ISSN 2664-4142 (Print) & ISSN 2664-6749 (Online)
South Asian Research Journal of Pharmaceutical Sciences
Abbreviated Key Title: South Asian Res J Pharm Sci

| Volume-8 | Issue-5 | Nov-Dec- 2026 | **DOI: https://doi.org/10.36346/sarjps.2026.v08i05.002**

Original Research Article

Herb MaRS Guided Identification and Prioritization of Chemical Markers in Marketed Herbal Products for Rheumatoid Arthritis

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Article History
Received: 27.08.2026
Accepted: 14.10.2026
Published: 14.11.2026

Abstract: Standardization of herbal products is a challenging area because of complexity in raw material identification and quality, extract consistency and diverse nature of phytoconstituents in them. The use of chemical markers in standardization is an important approach for overcoming the problems with raw materials to finished products in various stages of development. This study is aimed to identify and prioritize suitable chemical markers for medicinal plants used in rheumatoid arthritis herbal products marketed in India, with the objective of supporting product standardization and quality control. For the study most repeating medicinal plants are considered that are listed on the product labels were subsequently evaluated through a comprehensive review of available phytochemical and pharmacological information. The assessment carried out using Herbal Chemical Marker Ranking system (Herb MaRS) on a scale of 0-8 by considered factors like biological activity, commercial availability of reference standard, analytical method and its concentration in the plant. A total score of 8 indicated the highest suitability as a chemical marker, whereas score of 4 represented adequate suitability. The study identified several potential chemical markers with total 8 points were Boswellic acids (beta-boswellic acid, Acetyl-β-boswellic acid, 11-Keto-β-boswellic acid, 3-O-Acetyl- 11-keto-β-boswellic acid and Resveratrol (*Boswellic seerata roxb*), 1,8 cineole (*Moringa oleifera*), Rhoifolin (*Commiphora mukul*). These chemical markers may further consider in the standardization of the anti-rheumatic herbal products available in the market.

Keywords: Chemical Markers, Herbal Marker Ranking System (Herb MaRS), Rheumatoid Arthritis, Herbal Products, Standardization, Phytoconstituents.

INTRODUCTION

Rheumatoid arthritis is multifaceted inflammatory condition occurring in synovium initially, later spread to extraarticular organs. The pathogenesis of RA is multifactorial, needs individualized treatment based on patient condition. The multifaceted nature of RA has also generated considerable interest in medicinal plants and herbal preparations as potential complementary approaches for the management of inflammatory and rheumatic conditions.

Herbal medicines are the major sources for treating various diseases / disorders from ages. More than 80% of world population rely on herbal sources for their primary healthcare purpose. Global market value for herb based products has drastically increased from last decade with a 250 billon dollar to 515 billion dollar by the end of 2034 with a CAGR of 8.1. India alone holds nearly 30% of global market with a value of 64.8 Billion dollars. Despite of increasing market value herbal products facing a key in their standardization because of complexity in their composition, uncertainty in crude drug identification and concentration of chemical constituents, limited pharmacokinetic and pharmacodynamic information, posing a risk in their quality, safety and efficacy.

Standardization refers to the establishment of appropriate quality control procedures, specifications and regulatory requirements to ensure that herbal medicinal products maintain consistent chemical composition, potency, quality, and

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therapeutic characteristics between different batches. An important component of standardization is the authentication of raw plant materials and verification of the botanical identity of the ingredients present in finished products. Various approaches can be used for this purpose, including macroscopic, microscopic and physical evaluation, chromatographic and spectroscopic fingerprinting, chemical-marker analysis, and DNA-based identification techniques. Where Morphological, microscopical and DNA finger printing helps in authentication of crude drug, while chemical marker analysis helps in overcoming the problem associated with chemical composition variation due to geography add extension to the proper authentication and quality check at both crude drug level and finished product to improve product quality and efficacy. So in standardizing the herbal products, analysis of chemical marker compound is one of the promising area, even though it is a poly herbal formulation.

