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A Brief Review on Formulation and Evaluation of Ethosomal Gel for Arthritis with Herbal Extracts

Nabamita Basu[1]*, Narasimha Buragommula[1], Hephzibha B[1], Jagadeesh Induru[2]

***Assistant Professor, [1]Department of Pharmaceutics, Gokaraju Rangaraju College of Pharmacy, Hyderabad, Telangana - 500090**

[2]Associate Professor, Department of Pharmaceutics, Gokaraju Rangaraju College of Pharmacy, Hyderabad, Telangana - 500090

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***Corresponding Author Email:** sbasu151276@gmail.com

Abstract

The aim of this article is to gather information about Rheumatoid Arthritis (RA) and its consequences for various uses in medical field with herbal formulation mainly plants extracts. Now a days phytoconstituents from many plants are tremendously used in combination with chemical constituents to decrease its adverse effects and contraindications. Ethosomes drug delivery is one of the most challenging works in research to improve the skin penetration capacity of various drugs. The rate of absorption is high while promoting the drug through this delivery system. Much research is going on to improve the systemic aabsorption of drug through trans-dermal drug delivery system. Various plants extracts are also incorporated in ethosomal vehicle system to treat many types of disorders like Rheumatoid arthritis using gel formulation. Oral drug delivery causes lots of adverse effects and contraindications, hence, to reduce these, a mode of drug delivery by topical administration is effective in treating many disorders. Several plant's secondary metabolites are also playing vital role in curing many disorders and no side effects have been reported. Novel modified lipid carriers as ethosomes which have been suggested to have improved vesicular properties and skin penetration capacity.

Keywords

Ethosomes, Rheumatoid Arthritis (RA), Herbal extracts.

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INTRODUCTION:

Ethosomes are lipid-based carriers developed by Touitou et al. in 1997, that enhance drug delivery through the skin. They consist of ethanol, phospholipids, and water, and have been shown to improve skin penetration of various drugs [1]. The high ethanol concentration in ethosomes acts as a permeation enhancer, affecting the stratum corneum intercellular region, the skin's outermost layer. Ethosomes are characterized by their soft and malleable vesicle structure, allowing them to be easily absorbed into the skin. The size of ethosome vesicles can be adjusted from nanometres to microns, providing versatility in drug delivery. The presence of ethanol in high concentrations disrupts the organization of the skin lipid bilayer, enabling the vesicles to penetrate the stratum corneum effectively. This results in a less tightly packed lipid membrane, leading to improved drug distribution within the stratum corneum lipids. Studies have demonstrated the efficacy of ethosomes in delivering drugs to deep layers of the skin and even into systemic circulation. Ethosomes have also been shown to enhance chemical delivery in permeation experiments using fluorescent probes, with increased depth and activity compared to classic

liposomes or hydroethanolic solutions. Ethosomes are effective carriers for dermal and transdermal drug delivery, offering potential applications in pharmaceutical and cosmetic formulations [2].

ETHOSOMAL GEL MECHANISM OF ACTION:

The ethosomal drug delivery system involves a synergistic interaction between ethanol, vesicles, and skin lipids, enhancing the delivery of active ingredients. Ethanol interacts with lipid molecules in the stratum corneum, making it more permeable. The "Ethosome effect" allows ethosomes to penetrate and fuse with skin lipids, creating new pathways for drug penetration into deeper layers. Ethanol contributes to the soft and flexible characteristics of ethosomes, allowing them to penetrate more easily. The drug released from ethosomes can reach deeper layers of the skin and potentially be absorbed into systemic circulation. This mechanism provides a promising approach for improving transdermal drug delivery.

