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DEVELOPMENT, STANDARDISATION AND ACCELERATED STABILITY STUDIES OF A POLYHERBAL FORMULATION

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Abstract

Rheumatoid Arthritis is a chronic, inflammatory, systemic autoimmune disease characterized by pain, swelling and stiffness. Allopathic medications have been prescribed to alleviate symptoms of this disease that results into associated side effects like gastrointestinal disorders, cardiovascular risks, immunodeficiency, hepatotoxicity and renal toxicity. The use of herbal medicine is becoming popular due to toxicity and side effects of allopathic medicine. Several herbs have been used traditionally for relieving the symptoms of rheumatoid arthritis. The antiarthritic polyherbal formulation composed of four medicinal plants namely *Imbatiens balsamina*, *Capparis decidua*, *Dioscorea alata*, *Onosma bracteatum*. The aim of the study was to develop a polyherbal formulation (polyherbal capsules) to treat Rheumatoid arthritis to set up its quality control standards by evaluating its physicochemical, phytochemical and formulation parameters. The optimized batch was subjected to accelerated stability studies to ascertain the stability of the formulation.

KEYWORDS: Rheumatoid arthritis, Standardization, Polyherbal formulation, Evaluation.

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INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic, inflammatory autoimmune disorder where the immune system targets and attacks the joints. It is a disabling and painful inflammatory condition, which can lead to substantial loss of mobility due to pain and joint destruction. RA is a systemic disease, often affecting extra-articular tissues throughout the body [1]. As a joint

disease, it is commonly polyarticular and its involvement of many joints distinguishes rheumatoid and other inflammatory arthritis from non- inflammatory arthritis (i.e) osteo arthritis [2, 3]. RA has a world-wide distribution and affects 0.5-1% (with a female preponderance) of the population. RA is a significant cause of disability and mortality and carries a high socio-economic cost. The most common age of onset is between 30 and 50 years. The goals of treatment in RA are to control inflammation, prevent progressive joint destruction, preserve and improve activities of daily living, and alleviate pain. The drugs commonly in use for the treatment of RA include, Glucocorticoids, non steroidal anti-inflammatory drugs, disease modifying anti rheumatic drugs. Their prolonged duration of treatment is associated with many adverse reactions, apart from the GI disorders, immunodeficiency and humoral disturbances. Accordingly, reducing the side effects and cost should be considered while designing improved therapeutics for RA, besides enhancing medicinal effectiveness [4]. Alternative treatments based on plant products and herbal mixtures are becoming increasingly popular in India, US and other countries [5]. Medicinal plants like *Boswellia serrata, Berberis vulgaris, Syzygium aromaticum, Curcuma longa, Commiphora mukull, Allium cepa, Vitis vinifera*, etc. are used widely as an alternative[6].A standardized polyherbal formulation in a convenient dosage form along with a scientific evaluation would contribute significantly in the treatment of rheumatoid arthritis. The present investigation is an attempt in this direction. The study includes raw materials analysis for varying its identity and quality, formulation development of polyherbal capsules, standardization of the formulation and stability studies of the formulation under accelerated conditions.

MATERIALS AND METHODS

Based on the extensive review of literature, four active ingredients were selected for the formulation of polyherbal capsules to treat Rheumatoid arthritis. Four ingredients which were used as crude herbal drugs are *Capparis decidua, Dioscorea alata, Imbatiens balsamina, and Onosma bracteatum*

RAW MATERIALS

Capparis decidua, Dioscorea alata, Imbatiens balsamina, and Onosma bracteatum were obtained from Absa Herbals, Chennai. Samples like *Capparis deciduas (whole plant), Dioscorea alata (tuber part) , Imbatiens balsamina (whole plant, and Onosma bracteatum (whole plant)* were authenticated with the help of Dr. P.Jayaraman, Director, Plant Anatomy & Research Centre, Tambaram, Chennai

COMPOSITION OF THE CAPSULES

Active ingredients	**Mg/ capsules**
Capparis decidua	180mg
Dioscorea alata	90mg
Imbatiens balsamina	75mg
Onosma bracteatum	75mg

EVALUATION OF QUALITY CONTROL PARAMETERS FOR RAW MATERIALS[7-14.]