Identification of chemical markers challenging because of complexity in composition, acts like active, analytical, negative, group and surrogate markers. In identifying all these categories Herb MaRS providing guidelines for prominent markers, with a 0 to 8 score. The scoring is given by considering biological activity given with 0 to 2 points, in relation to treating or managing symptoms with 1 point, based on the concentration of marker concentration in product or crud drug points given from 0-3 (Low (<5 µg/g or ppm) – 1 point, Moderate (5--50 µg/g or ppm) – 2 points, High (>50 µg/g or ppm) – 3 points), If the standard commercial sample available will be given with 1 point and 1 point is given for availability of analytical method either of HPLC, HPTLC, MS, GC-MS, etc. the compound that giving total 8 points were most suitable as a markers, and in between 4-7 were considered as moderate or optimal markers and compounds that were giving 3 or below points will not be considered as chemical markers in herbal product analysis.

METHODS

1. Study Design and Data Collection Methods

For the study seven poly herbal products were identified with reference to highly sold products in treating rheumatoid arthritis from market survey without considering type of formulation. Most repeated plants across the products were listed. And from this list seven plants are prioritised for further study that are having the capability to treat rheumatoid arthritis by conducting literature survey. For the selected plants literature survey is carried out and data collected for chemical constituent to apply Herb MaRS ranking to identify the potential chemical marker in standardizing herbal product. The selection criteria for the chemical marker includes evidence of biological activity, ease of treating or managing symptoms of RA, concentration of compound in plant and product, availability of standard and analytical method for the compound and toxicity of the compound [1].

Table 1

S.No	Product name	Indication	Vernacular name	Scientific name
1	Relief arthrite	Anti-rheumatoid arthritis	Kunduru	Boswelliaserrataroxb
			Kostasthasativa	Brachyura (crab shell)
			Draksha	Vitisviniferalinn
			Haridra	Curcuma longa linn
			Marica	Piper nigrumlinn
			Vetasa	Salix caprea
2	Rumalaya forte	Anti-rheumatoid arthritis	Gokshura	Tribulaterrestris
			Guduchi	Tinosporacordifolia
			Shallaki	Boswelliaserrata
			Guggulu	Commiphorawightii
			Rasna	Alpiniagalanga
			Yastimadhu	Glycyrrhizaglabra
			Nirgundi	Vitexnegundo
			Sunthi	Zingiberofficinale
3	Flexibility	Anti-rheumatoid arthritis	Motha	Cyperusrotundus
			Ashwagandha	Withaniasomnifera
			Rama tulasi	Ocimum sanctum
4	Rheumatill R	Anti-rheumatoid Arthritis	Prasarini	Paederiafoetida
			Salaiguggulu	Boswelliasarrata
5	Plant based joint care by simply herbal	Anti-rheumatoid Arthritis	Moringa	Moringaoleifera
			Eucalyptus	Eucalytusglobulus
			Burdock	Arctiumlappa
			Willow bark	Salix alba
			Drumstick	Moringaoleifera
			Green amla	Phyllanthsemblica

			Green tumeric	Curcuma longa
			Alfa alfa	Medicagosativa
			Aloevera	Aloe vera
6	Holo arthritis	Anti-rheumatoid Arthritis	Elemental calcuim	Calcium
			Elemental magnesium	Magnesium
			Elemental phosphorus	Phosphorus
			Nanocurcuminoids	Curcuma longa
			Vitamin D3	Cholecalciferol
			Vitamin K2 7	Menaquinone-7
7	Bio joint artho	Anti-rheumatoid Arthritis	Gokhru	Tribulusterrestries
			Bael	Aeglemarmelos
			Saptaparni	Alstoniascholeris
			Prikshanaparni	Urariapicta
			Sigru	Moringaolefera
			Punarnava	Bohreviadiffusa

Methods

1. Study Design and Data Collection Methods

By analysing the herbal product market in india we found seven poly herbal formulations. We find out mostly used are seven polyherbal formulation that are giving the anti rheumatoid activity. From this labels of this herbal products we identified plants in this products and from this identified plants list are given in the table (Table 1). In this plants we identify the most repeating plants through the seven herbal products and then we have performed literature survive from this plant matrial and they identified the potential compounds in this plants and scored those potential markers according to the Herb Mars scale.