RHEUMATOID ARTHRITIS:

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation, synovial damage, cartilage damage, and joint deformities. The incidence rate of RA is approximately 1% of the total population, with a higher prevalence in females compared to males. RA can lead to severe disability and reduce life expectancy by 10 to 15 years. The exact pathogenesis of RA is not fully understood, but it involves a combination of physical and chemical properties, genetic factors, and environmental influences. The over-expression of autoantibodies, such as Rheumatoid Factors (RFs) and anti-citrullinated protein antibodies (ACPAs), play a significant role in the development of RA. These autoantibodies activate B cells, T cells, and macrophages, leading to the production of inflammatory factors, primarily tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1 (IL-1). The inflammatory factors released in RA contribute to the destruction of cartilage and bone. TNF-α stimulates the abnormal proliferation of fibroblast-like synoviocytes (FLS), resulting in the overexpression of cathepsin and matrix metalloproteinases (MMPs). This leads to the breakdown of collagen and proteoglycans, ultimately causing cartilage and bone destruction. IL-6 activates the Janus kinase (JAK)/activator of transcription (STAT) pathway through the glycoprotein p130 receptor, promoting inflammation. IL-1 and TNF-α activate the nuclear factor Kappa-B (NF-κB) pathway, leading to the upregulation of cyclooxygenase 2 (COX-2) and proinflammatory cytokines MMPs, which contribute to cartilage degeneration and synovial erosion.

Altogether, the pathogenesis of RA involves a complex interplay of genetic factors, environmental influences, and inflammatory processes mediated by various cytokines, leading to joint damage and functional impairment in affected individuals [3].

TREATMENT STRATEGIES:

Over the past decade, the treatment of rheumatoid arthritis (RA) has evolved, with NSAIDs being an effective therapy for early management. Disease-modifying antirheumatic drugs (DMARD), which inhibit cyclooxygenase activity and block prostanoids and eicosanoids, have shown potential benefits in early management. However, treatment options are limited, and side effects which include weight loss, diarrhoea, skin rash, and alopecia. Cytokine research has led to the development of anti-cytokine therapy, such as Etanercept and Infliximab, which bind with TNF-α and decrease its bioavailability. Gene therapy is also being explored, but its full potential remains unexplored [4] [5] [6].

ALTERNATIVE APPROACHES:

Alternative treatments like dietary modifications, nutritional supplements, and botanicals as well as polyherbals are being explored due to the limitations and risks of conventional therapy. These treatments vary in response and are generally free of side effects [6][7].

PREPARATION OF ETHOSOMAL GEL HERBAL FORMULATIONS:

The complex process of ethosomal gel herbal formulation involves the following critical steps or unit operations.

- Preparation of herbal extract
- Extraction of crude drugs
- Preliminary phytochemical screening of herbal plant extract
- Preparation of ethosomes
- Characterization of prepared herbal ethosomal gel.

PREPARATION OF HERBAL EXTRACT:

The leaves of vitex negundo and cardiospermum were processed to remove earthy matter and residual materials carefully from the leaves, then cleaned and shaded dried. Coarse powdered leaves of *cardiospermum Halicacabum* was extracted with methanol by cold maceration process for seventy-two hours. Coarse powdered leaves of vitex negundo was extracted with methanol by cold maceration process for seven days. Both the extracts were then

filtered and concentrated under reduced pressure in IKA Rotatory evaporator (Model No. RN 10 digital V, ILMAC Germany) at 40^{0}C and stored at 4-8^{0}C for further use [8]. Further, the specific extraction methods used for some crude drugs are given in Table 1. In addition, the preliminary phytochemical screening of herbal plant extract to find the probable category of constituents is depicted in Table 2.