For the crude herbal drugs (*Capparis decidua, Dioscorea alata, Imbatiens balsamina and Onosma bracteatum*), the list of tests carried out include foreign organic matter, loss on drying, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, limit tests for heavy metals, microbial load analysis, fluorescence analysis.

FORMULATION DEVELOPMENT OF CAPSULES

Five trial batches (each trial batch size: 500 capsules) were formulated by varying the composition of the excipients proportions such as Talc, Colloidal silicon dioxide, Starch, Dicalcium Phosphate, Lactose (Table 1). The granules were evaluated for the flow property characteristics.

Table 1: Trial batches

S. No	Ingredients	Quantity (mg)/Capsule				
		T-I	T-II	T-III	T-IV	T-V
1	*Capparis decidua*	75	75	75	75	75
2	*Dioscorea alata*	75	75	75	75	75
3	*Imbatiens balsamina*	90	90	90	90	90
4	*Onosma bracteatum*	180	180	180	180	180
5	Talc	-	3.5	1.75	1.75	-
6	Lactose	-	-	-	-	3.5
7	Dicalcium phosphate	3.5	-	1.75	-	-
8	Starch	-	-	-	1.75	-
9	Colloidal silicon dioxide	1	1	1	1	1
10	Sodium methyl paraben	0.26	0.26	0.26	0.26	0.26
11	Sodium propyl paraben	0.1	0.1	0.1	0.1	0.1
12	Pronopol	0.1	0.1	0.1	0.1	0.1
13	Sodium benzoate	0.3	0.3	0.3	0.3	0.3

PREPARATION OF CAPSULE POWDER

All ingredients were weighed according to the formulae separately.

The pulverized crude drugs (into powder form) of *Capparis decidua, Dioscorea alata, Imbatiens balsamina and Onosma bracteatum* were mixed well for 20 minutes. The preservatives - sodium methyl paraben, sodium propyl paraben, sodium benzoate and bronopol as specified in each formula were added to the mixed powder. Then, the mixed powder were transferred to the polythene bags and labelled accordingly and then the samples were taken up for quality control tests.

EVALUATION OF THE CAPSULE POWDER [13]

BULK DENSITY (ρ_b)

It is determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder or through a volume measuring apparatus into a cup. It is expressed in g/ml and is given by

$$\rho_b = M/V_o$$

Where M is the mass of powder and V_o is the bulk volume of the powder.

TAPPED DENSITY (ρ_t)

The tapped density was measured by tapping the powder to constant volume. It is expressed in g/ml and is given by

$$\rho_t = M/V_t$$

Where, M is the mass of powder and V_t is the tapped volume of the powder.

COMPRESSIBILITY INDEX AND HAUSNER RATIO

The compressibility index has been proposed as an indirect measure of bulk density, size and shape, surface area, moisture content and cohesiveness of materials. All these are closely related to predicting the powder flow characteristics. The compressibility index and the Hausner's ratio are determined by measuring both bulk volume and the tapped volume of the powder.

Compressibility index = 100 x $(V_o-V_t)/V_o$

Hausner's Ratio = V_o/V_t

ANGLE OF REPOSE

For determination of angle of repose (Θ), the blends were poured through the walls of a funnel, which was fixed at a position such that its lower tip was at a height of exactly 2.0 cm above a hard surface. The drug or the blends were poured till the time when upper tip of the pile surface touched the lower tip of the funnel. Angle of repose was calculated using following equation.

The angle of repose Θ, was calculated by the formula,

$\tan \Theta = h/r \rightarrow \Theta = \tan^{-1}(h/r)$

Where, Θ is the angle of repose, h is the height in cm and r is the radius in cm.

CAPSULE FILLING

Only the powders of the optimized formulation were filled in "0" size capsules to an average net content weight of 520 mg. The capsules were then dedusted, transferred into

polybags, labelled and the samples were evaluated as per the testing requirements. From the final trial, samples were taken for accelerated stability studies as per the testing requirements.

STANDARDISATION OF THE FINISHED PRODUCT

The developed capsule formulation was evaluated for the following parameters for the purpose of standardization: evaluation of capsules, physicochemical parameters, phytochemical studies, heavy metal analysis and microbial load analysis, fluorescence analysis and pesticide residues.

