International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2023; 11(3): 38-45
Received: 28-02-2023
Accepted: 31-03-2023

Shefali Joshi
Assistant Professor, Sharda School of Pharmacy, Pethapur, Gandhinagar, Gujarat, India

Khushboo Vaghela
Associate Professor, Sharda School of Pharmacy, Pethapur, Gandhinagar, Gujarat, India

Divyakant Patel
Head of the Institute, Sharda School of Pharmacy, Pethapur, Gandhinagar, Gujarat, India

Hardik Soni
Assistant General Manager, R&D, Vasu Healthcare Pvt. Ltd. Vadodara, Gujarat, India

Maitreyi Zaveri
Associate Professor, K.B. Institute of Pharmaceutical Education and Research, Nr. Gh-6 Circle, Sector-23, Gandhinagar, Gujarat, India

Corresponding Author:
Shefali Joshi
Assistant Professor, Sharda School of Pharmacy, Pethapur, Gandhinagar, Gujarat, India

A detailed quality control assessment of polyherbal pain reliever cream

Shefali Joshi, Khushboo Vaghela, Divyakant Patel, Hardik Soni and Maitreyi Zaveri

DOI: https://doi.org/10.22271/flora.2023.v11.i3a.868

Abstract
Musculoskeletal injuries are most common risk factors for sport persons. Pain and swelling are the most common associated symptoms of such injuries. A use of non-steroidal anti-inflammatory drugs (NSAIDs) has been routine in the management of musculoskeletal inflammation and pain. Although effective at reducing pain and inflammation, NSAIDs may not be appropriate to use frequently or longer time due to their known side effects. Herbal and Ayurveda products are widely perceived as safe due to their natural origin and long historical clinical use. Hence in the present study, different quality control parameters for raw material and finished product (polyherbal pain reliever cream) were evaluated like Acid value, Saponification Value, Free fatty acid determination, Viscosity, Specific gravity, Refractive index, Moisture content, Analysis of heavy metals performed. Finger printing profile for the Oils which includes raw ingredients & finish products both. All data from results suggest that cream and its composition were consistent with various quality and purity parameters such as organoleptic characters, physicochemical parameter, fingerprinting analysis, Quantification of methanol in formulation, Heavy metal analysis and Microbial analysis.

Keywords: Polyherbal pain reliever cream, quality control, musculoskeletal injuries

Introduction
Herbal medicines are prepared from a variety of plant material leaves, stems, root, bark, so they usually contain many biologically active ingredients and are used primarily for treating mild or chronic ailments [1-2]. Rheumatoid arthritis means inflammation of joint. Rheumatoid arthritis is the most common systemic inflammatory disease, and is characterized by symmetrical joint involvement [3-4].
Polyherbal pain reliever cream is developed by Vasu Research Centre and manufactured & marketed by Vasu Healthcare Pvt. Ltd., Vadodara

Table 1: Ingredients of Polyherbal Pain Reliver Cream

Ingredients	Part Used	Quantity
Mahanarayan oil	Formulation	8.0%
Nirgundi oil	Formulation	4.0%
Ricinus communis (Erand) oil	Seed	1.6%
Eucalyptus globulus (Nilgiri) oil	Leaves	1.6%
Vateria indica (Sarjras)	Oleo resin	8.0%
Aloe vera (Kumari)	Leaves	4.0%
Mentha sylvestris (Pudina)	Satva	4.0%
Cinnamomum camphora (Karpoor)	Satva	4.0%

As in the ethnomedicinal-based review, all the selected plants were reviewed from the ancient literature for all mentioned activities. The following Table 2 shows the selected plant and its literature review

Table 2: Selection of plants on basis of literature review

Ingredients	Part Used	Activity mentioned	Reference
Mahanarayan oil	Formulation	soothes sore muscles & joints	Ram Narayan Sharma [5-6]
Nirgundi oil	Formulation	vata roga	Gopinadh Gupt. Bhart-Bhaishjy-Ratnakar[7-8]
Ricinus communis (Erand) oil	Seed	Anti-Arthritic Activity, Anti-inflammatory	Wilson E *et al* [9] Banejees *et al* [10] Ilavarasan R *et al* [11]
Eucalyptus globulus (Nilgiri) oil	Leaves oil	Analgesic and anti-inflammatory activity	Silva *et al* [12] Jio S [13]
Vateria indica (Sarjras)	Oleo resin	chronic rheumatism and neuralgia	Ashok Sheth [14]
Aloe vera (Kumari)	Leaves	Anti-inflammatory	Vazquez *et al* [15]
Mentha sylvestris (Pudina)	Satva	Anti-inflammatory Analgesic activity	Atla *et al* [16] Galeotti *et al* [17]
Cinnamomum camphora (Karpoor)	Satva	Ant arthritis, Anti-inflammatory	Hye Ja Lee *et al* [18]