Table 1: Extraction methods of crude drugs

S. No	Crude drug name	Method used	Reference
1	Annona montana	Maceration (polar extract), Soxhlet extraction method (non-polar extract)	[9]
2	Abrus precatorius	Soxhlet extraction method	[10]
3	Aristolochia bracteolata	Soxhlet extraction method	[11]
4	Alpinia conchigera	Aqueous extraction method, Super critical fluid extraction method	[12]
5	Boswellia serrata	Soxhlet extraction method	[13]
6	Borassus flabellifer	Centrifugation method, Fermentation method	[14]
7	Cardiospermum halicabum	Soxhlet extraction method	[15]
8	Commiphora mukul	Cold maceration	[16]
9	Calluna vulgaris	Super critical fluid extraction method	[17]
10	Cistus laurifolis	Maceration	[18]
11	Justicia gendarussa	Cold maceration	[19]
12	Moringa oleifera	Maceration by orbital shaker machine	[20]
13	Nyctanthes arbortristis	Steam distillation maceration	[21]
14	Premna serratifolia	Soxhlet extraction	[22]
15	Phyllantus emblica	Maceration	[23]
16	Vitex negundo	Microwave extraction, ultra sonification extraction, super critical fluid extraction	[24]
17	Vernonia anthelmintica	Soxhlet extraction method	[25]

Table 2: Preliminary phytochemical screening of herbal plant extract:

No	Phytochemicals	Test	Reference
1	Alkaloid	Dragondroff's test Mayers test	[26][27]
2	Flavonoids	Shinoda test	[26][27]
3	Carbohydrates	Fehlings test	[26][27]
4	Glycosides and Saponins	Lead acetate test	[26][27]
5	Tannins	Lead acetate test	[26][27]
6	Proteins	Ninhydrin test, Biuretic test	[26][27]
7	Quinone's	Sodium hydroxide test	[26][27]

METHODS OF PREPARATION OF ETHOSOMES:

COLD METHOD:

This method is widely used to prepare ethosomes. It was introduced in year 1996 by Touitou. In this method, initially phospholipid, herbal drug extract and other lipid material were dissolved in ethanol with vigorously stirring at room temperature in covered vessel, then propylene glycol at 40^{0}C during stirring heat mix up to 30^{0}C and water at 30^{0}C were added by drop wise/streamline/syringe pump. Then stirred for 5-30 min in covered vessel at speed of 700-2000 rpm by using overhead /magnetic stirrer to get ethosomal suspension. It was subjected to sonification or extrusion to get required particle size [28] [29].

HOT METHOD:

In this method aqueous and organic phases were prepared separately. The phospholipid was dispersed in water at 40^{0}C to form colloidal suspension. In another vessel ethanol was taken, propylene glycol was added to it at 40^{0}C. The organic phase was mixed with aqueous phase drop wisely. Plant extract was dissolved in suitable solvent depending on solubility and accordingly added. Thus, ethosomes are made [28] [30].

THIN-FILM HYDRATION METHOD:

This method involves the extension of the conventional liposome preparation technique using a unique approach. Instead of hydrating the lipid film with a purely aqueous solution, a hydroethanolic solution is used [14] [28][31]. The procedure is as follows:

- **Dissolution of Phospholipid**: The process begins by dissolving the phospholipid in chloroform. The chloroform can be used alone or in combination with methanol. The ratios for the chloroform-methanol mixture can be 3:1 or 2:1, depending on the specific experiment. This is done in a clean and dry round-bottom flask.
- **Evaporation of Organic Solvents**: The mixture containing the phospholipid is subjected to a rotary vacuum evaporator. This process removes the organic solvents (chloroform and methanol) at a temperature higher than the lipid's phase transition temperature. This step ensures the removal of residual solvents.
- **Removal of Solvent Traces**: Any remaining traces of solvents in the deposited lipid film were eliminated by keeping the lipid film under vacuum conditions overnight. This extended vacuum period ensures the complete removal of solvents.
- **Hydration with Hydroethanolic Solution**: The lipid film, now free from organic solvents, is hydrated using a solution composed of water and ethanol. Alternatively, a phosphate-buffered saline (PBS) solution can be used in combination with ethanol. The hydration process is conducted at a temperature suitable for the specific phospholipid's properties.
- **Rotating and Heating**: During hydration, the lipid film is both rotated and heated. The rotation aids in the uniform distribution of the lipid film in the hydroethanolic solution. The temperature for this step is determined by the nature of the phospholipid being used. The lipid film is maintained at this temperature for a designated period, which can vary. The hydration time could be 30 minutes, 1 hour, or even as long as 6 hours, depending on the experimental requirements.