EVALUATION OF CAPSULES [7]

The polyherbal capsules evaluated for organoleptic characteristics, uniformity of weight, disintegration test and moisture content. Uniformity of weight, disintegration test and determination of moisture content of the capsules were carried out as per Indian Pharmacopoeia 2010 procedures.

PHYSICOCHEMICAL PARAMETERS[7]

P^H OF 1%W/V SOLUTION OF THE POLYHERBAL FORMULATION

1 gm of capsule powder was taken and dissolved in 100 ml demineralized water. The pH value of the solution was determined by means of a digital pH meter.

DETERMINATION OF TOTAL ASH VALUE

3 gm of the sample was weighed in a tared silica dish and was incinerated at a temperature of 600°C until the sample turned white which indicated the absence carbon. Then, the ash was cooled and weighed. The percentage of the total ash was calculated.

DETERMINATION OF ACID INSOLUBLE ASH VALUE

The ash was boiled with 25 ml of 2 M hydrochloric acid for 5 minutes insoluble matter was collected on an ashless filter paper, washed with hot water, ignited, cooled in desiccators and weighed. The % of acid-insoluble ash was calculated.

EXTRACTIVE VALUES

DETERMINATION OF WATER SOLUBLE EXTRACTIVE

5 gm of the formulation as weighed, coarsely powdered and macerated with 100 ml of chloroform water in a closed flask for 24 hours; it was shaken frequently for six hours and allowed to stand for 18 hours. It was then filtered rapidly, taking precautions against loss of solvent; 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish and it was dried at 105ºC to constant weight. The percentage of water soluble extractive value was calculated.

DETERMINATION OF ETHANOL SOLUBLE EXTRACTIVE VALUE

Procedure for water soluble extractive was followed for the determination of alcohol soluble extractive but the solvent used was 90 % ethanol instead of chloroform water.

PHYTOCHEMICAL STUDIES

The phytochemical investigation of those extracts for linking to its pharmacological actions.

PRELIMINARY PHYTOCHEMICAL EXAMINATION[15]

PREPARATION OF THE EXTRACTUM

Aqueous extract was prepared from the developed polyherbal powder sing the water solvent system. The solvent was selected based on the extractive values. Polyherbal capsule powder was successfully extracted with water using maceration method. Then, the aqueous extract was subjected to evaporation on water bath to dryness. The resultant extract was used for the preliminary phytochemical evaluation. The standard qualitative tests were carried. The extract was tested for alkaloids, flavanoids, saponins, tannins, carbohydrates, starch, gums and mucilage.

QUANTITATIVE ESTIMATION OF SOME PHYTOCHEMICALS

ESTIMATION OF TOTAL ALKALOIDS {16}

5 gm of the capsule powder were weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to 1/4th of the original volume.

Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

ESTIMATIONOFTOTALSAPONINS [17]

20 gm of the capsule powder were weighed and 100 ml of 20 % ethanol was added. Then the sample was heated over a hot water bath for 4 hours with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The combined extract was reduced to 40 ml over water bath at about 90ºC. The concentrate was treated with 20 ml of diethyl ether and the aqueous layer was recovered while the ether layer was discarded. This process of purification was repeated three times and then 60 ml of n-Butanol was added and extracted. The extract obtained was then washed two times with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath for evaporating the solvent. After evaporation the samples were dried in an oven to a constant weight and the saponin content was calculated as percentage.

ESTIMATION OF TOTAL TANNINS [18]

Tannin content present in the formulation was estimated by Tocklai-Lowenthal method which is basically an Oxidimetric titration. The infusion of the sample of extract was prepared by boiling 5 g sample with 400 ml water for 1 hour, cooling and making up the volume of the extract to 500 ml. The separation of tannins and non-tans was effected by gelatin. The following reagents were used.1. *Gelatin solution*: 25 g gelatin was soaked for 1 hour in saturated sodium chloride solution. The mixture was then warmed until the gelatin got dissolved and after cooling the solution was made up to 1 litre with saturated sodium chloride.2. *Acid sodium chloride*: 25 ml of conc. sulfuric acid was added to 975 ml saturated sodium chloride solution. 100 ml of the sample infusion was mixed with 50 ml of the gelatin solution, 100 ml of acid sodium chloride and 20 g

of powdered kaolin. After shaking for several minutes and allowing settling, the mixture was filtered and an aliquot of the filtrate withdrawn for titration. 25 ml of the non-tan filtrate is equivalent to 10 ml of the original infusion.

