WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 1, 849-870 Research Article ISSN 2278 – 4357

STUDY OF POLYHERBAL ETHOSOMAL GEL FOR GOUTY LIKE ARTHRITIS

Varsha K.* and Arun Kumar K. V.

Kerala University of Health Sciences, Management and Principal of Rajiv Gandhi Institute of Pharmacy, Trikaripur, Kasaragod (DIST), Kerala.

Article Received on
03 Nov. 2018,

Revised on 24 Nov. 2018,
Accepted on 14 Dec. 2018,

DOI: 10.20959/wjpps20191-12870

***Corresponding Author**
Varsha K.
Kerala University of Health Sciences, Management and Principal of Rajiv Gandhi Institute of Pharmacy, Trikaripur, Kasaragod (DIST), Kerala.

ABSTRACT

This work aim to develop a poly herbal ethosomal gel for the management of gouty like arthritis. Gouty arthritis is a sudden, painful attack of joint inflammation due to over production and deposition of uric acid crystals in the joints. This acid can form needle-like crystals in a joint and cause of a sudden onset of a painful, hot, red, swollen joint particularly in the first metatarsophalangeal joint. Ethosomes are novel vesicular carriers that offer better skin penetration and enhanced therapeutic response. *Rubia cordifolia* Linn and *Tinospora cordifolia* Wild are two important herbal drugs with potent anti-gouty arthritic activity. The extracts of these two plants are incorporated into ethosomal vesicles which are prepared by the method developed by Touitou *et al.* Four different formulations were developed by varying the concentration of soya lecithin and ethanol. The prepared ethosome formulations were optimized by analyzing the morphology of the system by SEM analysis, from the particle size determination and zeta potential determination using Malvern zeta sizer nano essential and from the drug release profile obtained from free radical scavenging assay. The ethosomal formulation F4 containing 1g soyalecithin and 20ml ethanol was found to be optimized. The optimized ethosomal dispersion is incorporated into carbopol based gel. This ethosomal gel is then evaluated in terms of physical appearance, pH, spreadability, viscosity, swelling index, homogeneity, extrudability, washability and stability. The pH of the gel system was found to be compatible with skin pH. It shows good flow properties and spreadability that is essential for an ideal gel. The drug loaded ethosomal gel is subjected to animal study using MSU induced gout air pouch model to evaluate the anti gouty arthritic potential of the formulation and it was compared with standard diclofenac gel that is generally used to treat the

inflammation associated with gouty like arthritis. Stability studies indicated that the formulation was stable over a period of 3 months at 25±2^{0}c and 2-8±3^{0}c

KEYWORDS: Gouty arthritis; ethosomes; drug extract; inflammation; air pouch.

INTRODUCTION

Gouty arthritis[1,2] is a type of inflammatory arthritis with characteristic symptoms such as swollen joints, redness, tophi, joint, warmth and tenderness of the joint especially in the metatarsal phalangeal joint, connective tissue and parenchymal organs including kidney. It is caused by the accumulation of uric acid crystals in the joints. The etiological factors for the development of gouty arthritis include hyperuricaemia, genetic factors, dietary factors, alcohol consumption, metabolic syndrome, hypertension, obesity and long-term use of various medication. The management of gouty arthritis mainly focus to lower down the serum urate level. The treatments available today such as NSAIDs, corticosteroids, xanthine oxidase inhibitor etc. offer a better quality of life for gout sufferers. But when these synthetic drugs are used topically, the patients may have severe side effects such as rashes, blistering of skin and other allergic reactions. Other adverse reactions caused by these drugs include stomach ulcer, heart burn, wheezing, sore throat, liver or kidney problems, pain and bleeding when urinating etc. Hence an attempt was made to formulate a novel based herbal analgesic anti-inflammatory gel for gouty arthritis which is effective interms of safety and control inflammation and pain better than the chemical based analgesic anti-inflammatory gel. The incorporation of phytoconstituents into nanotechnology will offer a sustained delivery of components and hence avoid repeated administration and increases the patient compliance by reducing the adverse effects and toxicity of the drugs. The aim of the present research work was to develop an effective and safe formulation for gouty arthritis that will eliminate the harmful side effects of synthetic drugs. Through the literature survey on Rubia cordifolia and Tinospora cordifolia were selected based on their anti-gouty arthritic activity for the formulation. Rubia cordifolia is commonly called manjetti. The roots of the plant Rubia cordifolia is reported to be active against gouty arthritis based on the traditional information from Ayurveda. The various active principles the manjishta root reduces the uric acid level in blood, act as a blood purifier and also reduces the edema.[3,4] Tinospoa cordiolia[5,6] is a well-known herb, commonly called as chittamrithu. The leaves the plant found to be very effective in curing the gouty arthritic condition. It is anti-inflammatory, anti-oxidant drug. Many

research works have been conducted to establish the use of chittamrithu for gout and rheumatic conditions.

This research work is mainly focusing to develop an ethosomal gel loaded with herbal extracts for the management of inflammation associated with gouty like arthritis. Ethosomes[7] are nano sized drug delivery systems with enhanced skin penetration and targeted and controlled drug release compared to other vesicular systems. The leaf extract of *Tinospora cordifolia* and root extract of *Rubia cordifolia* was incorporated into the ethosomes which will protect the encapsulated drug from degradation and also enhances the penetration power. This drug loaded ethosome is then incorporated into a carbopol based gel in order to develop safe and effective herbal remedy for gouty arthritis.

MATERIALS AND METHODS

Collection of plants

The roots of Rubia cordifolia (Linn) and leaves of Tinospora cordifolia (Wild) were purchased from Prasad pharmacy who is a regional vendor in Kasargod district, Kerala (India) in the month of October 2017. The plant materials were identified and authenticated by Dr. A Rajagopalan, Professor Department of Horticulture, Padannakad College of Agriculture, Padannakad, Kasargod, Kerala.

Preparation of plant extracts[8]

The plant materials were thoroughly washed under running tap water, which was air dried at room temperature for 30-45 days. These dried plant materials were then milled to fine coarse powder by an electronic blender and after that kept in hermetically sealed compartments until further use. The process of extraction is carried out using solvents such as ethanol, chloroform, petroleum ether and water. The solvent selection is based on the polarity. 10gm of coarsely powdered dried roots of *R.cordifolia* and dried leaves of *T. cordifolia* were macerated in different conical flasks containing 100 ml of ethanol, chloroform, oil ether and waterand kept for overnight with occasional agitation. The extract from each sample (ethanol, chloroform oil ether and water) was separated through Whatman No: 1 filter paper and evaporated to dryness. The so formed dried extract is then utilized to identify different naturally occurring phyto constituents came out into the various solvents by the standard procedures.

