Scientific African 19 (2023) e01557

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Evaluation of *Terminalia macroptera* (Combretaceae) Guill. & Perr stem bark extract incorporated into an emulgel for the potential management of rheumatoid arthritis

Adeola Tawakalitu Kola-Mustapha [a,b,*], Suleiman Olubusayomi Taiwo [a,c], Abimbola Rofiat Isiaka [d], Sherifat Omowunmi Amao [d], Ismail O. Ishola [d], Yusuf Oluwagbenga Ghazali [e], Sukurat O. Usman [f]

[a] *Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Nigeria*
[b] *College of Pharmacy, Alfaisal University, Riyadh Saudi Arabia*
[c] *Ekiti State University Teaching Hospital, Ado-Ekiti, Ekiti, Nigeria*
[d] *Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria*
[e] *Department of Pharmaceutics, University of the Western Cape, South Africa*
[f] *Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Nigeria*

ARTICLE INFO

Article history:
Received 21 August 2022
Revised 21 December 2022
Accepted 17 January 2023

Editor: DR B Gyampoh

Keywords:
Herbal emulgel
Terminalia macroptera
Anti-inflammatory
Rheumatoid arthritis

ABSTRACT

Background: Rheumatoid Arthritis (RA) is a chronic disease which causes inflammation and damage to the joint. The goals of treatment are to stop inflammation, relieve symptoms, improve physical function and overall well-being. The purpose of this study was to evaluate *Terminalia macroptera* stem bark (TMB) ethanol extract and formulate it into an herbal emulgel (TME) for the potential management of RA.

Methods: Phytochemical analysis and anti-inflammatory activity of TMB were first evaluated using standard analytical methods and complete Freund's adjuvant (CFA) induced arthritis model in rats respectively. Post-formulation, physical characterization of the carbopol 940 based herbal emulgels (TME) and the reduction in the induced rat paw sizes by the herbal emulgels were evaluated using diclofenac emulgel as the positive control.

Results: Phytochemical screening revealed the presence of flavonoids, tannins, terpenoids, saponins, and alkaloids. Inflammatory activity of the extract gave the highest percentage inhibition with TMB (50 mg/kg). The formulated herbal emulgels had good spreadability, extrudability, pH ranging from 4.5±0.2 to 6.9±0.4 and viscosity ranging from 0.36 ± 0.20 to 8.37 ± 0.65 Pas at 6 rpm and 0.26 ± 0.01 to 10.67±0.96 Pas at 12 rpm. TME emulgel significantly ($p < 0.05$) reduced oedema formation and arthritic index induced by complete Freund's adjuvant in rats. TME showed dose-dependent anti-inflammatory activity comparable with commercial diclofenac emulgel.

Conclusion: TMB showed an excellent inhibitory activity on the induced paw of the test animals which makes it a suitable candidate in a topical herbal emulgel formulation (TME) for the potential use in the management of RA.

* Corresponding author.
E-mail address: kolamustapha.at@unilorin.edu.ng (A.T. Kola-Mustapha).

https://doi.org/10.1016/j.sciaf.2023.e01557

Background

Rheumatoid arthritis (RA) is a long-term chronic autoimmune inflammatory disease that affects primarily the joints and leads to irreversible joint deformity, disability, and reduced quality of life. The symptoms of RA include pain, stiffness, edema, and joint deterioration [1]. Around 1% of the world's population suffers from rheumatoid arthritis with main cause unknown [2]. Genetics is one of the major risk factors of RA as family history increases the risk to about three to nine times [3]. It accounts for 40-65% and around 20% of cases of seropositive (presence of rheumatoid factors and anti-CCP antibodies) and seronegative RA respectively [1]. Genes such as the Major Histocompatibility Complex (MHC) antigen, HLA-DR4 (Human Leukocyte Antigen) and HLA-DRB1 (identified as the strongest known genetic risk factor for RA [4]) allele have been indicated in the pathogenesis of RA where their involvements among ethnic groups differ [5]. Obesity [6], lifestyle, hormonal effect; and environmental factor such as cigarette smoking, exposure to gases (nitrates (NO_2), ozone (O_3), sulphur dioxide (SO_2), and carbon monoxide (CO)), chemicals and second-hand smoke [2,7] are other risk factors associated with RA. RA is the most common autoimmune disease [2] which affects 0.5–1% of the population at any age with a female to male ratio of 2:1 [3]. It is of note that, RA usually manifests between the ages of 30 and 60, it can also be seen in young adults, teens, and even children [8]. The average person does not develop symptoms of RA until they reach their 60's. Although there is no cure for RA; the available treatment options used in its management have numerous side effects (of which medicinal plants pose a great advantage of fewer or no side effects [9]) and are frequently not cost effective [10].

There has been a recent increase in the interest in medicinal plants in the management of RA and it has been documented that most of the patients with poorly managed RA-associated chronic pain often use herbal medications as alternative [11]. Many herbal materials have been used in the management of RA, examples include feverfew (*Tanacetum parthenium*), flaxseed oil, borage seed oil (*Borago officinalis*), curcumin (*diferuloyl methane*), evening primrose oil (*Oenothera blennis*), blackcurrant seed oil (*Ribes nigrum*) and capsaicin [10]. Also, numerous traditional Chinese medicines with anti-inflammatory, immunomodulatory, or cartilage-protective effects have also been widely used in recent years [12,13]. Research have shown that flavonoids, steroids, terpenoids and fatty acids are phytochemicals that are useful in the management of RA [14].

Terminalia macroptera is a specie of flowering plant in the Combretaceae family which grows up to 1400 meters in deciduous open woods and bushy grassland. It is known by the Hausa common name 'kwandari'. The plant is native to African countries- Ghana, Senegal, Benin, Sudan, Burkina Faso, Nigeria and Uganda [15]. Its various morphological parts such as the leaves, stem bark and root have been reported to be used in traditional medicine in Nigeria against several ailments. The leaves decoction is used against ringworm, hepatitis, and skin diseases while its stem bark is used in the treatment of tuberculosis, edema, gastritis, cough, hepatitis and in the management of pain [16]; and the root decoction for treatment of urethral discharge and urinary troubles [17]. Furthermore, the various parts of the plant have been researched to possess anti-*Helicobacter pylori* activity and antimicrobial activity [17].

