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Research Article

STANDARDIZATION STUDY OF POLYHERBAL FORMULATION: ARTHRUM OINTMENT

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ABSTRACT

To access the quality of drug the standardization is very critical. As per WHO guidelines, 80% of the peoples have relied on traditional medicine in a rising nation. Most of the conventional system is valuable but a lack of standardization. The parameters studied are physicochemical parameters, heavy metal analysis, aflatoxin content, pesticide residue and microbial analysis. These values will help to obtain the batch-to-batch variation in Arthrum ointment. This is used to treat different types of pain. We calculated and discussed physicochemical parameters, heavy metal analysis, aflatoxin content, pesticide residue and microbial analysis. The scientific method for its quality and safety evaluation is not yet to be documented. Hence in the current work, an attempt has been made to evaluate the quality parameters to be used for its preparation and processing.

Keywords: Standardization, Arthrum ointment, polyherbal formulation

INTRODUCTION

Ayurveda is the oldest traditional system of medicine. Different herbs are used to prepare Ayurvedic Single drug or polyherbal formulation. In recent years there has been a tremendous demand for herbal drugs especially in developed countries and this demand is increasing every day in the world market. Traditional medicines stood the test of time for their cultural acceptability and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body.

Today traditional medicines, however, are manufactured on a large scale in mechanical units, where manufacturers come across many problems such as non-availability of good quality raw materials, and proper methodology for standardization, etc., [1] Exploring traditional medicines in the context of modern science is the need of the hour for their optimum and proper utilization. Reproducible efficacy and safety of traditional products is based on reproducible quality. If traditional products are to be regarded as rational drugs, they have to be standardized and their pharmaceutical quality must be approved.[2] Also, their composition needs to be well documented in order to obtain reproducible results in pharmacological, toxicological and clinical studies.[3]

World Health Organization (WHO) encourages, recommends and promotes traditional remedies in natural health care programmers since these drugs are readily available at little cost, safe and people have confidence in them. The WHO assembly in some resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying appropriate standards.

Arthrum ointment is polyherbal formulations consist of *Gaultheria fragrantissima, Cedrus deodara,* Sat peppermint, *Camphora officinarum, Eucalyptus globules* and *Capsicum annum* ingredients in ointment form it supposed to have multi-faceted action in all types of pain.

The present study revealed a scientific evaluation and standardization of polyherbal Ayurvedic ointment for physicochemical analysis, Heavy metal content, Aflatoxin content, Pesticide residue and Microbial analysis. This study is useful for maintaining consistency and quality of formulation. These values should help to develop new pharmacopoeial standard to overcome batch to batch variation in the traditional preparation.

MATERIAL AND METHODS

Drugs and Chemicals

Arthrum ointment is a proprietary Ayurvedic poly-herbal formulation of Vital Care Pvt. Ltd., Vadodara. All other chemicals and solvents were of analytical grade obtained from Merck, SISCO and SD-fine chemicals, India.

Physicochemical Parameters [4, 5]

Description : The general apparence, its visual identity is essential for consumer acceptance for control of batch to batch uniformity and for monitoring trouble free manufacturing. The control of the General apparence of ointment involves the measurement of colour, odour etc.

Determination of pH: The pH of various formulations was determined by using Digital pH meter. One gram of ointment was dissolved in 100 ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were taken.

Determination of Loss on drying: Loss on drying was determined by placing ointment in petridish on water bath and dried for 105^{O}C.

*Determination of Spreadibility***:** Spreadibility is a term expressed to denote the extent of area to which the ointments readily spreads on application to skin or affected part. The spread ability was expressed in terms of times in seconds taken by two slides to slip off from ointment and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, result the better spread ability. Spread ability was calculated by using the formula.

$$S = (M.L/T)$$

Where, S = Spreadibility, M = Weight tied to upper slide, L = Length of glass slides and T = Time taken to separate the slides

Determination of Acid Value: The acid value defined as the amount of potassium hydroxide (KOH, in milligrams) necessary to neutralize the free fatty acids contained in 1 g of oil, is one of the important parameters related to the oil quality. A high AV indicates the deterioration of oil in ointment, which affects its nutritive value.

Heavy Metal analysis [6]

Preparation of samples by acid digestion method: Accurately weighed 2 g of each sample of Arthrum ointment was taken in kjeldahl flask. Acid mixture of HNO3:HClO4 (4:1) was added in the flask and heated continuously till the solution is colourless. The sample was then transferred in a 25 ml volumetric flask and the volume was made-up with distilled water. Reagent blank was synchronously prepared according to the above procedure. The standards of Lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) were prepared as per the protocol in the manual and the calibration curve was developed for each of them.