Preliminary Phytochemical Screening[9]

The phytochemical screening of the root extract of *Rubia cordifolia* andleaf extract of *Tinospora cordifolia* was performed as per the standard procedures.

Extraction of plant materials[10]

The collected plant parts of *R.cordifolia* (root), *T.cordifolia* (leaves) were shade dried at room temperature and coarsely powdered. All these drugs were individually extracted by continuous hot extraction (soxhlation) using 95% ethanol in soxhlet apparatus. The process goes on 2-4 days until the solvent present in siphon tube becomes colorless. Ethanol retained within the extract can be recovered by distillation process and was then air dried and concentrated. This concentrated extracts of the three different drugs were then subjected to different formulations.

Preformulation study

Drug - excipient compatibility studies by FT-IR

The compatibility studies were performed to detect the existence of any sort of interaction between the active constituents and the excipients utilized in the formulation of gel system. Fourier-transform infrared spectra were obtained by using JASCO FT-IR 4700 L spectrometer. The dried pure drug sample was grounded and then mixed thoroughly with KBr. The KBr powder was used as blank for background correction in FT-IR studies.

Preparation of drug loaded ethosomes[11,12]

Composition of developed ethosome

Formulation Code	Tinospora Cordifolia (mg)	Rubia Cordifolia (mg)	Lecithin (g)	Ethanol (mL)	Propylene Glycol (mL)	Water (mL)
F1	150	150	0.5	10	5	25
F2	150	150	1	20	5	25
F3	150	150	0.5	10	5	25
F4	150	150	1	20	5	25

Preparation of Ethosomes[13]

Ethosomes were set up by the technique created by Touitou *et al*. Four formulas were produced by varying the concentration of soyalecithin and ethanol. The ethosomal prepared here comprised of 1-4% phospholipids, 10-50% ethanol, 10% propylene glycol, drug and water to 100% w/w. Phospholipid and drug were dissolved in ethanol-propylene glycol mixture. The mixture was heated to 30ºC in a water bath. The double distilled water heated to

30ºC was added slowly in a fine stream with constant mixing (Mechanical stirrer, Remi Equipment, Mumbai) at 700 rpm in a closed vessel. Mixing was continued for an additional 5 min. The system was kept at 30ºC throughout the preparation. The preparation was sonicated at 4ºC using probe sonicator (at 40 W, Imeco, Ultrasonics, India) in 3 cycles of 5 minutes with 5 minutes rest between the cycles.

Preparation of ethosomal gel[14,15]

The optimized Ethosomal formulation was used for the preparation of 1% carbopol gel. The required amount of carbopol 934 (1% W/W) was weighed and slowly sprinkled into a 500ml beaker containing distilled water with continuous stirring using mechanical stirrer (at the minimum speed to avoid entrapment of air) to get a transparent dispersion. After complete homogenization of the carbopol polymer with distilled water prepared ethosomal dispersion is added into the above mixture slowly with continuous stirring followed by addition of 10% propylene glycol. Required amount of preservatives were taken in a beaker and is dissolved by heating it over a water bath and is then added to the above mixture. The above dispersion is then neutralized using triethanolamine with continuous stirring for adjusting the skin pH (6.8-7) and to obtain a gel at required consistency.

Evaluation[15-20]

1. Vesicle size of Ethosomes

The average particle size of the ethosomes was measured using dynamic light scattering with a computerized Malvern zeta sizer nano essential.

2. Vesicle shape of Ethosomes

The shape and morphological characteristics of optimized ethosomes were obtained from SEM photographs. The morphology of prepared drug loaded ethosomes can be well studied by the SEM analysis. The formulations were placed into circular aluminium stubs using double adhesive carbon tape and coated with gold in Hitachi ion sputter E-1010 vaccum evaporator. Then it was observed in Hitachi SU6600 FE SEM (Field Emission Scanning Electron Microscope) having acceleration voltage of 10.0Kv and magnification of 50.0k.

3. Zeta potential

Zeta potential was determined using Malvern zetasizer nano essential. Zeta potential of the ethosomal formulation is related to the stability of ethosomal vesicle. The high value of zeta

potential indicating the high degree of repulsion between the vesicle and excellent stability i.e the dispersion will resist aggregation.

A. Evaluation of ethosomal gel[15,21,22]

Physiochemical Evaluation

- **Physical examination**

The prepared herbal ethosomal gel was procedure evaluated for their colour, odour and transparency.

- **pH**

2.5gm of gel was accurately weighed and dispersed in 25ml of distilled water and stored for two hours. The measurement of pH of each formulation was carried out in triplicate and the average values are represented. The pH of dispersions was measured using pH meter. pHmeter was calibrated using buffers of pH4 and pH7 prior to measurements.

- **Spreadability**

Test formulations 0.5 gm each were placed with in a circle of 1 cm diameter pre marked on a glass plate over which a second glass plate was placed. A weight of 5 gm was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to spreadability of the formulation was noted.

- **Viscosity**

Viscosity is a rheological parameter for semisolid dosage forms. Viscosity of the gel was determined using Brookfield viscometer (DV-1 programmable rheometer) at 6 rpm and at 25^0c. 200 g of the gel was taken in a beaker and spindle was dipped in it for about 5 minutes and then the reading was taken.

- **Swelling Index**

Swelling of the polymer depends on the concentration of the polymer, ionic strength and the presence of water. To determine the swelling index of prepared topical gel, 1 gm of gel was taken on porous aluminum foil and then placed separately in a 50 ml beaker containing 10 ml 0.1N NaoH. Then samples were removed from beakers at different time intervals and put it on dry place for some time after it reweighed. Swelling index was calculated as follows:

$$\text{Swelling Index (SW) \%} = \frac{Wt - W0}{W0} * 100$$

Where, (SW) % = Equilibrium percent swelling, Wt = Weight of swollen gel after time t, Wo = Original weight of gel at zero time.

- **Homogenicity**

Developed ethoosomal gel was tested for homogenicity by visual inspection after the gel has been set in the container. This was tested for their appearance and presence of any aggregates.

- **Extrudability studies**

The gel formulation were filled in standard capped collapsible aluminium tubes and sealed by crimping to the end. The weight of tubes were recorded and the tubes were placed between two glass slides and were clamped. 500gm were placed over the slides and then the cap was removed. The amount of extruded gel was collected and weighed. The percent of extruded gel was calculated as:

When it is greater than 90% then the extrudability is excellent.

When it is greater than 80% then the extrudability is good.

When it is 70% then the extrudability is fair.

- **Washability**

The product was applied on hand and was observed under running water.

