



Herbal emulgels incorporated with *Cola millenii* K. Schum stem bark ethanol extract potentially for the management of rheumatoid arthritis in-vitro

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ABSTRACT

Background: Rheumatoid Arthritis (RA) is a chronic autoimmune disease characterized by inflammation and damage of the joints. The current, prevalent treatment options of RA are usually deficient in efficacy, cost-effectiveness and/or are frequently toxic. This is a formulation-oriented study, designed to formulate *Cola millenii* stem bark extract in an emulgel, suitable for use in the treatment of inflammation associated with RA.

Method: The phytochemical analysis and anti-inflammatory activity of *Cola millenii* stem bark ethanol extract was evaluated using standard analytical methods and Enzyme-Linked Immunosorbent Assay respectively. Inhibitory activity on enzyme/chemokine-substrate reactions involving inflammatory mediators, TNF- α , IL-10, and COX-2 indicated in the pathophysiology of RA were studied. Physical characterization of the formulated emulgels and post-formulation anti-inflammatory studies, using diclofenac emulgel as the positive control were evaluated.

Results: The phytochemical analysis of the extract showed the presence of the following phytochemicals: saponins, steroids, anthocyanins, and terpenoids. The extract at concentrations, 2, 4, 6, and 8% w/v, showed dose-dependent anti-inflammatory activity comparable with diclofenac. The formulated emulgels showed good physical characteristics including, easy application and removal, good extrudability, spreadability, pH ranging from 5.06 ± 0.05 to 4.34 ± 0.06 and viscosity ranging from 3.08 ± 0.3 to 3.74 ± 0.4 . The formulated emulgels also showed dose-dependent anti-inflammatory activity comparable ($p < 0.05$) with diclofenac emulgel.

Conclusion: The exhibited inhibitory activity of the plant on mediators of inflammation like TNF- α , IL-10, and COX-2 makes it a suitable candidate for formulation in a topical herbal formulation for use in the management of rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune disease characterized by progressive inflammation of the synovium in joints, causing pain, loss of function and deformity (Guo et al., 2018). It was estimated that as of 2016, genetic factors accounted for between 40% and 65% seropositive and about 20% seronegative cases of rheumatoid arthritis (RA) respectively (Suresh and Shetty, 2018). A family history of RA increases its risk to about four-fold. Inheritable genes such as the Major Histocompatibility Complex (MHC) antigen HLA-DR4 have been indicated in the pathogenesis of RA; the significance of this antigen is believed to vary among different ethnic groups (Younis and Al-Bustany, 2017). About a hundred genes have been associated with the occurrence of RA, most of which involve the Human Leukocyte Antigen complex (particularly HLA-DRB1) which functions in the recognition of

'self' versus 'non-self' molecules. The HLA-DRB1 allele has been identified as the strongest known genetic risk factor for RA (Liao et al., 2009).

Other risk factors, unrelated to genetics, have been identified and established in relation to RA (Firestein and McInnes, 2017; Suresh and Shetty, 2018). Exposure to sources of silica poisoning for example, has been suggested as a possible environmental risk factor for RA (Pollard, 2016).

RA has a worldwide prevalence of about 0.5 - 1%, making it the most common autoimmune disease (Pollard, 2016). RA reduces life-expectancy by about 12 years, complications including excessive joint damage, functionality loss and hospitalization increased mortality (Pollard, 2016).

The pro-inflammatory cytokine, TNF- α is about the most important among the cytokines indicated in RA and regulation of this protein produces an impressive control of the disease progression

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through direct inhibition of the cytokine and indirect modulation of the activities of other cytokines (Brennan and McInnes, 2008). Moreover, pleiotropic cytokines like IL-10 and IL-4 have also produced active roles in the progression of the disease (Shams and Ali, 2017).