Table 1

S.No	Product name	Indication	Vernacular name	Scientific name
1	Relief arthrite	Anti-rheumatoid arthritis	Kunduru	Boswellia serrata roxb
			Kostastha sativa	Brachyura (crab shell)
			Draksha	Vitis vinifera linn
			Haridra	Curcuma longa linn
			Marica	Piper nigrum linn
			Vetasa	Salix caprea
2	Rumalaya forte	Anti-rheumatoid arthritis	Gokshura	Tribula terrestris
			Guduchi	Tinospora cordifolia
			Shallaki	Boswellia serrata
			Guggulu	Commiphora wightii
			Rasna	Alpinia galanga
			Yastimadhu	Glycyrrhiza glabra
			Nirgundi	Vitex negundo
			Sunthi	Zingiber officinale
3	Flexibility	Anti-rheumatoid arthritis	Motha	Cyperus rotundus
			Ashwagandha	Withania somnifera
			Rama tulasi	Ocimum sanctum
4	Rheumatill R	Anti-rheumatoid Arthritis	Prasarini	Paederia foetida
			Salai guggulu	Boswellia sarrata
5	Plant based joint care by simply herbal	Anti-rheumatoid Arthritis	Moringa	Moringa oleifera
			Eucalyptus	Eucalytus globulus
			Burdock	Arctium lappa
			Willow bark	Salix alba
			Drumstick	Moringa oleifera
			Green amla	Phyllanths emblica
			Green 96teroida	Curcuma longa
			Alfa alfa	Medicago sativa
			Aloevera	Aloe vera
6	Holo arthritis	Anti-rheumatoid Arthritis	Elemental 96teroid	Calcium
			Elemental magnesium	Magnesium
			Elemental phosphorus	Phosphorus

			Nanocurcuminoids	Curcuma longa
			Vitamin D3	Chole calciferol
			Vitamin K2 7	Menaquinone-7
7	Bio joint artho	Anti-rheumatoid Arthritis	Gokhru	Tribulus terrestries
			Bael	Aegle marmelos
			Saptaparni	Alstonia scholeris
			Prikshanaparni	Uraria picta
			Sigru	Moringa olefera
			Punarnava	Bohrevia diffusa

2. Identification of Medical Plants and Their Evidence of Safety and Efficacy:

From the selected seven herbal products most repeating plants that with antirheumatic potential were selected for further study. Literature survey is conducted for the selected plants across search engines such as Pub med, Research gate, Google scholar, Web of sciences. Full length articles without any limitation related to year of publishing were considered.

Table 2

Plant used	Activity	Evidence of efficacy	Evidence of safety
Boswellia serrata roxb	Anti rheumatoid activity	Anti rheumatoid activity i.e in vivo and in vitro	The dose greater than the lethal dose i.e.5000mg kg (-1) may produce toxicity
Tribula terrestris	Anti rheumatoid activity	Anti rheumatoid activity i.e in vivo	The 97teroida terrestris exhibits the genotoxic effect particularly when administered in higher doses
Cyperus rotundus	Anti rheumatoid activity	Anti rheumatoid activity i.e in vivo	Animal toxicological evaluations,clinical trails and traditional use
Vitex negundo	Anti rheumatoid activity	Anti rheumatoid activity i.e in vivo	Similarly, vitex negundo was lead to severe toxic conditions at higher doses i.e. particularly hepatotoxic activities by overloading hepatic processing enzymes and creating an unsafe accumulation of toxic drug metabolites
Moringa oleifera	Anti rheumatoid activity	Anti rheumatoid activity i.e in vivo	Moringa oleifera consider to be non toxic that the dose exceeding 3000mg / kg of body weight should be avoided
Withania somnifera	Anti rheumatoid activity	No evidence	No evidence
Commiphora mukul	Anti rheumatoid activity	Anti rheumatoid activity i.e in vivo and in vitro	It promote the DNA intercalation and chromosomal aberrations due to toxicity

3. Identification of Potential Marker Compounds:

From these selected medicinal plants we have collected the literature survey, this literature survey reveals that the following compounds are giving the more prominent pharmacological activity at the molecular level.