Topical formulations, which are available in semi-solid and liquid forms; are preparations applied to the skin or mucous membrane for their local or systemic action. In most cases, they are medicated, and the non-medicated ones are often used as skin protective and moisturizers. The skin which provides an ideal site for drug delivery for both local and systemic effects; also acts as a mechanical barrier, mediated by the stratum corneum, to the entry of many drug substances. Emulgels (mixture of pharmaceutical emulsion and gel) as an example of topical formulations, has good cutaneous penetration and offer advantages of better stability, controlled release, incorporation of hydrophobic drugs and better loading capacity [18]; in addition to the general advantages of topical formulations such as ease of administration, low cost and fewer toxic effects [19,20]. The basic components of emulgels are the active ingredient(s), solvents (polar and non-polar), emulsifiers, gelling agents, permeation enhancers and preservatives. They are prepared in three basic steps - formulation of emulsion (oil-in-water or water-in-oil), formulation of gel base and incorporation of emulsion into gel base with continuous stirring [21].

The purpose of this study was to explore the anti-inflammatory activity of *Terminalia macroptera* in a formulation-oriented manner to yield an herbal-based product as a viable and suitable alternative in the treatment of RA.

Materials and methods

Materials

Stem bark of *Terminalia macroptera*, Ethanol (BDH Chemicals Ltd., Poole England), Tween 20 (BDH Chemicals Ltd., Poole England), Span 20 (BDH Chemicals Ltd., Poole England), Methyl paraben (BDH Chemicals Ltd., Poole England), Ethyl paraben (BDH Chemicals Ltd., Poole England), Carbopol 940 (Guangdong Chemical Reagent, China), Liquid paraffin (BDH Chemicals Ltd., Poole England), Propylene glycol (BDH Chemicals Ltd., Poole England), Triethanolamine (BDH Chemicals Ltd., Poole England), Clove oil (ASHIFAUL-HAQI Ltd, Nigeria), Mentha oil (ASHIFAUL-HAQI Ltd, Nigeria), Distilled water (Department of Chemical Engineering, UNILORIN), Water bath (Fisher Scientific Company, USA), Analytical weighing balance (Ohaus, USA), Vortex mixer (Microfield Instrument, England), Refrigerator (LG, South Korea), pH meter (Hanna, England), Viscometer (NDJ-5S, China)

Method

Collection of plant

The stem barks of *T. macroptera* were collected along Ilorin-Ekiti road, Kwara State, Nigeria in March 2020. The plant was identified by Mr Bolu Ajayi and authenticated at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Nigeria where a voucher specimen (UILH/001/1230) was deposited.

Extraction from T. macroptera *stem bark*

The powdered material (800 g) was macerated with 96% ethanol for 72 h at room temperature, with intermittent swirling of the content. The resultant solution was filtered through a Whatman filter paper 125 mm (No 1) and concentrated in vacuum at 30°C using the rotary evaporator to obtain the ethanol extract which was reddish brown in colour. After evaporation, the extract was stored at −20°C until use. The percentage yield was calculated for *Terminalia macroptera* stem bark.

Characterization of extract

The physical characteristics such as the colour, odour and texture of *Terminalia macroptera* stem bark extract was examined, and the pH meter (Hanna, England) was dipped into the undiluted extract at room temperature 25 ºC and the reading recorded. The pH was conducted in triplicates.

Phytochemical analysis of extract

Qualitative assays were done to check for the presence of various phytochemicals in the ethanol extract of *Terminalia macroptera* stem bark following standard procedures [22]. Assays were done to determine levels of phytochemicals (phenolics and flavonoids) found present in the ethanol extract of *Terminalia macroptera* stem bark.

Quantitative analysis.

Determination of total phenolic content. The total phenolic content (TPC) of the extract was determined by modified Folin-Ciocalteu reagent method [23]. Deionized water (0.5 mL) and 0.125 mL of a known dilution of *T. macroptera* extract were added to a test tube. Folin-Ciocalteu reagent (0.125 mL) was added to the solution. It was allowed to react for 6 min. Followed by, 1.25 mL of 7% sodium carbonate solution which was aliquoted into the test tubes, and the mixture was diluted to 3 mL with deionized water. The color developed for 90 min, and the absorbance was measured at 760 nm using UV-Vis spectrophotometer. All determinations were performed in triplicates. The measurement was compared to a standard curve of prepared gallic acid solutions and expressed as milligrams/g of gallic acid equivalents.

Determination of total flavonoid content. Total flavonoid was estimated using the method of Miliauskas *et al* [24]. Concentrations of 0.3 mg/mL of the extract in methanol was prepared, while Quercetin concentrations of 0.03, 0.06, 0.09, 0.12 and 0.15 mg/ mL also prepared in methanol were used to obtain the calibration curve. 2.0 mL of 2% Aluminium Chloride ($AlCl_3$) in ethanol was added to 2.0 mL of each preparation and allowed to stand at room temperature for 60 min. The absorbance was measured at 420 nm after 60 min. The estimation of total flavonoids content in the crude extract was carried out in triplicate and the result averaged. The total flavonoid content was calculated as Quercetin equivalent (mg/g) using the equation based on the calibration curve: y=0.2267x, where y is the absorbance and x is the concentration incorporated into the following formula:

$$\mathbf{T_{(QE)}} = \frac{\mathbf{(C\ x\ V)}}{\mathbf{M}}$$

Where: T= Total Flavonoid content (Quercetin equivalent), mg/g plant extract; C = Concentration of Quercetin from standard curve mg/mL; V = Volume of extract used during the assay (mL); M = Mass of extract used during assay (g).

Qualitative analysis. Various phytochemical investigations were performed on the ethanol extract of *T. macroptera* following standard procedures as described by Kokate [22]. Colour intensity was used to categorize the presence of each phytochemical into copious, moderate or slight (trace).

Test for flavonoids. To a test tube containing 1 mL of extract, a few drops of dilute sodium hydroxide (NaOH) solution was added. An intense yellow colour produced in the extract which becomes colourless on addition of few drops of dilute acid indicates the presence of flavonoids.

Test for anthraquinones (Borntrager's reaction). To 2 mL of chloroform extract, dilute (10%) ammonia solution was added. A pink-red colour in the ammoniacal (lower) layer indicates the presence of anthraquinones.

Test for Tannins. To a test tube containing the extract, 1 mL of 5% Ferric chloride was added. The presence of tannin is indicated by the formation of bluish black or greenish black precipitate.

Test for Saponins. The extract was diluted with 20 mL distilled water and was agitated in a graduated cylinder for 15 min. The formation of 1 cm layer of foam indicates the presence of saponin.