- ***In-vitro* drug release from hydrogen peroxide free radical scavenging assay[23,24]**

Hydrogen peroxide assay

A solution of hydrogen peroxide (20 mM) was prepared in PBS (pH 7.4). Various concentrations of 1 ml of the samples or standards in methanol were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against blank solution that contained extracts in PBS without hydrogen peroxide. IC50 value is the concentration of the sample required to scavenge 50% free radical. The above experiments were performed (in triplicate) and the percentage inhibition was calculated using the following formula:

$$\%\text{scavenged } [H_2O_2] = [(A_0 - A_1)/A_0] \times 100$$

Where, A_0 -the absorbance of the standard (ascorbic acid)

A_1 was the absorbance of samples.

B. In vivo animal activity

a. Skin irritation study[25]

Hair on the back of each rabbit in a territory of roughly 10 × 15 cm was shaved without harming the skin 24 h before testing. 0.5 g ethosomal gels were applied to the shaved zone and secured with a dressing patch held by a wrap. The fix was evacuated 24 h later and the skin cleaned utilizing dressing absorbed warm water. The skin reaction was determined as per OECD rules at 1, 24, 48 and 72 h. The mean scores at 24, 48 and 72 h were utilized to acquire the primary irritation indice

b. MSU induced gout air pouch model[26,27,28]

To perform the biological evaluation of the ethosomal formulation, the monosodium urate crystals were synthesized by an established procedure. The crystals were suspended in PBSat a concentration of 10mg/ml. The crystals were sonicated to get rod shaped crystals and sterilized by autoclaving at 121^0C for 30 minutes.

The subcutaneous air pouch model was used for the evaluation of anti-gout activity. The rats were anesthetized, the dorsal area was shaved, and 10 ml sterile air was injected subcutaneously. Sterile air was injected in air pouch every 2 days to maintain pseudo gout conditions. After 6 days rats were randomly divided into 3groups. Then the rats are injected with 10ml MSU (1mg/ml) into the subcutaneous air pouch.

- Group 1 is taken as normal.
- Group 2 is given with standard formulation
- Group 3 is given with test formulation

After 24 hours of MSU administration the treatment was given to group 2 and 3.

Evaluation of anti-gout activity[28]

The rats are sacrificed and the following parameters are evaluated.

- **Measurement of exudate volume of air pouch and leukocyte count**

The pouch exudate was collected from all groups. The exudate volume was measured immediately after collection. Inflammatory exudate harvested from each animal was placed into heparinized saline. An aliquot of the dilute exudate was used to count leukocyte.

- **Histopathological studies**

The skin from air pouch was excised and immediately immersed in 10% buffered formalin, dehydrated in graded concentrations of ethanol, immersed in xylene, and embedded in paraffin. The 5µm thick sections of skin were cut by microtome and mounted on slides using commercial Baker's mounting fluid. The paraffin wax was removed by warming the slide gently until the wax melted and then washed with xylene followed by washing with absolute alcohol and water. The sections were stained with hematoxylene-eosin to determine histopathology. The slides were analyzed at 100 fold magnification by optical microscope.

C. Stability study of optimized formulation

The stability study of formulated gel was conducted as per the ICH guidelines. The optimized herbal ethosomal gel was sealed in amber colored bottles with cap covered by aluminium foil and these packed formulations was stored in different temperature viz a) room temperature(R.T) b) 2^0C-8^0C±3^0C. The formulation was checked for appearance, pH, and viscosity before and after the stability studies.

RESULTS AND DISCUSSIONS

Authentication ofcollected plantmaterials

The collected plant materials were identified and authenticated by Dr.ARajagopalan, Professor, Department of Horticulture, College of Agriculture, Padannakkad, Kasaragod.

Physico – chemical parameter

After the collection of plant materials, they were shade dried and powdered coarsely in an electronic blender and stored in air- tight containers until further use. Physico – chemical parameters of both plants were tabulated in Table 1. Parameters such as ash values, extractive values and moisture content were estimated.

Table 1: Physico-chemical parameters of Rubia cordifolia and Tinospora cordifolia.

Sl. No	TEST	Rubia cordifolia	Tinospora cordifolia
1	Total ash(%w/w)	7.97667±0.055(NMT 10%w/w)	6.42667±0.097 (NMT 10%w/w)
2	Acid insoluble ash(%w/w)	4.27667 ±0.24 (NMT 5%w/w)	1.9166±0.015 (NMT 3%w/w)
3	Water soluble ash(%w/w)	1.069±0.114 (NMT 5%w/w)	0.6266±0.00513 (NMT3%w/w)
4	Water soluble extractive value(%w/w)	20.22667±0.335 (NLT 10%w/w)	6.87±0.02 (NLT 2%w/w)
5	Alcohol soluble extractive value(%w/w)	35.35±0.173 (NLT 10%w/w)	12.549±0.0108 (NLT 10 %w/w)
6	Moisture content(%w/w)	13.8133 ±0.315 (NLT 10%w/w)	11.84±0.65818 (NLT 8%w/w)

All values are expressed in % w/w

Preliminary phytochemical screening

Standard procedures were followed inorder to determine the therapeutically active constituents present in the extracts and the results obtained were mentioned in Table 2&3.

Phytochemical test on various extracts of *Rubia cordifolia* root powder

Phytochemical Test	Ethanolic extract	Chloroform extract	Petroleum ether extract	Aqueous extract
Alkaloid	+++	+	+++	++
Flavonoid	+	-	-	-
Anthraquinone	+++	+	+	+
Glycosides	-	-	-	+
Tannins	+++	-	+++	+++
Carbohydrate	++	+	+++	++
Saponin	+	-	-	-
Phenol	++ +	+	+	++ +
Steroid	+	-	+	-
Terpenoid	++	+	+	+

(+++) highly present, (++) moderately present, (+) present, (-) absent

Table 3 : Phytochemical test on various extracts of *Tinospora cordifolia* leaf powder.

Phytochemical Test	Ethanolic Extract	Chloroform Extract	Petroleum ether extract	Aqueous Extract
Alkaloid	+++	+	+	+
Flavonoid	+	+	+	+
Anthraquinone	+	+	+	+
Glycosides	+	+	+	-
Tannins	++	-	-	-
Polysaccharide	+++	++	++	+++
Saponin	+	+	+	+
Phenol	+	+	+	+
Steroid	+++	+	+	+
Terpenoid	+++	+	++	+++

(+++) highly present, (++) moderately present, (+) present, (-) absent

Extraction of plant materials

The extraction of dried roots of Rubia cordifolia and dried leaves of Tinospora cordifolia were carried out by continous hot Soxhlet extraction process by using ethanol as solvent. The extracts obtained were collected and concentrated which was then weighed and kept in a desiccator until it was used for further studies. The yield so obtained was shown in Table 4.