Detection: Then samples were analyzed for the presence of Pb, Cd, As and Hg using Atomic absorbance spectrophotometer (AAS) 6300 (by SHIMADZU).

Aflatoxins Content [7, 8]

To determine Aflatoxin the sample preparation was carried out by immunoaffinity column liquid chromatography as per the reported literature. Aflatoxins were determined by HPLC analysis by Waters Alliance 2695 HPLC instrument using a Luna C18 column (Phenomenex) of dimensions 4.6×150 mm×5 µ coupled with a Waters 2475 fluorescence detector containing Cobra cell. In this method, 40 µl of the samples were injected into the HPLC column heated at 40°. The mobile phase was taken as water: methanol solution (60:40, v/v) with 119 mg of potassium bromide and 350 µl of 4 M nitric acid were added to the 1 l of mobile phase for post column electrochemical derivatization of fluorescence detector. The flow rate was kept at 1 ml/min with a total runtime of 20 min where the retention times were found to be 7.5, 9.38, 11.44 and 14.5 min for aflatoxins G2, G1, B2 and B1, respectively. The excitation wavelength and the emission wavelength for fluorescent detection were set at 362 and 455 nm, respectively. The calibration standards were procured from Sigma Aldrich.

Pesticide residues [9]

To determine the pesticide residues, 2 g of each sample was extracted in Soxhlet apparatus with 150 ml hexane. Traces of water and oil were removed from hexane extract. After oil removal, this extract was concentrated on rotary evaporator under reduced pressure and this concentrated extract was transferred to clean-up column. The elute was collected carefully and made up to 5ml with hexane. Aliquots of above concentrate were injected into pre-calibrated GC machine equipped with 63Ni electron capture detector. Operation temperature was programmed at 195°C, 200°C, 220°C for column, injector, and detector, respectively. Purified nitrogen gas was used as carrier gas at flow rate of 60 ml/min. Limit of detection was 0.1 to 0.5 ppb for organochlorine pesticides analyzed. Periodically procedural blanks were used to check cross contamination. Recovery studies with purified samples indicated that overall recovery value exceeded 80%. Identification and quantification were accomplished using known amount of external standard procured from Sigma-Aldrich.

Microbial Analysis [10, 11]

a) *Total Microbial count*

Preparation of sample: About 10mg of sample were weighed and dissolved in DMF (dimethyl formamide) and used for activity studies.
Examination of sample: Total viable aerobic count in the sample was examined by using the plate count method by Digital colony counter.
For bacteria: Petri dishes of 10 cm diameter were used, mixture of 1 ml of the pre-treated preparation and 15 ml of liquefied casein soyabean digest agar was added in each of petridishes at not more than 45ºC. If necessary, dilute the pre-treated preparation so that a colony count of not more than 300 may be expected. Two Petri dishes for each sample were prepared using the same dilution and incubated at 30-40ºC for 5 days. The number of colonies form was calculated using the digital colony counter. Results were calculated using plate with the greatest no. of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.
For fungi: Proceed as described in the test for bacteria but sabouraud dextrose agar with antibiotic was used in place of soyabean digest agar and incubate the plates at 20-25º C for 5 days unless a more reliable count was obtained in shorter time. Results were calculated using the plates with not more than 100 colonies by using colony counter.

b) *Test for Escherichia coli*

Take 10 gm of sample and the volume made up to 100 ml with lactose broth. This mixture was incubated at 35-37ºC for 4 hrs. 1 ml sample from this to 100 ml MacConkey broth and incubated at 43-47ºC for 24 hrs. Subculture was prepared and inoculated on MacConkey agar media and incubated at 43-47 ºC for 24 hrs. Growth of red, generally non-mucoid colonies of Gram negative rods indicated the possible presence of *E. coli.*

c) *Test for Salmonella typhimurium*

Take 10 gm of sample and the volume made up to 100 ml with lactose broth. This mixture was incubated at 35-37ºC for 4 hrs. A further 10 ml of this sample was taken in 100 ml of tetrathionate bile brilliant green broth and incubated at 42-43ºC for 18-24 hrs. 1 ml of sample was taken from it and plated on a xylose lysine deoxycholate agar media and incubated at 35-37ºC for 24 hrs. Well developed, red with or without black centre colonies indicated the presence of *S. thyphi.*

d) *Test for Pseudomonas aeruginosa*

0.1ml of the solution was pipetted out from SCDB and streaked

onto Cetrimide agar plates to check for the presence of *Pseudomonas aeruginosa*, The plates were then inverted and incubated at 37° C for 18-24 hours and were then observed for fluorescence colonies under UV.