Table 1
Formulation of emulgel from ethanol extract of *Terminalia macroptera* stem bark.

INGREDIENTS	COMPOSITON (%W/W)										
	TME1	TME2	TME3	TME4	TME5	TME6	TME7	TME8	TME9	TME10	TME11
Extract	0	0.5	1.0	1.5	2.0	0.5	2.0	0.5	2.0	0.5	2.0
Alcohol	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Span 20	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Tween 20	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Ethyl paraben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Liquid paraffin	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Carbopol 940	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Clove oil	-	-	-	-	-	10.0	10.0	-	-	10.0	10.0
Mentha oil	-	-	-	-	-	-	-	6.0	6.0	6.0	6.0
Propylene glycol	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Test for cardiac glycosides. To a test tube containing 5 mL of extract was added 2 mL of glacial Acetic acid containing a drop of Ferric chloride ($FeCl_3$) solution. It was then underplayed with 1 mL concentrated sulphuric acid (H_2SO_4). A brown ring at the interface indicates a de-oxy sugar characteristic of cardenolides.

Test for steroids. The extract (1 mL) was dissolved in 10 mL chloroform and equal volume of concentrated sulphuric acid (H_2SO_4) was added down the side of the test tube. The upper layer turns red and sulphuric acid layer show yellow with green fluorescent. This indicates the presence of steroids.

Assay of anti-inflammatory activity of extract

The anti-inflammation assay was done using animal models. Albino Wistar rats were used for the anti-inflammatory study and were maintained under standard environment conditions. The animals were allowed to get acclimatized with laboratory conditions for a period of seven days before the experiment and were deprived from food 4 h before experiment, after which they were taken for the experiment. Using the Carrageenan-induced rat paw edema method, twenty (20) rats were divided into four groups with five rats each. Three test groups were pre-treated orally with *T. macroptera* stem bark extract (50, 100 and 200 mg/kg) one hour before administration of carrageenan. Acute inflammation was produced, one hour after oral administration of the drugs, by sub-plantar injection of 0.1 mL of 1% suspension of carrageenan in normal saline in the right hind paw of the rats. The paw circumference was measured over 24 h after the carrageenan injection. The difference between the readings will be taken as the volume of edema and the percentage anti-inflammatory activity was calculated. The percentage value of edema inhibition was calculated using the formula:

$$\%\ inhibition = 1 - \left({}^{y\ -\ x}/_{b\ -\ a}\right)\ x\ 100$$

Where, x= Initial paw thickness of test group animal, y= Paw thickness of test group animal after treatment, a= Initial paw thickness of control group animal, b= Paw thickness of control group animal after treatment [25].

Emulgel formulations

The emulgels were prepared (Table 1) following the method described by [21] with slight modifications:

The plant extract was dissolved in alcohol, the preservatives (methyl paraben and propyl paraben) were dissolved in propylene glycol, and both solutions were added to a solution of tween 20 in purified water to make the aqueous phase. The oily phase was made by dissolving span 20 in light liquid paraffin and adding the mentha oil and/or clove oil (permeation enhancer). Both phases were then heated to about 75 oC and mixed with constant stirring until it cools to room temperature. The gelling agent was prepared by adding carbopol to purified water at a concentration of 1% with constant stirring at a moderate speed. The pH was then measured and adjusted to 6-6.5 using triethanolamine. The gel and emulsion were then mixed at a ratio of 1:1 and glutaraldehyde was added while mixing them to form the emulgel. The emulgels were then packaged in jars and well covered till further analysis.

Emulgel analysis

Physical characterization*:* Physical characteristics of the formulations like colour, homogeneity, consistency and texture were examined [26].

Extrudability*:* Extrudability of the emulgels were measured by applying a constant defined force to the emulgels, packed in a collapsible aluminum tube, measuring the length of ribbon extruded from the container and calculating the extrudability using the formula:

$$\text{Extrudability} = \frac{\text{Weight of load (in grams)}}{\text{Area of ribbon extruded (in cm squared)}}$$

Spreadability*:* The spreadability was done using two glass slides of standard dimension. The herbal emulgel formulation was placed over one of the slides and the other slide was then placed on the top of the gel, such that the gel is sandwiched

between the slides. A known weight (100 g) was placed upon the upper slide and how fast the slide slips off the other was determined. Spreadability was calculated using the formula:

$$S = M.L/T$$

Where, S = Spreadability
M = Weight tide to the upper slide
L = Length of a glass slide
T = Time taken to separate the slide completely from each other.

pH determination: The pH was measured in triplicate using a digital pH meter (Hanna, England) and the average values calculated and recorded.

Determination of viscosity: The estimation was done using an NDJ-5S viscometer (Spindle type no.3) at 6 and 12 rpm. Substantial quantity of the emulgel was put in a beaker and the spindle dipped in it for about 5 mins and then readings taken.

Swelling index: this is defined as the volume (mL) taken up by the swelling of 1g of herbal material under specified conditions. This was done by transferring 1 g of the emulgel to a 25 mL stoppered cylinder, filling the cylinder up to 20 mL mark with water, agitating gently occasionally for 24 hour and allowed to stand. The volume occupied by the swollen was then measured. The swelling index was calculated using the formula:

$$\text{Swellingindex}(SW)\% = [(Wt. - -Wo)/Wo]x100$$

Where {SW} % = Equilibrium perfect swelling.
Wt. = Weight of swollen emulgel after time t,
Wo = original weight of emulgel at zero time.

Skin irritation test: The intact skin of Wistar rats of either sex with average weight 150– 200 g was used. The hairs were removed from the rat three days before the experiment. Prepared gel formulations were used on the test animal and gel base on control group. The animals were treated daily for 7 days, and erythema and edema on the treated skin were examined [27].

Assay of anti-inflammatory activities of formulated emulgels

Complete Freund's adjuvant-induced arthritis in rats

Ninety-six Wistar rats were randomly divided into 12 groups (n = 8) and initial paw diameter were measured with vernier caliper and treated as follows:

Group I: vehicle (10% clove oil + 6% MO; control)
Group II: 0.5% *T. macroptera* extract in saline
Group III: 1% *T. macroptera* extract in saline
Group IV: 1.5% *T. macroptera* extract in saline
Group V: 2% *T. macroptera* extract in saline
Group VI: 0.5% *T. macroptera* extract in 6% MO
Group VII: 0.5% *T. macroptera* extract in 10% clove oil
Group VIII: 2% *T. macroptera* extract in 6% MO
Group IX: 2% *T. macroptera* extract in 10% clove oil
Group X: 0.5% *T. macroptera* extract in 10% clove oil and 6% MO.
Group XI: 2% *T. macroptera* extract in 10% clove oil and 6% MO.
Group XII: Diclofenac emulsion

0.1mL CFA (10 mg/kg of heated-killed Mycobacterium tuberculosis in 1 mL paraffin oil) was injected into the tarsal of the right-hind paw. Treatment was done through topical application to the inflamed paw for 35 consecutive days as well as recording of paw diameter weekly using the screw gauge vernier caliper.