Table 4: Percentage yield of the extracts.

Sl. No	Plants	Solvent	Weight of dry powder (g)	Weight of dry extract (g)	Percentage Yield (% w/w)
1	R.cordifolia	Ethanol	50	12.3	24.6
2	T.cordifolia	Ethanol	50	12.09	23.62

Table 5: Physical characteristics of the extracts.

Sl. No	Extract	Colour	Odour	Consistency
1	R. cordifolia	Brick red	Characteristic	Thicksemisolid
2	T. cordifolia	Darkgreen	Characteristic	Thicksemisolid

Preformulation study

Drug-Excipient Compatibility Studies by FT-IR

The compatibility studies were carried out to determine the chemical interaction of the drug with the excipients used in the formulation. Fourier-transform infra red spectra were obtained by using JASCOFT-IR4700 L spectrometer. The FT-IRresults obtained are shown below.

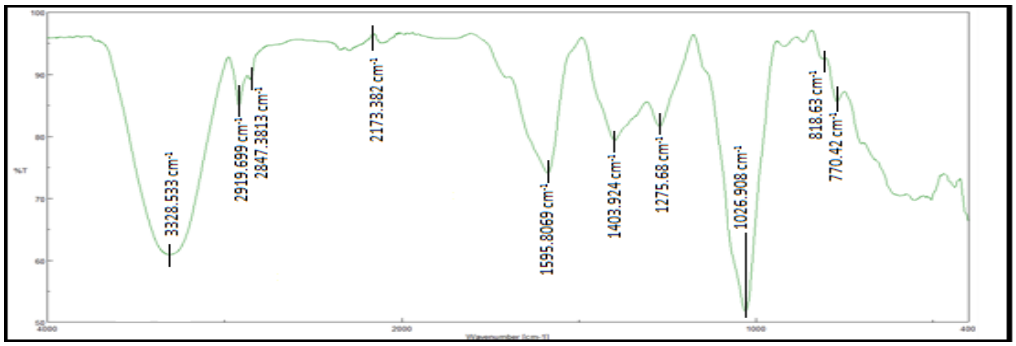


Figure 1: FT-IR spectrum of *Rubia cordifolia* (sample A).

Sample (A): 3328.53 cm^{-1} (O-H stretching, H bond), 2919.69 cm^{-1} (C-H stretching), 2847.3813 cm^{-1} (C-H stretching), and 2173. 382 cm^{-1} (-C=C- stretching), 1595.8064 cm^{-1}(N-H bend), 1403.924 cm^{-1}(C-H3 bend), 1275.68 cm^{-1} (C-N stretching), 1026.908 cm^{-1}(C-N stretching).

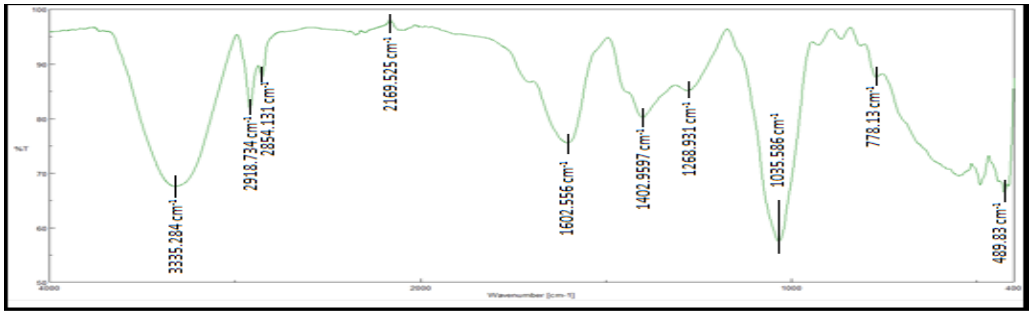


Figure 2: FT-IR spectrum of Tinospora cordifolia (sample B).

Sample B: 3335.28 cm^{-1} (O-H stretching, H bond), 2918.73 cm^{-1}(C-H stretching), 2854.13 cm^{-1}(C-h stretching), 2169.52 cm^{-1}(-C=C-stretching), 1602.556 cm^{-1}(N-H bend), 1402.95 cm^{-1}(C-H3 bend), 1268.931 cm^{-1}(C-N stretching), 1035.58 cm^{-1}(C-N stretching).

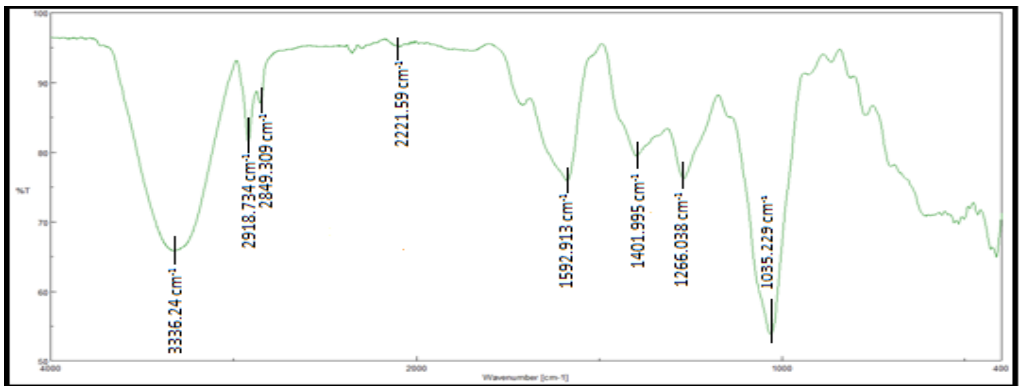


Figure 3: FT-IR spectrum of drug samples+ soya lecithin+ carbopol 934 (sample C).

Sample C: 3336.24 cm^{-1}(O-H stretching), 2918.73 cm^{-1}(C-H stretching), 2849.3 cm^{-1}(C-H stretching), 2221.59 cm^{-1}(C-H stretching), 1592.91 cm^{-1} (-C=C-sretching), 1401.99 cm^{-1}(N-H bend), 1266.03 cm^{-1}(C-H3 bend), 1035.22 cm^{-1}(C-N stretching).

Formulation of poly herbal ethosomal gel for gouty like arthritis

Preparation of drug loaded ethosome

The ethosomes were prepared by the classic method developed by Touitou *et al*. 4 formulations named F1- F4 were prepared by varying the concentration of soya lecithin and ethanol. The drug loaded ethosomal dispersion was found to be yellowish brown in color.