e) *Test for Staphylococcus aureus*

0.1 ml of the solution was pipetted out from SCDB and streaked onto Vogel-Johnson Agar Medium to check out the presence of *Staphylococcus aureus***.** The plates were then inverted and incubated at 37° C for 18-24 hours and were then observed for typical black colonies surrounded by yellow zones if any.

OBSERVATION AND RESULT

Table 1: Physicochemical analysis of Arthrum ointment

Parameter	Test Result
Description	
Colour	Yellow
Odour	Characteristic
Physicochemical parameters	
Spredability (Seconds)	13
Acid Value (mg KOH/g)	4.52
pH	7
Loss on Drying (% w/w)	2.55

Arthrum ointment has yellow colour and characteristic odour. Acid value, Spredability, Loss on drying of Arthrum ointment were found in uniform. The pH of Arthrum ointment was Neutral.

Table 2: Heavy metal analysis of Arthrum ointment

Sr. No.	Heavy metal	Test Method	Limit	Test Result
1	Lead	API Part-I, Vol.-VIII, 2011 (ICP-MS)	10 ppm	0.48 ppm
2	Cadmium	API Part-I, Vol.-VIII, 2011	0.3 ppm	BLQ (LOQ 0.1)
3	Arsenic	API Part-I, Vol.-VIII, 2011	3 ppm	BLQ (LOQ 0.1)
4	Mercury	API Part-I, Vol.-VIII, 2011	1 ppm	BLQ (LOQ 0.1)

Results indicated that concentration of cadmium, arsenic and mercury in Arthrum ointment was not in detectable amount. Lead shows in minor quantity however it was lesser than prescribed limit.

Table 3: Aflatoxin content and pesticide analysis of Arthrum ointment

Name of Test	Method of Test	Limit	Test result
Aflatoxin Content			
Aflatoxin B1	API Part I, Vol.-VI, 2008	0.5 PPB	ND
Aflatoxin B2		0.1 PPB	ND
Aflatoxin G1		0.5 PPB	ND
Aflatoxin G2		0.1 PPB	ND
Pesticide Residue			
Alpha-Lindane	AOAC 2007.01/QuEChERS Method GC/LC-MS/MS	0.600 mg/kg	BLQ (LOQ 0.01)
Alachlor	As Mentioned Above	0.050 mg/kg	BLQ (LOQ 0.01)
Aldrin	As Mentioned Above	0.050 mg/kg	BLQ (LOQ 0.01)
Dieldrin	As Mentioned Above		BLQ (LOQ 0.01)
Atrazine	As Mentioned Above	0.010 mg/kg	BLQ (LOQ 0.01)
Beta-Lindane	As Mentioned Above	0.600 mg/kg	BLQ (LOQ 0.01)
Chlorpyrifos	As Mentioned Above	0.200 mg/kg	BLQ (LOQ 0.01)
Cis-Chlordane	As Mentioned Above	0.050 mg/kg	BLQ (LOQ 0.01)
Cypermethrin-2	As Mentioned Above	0.200 mg/kg	BLQ (LOQ 0.01)
Cypermethrin-3	As Mentioned Above	0.200 mg/kg	BLQ (LOQ 0.01)
Cypermethrin-4	As Mentioned Above	0.200 mg/kg	BLQ (LOQ 0.01)
Cypermethrin-1	As Mentioned Above	0.200 mg/kg	BLQ (LOQ 0.01)
Delta-Lindane	As Mentioned Above	0.600 mg/kg	BLQ (LOQ 0.01)
Deltamethrin-1	As Mentioned Above	0.500 mg/kg	BLQ (LOQ 0.01)
Deltamethrin-2	As Mentioned Above	0.500 mg/kg	BLQ (LOQ 0.01)
Diazinone	As Mentioned Above	0.050 mg/kg	BLQ (LOQ 0.01)
Dichlorvos	As Mentioned Above	1.000 mg/kg	BLQ (LOQ 0.01)
Dieldrin	As Mentioned Above	0.500 mg/kg	BLQ (LOQ 0.01)
Endosulfan	As Mentioned Above	3.000 mg/kg	BLQ (LOQ 0.01)
Endosulfan II	As Mentioned Above		BLQ (LOQ 0.01)
Endosulfan sulphate	As Mentioned Above		BLQ (LOQ 0.01)
Ethion	As Mentioned Above	2.000 mg/kg	BLQ (LOQ 0.01)
Fenvalerate-1	As Mentioned Above	0.100 mg/kg	BLQ (LOQ 0.01)
Fenvalerate-2	As Mentioned Above	0.100 mg/kg	BLQ (LOQ 0.01)
Gamma-Lindane	As Mentioned Above	0.600 mg/kg	BLQ (LOQ 0.01)