Statistical analysis

Results obtained were expressed as Mean ± SEM. Statistical comparison was done by one-way analysis of variance (ANOVA). Two-way ANOVA followed by Bonferroni post hoc multiple comparison test was used for anti-inflammatory study. Statistical analysis were carried out using Graph-pad prism version 6 (GraphPad Software, Inc. CA, USA). The statistical significance is set at $p < 0.05$.

Ethical approval

Ethical approval to execute this study was sought and obtained from the University Ethical Review Committee of the University of Ilorin, Nigeria. The approval (with reference ID: UERC/ASN/2022/2338) was issued prior to the commencement of the research.

Table 2
Qualitative phytochemical constituents of ethanol extract of *Terminalia macroptera* stem bark.

Phytochemicals	Observation
Alkaloids	+
Tannins	+
Flavonoids	+
Glycosides	-
Anthraquinones	-
Saponins	+
Steroids	-
Phenolics	+

Note: + = present; - = absent.

Table 3
Quantitative estimation of ethanol extract of *Terminalia macroptera* stem bark.

Phytochemicals	Observation (mg/g)
Flavonoids	193.60 ± 6.34
Glycosides	0.00 ± 0.00
Anthraquinones	0.00 ± 0.00
Steroids	0.00 ± 0.00
Phenolics	164.87 ± 10.37
Saponins	134.25 ± 4.22
Tannins	367 ± 9.73

Data presented as Mean ± SEM ($n = 3$).

Table 4
Results of the anti-inflammatory assay of the extract as reduction in paw size of rats (mm).

Treatment	Paw size (mm) at time (h)									
	0 h	1 h		2 h		3 h		4 h		24 h	
	Mean ± SEM	Mean ± SEM	In (%)	Mean ± SEM	In (%)	Mean ± SEM	In (%)	Mean ± SEM	In (%)	Mean ± SEM	In (%)
Control	1.22±0.01	1.57±0.06		1.57±0.02		1.67±0.01		1.62±0.02		1.53±0.03	
TMB (50mg/kg)	1.18±0.03	1.67±0.04	37.73	1.78±0.00	67.92	1.81±0.03	38.51	1.75±0.03	42.5	1.46±0.04	10.63
TMB (100mg/kg)	1.21±0.02	1.84±0.07	80.18	1.82±0.01	73.58	1.75±0.05	21.48	1.70±0.07	24.16	1.50±0.08	5.31
TMB (200mg/kg)	1.23±0.00	1.87±0.01	81.13	1.85±0.03	73.58	1.81±0.02	28.14	1.76±0.01	31.68	1.52±0.04	9.57

Data presented as Mean ± SEM ($n = 3$)
KEY: In = Inhibition

Results

Extract characterization

Crude extraction

The ethanol extract yield obtained was 22.7 % (w/w). Usman et al., 2017 [16] had similar yield.

Appearance and pH

The ethanol extract of *Terminalia macroptera* stem bark obtained was brown in colour, smooth to touch and had a characteristic odour. The pH of the ethanol extract *Terminalia macroptera* stem bark was 6.1 ± 0.15.

Phytochemical screening

Phytochemical constituents in ethanol extract of *Terminalia macroptera* stem bark is presented in Table 2.

Quantitative analysis of the extract

Quantitative estimation of the ethanol extract of *Terminalia macroptera* stem bark is presented in Table 3.

Anti-inflammatory activity of extract

The anti-inflammatory assay of the extract is presented in Table 4.

Table 5
Physical characterization of emulgels.

Emulgels	Appearance	Colour	Odour	Ease of application	Ease of removal	Homogeneity
TME1	Glossy, less viscous	White	Characteristic odour	Very easily applied	Very easily removed	Homogeneous
TME2	Glossy, viscous	Cream	Characteristic with bark odour	Very easily applied	Very easily removed	Homogeneous
TME3	Glossy, viscous	Light brown	Characteristic with bark odour	Very easily applied	Very easily removed	Homogeneous
TME4	Glossy, viscous	Caramel brown	Characteristic with bark odour	Very easily applied	Very easily removed	Homogeneous
TME5	Glossy, viscous	Brown	Characteristic with bark odour	Very easily applied	Very easily removed	Homogeneous
TME6	Glossy, viscous	Cream	Bark with aromatic odour	Very easily applied	Easily removed	Homogeneous
TME7	Glossy, viscous	Dark brown	Bark with aromatic odour	Very easily applied	Easily removed	Homogeneous
TME8	Glossy, viscous	Cream	Bark with minty odour	Very easily applied	Easily removed	Homogeneous
TME9	Glossy, viscous	Reddish brown	Bark with minty odour	Very easily applied	Easily removed	Homogeneous
TME10	Glossy, viscous	Cream	Bark with minty and aromatic odour	Very easily applied	Easily removed	Homogeneous
TME11	Glossy, viscous	Reddish brown	Bark with minty and aromatic odour	Very easily applied	Easily removed	Homogeneous

Table 6
Evaluation parameter for *Terminalia macroptera* stem bark ethanol extract emulgels

Emulgel	pH	Spreadability (gcm/s)	Extrudability (g/cm^2)	Swelling index (%)
TME1	6.9	30.0	1041.67	55
TME2	6.8	27.0	1136.36	50
TME3	6.5	35.0	961.54	42
TME4	6.7	28.0	961.54	60
TME5	5.8	29.5	1086.95	65
TME6	6.8	25.0	892.86	56
TME7	6.5	35.7	1250	48
TME8	6.2	33.0	1000	60
TME9	5.0	31.0	862.07	68
TME10	6.2	28.0	1388.88	50
TME11	4.5	37.0	1250	65

Table 7
Viscosity of the formulated emulgels at 6 and 12 rpm.