Table 3

Plant name	Isolated chemicals	Activity	Concentration
Boswellia serrata roxb	Boswellic acids (β-boswellic acid, acetyl-β-boswellic acid [ABA], 11-keto-β-boswellic acid [KBA], acetyl-11-keto-β-boswellic acid [AKBA]	Anti inflammatory, anti-cancer, anti-rheumatoid arhtitis	1.25µg/ml
	Chitosan	Anti inflammatory, anti-cancer, anti-rheumatoid arhtitis	25.6Mv
	resveratrol	Anti inflammatory, anti-cancer, anti-rheumatoid arhtitis	50µM
Tribula terrestris	97teroidal saponins	Anti-inflammatory, anti-oxidant, anti-diabetes	250µg/ml
	tinosporasides	Anti-inflammatory, anti-oxidant, anti-diabetes	500µg/ml
	guggulsterones	Anti-inflammatory, anti-oxidant, anti-diabetes	500mg/kg
Cyperus rotundus	alpha-cyperone	Anti-inflammatory, anti-microbial, anti-fungal	0.75,1.5 0r 3µM
	withaferin A(withanolides)	Anti-inflammatory, anti-microbial, anti-fungal	300mg/kg
	tinosporasides (tinosporic acid)	Anti-inflammatory, anti-microbial, anti-fungal	500µg/ml

Vitex negundo	iridoid glycosides	Anti-inflammatory, anti-oxidant, anti-fungal	50µg/ml
	triterpenoids (taraxasterol)	Anti-inflammatory, anti-oxidant, anti-fungal	100mg/kg
Moringa oleifera	Isothiocyanates	Anti-inflammatory, anti-oxidant, anti-diabetes	375µg/ml
	1,8-cineole	Anti-inflammatory, anti-oxidant, anti-diabetes	400mg/kg
	Arctigenin	Anti-inflammatory, anti-oxidant, anti-diabetes	50µM
Withania somnifera	Mineral (Macromineral)	Not available	0.1mg/25µL
	Curcumin	Anti-inflammatory	25µg/ml
	withaferin A (withanolides)	Anti-inflammatory, anti-oxidant	20ng/ml
Commiphora mukul	Rutin	Anti-inflammatory, anti-rheumatoid arthritis, anti-obesity	15,30,45µg/kg
	Alkaloids	Anti-inflammatory, anti-rheumatoid arthritis, anti-obesity	5mg/ml
	Rhoifolin	Anti-inflammatory, anti-rheumatoid arthritis, anti-obesity	20mg/kg

4. Selection of the Most Suitable Markers Compounds for Product Standardization:

For the selected compounds scoring has given with the following criteria, compounds with Anti-Rheumtoid activity and having potential to treat are given with a score of 2. The compounds doesn't show activity or treating symptoms were 0. We are given score 3 for the compounds that are having > 50µg/g. Concentration between 5-50µg/g given with a score of 1 and compounds which are concentration < 5µg/g. The compound which doesn't having any significant concentration of plants were given with 0. The compounds having any analytical methods given with a score of 1. The compound which does not having existing analytical method will be given with a score of 0. The compounds having analytical methods like High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), Enzyme Linked Immunosorbent Assay (ELISA), Scanning Electron Microscopy (SEM), Thin Layer Chromatography (TLC), Gas Chromatography -Mass Spectroscopy (GC-MS), Mass Spectroscopy (MS) are given with a score of 1. The compounds which does not have analytical methods validation previously given with scored 0. Based on evaluation market sample standards we are given a score of 1or 2. If the standard available in the market with a score of 2. The compound which foedoes not having standard scaorscore as 0. By following the above criteria we have given the scoring to the identified chemical markemarkers compound which is listed in the table.