THE REVERSE PHASE EVAPORATION METHOD:

This specialized method is designed for producing large unilamellar vesicles and involves several distinct steps:

- **Organic Phase Preparation:** The process begins by dissolving the phospholipid in diethyl ether, creating an organic phase. Diethyl ether serves as the solvent for the phospholipid.
- **Emulsion Formation:** The organic phase is mixed with an aqueous phase at a specific volume ratio of 3:1 (organic phase to aqueous phase) in an ultrasonic bath. This mixing results in the formation of a water-in-oil emulsion. The ultrasonic bath facilitates the emulsification process, where small water droplets become dispersed in the organic solvent due to the ultrasonic waves [28] [32].
- **Cooling:** The emulsion is maintained at a low temperature of 0°C during the ultrasonic mixing process. This cooling helps in controlling the emulsion formation and stabilizing the system.
- **Emulsion Stability:** The ultrasonic treatment for 5 minutes at 0°C contributes to the stability of the water-in-oil emulsion. It ensures that the water droplets are uniformly dispersed within the organic phase.
- **Solvent Removal:** Following the emulsification step, the organic solvent (diethyl ether) is removed from the emulsion under reduced pressure. This process typically involves using a vacuum to evaporate the solvent. As the solvent evaporates, a gel-like structure is formed due to the presence of the phospholipid.
- **Gel to Colloidal Dispersion Transition:** The gel resulting from the removal of the organic solvent gradually transforms into a colloidal dispersion with the application of vigorous mechanical agitation. This agitation breaks down the gel structure, allowing the vesicles to form in the aqueous phase. The transformation from gel to colloidal dispersion likely involves the phospholipid molecules reorganizing into bilayers to create vesicles.
- The reverse-phase evaporation method is an infrequently used technique designed for generating large unilamellar vesicles. It involves dissolving phospholipids in diethyl ether, creating an organic phase mixed with an aqueous phase in a 3:1 v/v ratio using ultrasonic agitation at 0°C for 5 minutes. This forms a water-in-oil emulsion, which transforms into a gel upon removal of the organic solvent under reduced pressure. With vigorous agitation, this gel becomes a colloidal dispersion.

TRNSMEMBRANE pH GRADIENT METHOD:

In the transmembrane pH-gradient method, a drug is "actively" loaded into the ethosomal system based on pH differences between the acidic interior of the internal phase and the basic exterior of the external phase. This method is suitable for water-soluble drugs with protonizable amine functions. The procedure involves three stages:

1. preparing a blank ethosomal system using an acidic buffer,
2. actively loading the drug by stirring it into the empty ethosomal suspension, and
3. inducing a pH gradient by adding a sodium hydroxide solution to establish a pH difference between the internal and external phases.

The ethosomal system is then incubated at 30°C–60°C, allowing the unionized drug to traverse the ethosomal vesicle bilayer and become entrapped [28] [33] [34].

Factors like drug properties, pH of phases, temperature, and incubation duration must be considered before preparing ethosomal systems using this method. This technique provides a unique way to actively load water-soluble drugs into ethosomal vesicles by leveraging pH gradients between internal and external phases, ultimately enhancing drug encapsulation efficiency.

ETHOSOMAL GEL:

Ethosome gel, also known as ethosome-based gel, is a specialized type of topical drug delivery system designed to improve the delivery of active substances, such as drugs or cosmetic ingredients, through the skin. Ethosomes are phospholipid-based nanocarriers that can encapsulate both hydrophilic and lipophilic substances, enhancing their penetration into the skin's deeper layers.

Ethosomal gels commonly made with gel-forming agents like Carbopol and hydroxypropyl methylcellulose, along with their various grades. These polymers are proven to be compatible with ethosomal systems, offering the necessary viscosity and bioadhesive characteristics. This compatibility ensures that ethosomal gels maintain their desired properties, facilitating controlled drug delivery and enhancing adherence to target sites. In general, ethosomal gels are assessed based on pH, viscosity, spreadability, and extrudability. [35] [36].