The current, prevalent treatment options of RA are deficient in efficacy, cost effectiveness and/or are frequently toxic (Soeken et al., 2003). Research has shown that patients with chronic pain such as seen in RA as well as those dissatisfied with current therapy are likely to seek alternative care or herbal remedies (Soeken et al., 2003; Tamhane et al., 2014). An estimated 60 - 90% of patients suffering from arthritis seek complementary and alternative medicine with herbal remedies and chiropractic procedures among the most commonly sought (Rao et al., 1999; Tamhane et al., 2014).

Recently, the demand for herbal medicines has increased due to their lower tendency to cause side effects and complications when compared with synthetic drugs (Boyd et al., 2019). Consequently, phytopharmaceutical studies of medicinal plants have increased worldwide to further characterize and provide more information on the medicinal values of such plants (Garg and Adams, 2012). Examples of herbal materials commonly used in RA include herbal oils containing γ -linoleic acid including borage seed oil, evening primrose oil, and blackcurrant seed oil. Other herbal preparations include capsaicin, curcumin, feverfew, and flaxseed oil (Soeken et al., 2003). Phytoconstituents such as terpenoids, steroids, flavonoids, and fatty acids are among chemicals that have been studied for their potential use in RA (Kumar et al., 2015).

Cola millenii K. Schum (Sterculiaceae) also known as monkey cola, *Obi Edun* (Yoruba), *Uto* (Ibo), and *Ivyureanchere* (Ebira). It is a tree that grows in the wild in tropical and subtropical regions, suspected to have originated from Indonesia (Akinnibosun and Adewunmi, 2019). Members of the *Cola* genus all contain caffeine, kolatin alkaloids, pro-anthocyanins, magnesium, sodium, potassium bromide, cobalt, cesium, zinc, and selenium (Akinnibosun and Adewunmi, 2019). Sugar, starch, crude fat, crude protein, ascorbic acid, iron, and manganese have also been shown to be present in plant parts of *Cola* species (Nwokocha and Williams, 2018). Terpenoids found in the leaf, stem bark, and seeds of the plant possess anti-hepatotoxic, and antiseptic properties (Borokini, 2012). They are also reported to be actively cardiotonic and diuretic (Olaleye, 2007). Alkaloids found in *Cola millenii* have been reported to have analgesic, antiplasmodial, and bactericidal effects (Okwu and Okwu, 2004), explaining the bases for the folkloric use of *Cola millenii* in the treatment of various diseases in monkeys. High concentration of saponins found in leaf, stem bark, seed, and pulp which have hypotensive, cardiac depressant, anticarcinogenic, expectorant, and emulsifying properties (Borokini, 2012; Olaleye, 2007) may validate the use of such plant parts in the management of cardiovascular diseases and other applications.

Topical preparations are medicated or unmedicated formulations for external application meant for local or systemic actions, following absorption. The barrier function of the skin is mediated by a membranous structure - stratum corneum. The permeability of which is regulated by a robust, hydrophobic, and tortuous inter-cellular apparatus. Emulgels are gel-variants formulated to allow for the incorporation of hydrophobic drugs (or excipients) in the highly polar colloidal networks of gels (Hardenia et al., 2014). Emulgels typically contain in addition to the active ingredient; polar and non-polar solvents, preservatives, emulsifiers, gelling agents, and permeation enhancers which interact with the skin to increase the rate of absorption of the drug from the site of application (Yadav et al., 2016). The formulation of emulgels involves an initial stage of making an oil-in-water emulsion using fusion method to incorporate the hydrophobic constituents of the formulation in a hydrophilic continuous phase, a subsequent incorporation of the emulsion in a gel base and adjusting the pH of the formulation (Mohamed, 2004). In addition to possessing the desirable properties of gels (high drug carrying capacity, efficient deposition of drug and cosmetic acceptability), emulgels allow the incorporation of hydrophobic drugs which were once considered unsuitable for delivery in gel bases. Emulgels also allow

for the incorporation of hydrophobic excipients that improve the pharmaceutical parameters of formulations, are inexpensive, thus suitable for small-scale as well as large-scale production and are relatively easy to formulate. The concept is thus rapidly garnering popularity among drug formulators and researchers alike. This study attempts to explore the anti-inflammatory activity of *Cola millenii* in a formulation-oriented manner to yield a herbal emulgel as a viable and suitable alternative in the treatment of RA.