EVALUATION

Evaluation of drug loaded ethosome

A. Vesicle size of ethosome

The average vesicle size of ethosomes in formulation F1, F2, F3 and F4 were determined by dynamic light scattering using Malvern zetasizer nano essential.

Table 6: Mean particle size of ethosomes.

Formulation code	Mean particle size (nm)
F1	214.12±0.8001
F2	317.87±0.815
F3	367.37±0.4483
F4	442.79±0.3755

B. SEM

Prepared ethosomes were subjected for morphological studies using Field emission scanning electron microscope having an acceleration voltage of 10.0 Kv and magnification of 100k. SEM photographs are shown in Figure 1.

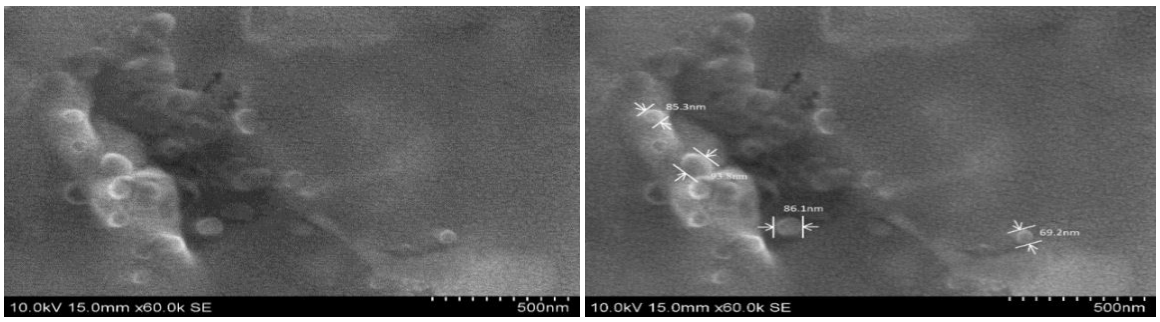


Figure 1: SEM photographs of formulated ethosome.

C. *In-vitro* drug release study – Anti-oxidant activity

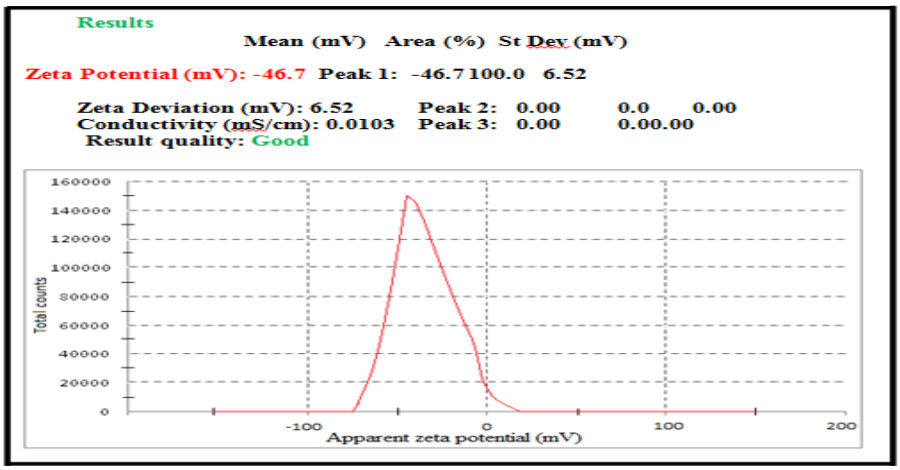
Hydrogen peroxide radical scavenging assay

The hydrogen peroxide scavenging activity of polyherbal ethosomal dispersion was evaluated and compared with Ascorbic acid and the results are given in Table 19. The IC_{50} values of ethosomal dispersions as well as standard Ascorbic acid were calculated and plotted.

Table 7: Results of hydrogen peroxide scavenging activity.

Concentration	IC_{50} values				
(µg/ml)	Standard	F1	F2	F3	F4
20	18.22±0.01	23.04±0.00	25.78±0.079	26.36±0.02	20.82±0.079
40	26.31±0.01	32.60±0.01	36.4±0.052	39.09±0.00	29.49±0.052
60	32.12±0.01	37.43±0.01	41.11±0.011	44.83±0.01	35.84±0.011
80	43.23±0.02	49.07±0.02	50.003±0.0061	55.08±0.04	45.35±0.006
100	52.12±0.01	56.04±0.00	58.69±0.032	62.03±0.00	55.027±0.03

D. Zeta potential



E. Optimization of ethosome

The ethosomal formulation F4 showed the highest *In-vitro*drug release profile in the hydrogen peroxide free radical scavenging assay. The vesicle size and vesicle morphological characters of F4 were also suitable for ethosomal gel preparation.

Evaluation of ethosomal gel

A. ORGANOLEPTIC EVALUATIONS

The organoleptic parameters like colour, odour, texture and physical appearance were evaluated by visual inspection and the results are shown in table 8.

Table 8: Organoleptic evaluations of drug loaded ethosomal gel.

Parameters	Observation
Color	Yellowish orange
Odor	Characteristic
Appearance	Clear and translucent

B.PHYSICO-CHEMICAL EVALUATIONS

1. pH

0.3%ww ethosomal gel is dissolved in distilled water and the pH was determined at room temperature by using a digital pH meter and the values are given in Table 9. The pH of the formulations was found to be 5.72±0.11355, which lies within the normal pH range of skin (4.5-5.5).

Table 9: Evaluation of pH of developed ethosomal gel.

Formulation code	Trial			Average pH
F4	1	2	3	5.72±0.11355
	5.84	5.62	5.7	

***All values are expressed as average ± SD (n=3)**

2. Spreadability

Spreadability is the term used to express the ability of a gel to readily spread on application to the skin. 0.5 gm of test formulation was placed with in a circle of 1 cm diameter pre marked on a glass plate over which a second glass plate was placed. A weight of 5 gm was allowed to rest on the upper glass plate for 5 min. the increase in the diameter due to spreadability of the formulation was noted.

Table 10: Evaluation of Spreadability of developed ethosomal gel.

Formulation code	Trial			Average spreadability (gcm/sec)
F4	1	2	3	4.4966±0.351
	4.26	4.33	4.9	

***All the values expressed as mean±SD (n=3)**

The spreadability of optimized ethosomal gel was found to be 4.4966±0.351, which indicate good spreadability.

3. Viscosity

The viscosity was determined by Brookfield viscometer. All the formulated gels were sheared at 6 rpm for 5 min which is shown in Table 11.

Table 11: Evaluation of viscosity of developed ethosomal gel.

Formulation code	Trial			Average viscosity(cps)
F4	1	2	3	11535.8±3.0512
	11539.3	11534.4	11535.7	

***All the values expressed as mean ± SD (n=3)**

4. Swelling index

Table 12 Evaluation of swelling index of developed ethosomal gel.