Table 4

Plant used	Isolated chemicals	Evidence of biological activity	Level of evidence of biological activity (Anti-PA-2, sympt-1, No-0)	Concentration of compound	Reference standard	Analytical 98teroi	Herbs MaRS score
Boswellia serrata roxb	Boswellic acids (β-boswellic acid, acetyl-β-boswellic acid [ABA], 11-keto-β-boswellic acid [KBA], acetyl-11-keto-β-boswellic acid [AKBA]	1	2	3	1	1	8
	Chitosan	1	1	3	1	1	7
	resveratrol	1	2	3	1	1	8
Tribula terrestris	98teroidal saponins	1	1	3	0	1	6
	tinosporasides	1	2	3	0	1	7
	guggulsterones	1	2	1	1	1	6
Cyperus rotundus	alpha-cyperone	1	2	1	0	1	5
	withaferin A(withanolides)	1	2	1	1	1	6
	tinosporasides (tinosporic acid)	1	2	1	0	1	5
Vitex negundo	iridoid glycosides	1	2	2	0	1	6
	triterpenoids (taraxasterol)	1	2	3	0	1	7
Moringa oleifera	Isothiocyanates	1	2	1	0	1	5
	1,8-cineole	1	2	3	1	1	8
	Arctigenin	1	2	2	0	1	6

Withania somnifera	Mineral (Macromineral)	1	1	3	1		7
	Curcumin	1	2	2	1	1	7
	withaferin A (withanolides)	1	1	1	1	1	5
Commiphora mukul	Rutin	1	2	2	0	1	6
	Alkaloids	1	1	1	1	1	5
	Rhoifolin	1	2	3	1	1	8

5. Availability of Analytical Methods:

Analysis of specific herbal products or raw material helps in identification of products, specification of raw materials, standardization of products and product stability. Analytical methods for identification and qualification of products. The analytical methods include High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), Enzyme Linked Immunosorbent Assay (ELISA), Scanning Electron Microscopy (SEM), Thin Layer Chromatography (TLC), Gas Chromatography -Mass Spectroscopy (GC-MS), Mass Spectroscopy (MS) were considered in ranking of markers.

Table 5

Plant name	Isolated chemicals	Commercial sources	Analytical methods
Boswellia serrata roxb	Boswellic acids (β-boswellic acid, acetyl-β-boswellic acid [ABA], 11-keto-β-boswellic acid [KBA], acetyl-11-keto-β-boswellic acid [AKBA]	Sigma-Aldrich	RP-HPLC
	Chitosan	Sigma-Aldrich	NMR
	resveratrol	Sigma-Aldrich	RH-HPLC
Tribula terrestris	steriodal saponins	Not available	UPLC-QTOF
	tinosporasides	Not available	HPLC
	guggulsterones	Sigma-Aldrich	HPTLC
Cyperus rotundus	alpha-cyperone	Not available	ELISA
	withaferin A(withanolides)	Sigma-Aldrich	SEM
	tinosporasides (tinosporic acid)	Not available	GC-MS
Vitex negundo	iridoid glycosides	Not available	HPLC
	triterpenoids (taraxasterol)	Not available	LC/MS
Moringa oleifera	Isothiocyanates	Not available	ELISA
	1,8-cineole	Sigma-Aldrich	GC-MS
	Arctigenin	Not available	ELISA
Withania somnifera	Mineral (Macromineral)	Sigma-Aldrich	RP-HPLC
	Curcumin	Sigma-Aldrich	HPTLC
	withaferin A (withanolides)	Sigma-Aldrich	UPHLC-MS
Commiphora mukul	Rutin	Not available	HPTLC
	Alkaloids	Sigma-Aldrich	HPLC
	Rhoifolin	Sigma-Aldrich	TLC

RESULTS

Identification of Common Raw Materials in Anti-Rheumatoid Products

Analysis of the product information from the seven herbal products listed in India that they contained a total of seven plant species as ingredients. All expect [Rheumatill R],had atleast four plants listed in as raw material [table 1] [1]. The plant material were ranked based on the number of times they appeared on the different product labels. Out of the 7 plants materials assessed, *Boswellia serrata roxb* was the most common [60-70percentage] [4], Followed by *Commiphora mukul* [50 percent] and T*ribulus terrestris, Cyperus rotundus, Vitex negundo, and Moringa oleifera* were joined third highest with percentage occurrence of 30 percent[table 1]. These plants materials were then selected further analysis and marker identification. Rest of the medicinal plants were mentioned in only one product and were not considered for further analysis [43].