2. Materials and method

2.1. Materials

Stem bark of *Cola millenii*, Methanol (Guangdong Chemical Reagent, China), ethanol (BDH Chemicals Ltd., Poole England), liquid paraffin (BDH Chemicals Ltd., Poole England), span 20 (BDH Chemicals Ltd., Poole England), tween 20 (BDH Chemicals Ltd., Poole England), ethyl paraben (BDH Chemicals Ltd., Poole England), triethanolamine (BDH Chemicals Ltd., Poole England), methyl paraben (BDH Chemicals Ltd., Poole England), mentha oil (BDH Chemicals Ltd., Poole England), dimethyl sulfur oxide (DMSO) (Guangdong Chemical Reagent, China), carbopol 940 (Guangdong Chemical Reagent, China), hydrochloric acid (Guangdong Chemical Reagent, China), Ellman's reagent (Cayman Chemical, Ann Arbor, MI, USA), EIA kit (Cayman Chemical, Ann Arbor, MI, USA), Human-recombinant TNF- α ELISA kit (Cayman Chemical, Ann Arbor, MI, USA), Human-recombinant IL-10 ELISA kit (Cayman Chemical, Ann Arbor, MI, USA), Voltaren® emulgel (diclofenac).

2.2. Method

2.2.1. Collection of plant extraction

Cola millenii stem bark was collected from a location within Egbimo town, Ekiti State, Nigeria. The plant was identified at the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, University of Ilorin and authentication was done at the Forest Herbarium, Ibadan, Forestry Research Institute of Nigeria, Jericho Road, Ibadan with the voucher number FHI 112,883 and deposited for future reference.

2.2.2. Extraction from *Cola millenii* stem bark

Extraction was done by maceration of the weighed (Ohaus, USA) oven-dried and milled bark (1000 g) in 80% ethanol for 48 h. The extract obtained was filtered through a Whatman filter paper 125 mm (No 1) under vacuum at room temperature. The filtrate was concentrated by evaporation under reduced pressure in a rotary evaporator at 45 °C until the extracts became completely dry. After evaporation, the extract was stored at -20 °C until use.

The percentage yield was calculated for *Cola millenii* stem bark.

2.2.3. Characterization of extract

The ethanol extract of *Cola millenii* obtained was observed organoleptically and physical characteristics including texture, color, odor and pH were evaluated.

2.2.4. Phytochemical analysis of extract

Quantitative and qualitative analysis of *Cola millenii* extract was carried out.

Qualitative analysis of extract: qualitative assays were done to check for the presence of various phytochemicals in the ethanol extract of *Cola millenii* stem bark (Auwal et al., 2014; Gul et al., 2017; Hossain et al., 2013; Narasimhan, 2014; Obouayeba et al., 2015; Roghini, 2018).

Quantitative analysis of extract: assays were done to determine levels of phytochemicals found present in the ethanol extract of *Cola millenii* stem bark. Saponins were estimated using the method of Ejikeme and co-workers (Ejikeme et al., 2014).

Table 1Formulation of emulgel from ethanol extract of *Cola millenii* stem bark.