Formulation code	Trial			Average swelling index
F4	1	2	3	56.033± 0.904
	57.6	56.32	55.02	

***All values are expressed as mean±SD (n=3)**

5. Homogeneity

Homogeneity was confirmed by checking visually and by touch. The prepared gel was free of aggregates and the extract was uniformly distributed**.**

6. Washability: The gel when applied on the skin, it was easily removed by washing with tap water.

7. Extrudability

The gel easily extrude from the collapsible tube.

Table 13: Evaluation of homogeneity, washability and extrudability of ethosomal gel

Parameters	Observation
Homogeneity	Homogenous
Extrudability	Excellent
Washability	Good

8. *In-vitro* drug release from ethosomal gel

Hydrogen peroxide radical scavenging assay

The hydrogen peroxide scavenging activity of polyherbal ethosomal gel was calculated from the IC_{50} values of Ascorbic acid and the results are given in Table 14. The IC_{50} values of ethosomal dispersions as well as ethosomal gel are compared and graph is plotted.

Table 14: Results of hydrogen peroxide radical scavenging assay.

Concentration	IC_{50} values	
	Standard	Ethosomal gel
20	18.22±0.01	21.92±0.011
40	26.31±0.01	33.35±0.027
60	32.12±0.01	38.91±0.027
80	43.23±0.02	47.41±0.0021
100	52.12±0.01	58.353±0.0339

***All values are expressed as mean±SD (n=3)**

C. In vitro animal study

1. Skin irritation study

Table 15: Skin irritation study of prepared ethosomal gel.

Sl.No	Treatment	Day 1	Day 2	Day3	Day 4	Day 5	Day 6	Day 7
1	Control	A	A	A	A	A	A	A
2	F4	A	A	A	A	A	A	A

A (no reaction)

2. MSU Induced Gout Air Pouch model

MSU induced gout air pouch model is the model used for the evaluation of anti-gouty arthritic activity of prepared ethosomal gel.

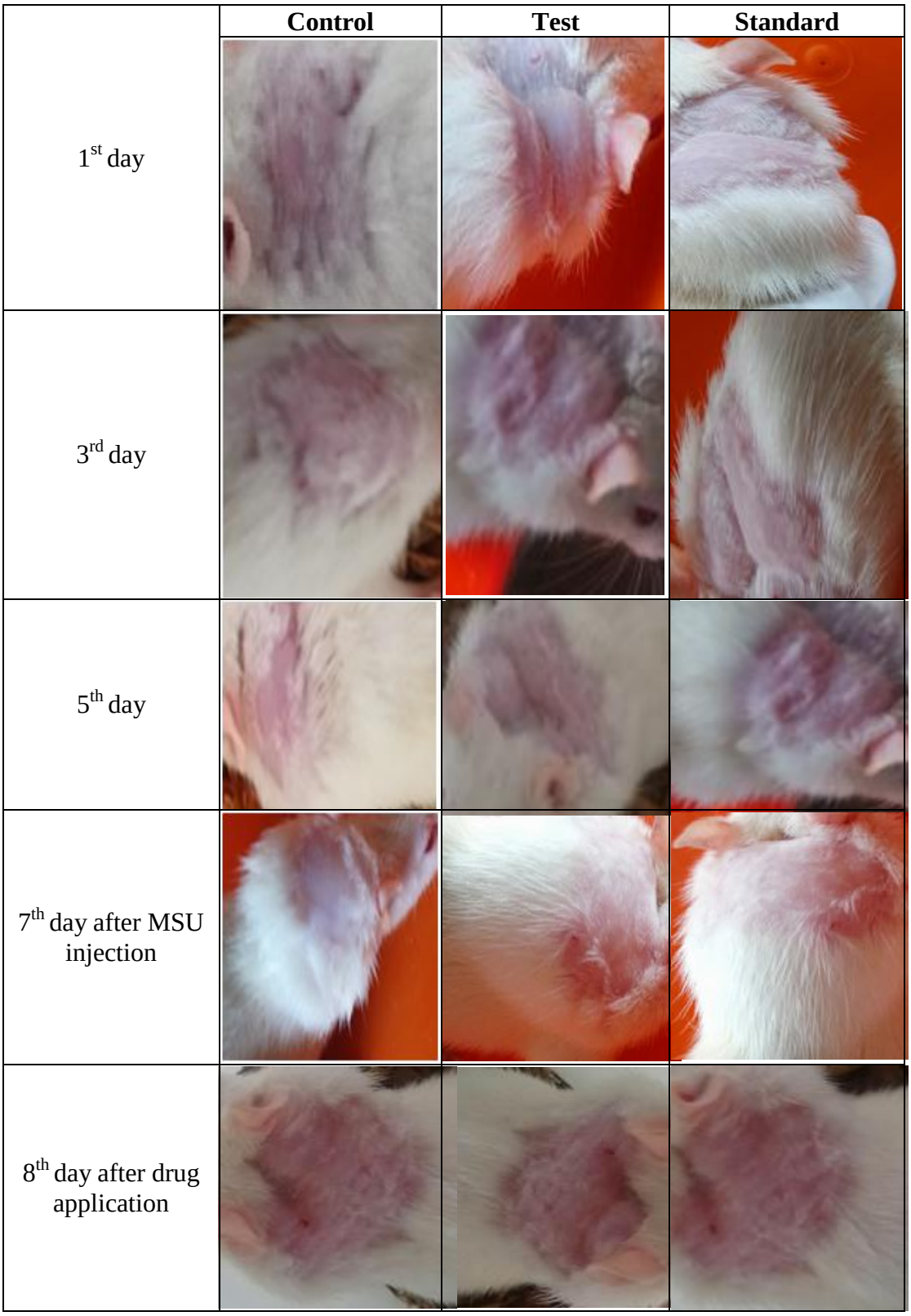


Figure 2: Various stages of animal activity.

- **Measurement of exudate volume and leukocyte count**

Table 16: Percentage reduction in exudate volume & leukocyte count

Group	Percentage reduction in exudate volume (%)	Percentage reduction in leukocyte count (%)
Control	-	-
Standard	32.87±1.82[a]	20.667±0.35[a]
Test	29.67±1.252[a]	18.138±0.338[a]

***All the values are expressed in mean±SD, (n=6 in each group). [a]p<0.05 significant difference in values when compared with control.**

- **Histopathology**

The rat skin section separated after sacrificing the animal was subjected to histopathological examination. The results are given below.