Viscosity (mPas)											
RPM	TME1	TME2	TME3	TME4	TME5	TME6	TME7	TME8	TME9	TME10	TME11
6	358.1±0.20	395.8±0.06	450.8±0.08	8079.6±0.57	1121.2±0.10	1416.7±0.09	1315.8±0.47	543.6±0.00	8371.2±0.65	2116.3±0.20	731.1±0.09
12	255.0±0.01	268.0±0.03	302.1±0.50	5118.4±0.40	646.8±0.00	840.0±0.06	968.2±0.2	448.9±0.00	5611.7±0.00	10668.0±0.96	535.0±0.00

Emulgel characterization

Physical characterization

The physical properties of the emulgels formulated were observed, including the appearance, colour, odour, homogeneity, ease of application and removal. The results obtained from physical characterization are presented in Table 5**.**

Extrudability

The extrudability of the formulated herbal emulgels is presented in Table 6; ranging from 862.07-1388.88g/cm^2.

Spreadability

The spreadability of the formulated herbal emulgels is presented in Table 6 ranging from 25.0-37.0 gcm/s.

pH

The pH of each of emulgel was done in triplicate and the average of each is presented in Table 6 (4.5-6.9).

Swelling index

The swelling index of each emulgel is presented in Table 6; the swelling indices of the emulgel ranged from 42-68%.

Viscosity

The estimation was done using an NDJ-5S viscometer (Spindle size 3) at varying speed of 6 and 12 rpm. The results obtained is presented in Table 7.

Data presented as Mean ± SEM ($n = 3$)

Skin irritation study

The results obtained indicated that prepared emulgels did not produce irritation, redness, or edema on application and free from dermatological reaction.

Table 8
Time course effect of TME emulgels on CFA-induced arthritis in rats.

	Change in paw diameter (mm)				
Treatment	Week 1	Week 2	Week 3	Week 4	Week 5
TME 1	0.68±0.25	2.55±0.43	3.25±0.64	3.41±0.59	2.02±0.33
TME 2	0.68±0.10	1.75±0.31[a]	2.02±0.28[a]	1.69±0.38[b]	1.95±0.52
% inhibition	**-0.3**	**31.11**	**38.05**	**50.35**	**3.65**
TME 3	0.88±0.06	1.89±0.42	3.23±0.34	3.07±0.61	1.80±0.28
% inhibition	**-30.47**	**25.84**	**0.68**	**9.8**	**10.96**
TME 4	1.10±0.19	3.41±0.50	3.88±0.29	3.21±0.60	3.65±0.35
% inhibition	**-62.43**	**-33.86**	**-19.48**	**5.81**	**-80.26**
TME 5	0.59±0.03	1.02±0.44[b]	1.79±0.37[a]	2.43±0.62[a]	1.72±0.24
% inhibition	**12.13**	**59.78**	**45.11**	**28.64**	**15.3**
TME 6	1.22±0.30	3.45±0.51	4.09±0.86	3.97±1.15	3.04±0.79
% inhibition	**-80.18**	**-35.82**	**-25.69**	**-16.49**	**-49.95**
TME 7	0.70±0.13	1.39±0.27[a]	1.94±0.46[a]	1.57±0.26[b]	0.67±0.11[b]
% inhibition	**-3.25**	**45.09**	**40.5**	**53.81**	**66.73**
TME 8	0.63±0.08	1.39±0.50[a]	1.74±0.41[a]	3.44±1.61	1.94±0.66
% inhibition	**6.51**	**45.25**	**46.65**	**-1.06**	**4.54**
TME 9	0.73±0.12	1.41±0.44[a]	1.78±0.49[a]	1.80±0.54[b]	1.19±0.39[a]
% inhibition	**-7.99**	**44.62**	**45.24**	**47.24**	**41.16**
TME10	0.95±0.20	2.46±0.53	3.73±0.46	2.16±0.35[a]	2.37±0.44
% inhibition	**-40.83**	**3.22**	**-14.57**	**36.68**	**-17.18**
TME 11	0.85±0.22	2.03±0.82	2.90±0.83	2.09±0.58[a]	1.56±0.30
% inhibition	**-26.33**	**20.19**	**10.76**	**38.62**	**21.89**
Diclofenac Emugel	0.90±0.08	1.10±0.42[b]	1.79±0.37[a]	1.44±0.29[b]	1.58±0.46[a]
% inhibition	**-33.51**	**56.6**	**44.91**	**57.75**	**21.89**

Values are expressed as mean±SEM (n=6). [a]$p < 0.05$; [b]$p < 0.01$ Versus vehicle-treated control, statistical level of significance analysis by two-way ANOVA followed by Tukey post hoc multiple comparison test.

Anti-inflammatory assay of the emulgel

The result obtained is presented in Table 8.

Similarly in Fig. 1 below, photographs showing the effect of the treatments on CFA-induced arthritis in the rats are presented.

Discussion

There is a global rise in interest in the standardization of medicinal plants for pharmacological use and pharmaceutical formulation. Many of the plant species currently used for treatment of various illnesses have been in use for centuries in a limited part of the world without proper documentation and investigation. About 80% of the world's population- majorly those in developing countries, now use herbal medicines as part of their primary health care needs [28]. In traditional medicine, various parts of *Terminalia macroptera* plant have been used to treat more than thirty different human ailments in countries such as Nigeria, Senegal, Ghana, Mali, Sudan, and Uganda [29].

The phytochemical analysis of *T. macroptera* led to the identification of various chemical contents including ellagitannins, flavonoids, triterpenoids and related phenolics [30]. It was also reported by [31] that *Terminalia* species contain various secondary metabolites such as cyclic triterpenes and their derivatives (tannins, flavonoids, and phenolic acids). Phenolic compounds, terpenoids and flavonoids have been widely described for their anti-inflammatory, immunomodulatory [32], antinociceptive [33] and antioxidant properties (they contain hydroxyl group capable of capturing oxygen free radicals) [34]. According to Traore et al., 2019, the phytochemical study and characterization of *T. macroptera* stem bark extract by gas chromatography-mass spectrometry (GC-MS) analysis, showed the presence of the following chemical groups: poly-phenols, flavonoids, saponins, tannins gallic, cardiac glycosides, sterols, and terpenes. The study has successfully identified and reported 20 molecules accompanied by their mass spectra, their molecular weight, and their chemical structures [35].