Ingredient	Emulgel Formulations (% ^w / _w)			
	E0	E1	E2	E3
<i>Cola millenii</i> extract	0.00	0.50	1.00	1.50
Ethanol	2.50	2.50	2.50	2.50
Span 20	1.00	1.00	1.00	1.00
Tween 20	0.50	0.50	0.50	0.50
Methyl paraben	0.03	0.03	0.03	0.03
Propylene glycol	5.00	5.00	5.00	5.00
Light liquid paraffin	7.50	7.50	7.50	7.50
Mint oil	5.00	5.00	5.00	5.00
Carbopol 940	1.00	1.00	1.00	1.00
Purified water	100.00	100.00	100.00	100.00

2.2.5. Assay of anti-inflammatory activity of extract

For the anti-inflammatory assay, 2, 4, 6, and 8% w/v (g/100 mL) solutions of the extract were prepared in methanol. The positive control solution was prepared by dissolving 50 mg of diclofenac in 10 mL, methanol. The *in-vitro* anti-inflammatory activity of the extract was assayed by measuring its inhibition of the enzyme-substrate reactions involving the chemokines; TNF- α , Cox-2 and IL-10 (Kola-Mustapha et al., 2020).

2.2.6. Emulgel formulations

The emulgels were prepared Table 1 following the method described by Mohamed (Mohamed, 2004) with slight modifications, and packaged in closed jars till further analysis.

2.2.7. Emulgel analysis

Physical characterization: the general observable properties including texture, color, odor, homogeneity, consistency and general appearance of the emulgels formed were recorded.

Extrudability: the extrudability of the emulgels were measured by placing a 0.5 g load at the folded end of the tube, measuring the length of the ribbon of emulgel and calculating extrudability using the formula;

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

Spreadability: spreadability was measured by dropping 1 g of the emulgel on a glass slide, placing another glass slide of equal area, weighing 158 g on it gently and allowing the emulgel trapped between, to spread for 30 s after which the diameter of spread was measured, and the circumference calculated.

pH determination: the pH was measured using a digital pH meter (Hanna, England) and done in triplicate with the average values and standard errors of mean calculated and recorded.

Determination of viscosity: emulgel samples were placed at room temperature for 30 min. Viscosities were determined using NDJ-5S Digital Display Viscometer (Rinch, China). Number 4 spindle was attached then viscosity was determined at 25 °C and 60 rpm. The tests were done in triplicates and recorded.

2.2.8. Assay of anti-inflammatory activities of formulated emulgels

The anti-inflammatory activities of the emulgels formed were studied using Enzyme-Linked Immunosorbent Assay by measuring their inhibitory activities as *in-vitro* reactions involving COX-2, TNF- α , and IL-10. Graded concentrations of each emulgel (10, 20, 30, 40, and 50% w/v) were prepared by dissolving the emulgels in DMSO with agitation until complete dissolution was achieved. Positive control solution was prepared by dissolving diclofenac gel in methanol to obtain concentrations of 10, 20, 30, 40, and 50% w/v respectively (Kola-Mustapha et al., 2020).

2.2.9. Statistical analysis

Results are presented as mean $\pm$ standard error of mean (SEM). Statistical comparison was done by one-way analysis of variance (ANOVA). The differences between the %inhibition of the inflammatory mediators

Table 2Qualitative phytochemical constituents of ethanol extract of *Cola millenii* stem bark.

Phytochemicals	Observation
Saponins	+
Tannins	-
Phenolics	-
Phlobatannin	-
Steroids	+
Flavonoids	-
Coumarins	-
Anthocyanins	+
Amino acid	-
Terpenoids	+
Glycosides	-
Triterpenes	-
Alkaloids	-

Note: + = present; - = absent.

Table 3Quantitative estimation of ethanol extract of *Cola millenii* stem bark.

Phytochemicals	Quantity (mg/g)
Saponins	0.41 $\pm$ 0.08
Tannins	0.00 $\pm$ 0.00
Phenolics	0.00 $\pm$ 0.00
Steroids	108.10 $\pm$ 0.06
Flavonoids	0.00 $\pm$ 0.00
Coumarins	0.00 $\pm$ 0.00
Anthocyanins	0.35 $\pm$ 0.08
Terpenoids	9.21 $\pm$ 0.80
Alkaloids	0.00 $\pm$ 0.00

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

by the different emulgel formulations and control were also evaluated. The statistical analysis was carried out using the online Social science statistics one-way ANOVA calculator as well as graph-pad prism version 8.4.3 for windows and graph-pad prism version 6.00 for mac OS, Graph-pad Software, La Jolla, California, USA, www.graphpad.com. Significant differences were set at p values lower than 0.05.