Materials and Methods

Evaluation of quality control parameters for raw material and finished product

Physicochemical parameters

Physicochemical parameters like type, clarity, color, odor and taste of all the oils were determined.

Determination of acid value [19]

Weigh accurately about 10 g of the substance into a 250 ml flask and add 50 ml of mixtures of equal volumes of alcohol & solvent ether, which has been neutralized after the addition of 1 ml of solution of Phenolphthalein. Heat gently on a water bath, if necessary until the substance has completely melted. Titrate with 0.1N Potassium hydroxide, shaking constantly until a pink color which persists for 15 sec. is obtained. Note the number of ml required.

Determination of Saponification Value [19]

Weigh accurately about 2 g of the substance, into a 250ml borosilicate iodometric flask. Add 25.0 ml of 0.5 M methanolic potassium hydroxide and a few glass beads and boil under reflux condenser on a water-bath for 30 minutes, and cool at room temperature. Add 1 ml of phenolphthalein solution and titrate immediately with 0.5 M hydrochloric acid. Carry out a blank titration without the substance.

Quantitative estimation of free fatty acids [19]

Take 25 ml methanol and 25 ml diethyl ether in 250ml Iodine flask. Add 2-3 drops Phenolphthalein in Iodine flask. Mix it properly and neutralize with 0.1 N potassium hydroxide Add 1-2 gm of substance in it. Put the Iodine flask on water bath and reflux it for 15 to 20 min. Titrate it with 0.1 N potassium hydroxide till pink color appear.

Rancidity test (Kreis test) [19]

Mix 1 ml of melted fat and 1 ml of conc. HCL in a test tube. Add 1 ml of a 1% solution of phlorolucinol in ether and mix thorough with the free- acid mixture. A pink colour indicates that the fat is definitely oxidized.

Determination of specific gravity [20]

A calibrated 50 ml specific gravity bottle was taken. Rinsed it with water & dried then weighed specific gravity bottle on a balance and noted down its weight then filled the oil up to the mark in the bottle & took the weight of the bottle along with the oil. From the weight of the bottle + oil, the weight of empty specific gravity bottle was subtracted which gave the actual weight of the oil, thus weight of the actual filled oil divided by the volume of the specific gravity bottle gave me the weight per milliliter of the given oil.

Determination of Viscosity [21]

The Ostwal's Viscometer was cleaned with a soap solution & then rinsed it with acetone & dried it in an oven. Then at 25 °C water was added in the viscometer up to the "G" mark, and then raised the level of water up to "E" mark with the help of a bubbler. The time in second was noted down for the water to pass from "E" to "F" level. N = K x P x T (where K = constant, (Where N = 8.94 poise viscosity of water at 25 °C) thus calculated value of constant, then water was removed & oil was filled up to the mark "G" in the viscometer & found out the time taken for the oil to travel from "E" to "F" level & found out its viscosity from the following formula.

N = K x P x T Where, K = constant, P = wt/ml of oil, T = time in second

Determination of Refractive Index [22]

The refractive index of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of refraction of a beam of light passing from air to the substance. It varies with the wavelength of the light used in its measurement.

The cleanliness of the instrument should be checked frequently by determining the refractive index of distilled water, which at 25^0 is 1.3325.

The refractive index of oil sample was determined using Abbe's Refract meter 300918. The sample chamber containing the lens was opened and cleaned with acetone then plugged to source of light. The equipment was calibrated with a drop of water, after which a drop of the sample was added into the sample chamber and closed.

1. The adjustment knob was turned until the light and dark field.
2. Crossed the cross bar then readings were taken.

Identification test for Menthol [23]

Dissolve 10 mg in 1 ml of concentrated sulphuric acid and add 1 ml of a 1 percent W/V solution of vanillin in sulphuric acid; an orange-yellow colour is produced; on adding 1 ml of water the colour changes to violet (distinction from thymol). Dissolve a few crystals in 1 ml of glacial acetic acid, add three drops of conc. sulphuric acid and one drop of nitric acid; no green colour is developed (distinction from thymol). When triturate with about an equal amount of camphor, chloral hydrate or phenol, the mixture liquefies.