Heptachlor	As Mentioned Above	0.050 mg/kg	BLQ (LOQ 0.01)
Heptachlor epoxide	As Mentioned Above		BLQ (LOQ 0.01)
Malathion	As Mentioned Above	1.000 mg/kg	BLQ (LOQ 0.01)
o,p'-DDD	As Mentioned Above	1.000 mg/kg	BLQ (LOQ 0.01)
p,p'-DDD	As Mentioned Above		BLQ (LOQ 0.01)
o,p'-DDT	As Mentioned Above	1.000 mg/kg	BLQ (LOQ 0.01)
P,p'-DDT	As Mentioned Above		BLQ (LOQ 0.01)
P,p'-DDE	As Mentioned Above	0.500 mg/kg	BLQ (LOQ 0.01)
O,p'-DDE	As Mentioned Above		BLQ (LOQ 0.01)
Paraoxon methyl	As Mentioned Above	0.200 mg/kg	BLQ (LOQ 0.01)
Parathion	As Mentioned Above	0.500 mg/kg	BLQ (LOQ 0.01)
Permethrin-1	As Mentioned Above	3.000 mg/kg	BLQ (LOQ 0.01)
Permethrin-2	As Mentioned Above		BLQ (LOQ 0.01)
Phosalone	As Mentioned Above	0.100 mg/kg	BLQ (LOQ 0.01)

Result of Arthrum ointment in Aflatoxin and pesticide residue indicated that obtained values are either not detected or not in detectable amount.

Table 4: Microbial Analysis of Arthrum ointment

Sr. No.	Microbial analysis	Limit	Test Result
1	Total Microbial count	1000 CFU/ml	7 CFU/ml
2	Total Yeast & Mould count	100 CFU/ml	<10 CFU/ml
3	Presence of *Staphylococcus aureus*	Absent/ml	Absent
4	Presence of *Escherichia coli*	Absent/ml	Absent
5	Presence of *Salmonella Spp.*	Absent/ml	Absent
6	Presence of *Pseudomonas aeruginosa*	Absent/ml	Absent
7	Presence of *Staphylococcus aureus*	Absent/ml	Absent

Results showed that total Microbial count was 7 CFU/ml in Arthrum ointment. It was less than prescribed limit. *E. coli, Salmonella spp., Pseudomonas aeruginosa, Staphylococcus aureus* were not found in detectable amount in the formulation.

DISCUSSION

Plant materials are used throughout the developed and developing world as home remedies, in over-the-counter drug products, and as raw material for the pharmaceutical industry, and they represent a substantial proportion of the global drug market. The quality of herbal drugs is the sum of all factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product. Now a day the field of herbal drugs and formulation is very fast and there is still lot to explore on the subject of standardization of these. So, while developing an herbal formulation it is must to have all the related knowledge of that particular drug including all its standardization aspects in respect to various parameters via various techniques.

Arthrum ointment is Ayurvedic polyherbal formulation useful for analgesic effect. It has been standardized by the intervention of scientific quality Control measures in the traditional preparation describe in established texts. The physicochemical properties such as colour, odour, pH, loss on drying, Spreadibility and Acid value influences quality of herbal product, its tolerability by its consumers and also the activity of the product. From the result outcomes, there were no lumps found in the Arthrum ointments and there were of acceptable homogeneity. This might be because of good and powerful blending of the plant materials with ointment bases. The ointments had characteristic yellow colours with their characteristic odour. This makes the product promptly worthy and increase patient conformity. With the test on surface, it was discovered that a portion of the treatments with ointment were smooth when applied to the skin whiles others were gritty. A gritty ointment when applied to an influenced skin can exasperate the condition by causing scraped areas of the skin. An ointment for topical application is expected to be smooth without any grits. scent. This makes the item promptly worthy and increment understanding consistence. With the test on surface, it was discovered that a portion of the treatments were smooth when applied to the skin whiles others were lumpy. A dirty treatment when applied to an influenced skin can exasperate the condition by causing scraped areas of the skin. A salve for effective application is relied upon to be smooth with no corn meal.