3. Results

3.1. Extract characterization

3.1.1. Crude extraction

The ethanol extraction of *Cola millenii* stem bark gave 19% yield.

3.1.2. Appearance and pH

The ethanol extract of *Cola millenii* stem bark obtained had a brown syrupy appearance, smooth to touch and with a characteristic odor. The pH was 5.72 $\pm$ 0.18.

3.1.3. Phytochemical screening

Phytochemical constituents in ethanol extract of *Cola millenii* stem bark is presented in Table 2.

3.1.4. Quantitative analysis

Quantitative estimation of the ethanol extract of *Cola millenii* stem bark is presented in Table 3.

3.1.5. Anti-inflammatory activity of extract

The inhibitory activity of three graded concentrations of the extract as prepared against TNF- α , IL-10, and COX-2 were observed to be generally comparable with the control drug, diclofenac. The results obtained from the *in-vitro* anti-inflammatory activity assay of the various concentrations of the extracts are given in Table 4 and Fig. 1.

Table 4Percentage inhibition of TNF- α , IL-10, and COX-2 by ethanol extract of *Cola millenii* stem bark using diclofenac as positive control.

Enzyme	% inhibition 20 mg/mL	40 mg/mL	60 mg/mL	80 mg/mL	Control (Diclofenac)
TNF- α	24.94 $\pm$ 0.90 ^b	25.54 $\pm$ 0.74 ^b	26.24 $\pm$ 1.26 ^b	28.94 $\pm$ 1.42 ^b	52.33 $\pm$ 2.32 ^a
Cox-2	16.68 $\pm$ 0.57 ^b	19.29 $\pm$ 0.44 ^b	25.48 $\pm$ 0.85 ^b	30.57 $\pm$ 1.84 ^b	65.50 $\pm$ 3.86 ^a
IL-10	15.78 $\pm$ 0.38 ^b	15.18 $\pm$ 0.80 ^b	16.92 $\pm$ 0.36 ^b	19.20 $\pm$ 0.52 ^b	24.83 $\pm$ 0.77 ^a

Data presented as Mean $\pm$ SEM ($n = 3$).**Table 5**

Physical characterization of emulgels.

Emulgel	appearance	Color	Odor	Ease of application	Ease of removal	Homogeneity
E0	Glossy, less viscous	Slightly off-white	Minty odor	Very easily applied	Easily removed	Homogenous
E1	Glossy, viscous	Cream	Characteristic of bark with minty odor	Very easily applied	Easily removed	Homogenous
E2	Slightly glossy, more viscous	Light Brown	Characteristic of bark with minty odor	Very easily applied	Easily removed	Homogenous
E3	Least glossy, most viscous	Brown	Strong bark odor, with slight minty odor	Very easily applied	Easily removed	Homogenous

Table 6

PH, spreadability, extrudability and viscosity of emulgels.

Emulgels	pH	Spreadability (mm)	Extrudability (g/cm ²)	Viscosity (Pas)
E0	5.06 $\pm$ 0.05	27.9 $\pm$ 0.44	1052.63 $\pm$ 12.37	3.74 $\pm$ 0.40
E1	4.56 $\pm$ 0.05	30.4 $\pm$ 0.52	980.39 $\pm$ 13.21	3.25 $\pm$ 0.20
E2	4.55 $\pm$ 0.03	32.5 $\pm$ 1.29	917.43 $\pm$ 10.52	3.08 $\pm$ 0.30
E3	4.34 $\pm$ 0.06	29.2 $\pm$ 0.40	980.39 $\pm$ 11.54	3.46 $\pm$ 0.50

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

3.2. Emulgel characterization

3.2.1. Physical characterization

The physical features of the emulgels formed as observed organoleptically, including general appearance, color, odor, texture, ease of application and removal, and feel on skin are presented in Table 5.