Acidity of Menthol [23]

To 1 g in a 100 ml glass-stoppered conical flask add 20 ml of water, boil until dissolution is complete, cool, stopper the flask and shake vigorously for 1 min.

Add a few crystals of the substance under examination to initiate crystallization, shake vigorously for 1 min and filter. To 5 ml of the filtrate add 0.05 ml of methyl red solution and 0.05 ml of 0.01M sodium hydroxide; the solution is yellow.

Identification test for Camphor [24]
A drop of solution of vanillin (1:100) and sulphuric acid when added to powdered natural camphor produces immediately a yellow colour, changing to red, violet, and finally blue.

Residue on Evaporation [25]
Evaporate 2.0g on a water-bath and heat at 105^0 for 1 hr. The reside weights not more than 1.0 mg (0.05%)

Test for presence of Colophony - (Distinction from Sala and Shallaki resin) [26]
Dissolve 0.1 g in 10 ml of acetic anhydride by gentle heat, cool, and add I drop of sulphuric acid; a bright purplish-red colour, rapidly changing to violet, is produced. Shake 0.1 g of powder with 10 ml of light petroleum (b.p. 50°-60°c), and filter; shake 5ml of the filtrate with 10 ml of dilute solution of copper acetate; the petroleum layer assumes a bright bluish-green colour.

Determination of pH [27]
The pH value of a cream was determined potentiometrically by means of a glass electrode, a reference electrode and a digital pH meter. The pH meter was operated according the manufacturer's instructions. First the apparatus was calibrated using buffer of 4, 9 and 7 pH. The electrodes were immersed in the cream and measured the pH.

Determination of moisture content [28]
Karl fisher titration method was carried out by using Karl Fischer apparatus. In which sufficient quantity of methanol was added to dip the electrodes in the beaker and neutralize the methanol with Karl Fischer Reagent then10 ml water was added and the addition of Karl Fischer Reagent was started till end point to calculate the factor.

Heavy Metal Analysis [29]
Preparation of test solution
Sample preparation (Applicable for Finished product and raw material)

Vessel no.	Power		Ramp (Min)	° C (control)	Hold time (min)
	MAX	%			
8	800 W	100	20	180	10
16	1600 W	80	20	180	10

Accurately weight 0.5 g or 0.5 mL sample in PFA (Perfluoroalkoxy) Teflon vessels. Add 8mL 69% Nitric acid (HNO_3) along the sides of the vessel so as to remove the adhering matter. If the effervescence is formed, then let vessel open for 30 minutes in fumigation chamber. Close the vessels tightly and keep on the turner. Set the method parameters are as follows:
After digestion procedure is complete. Take out the vessels and keep it in fumigation chamber for 10 minute. Open the vessels slowly to release the pressure from the vessel. Leave the vessel undisturbed till the fumes are released competently. Transfer the solution in 50mL volumetric flask using funnel. Make up the volume with distilled water. Shake well and filter using what man filter paper No.1. Use this solution as a test solution. Prepare synchronously the blank solution.

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

Microbial Analysis [30]
Pretreatment of the Sample
Dissolve 5 g or 5 ml of the sample in 50 ml of buffered NaCl-peptone solution (pH- 7.0) or any other suitable medium having no antimicrobial activity under the condition of test and in Lactose Broth having a pH of 7.2 ± 0.2. If the product was not soluble in buffer then certain surface active agent like 0.1% w/v polysorbate 80 may be added to assist the suspension of poorly wet table substances. They are then incubated at 37 °C for 2 to 4 hours.

