were within acceptable limit. The herbal ointment useful for an anti-inflammatory and analgesic activity. From the above evaluation parameters it can be concluded that overall batches the F3 batch show all parameter in acceptable limit. Therefore it is considered as a good formulation.[14]

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CONFLICT OF INTEREST

Authors have declared that there is no conflict of interests. [14]

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