3.2.2. Extrudability

The extrudability of the emulgels formed from an aluminum foil tube is presented in Table 6 as measured in terms of the ratio of weight of load to area of ribbon extruded

3.2.3. Spreadability

The spreadability of the emulgels formed was presented in Table 6 as measured in terms of circumference of smear ($\emptyset$).

3.2.4. pH

The mean pH of the emulgels formed expressed as standard errors of mean are presented in Table 6.

3.2.5. Viscosity

The mean viscosity of each emulgel (Pas) is presented in Table 6

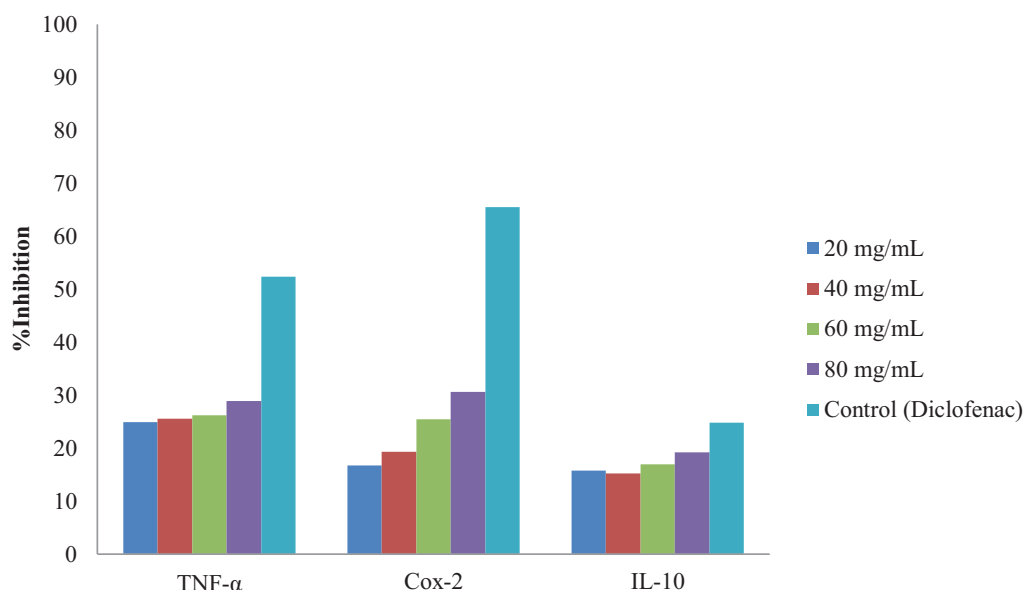
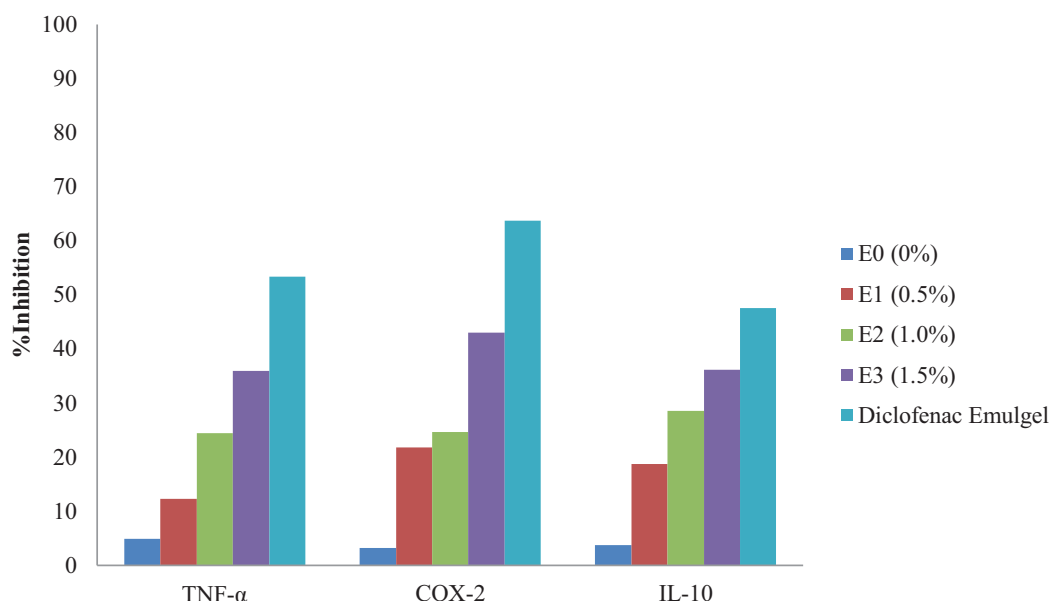