The Tocklai - Lowenthal method:

The following reagents were used:

(1) N/25 potassium permanganate;

(2) 1-5 g indigo carmine dissolved in 1litre water containing 50 ml sulfuric acid.

A 10 ml aliquot of the infusion is mixed with 25 ml of the indigo-carmine solution and the mixture diluted to 750 ml potassium permanganate was then run in from a burette 1 ml at a time with brisk shaking. As the titration proceeded, the blue of the Indigo-carmine passed through many shades to a final yellow with a faint pink tint at the rim. This was taken as the endpoint (Note: This needs considerable judgment on the part of the operator. The titre is affected to some extent by the amount of tannin in solution, the time taken in the titration and the vigor of shaking. Repeats often differ by 0-3 ml. or more, and the variation between different operators may amount to 1 ml.) The tannin titre was arrived at by subtracting the non-tan from the total titre. The equivalent weight factor is 0.0416.

ESTIMATION OF TOTAL FLAVONOIDS [19]

10gm of the sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No.42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

FLUORESCENCE ANALYSIS [12]

Samples were studied for any colour changes with different chemicals and solvents. The samples were observed under UV chamber in different wavelengths viz., shorter wavelength V (254 nm) and longer wavelength UV (366 nm.)

HEAVY METAL ANALYSIS [14]

PREPARATION OF SAMPLES AND STANDARDS BY ACID DIGESTION METHOD

Accurately weighed 2 g of sample was taken in Kjeldahl flask. Acid mixture of HNO_3:$HClO_4$ (4:1) was added in the flask and heated continuously till the solution was 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 [14]. The standard solutions of lead (Pb), arsenic (As) and mercury (Hg) were prepared as per the protocol and the calibration curve was developed for each of them.

Detection: Then samples were analyzed for the presence of lead, arsenic and mercury using Atomic Absorbance Spectrophotometer (AAS) (6300 SHIMADZU).

DETERMINATION OF PESTICIDAL RESIDUE BY TLC [19]

EXTRACTION OF COMMON PESTICIDE FROM CAPSULE POWDER:

10 gm of capsule powder were taken in a round bottom flask and added sodium sulfide with 100 ml n-hexane. It was refluxed for 1 hour and filtered. The filtrate extracted with 50 ml

and 25 ml of Acetonitrile. The Acetonitrile layer was mixed with 500 ml demineralized water with 2.5ml saturated sodium sulfide and then extracted with an n-hexane layer and evaporated on a water bath. This residue was used for the analysis of organochloro, organophosphate and carbamate pesticides by thin layer chromatography using reference standards (Accu standards, USA).

TLC details:

Sample solution: Residue in methanol

Development system: Benzene: Methanol (60: 40)

Stationary Phase: Silica gel 60 F254 TLC plate of 0.2 mm thickness.

The extracts were spotted along with reference standards and chromatogram was developed and analyzed under UV from 200 to 300 nm.

MICROBIAL LOAD ANALYSIS [20]

The following tests were carried out for the estimation of number of viable aerobic microorganisms present and for detecting the presence of designated microbial species in the polyherbal formulation which includes total aerobic viable count, [yeasts and moulds, *Escherichia coli, Clostridia, Salmonellae* and *Shigella]*. The tests were carried out as per the WHO guidelines [20].

ACCELERATED STABILITY STUDIES OF CAPSULES [21]

The stability study of capsules under accelerated conditions was done according to the ICH guidelines [21] for 3 months. The conditions of temperature and relative humidity were maintained at 40°C ± 2°C and 75% ± 5% RH respectively in the stability chamber.

The parameters studied included description, uniformity of weight, disintegration test, moisture content, pH of the 1% w/v solution of the formulation, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, and quantitative estimation of total alkaloids, total saponins, total tannins, total flavonoids and microbial load analysis.

The samples were analyzed for all the parameters except microbial load analysis at the beginning of the study and at the end of 30 days, 60 days and 90 days. The microbial load analysis was done at the beginning and at the end of 90 days.

RESULTS AND DISCUSSION

All the raw materials complied with the quality control standards. Hence, they were taken for the further formulation and development studies.