The anti-inflammatory assay of *T. macroptera* extract was done using the carrageenan- induced rat paw model (animal studies) to ensure the anti-inflammatory activity was by a mechanism suitable for the plant use in the management of RA. This model is also used to study non-steroidal anti-inflammatory drugs [36]. The effect of the extract was studied, and it was observed that TMB (50mg/kg) had the highest percentage (%) inhibition; a similar result obtained by Usman et al., 2017 [16]. This led to the design of experiment to formulate a topical preparation with the extract grading from 0.5-2% using a slightly modified version of the formulation method described by Mohamed (2004) [21]. The formula was developed for emulgels containing different concentrations of the plant stem extract- 0.5, 1, 1.5, and 2% w/w; and the permeation enhancer used were clove oil (10%) and/or mentha oil (6%). *Terminalia macroptera* extract showed low percentage inhibition (5.31 -10.63%) which might be due to poor permeation of the extract into the skin; and consequently, the highest active concentrations of the permeation enhancers were used. The herbal emulgels formed were generally glossy, homogenous, and were very easily

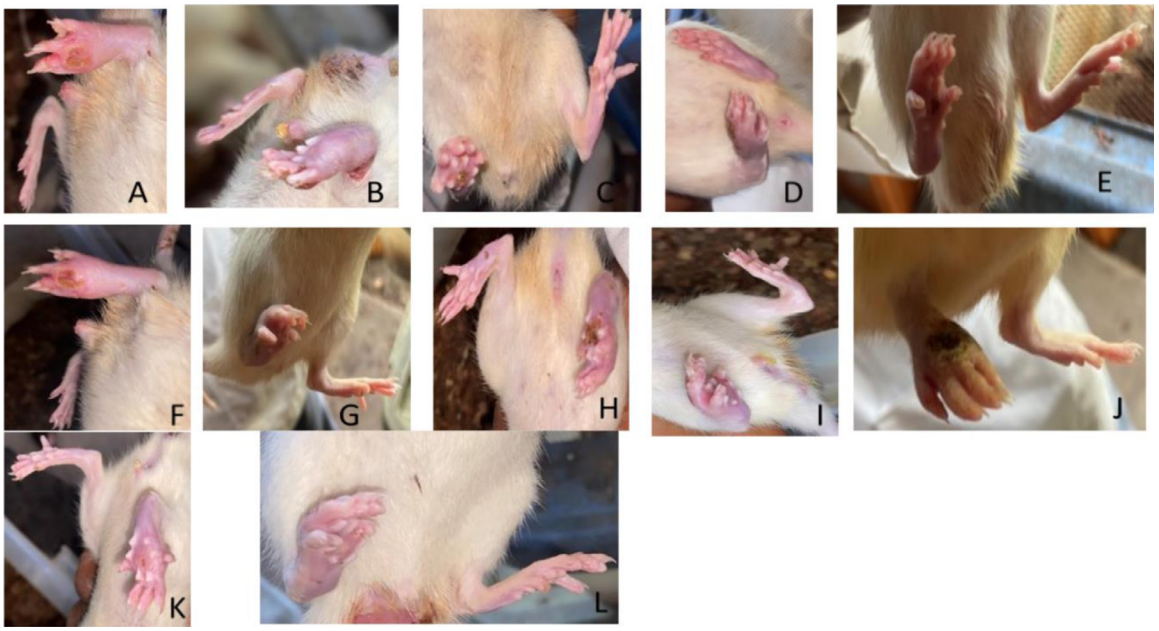

Fig. 1. The effect of the treatments on CFA-induced arthritis in the rats
(a) TME 1 (0% extract) (b) TME 2 (0.5% extract) (c) TME 3 (1% extract) (d) TME 4 (1.5% extract) (e) TME 5 (2% extract) (f) TME 8 (0.5% extract, 6% MO %) (g) TME 6 (0.5% extract, 10% CO) (h) TME 9 (2% extract, 6% MO) (i) TME 7 (2% extract, 10% CO) (j) TME 10 (0.5% extract, 6%MO &10% CO) (k) TME 11 (2% extract, 6%MO &10% CO) and (l) diclofenac emulgel.

applied and removed. All the emulgels formulated with mentha oil and clove oil had minty odour and aromatic odour as impacted by the respective permeation enhancers. The formulations containing the extract and no permeation enhancer(s) had a bark odour and the formulation with neither the extract nor permeation enhancers(s) had a characteristic odour. The colour of the emulgel without extract was white and those with extract varied from 'cream' to 'reddish brown'. The pH of the formulated emulgels were moderately/slightly acidic with TME11 having an acidic pH of 4.5. The pH of the formulations decreased with increasing concentration of the extract except for TME4 with pH 6.7. This can be related to the acidic nature of ellagitannins, flavonoids triterpenoids and phenol which are the major components of *Terminalia macroptera* [30]. Sultana et al., 2016 [37] suggests the pH of emulgel should be between 5-7 to mimic the skin condition and prevent irritation to the patient. The results of extrudability, spreadability and the swelling index of the emulgels are presented in Table 5. It was found that all the formulations showed excellent extrudability and this will aid easy application and promote patient acceptance [38]. The emulgels had different swelling index values (42-68%) and the variation may be dependent on the water uptake nature and chain strength of the polymer used as gelling agent- carbopol 940 [39].

The extent of area of the skin to which a topical formulation readily spreads on application is denoted by the spreadability; which is an ideal quality that must be met by topical formulations. The more a topical formulation spread over the skin, the higher the patient compliance and therapeutic effects [37]. All the formulations were of adequate spreadability. TME9 had the lowest spreadability of 862.07g/cm^2 and TME10 had the highest spreadability of 1388.88g/cm^2. The viscosity of all the formulations were measured and they showed decrease in viscosity with increase shear rate from 6 to 12 rpm which suggests shear thinning (pseudoplastic behavior) of the formulations [40]. This is because increasing the shear stress cause the normally disarranged molecules of the gelling material (carbopol 940) to align their long axes in the direction of flow, which in turn reduces the internal resistance of the material and hence decreases the viscosity [41].