Fig. 1. Percentage inhibition of TNF- α , COX-2, and IL-10 by graded concentrations of *Cola millenii* ethanol stem bark extract.

Table 7%Inhibition of TNF- α , Cox-2, and IL-10 by graded concentrations of the emulgels formed.

Mediators	% Inhibition E0 (0%)	E1 (0.5%)	E2 (1.0%)	E3 (1.5%)	C0 (Diclofenac emulgel)
TNF- α	4.88 $\pm$ 0.23 ^b	12.32 $\pm$ 0.61 ^b	24.46 $\pm$ 1.00 ^a	35.99 $\pm$ 1.56 ^a	53.33 $\pm$ 1.30 ^a
COX-2	3.18 $\pm$ 0.68 ^b	21.84 $\pm$ 1.39 ^b	24.64 $\pm$ 1.54 ^a	43.05 $\pm$ 1.20 ^a	63.72 $\pm$ 2.28 ^a
IL-10	3.76 $\pm$ 0.56 ^b	18.76 $\pm$ 0.41 ^b	28.59 $\pm$ 1.51 ^a	36.11 $\pm$ 0.46 ^a	47.61 $\pm$ 5.88 ^a

Data presented as Mean $\pm$ SEM ($n = 3$). Values carrying superscripts different from the control emulgel for each parameter are significantly different ($p < 0.05$).

**Fig. 2.** Percentage inhibition of the various formulations against mediators of pain and inflammation; TNF- α , IL-10, and COX-2.

3.3. Anti-inflammatory activities of emulgels

The anti-inflammatory activity of the emulgels, as measured by their inhibitory activity against TNF- α , IL-10, and Cox-2 was obtained and compared with that of diclofenac emulgel (Voltaren®). The results obtained are presented in Table 7 and Figure 2.

3.4. Statistical analysis

3.4.1. Pre-formulation

The anti-inflammatory activity of each concentration of *Cola millenii* extract prepared was obtained as the mean of its%inhibition against the three inflammatory mediators. The resulting average was used as the parameter for comparing the anti-inflammatory activities of the extract against diclofenac. On this basis, null hypothesis (H_0) and alternate hypothesis (H_A) were set and ANOVA was employed to compare the means.

H_0 = There is no difference between the means of the anti-inflammatory activities of 2, 4, 6, and 8% solutions of *Cola millenii* stem bark ethanol extract and diclofenac.

H_A = There is a difference between the means of the anti-inflammatory activities of 2, 4, 6, and 8% solutions of *Cola millenii* stem bark ethanol extract and diclofenac.

p value = 0.0498; p value summary = *

A p value < 0.05 as obtained implies that there is a statistically significant difference between the average anti-inflammatory activities of the extract at graded concentrations and the control, diclofenac. The alternate hypothesis is therefore taken, and the null rejected. In addition, the results obtained are statistically significant.

A post Dunett's multiple comparisons test was also done to compare the anti-inflammatory activities of *Cola millenii* stem bark ethanol extract solutions with diclofenac, a null hypothesis (H_0) and an alternate

hypothesis (H_A) was developed at each concentration of the extract solution.