Establishment of Evidence for Efficiency and Safety of Plant Products

Literature survey of seven medicinal plants of herbal products, gave evidence for the most of plants in support of their use for treating rheumatoid arthritis [44]. All the selected plants undergone through in-vivo studies but *Tribulus terrestris*, *Cyperus rotundus*, *Vitex negundo* and *Moringa oleifera* have extensive in-vivo studies whereas *Boswellia serrata* and *Commiphora* mukul undergo both in-vivo and invitro studies for anti-Rheumatoid arthritis. In all most common anti-

rheumatoid medicinal plants, among them is vitex negundo which widely proposed as most potent anti-rheumatoid medicinal plant [45]. Similarly, vitex negundo may leads to toxic condition at higher doses that is particularly hepatotoxic activities by over loading hepatic processing enzymes and creating an unsafe accumulation of toxic drug metabolites. The dose greater than the lethal dose of 2000 mg/kg may produce toxicity but genotoxicity and mutagenicity are not detected at the lethal dose. Increasing doses of *Vitex negundo* may affect the bio chemical markers of hepatotoxicity and renal toxicity. The *Tribulus terrestris* exhibits genotoxic effect when administered at higher doses, it may promote DNA intercalation and chromosomal aberrations due to toxicity. *Moringa oleifera* considered as non toxic at the lethal dose of 1585mg/kg, exceeding the above body weight of 2000mg/kg should be avoided [46].

Isolated Compounds from the Selected Plants and Their Evidence of Biological Activity and Toxicity

Several compounds with varying biological activities have been reported from selected medicinal plants [47]. Alkaloids are the main secondary metabolites in *Commifora mukul* having Guggulsterons, E-Guggulsterons, terpenes, terpenoids, in aqueous and organic acids reported as sum of the metabolites from the plants [48]. Common compounds reported from *Tribulus terrstris* having the isolated compounds of steroidal saponins, tinosporasides, guggulsterons. *Vitex negundo* having the iridoid glycosides, triterpenoids (taraxasterol). *Moringa oleifera* having the secondary metabolites of isothiocynates, 1,8-cineole, arctigenin. *Boswellia serrata roxb* having the boswellic acids contains β-boswellic acids, acetyl-11-keto-boswellic acids. *Cyperus rotundus* having alpha-cyperone is the present at the root of the plant as their primary metabolite. Most of the isolated compounds show anti-rheumatoid activity along with other activities like *Cyperus rotundus* show anti-pyretic activity and *Commifora mukul* shows the anti-obesity activity. It modulates thyroid function and fat metabolism to aid weight management. *Vitex negundo* acts as anti-pyretic, anti-microbial, anti-tumour, sedative and an analgesic which is beneficial for the management rheumatoid arthritis [49].

Available Analytical Methods Used

Advanced chromatographic and spectroscopic techniques coupled with mass spectroscopy were the main analytical methods used for the quantification of the metabolites from the plants. Approximately 1-10% of identified bioactive compounds from the selected plants had chromatographic and spectroscopic methods listed in pharmacopoeias. High Performance Liquid Chromatography is the most popular analytical technique which is used. HPLC was used for components of flavonoids, terpenoids, phenolic acids, coumarins. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) sub fraction, liquid- liquid partition, HPLC, UHPLC, gas chromatography, TLC and spectroscopic methods such as IR and NMR are the analytical methods used for the quantification of the anti-rheumatoid activity.

Thin Layer Chromatography (TLC) have the recent advances and changes in technology have significant improved resolution, sensitivity and reproducibility of technique. High Performance Thin Liquid Chromatography (HPTLC) is a fast separation technique and flexible enough to analyze a wide variety of samples. Ultra High Performance Liquid Chromatography (UHPLC). In comparison to HPLC, it gives reduce analysis time and have advantages then the conventional HPLC. Gas Chromatography used for the volatile oil extraction, sensitive detection, faster analysis and efficiency of the plant compound. The combination of spectroscopy and chromatographic techniques with high resolution techniques provide more useful information of structural compounds [50].