To summarize, the preparation of ethosomal gel involves several steps:

1. **Selection of Ingredients**: The formulation typically includes phospholipids (e.g., phosphatidylcholine), water, alcohol, and the active ingredient (drug or cosmetic compound) as shown in Table 3.

Table 3: Composition of ethosomes:

Chemicals	Examples	Uses	Reference
Phospholipids	Soyaphosphatidyl choline, Egg phosphatidyl choline, Dipalmityl phosphatidyl choline	Vesicle forming component	[7][36]
Polyglycols	Propylene glycol, transcutol	As a skin penetration enhancer	[7][36]
Alcohols	Ethanol Isopropyl alcohol	For providing the softness for vesicle membrane	[7][36]
Membrane modulator	Cholesterol	For providing the stability, fluidity, elasticity, and permeability to vesicle membrane	[7][36]

2. **Solvent Selection:** Ethosomal gels are often prepared using a mixture of ethanol and water as the solvent. The use of alcohol aids in the formation of ethosomes and ensures proper solubilisation of the active ingredient.
3. **Lipid Film Formation:** Initially, the phospholipids are dissolved in the alcohol-water mixture to form a thin lipid film. This film serves as the basic structure for the ethosomes.
4. **Hydration:** The lipid film is then hydrated with additional water, and the mixture is sonicated or subjected to other mechanical means to generate vesicles, which are small lipid-based spherical structures known as ethosomes.
5. **Incorporation of the Active Ingredient:** The active ingredient, whether hydrophilic or lipophilic, is added to the hydrated lipid film and mixed thoroughly to encapsulate it within the ethosomal vesicles.
6. **Gel Formation:** The ethosomal dispersion is then combined with suitable gelling agents to form the ethosomal gel. Common gelling agents include carbomer, xanthan gum, or other cellulose derivatives.
7. **Characterization and Quality Control:** The final ethosomal gel is subjected to various tests to ensure stability, consistency, and proper encapsulation of the active ingredient.

EVALUATION OF ETHOSOMAL GEL:

The evaluation of the ethosomal gel involved several tests to assess its physical properties, drug release characteristics, and therapeutic efficacy. Here is a summary of the evaluation methods and their purposes:

Spreadability: Spreadability of the gel was measured to determine how easily it could be spread on the skin. Gel is sandwiched between two slides, and a weight is placed on the upper slide for 5 minutes. The time taken for the two slides to separate was noted [8] [27].

Extrudability: Extrudability refers to how well the gel can be squeezed out of the tube. This test is performed using a lacquered aluminium collapsible tube. The amount of gel extruded through the tip when a constant weight is applied, is measured [8] [27].

Drug Content: Ethosomal suspensions are prepared in a 10 ml flask, shaken, and suitable dilutions made. Drug concentration is analysed using a UV spectrophotometer or suitable method [8].

***In vitro* Drug Diffusion**: This test evaluated the release and diffusion of the drug from the ethosomal gel. A sample of the gel was placed over a semipermeable membrane, which was immersed in a pH 7.4 phosphate buffer solution. The setup is placed on a magnetic stirrer at 37°C. Samples are withdrawn at specific time intervals for six hours, and the absorbance was measured at wavelength maxima [27].

***In vitro* Anti-arthritic Activity**: The ethosomal gel's potential anti-arthritic activity is assessed using the protein denaturation method. Test solutions are prepared with bovine serum albumin and pH adjusted to 6.3. The samples are incubated at 37°C and heated at 57°C. After cooling, phosphate buffer (pH 6.3) is added. The percentage inhibition of protein denaturation is calculated by comparing the optical density (OD) of the test solution to that of the test control (distilled water with bovine serum albumin). The result is then compared with diclofenac sodium, a known anti-arthritis drug [8] [27].

Rheological Studies: The viscosity of the ethosomal formulation is found using Brookfield viscometer or suitable Rheometer.