Primary Treatment
0.1 ml of the sample was pipette out from buffered NaCl-peptone solution and spread onto Soyabean Casein Digest Agar plates (SCDA) to determine the Total Bacterial Count and 0.1 ml onto Sabouraud‟s Dextrose Agar (SDA) having a pH range of 5.6 ± 0.2 for Total Fungal Count. SCDA plates were then inverted and incubated at 37°C for 2 to 4 hours after which the number of colonies were counted and multiplied by the dilution factor and were represented in the form of cfu/g/ml. (Dilutions are performed if necessary) whereas the SDA plates were inverted and incubated at 25°C for 3 to 4 days after which the number of colonies were counted and multiplied by the dilution factor and were represented in the form of cfu/g/ml. (Dilutions are performed if necessary) 5 ml from Lactose Broth having a pH of 7.2±0.2 was pipette out and transferred to both Nutrient Broth (NB) having a pH range of about 7.3±0.1 and Soyabean Casein digest broth (SCDB) having a pH range of about 7.3±0.2. Both the flasks are then incubated at 37°C for 18 to 24 hours

Test for *Esherichia coli*
1ml of the solution was pipetted out from (SCDB) and transferred into 5 ml of Mc Conkey broth (MCB) medium containing Duram‟s tube. The tubes were then incubated at 37 °C for 18 to 24 hours. They were then checked for the presence of acid and gas production. This shows the preliminary presence of *E. coli*.
If MCB was positive they are confirmed by Indole test which is by the addition of 0.5 ml of Kovac‟ s reagent and if there is deamination of Typtophan then a red colored ring is formed at the interface region. 0.1 ml from the positive MCB tube is streaked onto Eosine Methylene Blue plates and the plates are then inverted and incubated at 37 °C for 18 – 24 hours and are then observed for a green metallic sheen that confirms the presence of *E. coli*

Test for *Salmonella*
0.1ml of the solution was pipetted out from NB and transferred into 5 ml Tetrathionate broth Medium. The tubes were then incubated at 37 °C for 18 to 24 hours. They are then checked for the change in colour.
If TTB was positive they were confirmed by Triple Sugar Iron test wherein they were streaked and stab the inoculums to give it anaerobic conditions. The slants were then incubated at 37°C for 18 to 24 hours. Black coloured colonies that indicates the presence of *Salmonella* species is a positive test.

Test for *Staphylococcus aureus*
0.1 ml of the solution was pipetted out from (SCDB) and streaked onto Vogel-Johnson Agar Medium to check for the presence of *Staphylococcus aureus***.** The plates were then inverted and incubated at 37° C for 18 – 24 hours and are then observed for typical black colonies that were surrounded by yellow zones. They were also positive for coagulase test and

were further confirmed by gram staining.

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 colonies that show fluorescence when observed under UV.

Results

Organoleptic parameters

Table 3: Organoleptic characters of ingredients present in Polyherbal Pain Reliver Cream

Sr. No.	Name of ingredients	Colour	Odour	Taste
1	Mahanarayana Oil	Yellow	Characteristic	Characteristic
2	Nirgundi oil	Yellowish brown	Characteristic	Characteristic
3	*Aloe vera* (Kumari)	Colourless	Characteristic	Characteristic
4	*Eucalyptus globulus* (Nilgiri) oil	Colourless	Aromatic & camphorous	Characteristic
5	*Ricinus communis* (Erand) oil	Pale yellow	Characteristic	Characteristic
6	*Cinnamomum camphora* (Karpoor)	White	Strong aromatic	Characteristic
7	*Mentha sylvestris* (Pudina)	White	Pleasant	Characteristic
8	*Vateria indica* (Sarjras)	Light yellow	Fragrant	Characteristic

Acid value

Table 4: Acid value of Oils in Polyherbal Pain Reliver Cream

Sr. No.	Name of oils	Value (% oleic acid)
		*Practical
1	Mahanarayana Oil	5.606 ± 0.100
2	Nirgundi oil	1.169 ± 0.1300
3	*Eucalyptus globulus* (Nilgiri) oil	4.556 ± 0.04
4	*Ricinus communis* (Erand) oil	1.873 ± 0.100

* Data represents in Mean±SD; where n=3

Results indicated that the acid value of oils was found within the normal limits as shown in table 4. The acid value of Mahanarayan oil and *Eucalyptus globulus* Labill. were found to be comparatively higher compared to other oils.