The post formulation anti-inflammatory studies of the emulgels were done using the Complete Freund's adjuvant (CFA)-induced arthritic model and the results were presented in Table 8. It is well documented that the injection of CFA into the rat hind paw results in marked joint inflammation, characterized by release of various inflammatory mediators such as prostaglandins, reactive oxygen species (ROS), kinins, cytokines and substance P [42]. However, the pre-treatment of rats with the formulated emulgels (containing different concentration of *Terminalia macroptera* stem bark extract) produced significant dose dependent inhibition of oedema. Subcutaneous injection of CFA into the right hind paw produced an increase in the right paw diameter which peaked on day 21 (3.25±0.64) but significantly reduced by topical administration of TME 8 (0.5% extract in 6% MO) ($p < 0.05$) to 1.74±0.41mm by 46.65% when compared to vehicle treated control. TME 7 (2% extract in 10% CO) and TME 9 (2% extract in 6% MO) had significant % inhibition at week 3,4 and 5 as compared to the positive control (diclofenac emulgel). The peak inhibition of oedema was achieved with TME 7 (2% extract in 10% CO) at week 5 (66.73%) which was better as compared to the positive control (diclofenac emulgel) with 21.89% inhibition. From the results obtained, the combination of 6% MO and 10% CO failed to produce additive anti-oedematogenic effect compared to when

the enhancer were used individually. Emulgels are good topical drug delivery systems as supported by the results obtained from the post-formulation and the pre-formulation assay of the extract. The importance of permeation enhancers in the formulation of emulgels was also stressed as the emulgels prepared without permeation enhancers showed lesser activities as compared to the ones with permeation enhancers. The formulated emulgels were evaluated to have good characteristics and hence can potentially serve as a good topical delivery system for herbal preparations.

Conclusion

The *Terminalia macroptera* stem bark ethanol extract obtained showed anti-inflammatory activity and emulgels were successfully formulated with different concentrations of the extract as the active ingredient. The emulgels were evaluated for their physical characteristics, pH, spreadability, extrudability, viscosity and anti-inflammatory activity (using animal studies); which were all found to be satisfactory. The formulated emulgels (TME2, TME5, TME7, TME8 and TME9) showed comparable anti-inflammatory activity with that of the standard Non-Steroidal Anti-Inflammatory emulgel (diclofenac). The emulgels formulated with mentha and clove oil as permeation enhancers showed lower activities than those formulated with either of the permeation enhancers; it is therefore recommended that mentha oil or clove oil alone should be used as permeation enhancer in the formulation of topical herbal emulgel. The extract incorporated into emulgel formulation shows prospects in the management of rheumatic arthritis.

Ethics approval and consent to participate

Ethical approval to execute this study was sought and obtained from the University Ethical Review Committee of the University of Ilorin, Nigeria. The approval (with reference ID: UERC/ASN/2022/2338) was issued prior to the commencement of the research.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare no competing interests.

CRediT authorship contribution statement

Adeola Tawakalitu Kola-Mustapha: Conceptualization, Data curation, Formal analysis, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. **Suleiman Olubusayomi Taiwo:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Abimbola Rofiat Isiaka:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Sherifat Omowunmi Amao:** Supervision, Writing – original draft, Writing – review & editing. **Ismail O. Ishola:** Supervision, Formal analysis, Writing – original draft, Writing – review & editing. **Yusuf Oluwagbenga Ghazali:** Formal analysis, Writing – review & editing. **Sukurat O. Usman:** Conceptualization, Writing – review & editing.

Acknowledgements

The authors are grateful to the Technologists of the Department of Pharmaceutics and Industrial Pharmacy University of Ilorin and Department of Pharmacology, Therapeutics and Toxicology, College of Medicine University of Lagos for their laboratory support.

References

[1] JS Smolen, D Aletaha, IB. McInnes, Rheumatoid arthritis, Lancet 388 (2016) 2023–2038.
[2] KM. Pollard, Silica, silicosis, and autoimmunity, Front. Immunol. 7 (2016) 97.
[3] D Van der Woude, AH van der Helm-van, Update on the epidemiology, risk factors, and disease outcomes of rheumatoid arthritis, Best Practice Res. Clin. Rheumatol. 32 (2) (2018) 174–187.
[4] KP Liao, L Alfredsson, EW. Karlson, Environmental influences on risk for rheumatoid arthritis, Curr. Opin. Rheumatol. 21 (3) (2009) 279–283, doi:10.1097/BOR.0b013e32832a2e16.