Other evaluation tests like pH determination, skin irritation test, histopathological tests to know Acute Dissemenated Encephalomyelitis (ADEM) of herbal drug and bioavailability, formulation appearance and homogeneity were also performed. These tests provide valuable information on the physical characteristics of the gel, the release profile of the active ingredient, and its potential therapeutic efficacy for the treatment of arthritis [8] [27]. There are some modern equipment and analytical tools are being used for formulation and evaluation. Some herbal drugs used to treat Rheumatoid arthritis are shown in Table 4 for ready reference. Some past research work and patented literature on rheumatoid arthritis is presented in Table 5.

MODERN EQUIPMENT AND ANALYTICAL TOOLS

Some modern equipment and analytical tools are used to evaluate the physicochemical characterisation of ethosomal gel formulation [8][27][35], which include:

- The characteristics of the formulated ethosomes are determined using dynamic light scattering (DLS) with a Malvern Zeta Sizer Nano ZS instrument at room temperature. The ethosomal formulations are dispersed in HPLC water, and the DLS instrument was set at a 90° angle with a medium viscosity of 0.8862 cps and a refractive index of 1.361. The following parameters are measured in triplicates.
- Particle Size Distribution: DLS was used to determine the average size of the ethosomes, which gives an indication of their overall size range and distribution.
- Polydispersity Index (PDI): The PDI is a measure of the width of the particle size distribution. A lower PDI value indicates a more uniform size distribution, while a higher value suggests a broader range of particle sizes.
- Zeta Potential: The zeta potential provides information about the surface charge of the ethosomes. It indicates the stability of the particles; higher absolute zeta potential values usually indicate better stability due to increased repulsion between particles. All measurements were performed in triplicates to ensure accuracy and consistency of the results.
- Thermo gravimetric analysis (TGA): TGA was performed to measure the change in mass of the sample with respect to temperature change.
- Study of interaction between the drug and the excipients using FTIR spectroscopy
- Study of surface morphology of vesicles by Scanning Electron Microscope (SEM)
- Entrapment efficiency: It is an important parameter that assesses the delivery potentiality of a system.

  % drug entrapped = [(W –w) /W] ×100.

  Where 'W' is total drug content and 'w' is free drug content.
- Lipid to ethanol ratio: The differences in the flux values among the formulations are attributed to variations in the concentration of lipid to ethanol ratio used in each formulation. Optimizing the lipid and ethanol content is crucial to achieving enhanced flux and efficient drug delivery through the skin.

Table 4: Herbal drugs used to treat Rheumatoid arthritis.