Saponification Value

Table 5: Saponification Value Oils in Polyherbal Pain Reliver Cream

Sr. No.	Name of oils	Value (mgKOH/g oil)
		*Practical
1	Mahanarayana Oil	204.58 ± 0.66
2	Nirgundi oil	199.41 ± 0.708
3	*Eucalyptus globulus* (Nilgiri) oil	56.98 ± 1.321
4	*Ricinus communis* (Erand) oil	187.36 ± 0.32

Data represents in Mean± SD; where n=3

The saponification value of Mahanarayan oil were found to be comparatively higher compared to other oils as shown in table 5

Determination of free fatty acids

Table 6: Free fatty acids of Oils in Polyherbal Pain Reliver Cream

Sr. No.	Name of oils	Value (% oleic acid)
		*Practical (%)
1	Mahanarayana Oil	2.803 ± 0.137
2	Nirgundi oil	0.584 ± 0.067
3	*Eucalyptus globulus* (Nilgiri) oil	2.278 ± 0.201
4	*Ricinus communis* (Erand) oil	1.013 ± 0.136

*Data represents in Mean± SD; where n=3

Results indicated that the fixed oil value of Polyherbal Pain Reliver Cream was found within the normal limits as shown in table 6.Value of Mahanarayan oil and *Eucalyptus globulus* Labill oils were comparatively higher than other oils.

Rancidity test

Table 7: Rancidity value of Oils in Polyherbal Pain Reliver Cream

Sr. No	Name of Oils	Value
1	Mahanarayana Oil	Not rancid
2	Nirgundi oil	Not rancid
3	*Eucalyptus globulus* (Nilgiri) oil.	Not rancid
4	*Ricinus communis* (Erand) oil	Not rancid

*****Data represents in Mean± SD; where n=3

Results indicated that the rancidity of oils in Polyherbal Pain Reliver Cream, oils were found not rancid.

Specific gravity

Table 8: Specific gravity of Oils in Polyherbal Pain Reliver Cream

Sr. No.	Name of oils	Value (g/ml)
		***Practical**
1	Mahanarayana Oil	0.923 ± 0.007
2	Nirgundi oil	0.905 ± 0.003
3	*Eucalyptus globulus* (Nilgiri) oil	0.905 ± 0.003
4	*Ricinus communis* (Erand) oil	0.963 ± 0.005

* Means average of 3 readings

Results indicated that the specific gravity value of Polyherbal Pain Reliver Cream, oils were found within the normal limits as shown in table 8.

Viscosity

Table 9: Viscosity of Oils in Polyherbal Pain Reliver Cream

Sr. No.	Name of oils	Value (poise)
		***Practical**
1	Mahanarayana Oil	216.116 ± 1.200
2	Nirgundi oil	200.80 ± 0.67
3	*Eucalyptus globulus* (Nilgiri) oil	12.90 ± 0.661
4	*Ricinus communis* (Erand) oil	392.17 ± 0.44

* Data represents in Mean± SD; where n=3

Results indicated that the viscosity value of Polyherbal Pain Reliver Cream, oils were found within the normal limits as shown in table 9. The viscosity of *Ricinus communis* Linn. was found higher compare to other oils.

Refractive Index

Table 10: Refractive Index of Oils Polyherbal Pain Reliver Cream

Sr. No.	Name of oils	Value
		***Practical**
1	Mahanarayana Oil	1.464 ± 0.001
2	Nirgundi oil	1.464 ± 0.0005
3	*Eucalyptus globulus* (Nilgiri) oil	1.470 ± 0.0005
4	*Ricinus communis* (Erand) oil	1.479 ± 0.002

*****Data represents in Mean ±SD; where n=3

Results indicated that the density value of Polyherbal Pain Reliver Cream, oils were found within the normal limits as shown in table10.

Results of *Aloe vera*

Table 11: Results of *Aloe vera*

Parameters	Results
Identification	To 2 ml gel, add 5 ml alcohol-a cloudy precipitation of mucilage is formed.
Specific gravity	0.986 ± 0.09
Reflective index	1.341 ± 0.04
pH	3.9 ± 0.001
Moisture content	102.6 ± 0.004%
Water solubility	Miscible in all proportion
Alcohol solubility	Soluble in some proportions, further addition causes the precipitation of mucilage

Results indicates that, all the parameters complies with in house specification and suppliers specifications as shown in table11

Identification Test for Menthol

Table 12: Identification test for menthol

Tests	Results
10 mg in 1 ml conc. H SO 1 ml 1% W/V vaniline sulfuric acid On addition of 1 ml H SO	Orange-yellow colour produced Colour change to violet
Crystals in glacial acetic acid and few drop of H_2SO_4 1 drop of nitric acid	No green colour produced
When triturate with about an equal amount of camphor, chloral hydrate or phenol, the mixture liquefies.	Menthol confirms

From identification test of menthol, menthol confirms and on performing acidity test yellow colour produced, so menthol is acid in nature.