PREFORMULATION AND FORMULATION DEVELOPMENT STUDIES

Totally five trials of formulation were carried out using different choices of excipients considering different facets of manufacturing problems as well as quality defects in mind. All the resultant formulations were evaluated for their flow property (Table 2). Among the five trial batches, T-V batch was found to have good flow properties; hence the powders of this batch were filled in the capsules and standardized.

Table 2: Evaluation of Trial batches

Parameter	T-I	T-II	T-III	T-IV	T-V
Bulk density (g/cm^3)	0.31	0.32	0.36	0.38	0.47
Tapped density (g/cm^3)	0.43	0.44	0.45	0.46	0.51
Compressibility index (%)	25.8	23.7	20.0	17.3	7.14
Hausner's ratio	1.37	1.34	1.24	1.20	1.08
Angle of repose (degrees)	50.1	41.4	39.6	36.2	27.3

STANDARDISATION OF THE FINISHED PRODUCT

The final formulation (Batch size: 5000) was analyzed for its quality control parameters in three trials. The mean value was obtained and standard deviation was calculated. Wherever there were no official standard/specification, limits for each parameter was established based on trial and error analysis of Trial V capsules.

Table 3: Organoleptic characters of the capsule content

Parameters	Observations
Colour	Light brown
Odour	Slight aroma
Nature	Powder
Taste	Tasteless

The polyherbal capsules were evaluated for organoleptic characters which include colour, odour, taste and nature (Table 3). The uniformity of weight, disintegration time, moisture content and physicochemical parameters of the polyherbal formulation are given in the Table 4.

Table 4: Quality control tests of the polyherbal formulation

Parameters	**Results**
Uniformity of weight	522.6±1.30 mg
Loss on drying	5.60±0.15 %w/w
Disintegration time	6'64" ±0.19
pH	5.44±0.11
Water soluble extractive	22.5±0.43%w/w
Alcohol soluble extractive	7.50±0.06 %w/w
Total ash	12.4±0.45 %w/w
Acid insoluble ash	3.45±0.09%w/w
Total alkaloid content	7.15±0.15%w/w
Total tannin content	18.7±0.10%w/w
Total flavonoid content	4.19±0.16%w/w
Total saponins content	1.87±0.03 %w/w

The preliminary phytochemical screening of the formulation is given in Table 5.

Table 5: Preliminary phytochemical screening of the Aqueous extract of capsule content

S.No.	Chemical Constituents	Phytoconstituents
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Tannins	+
5	Carbohydrates	+
6	Phenols	+
7	Starch	+
8	Gums and mucilage	+
9	Sterols	+
10	Terpenoids	+
11	Glycosides	+
12	Fats and oils	+
13	Proteins	+

(Present (+), Absent (-))

The fluorescence analysis data (Table 6) gives the valuable input for the identification of the olyherbal capsules.

Table 6: Fluorescence analysis of capsule content

S.NO	CHEMICAL TREATMENT	SHORT UV	ORDINARY LIGHT	LONG UV
1.	Powder	Light brown	Light brown	Dark brown
2.	Powder+ water	Light brown	Yellow	Yellowish brown
3.	Powder+iodine	Light brown	Light brown	Colourless
4.	Powder+picric acid	Dark brown	Dark brown	Dark brown
5.	Powder+NaOH	Light brown	Yellowish brown	Fluorescent yellow
6.	Powder+ethanol	Light brown	Yellow	Yellow
7.	Powder+KOH	Light brown	Light brown	Colourless
8.	Powder+$FeCl_3$	Dark brown	Dark brown	Dark brown
9.	Powder+acetic acid	Light brown	Yellow	Dark yellow
10.	Powder+acetone	Light brown	Yellow	Yellow
11.	Powder+ammonia	Light brown	Greenish brown	Green
12.	Powder+Hcl	Light brown	Colourless	Colourless
13.	Powder+ HNO_3	Light brown	Yellow	Fluorescent yellow

Capsules were analyzed for the heavy metals, which included Arsenic, Lead and Mercury and the results are given in Table 7.

Table 7: Heavy metals analysis of the capsule content

Heavy metals	Results
Arsenic	Complies
Lead	Complies
Mercury	Complies

The polyherbal capsule were analysed for pesticide residues and the results were given in Table 8.