[5] K Younis, D. Al-Bustany, Prevalence of obesity in rheumatoid arthritis and its association with disease activity and latex positivity in a sample of patients in Erbil, ZJMS 21 (2017) 1726–1735, doi:10.15218/zjms.2017.027.
[6] J Feng, Q Chen, F Yu, Z Wang, S Chen, Z Jin, et al., Body mass index and risk of rheumatoid arthritis: a meta-analysis of observational studies, Medicine (Baltimore) 95 (8) (2016) e2859.
[7] M Essouma, JJ. Noubiap, Is air pollution a risk factor for rheumatoid arthritis, J. Inflamm. 12 (1) (2015) 1–7.
[8] Freeman J. RA facts: what are the latest statistics on rheumatoid arthritis? rheumatoidarthritis.org. 2018. https://www.rheumatoidarthritis.org/ra/facts-and-statistics/
[9] A Boyd, C Bleakley, DA Hurley, C Gill, M Hannon-Fletcher, P Bell, et al., Herbal medicinal products or preparations for neuropathic pain, Cochrane Database Syst. Rev. 4 (2019) CD010528, doi:10.1002/14651858.CD010528.pub4.
[10] KL Soeken, SA Miller, E. Ernst, Herbal medicines for the treatment of rheumatoid arthritis: a systematic review, Rheumatol. Oxf. 42 (2003) 652–659, doi:10.1093/rheumatology/keg183.
[11] A Tamhane, G McGwin, DT Redden, LB Hughes, EE Brown, AO Westfall, et al., Complementary and alternative medicine use in African Americans with rheumatoid arthritis, Arthritis Care Res. (Hoboken) 66 (2014) 180–189.
[12] J Pu, FF Fang, XQ Li, Z H Shu, YP Jiang, T Han, et al., Matrine exerts a strong anti-arthritic effect on type II collagen-induced arthritis in rats by inhibiting inflammatory responses, Int. J. Mol. Sci. 17 (9) (2016) 1410.
[13] XZ Zeng, LG He, S Wang, K Wang, YY Zhang, L Tao, et al., Aconine inhibits RANKL-induced osteoclast differentiation in RAW264. 7 cells by suppressing NF-κB and NFATc1 activation and DC-STAMP expression, Acta Pharmacol. Sin. 37 (2) (2016) 255–263.
[14] V Kumar, FA Al-Abbasi, A Verma, M Mujeeb, F. Anwar, Umbelliferone β-D-galactopyranoside exerts an anti-inflammatory effect by attenuating COX-1 and COX-2, Toxicol. Res. 4 (4) (2015) 1072–1084.
[15] H. Ibrahim, Pharmacognostic studies of the leaves of *Terminalia macroptera*, Niger. J. Nat. Prod. Med. 9 (2005) 14–18.
[16] S Usman, A Agunu, S Atunwa, S Hassan, A Sowemimo, K. Salawu, Phytochemical, and anti-inflammatory studies of ethanol extract of *Terminalia macroptera* Guill. & Perr. (Combretaceae) stem bark in rats and mice, Niger. J. Nat. Prod. Med. 13 (2) (2017) 147–156.
[17] O Silva, A Duarte, M Pimentel, S Viegas, H Barroso, J Machado, et al., Antimicrobial activity of Terminalia macroptera root, J. Ethnopharmacol. 57 (3) (1997) 203–207.
[18] P Peneva, V Andonova, B Pilicheva, M. Kassarova, In-vitro survey of ketoprofen release from emulgels, Medicine (Baltimore). 4 (1) (2014) 118–121.
[19] F Alexandrino-Junior, MC Freire, VD Lione, EA Cardoso, HR Marcelino, J Genre, et al., A functional wound dressing as a potential treatment for cutaneous leishmaniasis, Pharmaceutics 11 (5) (2019) 200.
[20] JQ Trinconi, VI Bonano, CR Espada, DC Miguel, JK Yokoyama-Yasunaka, SR. Uliana, Topical tamoxifen in the therapy of cutaneous leishmaniasis, Parasitology 145 (4) (2018) 490.
[21] Mohamed MI. Optimization of chlorphenesin emulgel formulation. 2004. Retrieved from https://link.springer.com/article/10.1208/aapsj060326
[22] CK. Kokate, Preliminary phytochemical screening, in: Practical Pharmacognosy, 4th ed., Nirali Prakashan P., 2000, pp. 107–111.
[23] K Wolfe, X Wu, RH. Liu, Antioxidant activity of apple peels, J. Agricul. Food Chem. 51 (3) (2003) 609–614.
[24] G Miliauskas, PR Venskutonis, TA. Beek, Screening of radical scavenging activity of some medicinal and aromatic plant extracts, Food Chem. 85 (2004) 231–237.
[25] R Gupta, J. Kaur, Evaluation of analgesic, antipyretic, and anti-inflammatory activity on *Cordia dichotoma* G. Forst. Leaf, Pharmacogn. Res. 7 (1) (2015) 126.
[26] VD Prajapati, PM Maheriya, GK. Jani, Carrageenan: A natural seaweed polysaccharide and its applications, Carbohydr. Polym. 105 (2014) 97–112, doi:10.1016/j.carbpol.2014.01.067.
[27] M Singh, V. Mittal, Formulation and evaluation of herbal gel containing ethanolic extract of ipomoea fistulosa, Int. J. Sci. Res. 3 (2014) 25–29.
[28] M. Ekor, The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety, Front. Pharmacol. 4 (2014) 177.
[29] DA Innalegwu, EJ Uyi, AO Amuda, N. Vivian, Phytochemical and anti-microbial study of seventy percent methanol leaf extract of terminalia macroptera against selected pathogenic bacteria, J. Adv. Med. Pharm. Sci. (2016) 1–8.
[30] BJ Arianne, BID Damer, B Jamila, B Asha, S Louanne, S Atlyn, et al., Molecular identification of Eimeria Species in broiler chickens in Trinidad, West Indies, Vet. Sci. 5 (2018) 12, doi:10.3390/vetsci5010012.
[31] M Haïdara, A Dénou, M Haddad, A Camara, K Traoré, A Aubouy, et al., Evaluation of anti-inflammatory, anti-pyretic, analgesic, and hepatoprotective properties of terminalia macroptera, Plant. Medi. Int. Open 7 (02) (2020) e58–e67.
[32] A Rodrigues, P Albuquerque, I Silva-Pereira, R Socodatto, CC Portugal, JB Relvas, et al., Antifungal and anti-inflammatory potential of eschweilenol C-rich fraction derived from Terminalia fagifolia Mart, J. Ethnopharmacol. 240 (2019) 111941.
[33] M Majid, RK Muhammad, AS Naseer, UH Ihsan, AF Muhammad, U Shafi, et al., Studies on phytochemical, antioxidant, anti-inflammatory, and analgesic activities of Euphorbia dracunculoides, BMC Complement. Altern. Med. 15 (2015) 349, doi:10.1186/s12906-015-0868-0.
[34] A Manosroi, P Jantrawut, E Ogihara, A Yamamoto, M Fukatsu, K Yasukawa, et al., Biological activities of phenolic compounds and triterpenoids from the galls of Terminalia chebula, Chem. Biodivers. 10 (2013) 1448–1463.
[35] Y. Traore, A. Toure, S. Meite, A. Ouattara, c. Bahi, K.G. Nathalie, A. Coulibaly, M Dosso, Phytomolecules of *Terminalia macroptera* stem bark extracts Guill *et* Perr (Combretaceae) and antibacterial activity, J. Pharmacog. Phytochem. 8 (2) (2019) 2255–2264.
[36] A Sowemimo, E Okwuchuku, FM Samuel, O Ayoola, I. Mutiat, *Musanga cecropioides* leaf extract exhibits anti-inflammatory and anti-nociceptive activities in animal models, Rev. Bras. Farmacogn. 25 (5) (2015) 506–512.
[37] SS Sultana, G Swapna, GS Lakshmi, S Swathi, GN Jyothi, AS. Devi, Formulation, and evaluation of herbal emulgel of Lantana camara leaves extract for wound healing activity in diabetic rats, Indo Am. J. Pharm. Res. 6 (8) (2016) 6404–6417.
[38] A Mahendra, D. Rasika, Formulation, and evaluation of topical anti-inflammatory herbal gel, Asian J. Pharm. Clin. Res. 12 (7) (2019) 252–255.
[39] R Ambala, SK. Vemula, Formulation, and characterization of ketoprofen emulgels, J. Appl. Pharm. Sci. 5 (7) (2015) 112–117.
[40] AT Kola-Mustapha, AO. Abioye, Formulation and Characterization of Gellan–DEAE-Dextran polyelectrolyte complex hydrogels, West Afr. J.Pharm. 27 (1) (2016) 1–14.
[41] SP Mulye, KA Wadkar, MS. Kondawar, Formulation development and evaluation of indomethacin emulgel, Der Pharm. Sin. 4 (5) (2013) 31–45.
[42] KE. McCarson, Models of inflammation: carrageenan- or complete freund's adjuvant (cfa)-induced edema and hypersensitivity in the rat, Curr. Protoc. Pharmacol. 70 (2015) 5.4.1–5.4.9.