Identification Test for Camphor

Table 13: Identification Test for Camphor

Test	Result
A drop of solution of vanillin (1:100) and sulphuric acid when added to powder of camphor	Produces immediately a yellow colour, changing to red, violet, and finally blue.

From identification of camphor test, it was natural camphor which differentiates from synthetic camphor.

Residue on Evaporation

Table 14: Residue on Evaporation

Evaporate 2.0g on a water-bath and heat at 105^0 for 1 hr. The reside weights not more than 1.0 mg (0.05%)	No residue	100% purity
	No reside	100% purity

Results indicate that both menthol and camphor having no reside, so it's having 100% purity.

Test for Presence of Colophony

Table 15: Test for Presence of Colophony

Test	Result
Dissolve 0.1 g in 10 ml of acetic anhydride by gentle heat, cool, and add 1 drop of sulphuric acid	a bright purplish-red colour, rapidly changing to violet, is produced
Shake 0.1 g of powder with 10 ml of light petroleum (b.p. 50°-60°), and filter; shake 5ml of the filtrate with 10 ml of dilute solution of copper acetate	Petroleum layer assumes a bright bluish-green colour

Solubility of *Vateria indica*

Table 16: Solubility of *Vateria indica*

In alcohol	Jelly mass form
In CCl4	Jelly mass form
In conc. H_2SO_4	Brick red color
In petroleum ether	Insoluble white ppt

Results indicates present of colophony in *Vateria indica* oleo-resin and not soluble in solvents.

Moisture Content determination by Karl-Fischer determination

Table 17: Moisture Content of oils Polyherbal Pain Reliver Cream

Sr. No.	Name of oils	Value
		*Practical (%)
1	Mahanarayana Oil	0.976 ± 0.010
2	Nirgundi oil	0.890 ± 0.050
3	*Eucalyptus globulus* Labill.	0.006 ± 0.005
4	*Ricinus communis* Linn.	0.123 ± 0.035

*Data represents in Mean ± SD; where n=3

Results indicated that the specific moisture content of Polyherbal Pain Reliver Cream, oils were found within the normal limits as shown in table 17. The moisture content in Mahanarayan oil was found higher than other oils.

Standardization of finished product (Polyherbal pain reliver cream)

Table 18: Standardization of Polyherbal Pain Reliver Cream

Parameters	Polyherbal pain reliver cream
Physicochemical Parameters,	
Colour	Creamish colour
Odour	Characteristic
Wt. of 5 filled tubes	176.466 g
Wt. of 5 empty tubes	25.528 g
Wt. of Net content	150.938 g
Avg. wt. of Net content	30.187 g
Acid value	6.96 ± 0.002
pH	5.81 ± 0.006
Moisture content	69.75 ± 0.004

Results indicated the results obtained from different standardization parameter of finished product Polyherbal Pain Reliver Cream, Creams as shown in table 18. All the parameters were found significant in formulation

Heavy metal analysis of raw materials and finished product

Analysis of Heavy metal like Lead (Pb), Cadmium (Cd), Mercury (Hg), Arsenic (As) were carried out using Atomic absorbance spectrophotometer (6300) by SHIMADZU. Results are shown in Table 19.

Table 19: Heavy metal analysis of raw materials and finished product

		Heavy Metals			
		Mercury	Lead	Cadmium	Arsenic
Sr. No.	Material Name	(Hg) (NMT ppm) 1	(Pb) (NMT ppm) 10	(Cd) (NMT 0.3 ppm)	(As) (NMT ppm) 3
1	Mahanarayana Oil	Absent	0.124	Absent	Absent
2	Nirgundi oil	Absent	0.078	Absent	Absent
3	Aloe vera gel	Absent	0.108	Absent	Absent
4	*Eucalyptus globulus* (Nilgiri) oil	Absent	Absent	Absent	Absent
5	*Ricinus communis* (Erand) oil	Absent	Absent	Absent	Absent
6	*Mentha sylvestris* (Pudina)	Absent	Absent	Absent	Absent
7	*Cinnamomum camphora* (Karpoor)	Absent	Absent	Absent	Absent
8	*Vateria indica* (Sarjras)	Absent	0.214	Absent	0.143
9	Polyherbal Pain Reliver Cream	Absent	0.427	Absent	0.168

Results suggested that Mercury was absent in formulation while concentration of Arsenic, Lead and Cadmium were significant. Readings obtained here were in limit indicated that formulation passed the limit test for heavy metal. Results of heavy metal analysis were shown in table 19.