The presence of grits may be a sign of physical instability. The loss on drying estimates the amount of moisture present in the polyherbal topical ointment. If the amount of moisture present in the products exceeds the acceptable limit, the excess water can facilitate the growth of both harmless and pathogenic microorganisms if present. It can likewise prompt both physical and chemical changes causing deterioration of the ointment. Arthrum ointment indicated 2.55% moisture contains. Higher the moisture contain can cause breakdown of both active substances into less potent or more poisonous substances and excipients utilized in the ointment formulation.

Acid value is the important parameter for ointment evaluation. Acid value of the formulation is 4.52. If acid value is more in the formulation, then chances of photo-oxidation and rancidity are more.

pH is a measure of how acidic or basic an aqueous solution is. It is calculated as the negative logarithm of hydrogen ion concentration. The pH of the ointment must fall in the range of the natural skin which is 4.0-7.0 with an average of 4.7.[12] The natural pH of the skin protects the skin against both pathogenic and contaminations and also promotes the growth of the microflora of the skin. The pH of the skin demolishes the protective functions of the skin and the microflora of the skin. Certain synthetic compounds and factors can alter the pH of the common skin and makes it vulnerable to endless infection of the skin. Arthrum ointment having 7 pH. Which demonstrate better substance similarity of ointment with skin.

Contamination by toxic metals can either be accidental or intentional. Contamination by heavy metals such as mercury, lead, copper, cadmium, and arsenic in herbal remedies can be

attributed to many causes, including environmental pollution, and can pose clinically relevant dangers for the health of the user and should therefore be limited. [13] The results of the present study show that examined formulation are within permissible limits.

Aflatoxins are very powerful liver carcinogen classified as class1 human carcinogens. The four major aflatoxins are B1, B2, G1 and G2. Approximately 40% of the productivity lost to diseases in developing countries is due to diseases exacerbated by aflatoxins. Many of people in these countries are not aware of the effect of consuming moulds products. Due to the poor hygienic condition and other socio-economic factors. These countries also have poorly developed infrastructures such as processing facilities, storage, transportation and skilled human resources.[14] The results indicate that although the natural occurrence of Aflatoxin in Arthrum ointment analyzed in this study was very less (Not detectable) fixed by the World Health Organization.

Medicinal plants are consumed worldwide for the treatment of several diseases and are important raw materials for the production of photochemical by pharmaceutical industries. The medicinal plant is susceptible to wide ranges of pests and diseases, which causes economic crop loss as well as quality of herbal products and is often treated with chemical pesticides to manage these problems. The presence of trace level of pesticide residues in the herbal plants, which impose serious health risks to human health.[15] the result of present study in pesticide residue for Arthrum ointment, is less than detectable amount.

Medicinal plants may be associated with a broad variety of microbial contaminants, represented by bacteria, fungi, and viruses. Inevitably, this microbiological background depends on several environmental factors and exerts an important impact on the overall quality of herbal products and preparations. Result of microbial analysis of Arthrum ointment indicates that It was less than prescribed limit. *E. coli*, *Salmonella spp.*, *Pseudomonas aerugenosa*, *Staphylococcus aureus* were not found in detectable amount in the formulation. Herbal drugs normally carry a number of bacteria and molds, often originating in the soil. Poor methods of harvesting, cleaning, drying, handling, and storage may also cause additional contamination, as may be the case with Escherichia coli or Salmonella spp. while a large range of bacteria and fungi are from naturally occurring micro flora, aerobic spore-forming bacteria that frequently predominate. Laboratory procedures investigating microbial contaminations are laid down in the well-known pharmacopeia, as well as, in the WHO guidelines. [16]

CONCLUSION

Present study revealed various standardization parameters such as physicochemical standards, Heavy metals analysis, Aflatoxin, pesticide residue and microbiological analysis were carried out. It very well may be inferred that it passes all the standardization parameters. The efficacy of the drug can be judged only by pharmacological activity. The study shows that contents of formulation present within the permissible limits as per WHO, all these investigations of Arthrum ointment are not specified anywhere. Therefore it could be helpful in the authentication of the Arthrum ointment.

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