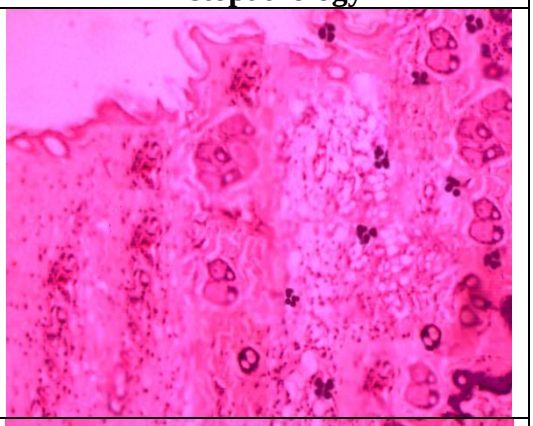
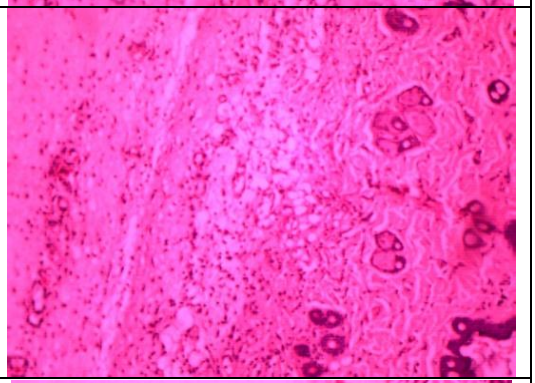
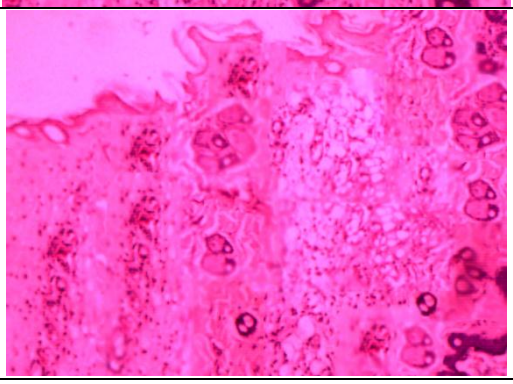
Group	Histopathology
Control	
Test	
Standard	

Figure 3: Results of histopathological examination of control, test and standard groups

4 Stability studies of ethosomal gel

The stability studies of ethosomal gel were performed at room temperature $25^0C\pm2^0C$ and at refrigeration temperature $2\text{-}8^0C\pm3^0C$ and the obtained results are given in table 17and 18.

Table 17: Stability studies of ethosomal gel at $25^0C\pm2^0C$.

Sl. No	Evaluation parameter	After one month observation	After two month observation	After three month observation
1	Colour	Yellowish orange	Yellowish orange	Yellowish orange
2	Appearance	Clear and translucent	Clear and translucent	Clear and translucent
3	pH	5.72±0.025	5.75±0.0062	5.79±0.0016
4	Homogenity	Homogeneous	Homogeneous	Homogeneous
5	Swelling index	56.73±0.037	58.13±0.11	59.68±0.398
6	Spreadability	4.53±0.035	4.81±0.0015	4.926±0.0052
7	Viscosity	1155.09±0.068	1156.89±0.026	1158.03±0.16
8	Extrudability	Good	Good	Good

Table 18: Stability studies of ethosomal gel at $2\text{-}8^0C\pm3^0C$

Sl. No	Evaluation parameter	After one month observation	After two month observation	After three month observation
1	Colour	Yellowish orange	Yellowish orange	Yellowish orange
2	Appearance	Clear and translucent	Clear and translucent	Clear and translucent
3	pH	5.71±0.0092	5.74±0.003	5.82±0.009
4	Homogenity	Homogeneous	Homogeneous	Homogeneous
5	Swelling index	55.38±0.037	57.028±0.053	58.929±0.153
6	Spreadability	4.61±0.0116	4.79±0.0092	4.90±0.0021
7	Viscosity	1154.83±0.04	1155.945±0.044	1158.078±0.029
8	Extrudability	Good	Good	Good

CONCLUSION

Novel drug delivery system is an area where huge number of research work is going on nowadays. NDDS refers to the approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effects. Medicinal plants posses a wide range of phytochemicals that cure disease or relieve pain. These phytoconstituents include alkaloids, flavonoids, tannins, saponinins, anthraquinones, and steroids etc. which are responsible for the therapeutic potency of a particular herb. Unlike the modern medicine, herbal medicine can work without many of the unpleasant side effects of the synthetic chemicals. When the phytoconstituents of herbal extracts are combined with the technique of novel drug delivery system miraculous results can be achieved, which include enhanced therapeutic value, reduced toxicity, enhanced bioavailabiliy and controlled release.

A novel herbal gel with anti-gouty arthritic activity is formulated by incorporating the drug extract into ethosomal vesicles. The high concentration of ethanol makes its deep penetration into the skin layers easier and the hydroethanolic core makes it suitable for hydrophilic as well as lipophilic compounds to be encapsulated. The drug loaded ethosomes were found to be stable in carbopol gel. The in-vitro models are used to evaluate its therapeutic potential in the gouty arthritic condition. From these it can be concluded that the formulated poly herbal ethosomal gel is found to be very effective in gouty arthritic condition.

ACKNOWLEDEMENT

Author expresses sincere thanks to the Kerala University of Health sciences, management and Principal of Rajiv Gandhi Institute of Pharmacy, Trikaripur, Kasaragod (DIST), Kerala, for giving all encouragement and valuable support to carry out this work.

REFERENCES

1. Renu Saigal and Abhishek Agrawal. Pathogenesis and Clinical Management of Gouty Arthritis. *Journal of the Association of Physicians of India*, 2015; 63: 56-63.
2. L.Barry Hainer, Eric Matheson, and R. Travis Wilkes. Diagnosis, Treatment, and Prevention of Gout. *American Family Physician,* 2014; 90: 831-836.
3. Anuradha Verma, Babita Kumar, Perwaiz Alam, Vijendra Singh and Shiv Kumar Gupta. *Rubia cordifolia* – a review on pharmaconosy and phytochemistry. *International Journal of Pharmaceutical Sciencesand Research,* 2016; 7(77): 2720-2731.
4. M. Devi Priyaand E.A Siril. Pharmacognostic Studies on Indian Madder (*Rubia cordifolia* L.). *Journal of Pharmacognosy and Phytochemistry*, 2013; 1(5): 112-119.
5. Kalpana Patni. The Amazing Pharmacological Properties of *Tinosporacordifolia*- A Short Review. *International Journal Of Ayurvedic Pharmaceutical Chemistry,* 2015; 4(2): 112-129.
6. Madhav mutalik, Maitreyee Mutalik. *Tinospora cordifolia* and its varied Activities: what is believed and what is known? *International Journal of Current Research and review*, 2011; 3(12): 94-109.
7. Tarun Parashar, Soniya, Roopesh Sachan, Vishal Singh, Gaurav Singh, Satyanand Tyagi, Chirag Patel and Anil Gupta. Ethosomes: a recent vesicle of transdermal drug delivery system. *International Journal of Research and Development in Pharmacy and Life Sciences,* 2013; 2(2): 285-292