Microbial analysis of raw materials and finished products

Table 20: Microbial analysis of raw materials and finished [product

Sr no	Sample name	TBC x 10^2cfu/g	TFCx 10^2 cfu/g	*E. coli*	*P. aergi nosa*	*S. aureus*	Sal. spp
1	Mahanarayana Oil	12	Absent	Absent	Absent	Absent	Absent
2	Nirgundi oil	16	Absent	Absent	Absent	Absent	Absent
3	*Aloe vera* gel	48	21	Absent	Absent	Absent	Absent
4	*Eucalyptus globulus* (Nilgiri) oil	59	Absent	Absent	Absent	Absent	Absent
5	*Globulus* Labill.	54	Absent	Absent	Absent	Absent	Absent
6	*Ricinus communis* (Erand) oil	56	Absent	Absent	Absent	Absent	Absent
7	*Mentha sylvestris* (Pudina)	52	Absent	Absent	Absent	Absent	Absent
8	*Cinnamomum camphora* (Karpoor)	26	Absent	Absent	Absent	Absent	Absent
9	*Vateria indica* (Sarjras)	12	Absent	Absent	Absent	Absent	Absent
10	Polyherbal Pain Reliver Cream	36	Absent	Absent	Absent	Absent	Absent

TBC: Total Bacterial Count; TFC: Total Fungal Count; E.c: Escherichia coli; P.a: *Pseudomonas aeruginosa;* S.a: *Staphylococcus aureus*; sal. spp*: Salmonella specie*

Discussion and Conclusion

For identification of raw materials their organoleptic characteristics study like clarity, colour, odour, test done and it complies with respect to standard monographs by Vasu Research Centre. Further measures for quality control were assured the purity of crude drugs taken in formulation by correlating with standard monographs, like Acid value, Saponification Value, Free fatty acid determination, Viscosity, Specific gravity, Refractive index, Moisture content were found under specified limits

Analysis of heavy metals like Lead (Pb), Cadmium *(*Cd), Mercury (Hg), Arsenic (As) were carried out using Atomic absorbance spectrophotometer. Results of them were under the limits. In finished product Polyherbal Pain Reliver Cream shown 0.427, 0.216 and 0.168 lead and arsenic respectively. Mercury and cadmium were absent in finished product. Along with all the experiment data to standardize the active ingredients being used in formulation it becomes clear that active ingredients were of significant quality. These standardized ingredients have been used to manufacture Polyherbal Pain Reliver Cream by Vasu Healthcare Pvt. Ltd. Finished products were again tested for quality control measures i.e. pH, Acid value microbial & heavy metal analysis. Further, it was found that even after boiling at high temperature oil did not loss its property; it might be presence of quality promising ingredients.

Microbial analysis for all Ingredients were performed to check quality of used drugs, Results of them are shown on various pathogenic species like *E.coli, Pseudomonas aeruginosa, Salmonella species & Staphylococcus aureus* were absent and bacteria as well as fungus count were in normal limits.

References

1. Venkata Rao. Modern approaches to herbal medicine. The Eastern Pharmacist. Edn. 2000;12:35-39.
2. Handa SS. Future Trends of Plants as Drugs. Pharmatimes. 1991;23(4):13-23.
3. Arthur A, Schuna. Pharmacotherapy a pathological approach. Edn 6, MCGRAW-HILL Medical Publishing Division; c2005. p. 1671-81.
4. Harris ED, Sledge CB, Firestein GS. Textbook of Rheumatology. Edn 6, New Delhi: McGraw-Hill, Medicinal Publication Divisio; c2001. p. 967-1000.
5. Ram Narayan Sharma. Ayurved Sarsangrah. Kolkata: Shree Baidhnadh Ayurved Bhavan Private Limited; c2004, 696.
6. Pharmacopial Standards for Ayurvedic Formulations, central Council for Research in Ayurveda and Siddha (Ministry Of Health And Family Welfare) New Delhip. 365.
7. Gopinadh Gupt. Bhart-Bhaishjy-Ratnakar. New Delhi: Narendhra Prakash Jain, Part 3. 201.
8. The Ayurvedic Formulary of India, Government Of India, Ministry Of Health And Family Welfare Department Of Indian System Of Medicine And Homoeopathy, New Delhi Prt I, Edn. 2006;2:151.
9. Wilson E, Rajamanickam GV, Vyas N, Agrawal A, Dubey GP. Herbs used in Siddha medicine for Arthritis. Indian Journal of Traditional Knowledge. 2007 Oct;6(4):678-686.
10. Banejee S, Bandhyopadhyay SK, Mukkherjee A, Sikdar S. Antiinflammatory activity of *Ricinus communis* in albino rat. Indian journal of Pharmacology. 1997;23(3):149.