Analytical Reference Standards

Approximately 1-10% of the identified isolated compounds from the selected plants had reference standard available from the review (Table 5) with purity levels is low ranging from 0.01% to 0.5%.

DISCUSSION

Boswellia Serrata Roxb

Boswellia serrata roxb extract is used in complementary medicine to help manage joint inflammation and symptoms associated with rheumatoid arthritis, though standard clinical guidelines typically consider evidence of rheumatoid arthritis. BSE consists of boswellic acids such as 11-keto-beta-boswellic acid (KBA) and acetyl -KBA which affect the cellular defence system by alteration in the levels of different cytokines. Anti inflammatory, antioxidant, antipyretic, analgesic activities of boswelliaserrata has been described in earlier studies. antiinflammatory effect of BSE were further supported by our histopathologyical findings of the joints. BSE showed a marked reduction in cartilage disruption, fibro-osseous proliferation, pannus formation, vascular proliferation which are the hall mark of rheumatoid arthritis.boswelliaserrata reduces cellular infiltrates in carrageenan -induced inflammation model of rheumatoid arthritis. along with other symptoms, decrease in weight and also has been observed in rheumatoid arthritis. BSE treatment showed a significant reduction in levels of several inflammatory madiators including TNF-α [51].

Tribulus Terristris

Tribulus terristris used as a traditional medicine and has been demonstrated to be effective in the treatment of variety conditions, including cardiovascular and fungal diseases. These effects are supoby a previous study that revealed antioxidantidant, antiedema, anti inflammatory activities of tribulaterristris.

The current study investigated the protective effects of ETT on cartilage and it's molecular mechanisms of action in an MIA -induced OA rat model. Micro-CT has revolutionized the application of animal models in arthritis research. Furthermore advance in 3D imaging techniques such as micro-cT, has provided accurate, non invasive tool for measuring micro structure. therefore, thpresent study performed micro -CT to confirm the cartilage protective effects of ETT treatment. The present study demonstrated that the BV /TV and Tb.sp compared with joint treatment.in vitro results supports the therapeutic usefulness of ETT for development as the treatment for OA [52].

Moringa Oleifera

Moringa oliefera leaf extract is promising natural supplement that may help reduce joint swelling, lower inflammation, decrease disease activity in people with rheumatoid arthritis. Rheumatoid arthritis is a heterogeneous pathological condition associated with elevation of certain pro-inflammatory and inflammatory mediators including IL-1,IL-6, TnF-a, which if not suppressed, will increase inflammation of macrophages into the flamed site with excessive production of auto-antibodies.

Another important mechanism in the pathogenesis of rheumatoid arthritis is the increase in the production of free radicles by the activated macrophages and neutrophils. Anti-inflammatory and anti-oxidant of *Moringa oliefera* leaves extract indicated a significant inhibition of nitric oxide (NO) production by macrophages cells, decrease in serum levels of IL-6, TnF-α in addition to inhibition of COX-2 pathway by inhibition of PGE2 production. Especially in human subjects is very essential and highly recommended. More over the use of leaves offers a good option for a source management that in more abundant and easy to get all the year around [53].

CONCLUSION

The herbal marker in identification system was successfully applied to select secondary metabolites for the standardization of anti-rheumatoid herbal products. Out of 27 plants metabolites evaluated, 15 were identified as suitable markers for standardizing these products [43]. Among the most suitable chemical markers identified in the herbal materials used in anti- rheumatoid formulations were boswellic acids, 1,8-cineole, resveratrol and rhoifolin. These markers can be used to determine botanical identify, assess raw materials quality, ensure batch-to-batch consistency, and monitor product stability. Future research should focus on validating the efficacy and identifying the specific phytoconstituents of herbal materials used in herbal medicine production [5]. Limited phytochemical and biological evidence for certain plants and their constituents constrained the application of Herb MaRS criteria for the systematic selection of appropriate chemical markers [54].

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