Table 8: Pesticidal residues

S.No	Pesticide groups	Pesticides		
		Sample1	Sample2	Sample3
1	Organochlorines	ND	ND	ND
2	Organophosphorus	ND	ND	ND
3	Carbamates	ND	ND	ND

ND –No spots were detected

The polyherbal capsules comply with the WHO Specification for the microbial load analysis (Table 9).

Table 9: Microbial load analysis

S.No.	Parameters	Results	WHO Specification
1	Total microbial count	120 cfu/g	NMT 1000cfu/g
2	Yeasts and moulds	Absent	NMT 100 cfu/g
3	*E.coli*	Absent	To be absent
4	*Salmonella*	Absent	To be absent
5	*Clostridia*	Absent	To be absent
6	*Shigella*	Absent	To be absent

ACCELERATED STABILITY STUDIES

Results of the stability studies at accelerated stability conditions are given in Tables 10, 11 and 12. The results showed that the formulation is stable under accelerated stability conditions

Table 10: Organoleptic parameters of capsules during the stability period

Parameter	Initial study	30 days	60 days	90 days
Colour	Light brown	NC	NC	NC
Odour	Slight aroma	NC	NC	NC
Nature	Powder	NC	NC	NC
Taste	Tasteless	NC	NC	NC

NC - no change

Table 11: Microbial load analysis during the stability study

S.No.	Parameters	Results		Specification
		Initial study	After 90 days	
1	Total microbial count	110cfu/g	130 cfu/g	NMT1000 cfu/g
2	Yeasts and moulds	Nil	Nil	NMT100 cfu/g
3	*E.coli*	Absent	Absent	To be absent
4	*Salmonella*	Absent	Absent	To be absent
5	*Clostridia*	Absent	Absent	To be absent
6	*Shigella*	Absent	Absent	To be absent

Table 12: Quality control tests during stability period

Parameters	Stability period			
	Initial study	After 1 month	After 2 months	After 3 months
Uniformity of weight	522.6±1.30 mg	526.4±0.45mg	525.7±0.83 mg	522.8±0.60 mg
Loss on drying	5.60±0.15 %w/w	5.73±0.04%w/w	5.72±0.04%w/w	5.67±0.18%w/w
Disintegration time	6'64" ±0.19	6'72''±0.32	7'06''±0.02	6'59''±0.02
pH	5.79±0.11	5.79±0.06	5.61±0.05	5.43±0.12
Total ash	22.5±0.43%w/w	15.7±0.15%w/w	14.5±0.20%w/w	13.7±0.15%w/w
Acid insoluble ash	7.50±0.06 %w/w	3.26±0.02%w/w	3.24±0.08%w/w	3.22±0.28%w/w
Water soluble Extractive value	12.4±0.45 %w/w	21.5±0.49%w/w	21.3±0.26%w/w	20.6±0.20%w/w
Alcohol soluble extractive	3.45±0.09%w/w	7.73±0.11%w/w	7.79±0.03%w/w	7.47±0.07%w/w
Total alkaloid content	7.15±0.15%w/w	7.25±0.07%w/w	7.38±0.06%w/w	7.05±0.05%w/w
Total Saponin content	18.7±0.10%w/w	10.8±0.06%w/w	10.5±0.35%w/w	10.7±0.20%w/w
Total tannin content	4.19±0.16%w/w	15.8±0.10%w/w	15.6±0.20%w/w	15.4±0.30%w/w
Total flavanoids	1.87±0.03 %w/w	4.59±0.28%w/w	4.09±0.02%w/w	4.13±0.16%w/w

CONCLUSION

The polyherbal formulation containing the plants like *Capparis decidua, Dioscorea alata, Imbatiens balsamina, and Onosma bracteatum* , the plants were reported for the treatment of rheumatoid arthritis in various literatures. So based on the literature evidence the plants were

selected. The polyherbal capsule was formulated and evaluated for phytoconstituents content and accelerated stability studies. The polyherbal extract shows the presence of alkaloids,flavonoids,tannins, terpenoids ,carbohydrates and also the exhibits the pharmacopoeial limits for its stability. Further the research work is needed to evaluate the invivo anti arthritic activity.

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