11. Ilavarasan R, Mallika M, Venkataraman S. Anti-inflammatory and free radical scavenging activity of *Ricinus communis* root extract. Journal of Ethnopharmacology. 2006;103(3):478-80.
12. Silva J, Abebe W, Sousa SM, Duarte VG, Machado MIL, Matos FJA. Analgesic and anti-inflammatory effects of essential oils of *Eucalyptus*. Journal of Ethnopharmacology. 2003;89:277-83.
13. Jio S, chen B, Gao W, Song H. studies on anti-inflammatory and analgesic activity of *Eucalyptus globules* seed. 27(3):223.
14. Ashok Sheth, The Herbs of Ayurveda. Bhavnagar (Gujarat): ALANKAR. 2005;4:1154.
15. Vazquez B, Avila G, Segura D, Escalante B. Antiinflammatory activity of extracts from *Aloe vera* gel. Journal of Ethanopharmacology. 1996 Dec;55(1):69-75.
16. Atta AH, Alkofahi A. Anti-inflammatory effect of *Mentha piperita*, J. Ethanopharmacol. 1998;60:1179.
17. Galeotti N, Lorenzo Di Cesare Mannelli, Gabriela Mazzanti, Alessandro Bartolini, Carla Ghelardi. Menthol: a natural analgesic compound. Neuroscience Letters. 2002 Apr 12;322(3):145-8.
18. Hye Ja Lee, Eun-A Hyun, Weon Jong Yoon, Byung Hun Kim, Man Hee Rhee, Hee Kyoung Kang, *et al*., Anti-inflammatory activity of *Cinnamomum camphora*. Journal of Ethnopharmacology; c2006. p. 208-16.
19. Lohar DR. Protocol for testing of Ayurvedic, Siddha & Unani Medicines. Government of India, Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory for Indian Medicines, Ghaziabad. 2007;103:124-128.
20. Lohar DR. Protocol for testing of Ayurvedic, Siddha & Unani Medicines. Government of India, Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory For Indian Medicines, Ghaziabad; c2007. p. 111-2.
21. Lohar DR. Protocol for testing of Ayurvedic, Siddha & Unani Medicines. Government of India, Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory For Indian Medicines, Ghaziabad; c2007, 123.
22. Lohar DR. Protocol for testing of Ayurvedic, Siddha & Unani Medicines. Government of India, Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory For Indian Medicines, Ghaziabad; c2007. p. 111-2.
23. The Ayurvedic Pharmacopoeia of India. Government Of India, Ministry of Health And Family Welfare Department of Indian System of Medicine And Homoeopathy, New Delhi. 2006;6(1):216.
24. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, Edn 45 Pune: Nirali prakashan. 2010 Jun;1:17-8.
25. Indian Pharmacopoeia. The Controller of Publication, Delhi. 2010;3:1649.
26. The Ayurvedic Pharmacopoeia of India. Government of India, Ministry of Health And Family Welfare Department of Indian System of Medicine And Homoeopathy, New Delhi. 2006;4(1):121.
27. The Ayurvedic Pharmacopoeia of India. Government Of India, Ministry Of Health And Family Welfare Department Of Indian System Of Medicine And Homoeopathy, New Delhi. 2006;6(1):291.
28. Indian Pharmacopoeia. The Controller of Publication, Delhi. 2007;1:93-4.
29. Indian Pharmacopoeia. The Controller of Publication, Delhi. 2010;3:1650.
30. Lohar DR. Protocol for testing of Ayurvedic, Siddha & Unani Medicines. Government of India, Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory For Indian Medicines, Ghaziabad; c2007. p. 47-52.