BIOLOGICAL SOURCE	PARTS USED	ACTIVE INGREDIENTS	USES	EXTRACT SOLVENT	REFERENCE
Annona montana	Leaves, fruits, seeds, bark, roots	Cyclomontanins A-D. Annomuricatin C, ((+)-Corytuberine	Antirheumatic, antiviral, astringent cardiodepressant	Methanol	[37]
Abrus precatorius	Fresh leaves	Triterpenoids (abrusosides A-D)	Treat cold, cough, convulsion, fever, rheumatism, conjuctives	Methanol	[38]
Aristolochia bracteolata	Whole plant	Ceryl alcohol, β-sitosterol, alkaloid myristic, aristolochinic acid, aristolactam magnoflorine.	Treat for fever, purgative, anthelmintic painful joints	Petroleum ether, chloroform, methanol	[39]
Alpinia conchigera	Rhizome	Galangoflavanoid, cis-1-acetoxyehavicol acetate, β-sitosterol diglucoside, β-sitsteryl arabinoside	Analgesic & anti-inflammatory	ethanol	[40]
Boswellia serrata	Oleogum resin	Pentacyclic triterpenoids, bosuellic acids	Cancer, inflammation, arthritis, asthma.	Petroleum ether	[41]
Borassus flabellifer	Male flower	Alkaloids, terpenoid, spirostance type steroid saponin & phenolic compounds	Anti-inflammatory, immunosuppressant.	Ethanol	[42]
Cardiospermum halicabum	Leaves	Saponins, alkaloids, (+)-pinitol, apigenium, luteolin and chrysoeriol.	Rheumatoid arthritis, Anti-inflammatory activity	methanol	[43]
Commiphora mukul	Stem	Guggusterone and gugulipid	Arthritis, obesity, & other disorders	Ethyl acetate	[44]
Calluna vulgaris	Aerial parts	Flavonol derivatives	Anti-inflammatory, anti-rheumatic, urinary tract infections	Ethyl acetate	[45]
Cistus laurifolius	Leaves	flavonoids	Inflammations include rheumatism	Ethyl acetate	[46]
Justicia gendarussa	Leaves	Sterols & flavonoids	Respiratory disorders, rheumatic arthritis.	Ethanol	[47]
Moringa oleifera	Flowers, leaves	Nitrile glycosides, campisterol	Anti-arthritis drug	Hydroalcoholic extracts	[48]
Nyctanthes arbortristis	Leaves & stems	B-sitosterol, nyctathoside.	Sciantica, Artritis and anti-inflammatory	Alcoholic extract	[49]
Premna serratifolia	Wood	Iridoid gilcosides, alakaloids phenolic compounds	Treat inflammatory, arthritis, rheumatism anorexia &jaundice	Ethanol	[50]

Phyllantus embica Syn embillica officinalis	Leaves, bark, or fruit	Flavonoids, ellagic acid, Gallic acid & vit-C	Osteoarthritis, rheumatoid arthritis	Water	[51]
Vitex negundo	Leaves	C-glycoside, casticin, essential oil, vitamin c, benzoic acid, flavone	Rheumatoid arthritis, antiseptic, ophthalmic, anti-gonorrhoeic, depurative, anti-inflammatory	Ethanol and water	[52]
Vernonia anthelmintica	Seeds, dried seeds	Alkaloids, steroids	Anti-inflammatory, anti-arthritic	ethanol	[53]

Table 5: Some past research work on rheumatoid arthritis [54][55].

S.No:	Title	Identifier
1	Treatment of pain with topical diclofenac	US10058519B2
2	Topical formulation comprising Comfrey and Tannic acid, and uses thereof	CA2669918C
3	Transdermal formulations of cannabidiol comprising a penetration enhancer and methods of using the same	CA2760460C
4	Compositions for the treatment of rheumatoid arthritis and methods of using same	AU2014352801B2
5	A Phase 2b Study to Evaluate the Efficacy and Safety of Mavrilimumab in Subjects with Moderate-to-Severe Rheumatoid Arthritis	2011–005634–19
6	A clinical study on Meganathi kuligai for UthiraVatha suronitham (Rheumatoid Arthritis)	CTRI/2020/03/ 024153
7	A Clinical Trial to study the Safety, Tolerability and Effect of Tocilizumab in Patients with Active Rheumatoid Arthritis taking Background Non-biologic DMARDs and having an Inadequate Response to Current Non-biologic DMARD and/or Anti-TNF Therapy	CTRI/2009/091/ 000402
8	The effect of corticotrophin (ACTH) in combination with methotrexate in newly diagnosed rheumatoid arthritis patients	NCT01948388
9	Study to assess the safety and efficacy of Enbrel administered by Sofusa Dose Connect for Rheumatoid Arthritis	NCT04559412
10	Curcumin for the Management of Periodontitis and Early Rheumatoid Arthritis: Killing Two Birds with One Stone	CTRI/2020/11/ 029047

CONCLUSION

In conclusion, the preparation and evaluation of ethosomal herbal gel formulation is complex phenomena, but having wide applications and hence the present article could be focused on Rheumatoid Arthritis treatment purpose. The same concepts can be extended to other disease conditions and delivery systems with suitable changes. The same approach can be a study of interest for targeted drug delivery also with additional features introduced for selectivity and specificity.

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