8. Namrta Choudhary, M. BSiddiquiand Sayyada Khatoon. Pharmacognostic evaluation of (Tinospora cordifolia Willd.) Miers and identification of biomarkers. *Indian Journal of Traditional Knowledge*, 2014; 13(3): 543-550.
9. M. Archana Gorle and S. Swati Patil. Evaluation of antioxidant and antiacne property of Rubia cordifolia. *Der Pharmacia Sinica*, 2010; 1(3): 59-63.
10. B.S Chandrashekar, S. Prabhakara, T. Mohan, D. Shabeer, Basavaraj Bhandare, M. Nalini, P.S Sharmila, D.LMeghana, Basanth Kumar Reddy, H.MHanumantha Rao, H. Sahajananda, K. Anbazhagan. Characterization of *Rubia cordifolia* L. root extract and its evaluation of cardioprotective effect in Wistar rat model. *Indian journal of pharmacology*, 2018; 50(1): 12-21.
11. Kuo Xu, Penglong Wang, Bin Yuan, Yatao Cheng, Qiang Li and Hiamin Lei. Structural and bioactive studies of terpenes and cyclopeptides from genus Rubia. *Chemistry Central Journal*, 2013; 7(81): 1-11.
12. Rupali Patil, Mahalaxmi Mohan, Veena Kasture and Sanjay Kasture. Rubia cordifolia: a review. *Oriental Pharmacy and Experimental Medicine*, 2009; 9(1): 1-13.
13. Navneet bhulli and Arvind sharma. Preparation of novel vesicular carrier ethosomes with glimepiride and their investigation of permeability. *International Journal of Therapeutic Applications*, 2012; 2: 1-10.
14. Nimisha, Zeeshan Fatima1 and Chanchal Deep Kaur. Formulation and performance evaluationof Berberis aristata extract loaded ethosomal gel. *Asian Journal of Pharmaceutics*, 2017; 11(3): 177-183.
15. Bala Tripura Sundari, P.Sailaja Rao, K. Sireeshaand Y. Krishna Sai. Formulation and evaluation of ethosomal gels of mangifera indica leaf extract. *Indo American Journal of Pharmaceutical Sciences*, 2017; 4(06): 1755-1761.
16. Li-Na Shena, Yong-Tai Zhanga, Qin Wangb, Ling Xub and Nian-Ping Feng. Enhanced in vitro and in vivo skin deposition of apigenin delivered using ethosomes. *International Journal of Pharmaceutics*, 2014; 460: 280–288.
17. Yong-Tai Zhang, Li-Na Shen, Ji-Hui Zhao and Nian-Ping Feng. Evaluation of psoralen ethosomes for topical delivery in rats by using in vivo microdialysis. *International Journal of Nanomedicine Dovepress*. 2014; 9: 669–678.
18. Sheo Datta Maurya, Sunil Kumar Prajapati, Anish Kumar Gupta, Gyanendra Kumar Saxena and Ram Chand Dhakar. Formulation Development and Evaluation of Ethosome of Stavudine. *Indian Journal of Pharmaceutical Education and Research*, 2010; 44(1): 102-108.

19. M.R Vijayakumar, A.Abdul Hasan Sathali and K.Arun. Formulation and evaluation of diclofanac potassium ethosomes. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2010; 2(4): 82-86.
20. Bhale Shweta1, Bhandari Govind, Pal Pradeep and S.CMahajan. Formulation and Evaluation of Ethosomes for Transdermal Delivery of Etodolac. *American Journal of Pharmtech Research*, 2013; 3(5): 513-523.
21. Nimisha, Zeeshan Fatima1 and Chanchal Deep Kaur. Formulation and performance evaluation of Berberis aristata extract loaded ethosomal gel. *Asian Journal of Pharmaceutics*, 2017; 11(3): 177-183.
22. Nimisha, Khushboo Srivastava and Amrit Kumar Singh. Formulation and Evaluation Of Seabuckthorn Leaf Extract Loaded Ethosomal Gel. *Asian Journal of Pharmaceutical and Clinical Research*, 2015; 8(5): 316-320.
23. Idir Moualek, Ghenima Iratni Aiche, Nadjet Mestar Guechaoui, Souad Lahcene and Karim Houali. Antioxidant and anti-inflammatory activities of *Arbutus unedo* aqueous extract. *Asian Pacific Journal of Tropical Biomedicine*, 2016; 6(11): 937-944.
24. Julie mariam Joshua, Athira anilkumar, C.U Verjina, T. Deepa Vasudeva and A.Saritha Surendran. Formulation and evaluation of antiaging phytosomal gel. *Asian Journal of Pharmaceutical Clinical Research*, 2018; 11(3): 409-422.
25. Sudipta Das, K. Pallab Haldar, and Goutam Pramanik. Formulation And Evaluation Of Herbal Gel Containing Clerondendron Infornatum Leaves Extract. *International Journal of Pharmatech Research*, 2011; 3(1): 140-143.
26. Daniela Jabes, Gianpaolo Candiani, Gabriella Romano, Cristina Brunati, Simona Riva, and Marco Cavaleri. Efficacy of Dalbavancin against Methicillin-Resistant *Staphylococcus aureus* in the Rat Granuloma Pouch Infection Model. *American Society for Microbiology*, 2004; 48(4): 1118–1123.
27. Jennifer Vandooren, Nele Berghmans, Chris Dillen, Ilse Van Aelst, Isabelle Ronsse, Liron Limor Israel, Ina Rosenberger, Jörg Kreuter, Jean-Paul Lellouche, Shulamit Michaeli, Erica Locatelli, Mauro Comes Franchini, K. Miren Aiertza, Laura Sánchez-Abella, Iraida Loinaz, R.Dylan Edwards, Louis Shenkman and Ghislain Opdenakker. Intradermal air pouch leukocytosis as an in vivo test for nanoparticles. *International Journal of Nanomedicine*, 2013; 8: 4745–4756.
28. Hardevinder pal singh, Ashok kumar Tiwari and Subheet Jain. Preparation, in vitro, in vivo characterization of elastic liposome encapsulating cyclodextrin – colchicine complex for topical delivery of colchine. *Yakugaku Zasshi*, 2010; 130(3): 397-407.