H_0 = There is no difference between the mean anti-inflammatory activity of *Cola millenii* stem bark ethanol extract at concentrations 2, 4, 6, and 8%; and control, diclofenac.

H_A = There is a difference between the mean anti-inflammatory activity of *Cola millenii* stem bark ethanol extract at concentrations 2, 4, 6, and 8%; and control, diclofenac.

Adjusted p value (summary):

2% vs diclofenac = 0.2756 (ns)

4% vs diclofenac = 0.2538 (ns)

6% vs diclofenac = 0.2471 (ns)

8% vs diclofenac = 0.2708 (ns)

The p values (> 0.05) obtained with every concentration of *Cola millenii* extract against diclofenac implies that there is no statistically significant (ns) difference between the anti-inflammatory activities of each extract solution with that of the control, diclofenac. The null hypothesis is therefore accepted and the alternate hypothesis, rejected at all concentrations of *Cola millenii* stem bark ethanol extract.

3.4.2. Post formulation

The mean of anti-inflammatory activity of each emulgel obtained as the average of their%inhibition was compared against the three inflammatory mediators. This resulting average was used as the parameter for comparing the anti-inflammatory activities of the emulgels against the control diclofenac emulgel. On this basis, null hypothesis (H_0) and alternate hypothesis (H_A) were set, and ANOVA was employed to compare the means.

H_0 = There is no difference between the means of the anti-inflammatory activities of the emulgels E0, E1, E2, E3 and the control diclofenac emulgel (C0).

H_A = There is a difference between the means of the anti-inflammatory activities of the emulgels E0, E1, E2, E3 and the control diclofenac emulgel (C0).

p value = 0.0044; p value summary = **

A p value of < 0.05 as obtained implies that there is a statistically significant difference between the average anti-inflammatory activities of the emulgels and the control gel. The alternate hypothesis is therefore taken, and the null rejected. In addition, the results obtained are statistically significant ("One-Way Repeated Measures ANOVA Calculator," n.d.).

A post linear trend test was done to test for linear variability (ergo dose-response relationship) in the mean anti-inflammatory activities of the formulated herbal emulgels, a null and alternate hypothesis was set.

H_0 = There is no linear variability in the mean anti-inflammatory activities of herbal emulgels E0, E1, E2, and E3.

H_A = There is a linear variability in the mean anti-inflammatory activities of herbal emulgels E0, E1, E2, and E3

p value = < 0.0001 p value summary = ****

The p value obtained (< 0.05) obtained indicates a statistically significant linear variability in the mean anti-inflammatory activities of the formulated herbal emulgels E0, E1, E2, and E3. The alternate hypothesis is therefore adopted and the null, rejected.

A post Dunnett's multiple comparison test was done, comparing the mean anti-inflammatory activities of the herbal emulgels formulated with the mean anti-inflammatory activity of diclofenac emulgel. A null and alternate hypothesis was developed for each herbal emulgel formulation; E1, E2, and E3.

H_0 = There is no statistically significant difference between the mean anti-inflammatory activities of each herbal emulgel E1, E2, and E3 and diclofenac emulgel, C0.

H_A = There is a statistically significant difference between the mean anti-inflammatory activities of each herbal emulgel E1, E2, and E3 and diclofenac emulgel, C0.

Adjusted p value (summary)

E1 vs C0 = 0.0276 (*)

E2 vs C0 = 0.0822 (ns)

E3 vs C0 = 0.0557 (ns)

The p value (< 0.05) obtained with the herbal emulgel E1 indicates a statistically significant difference between the mean anti-inflammatory activities of E1 and C0, the alternate hypothesis is therefore adopted for E1. The p values (> 0.05) obtained with the herbal emulgel formulations E2 and E3 implies that there is no statistically significant difference between the mean anti-inflammatory activities of each of E2 and E3; and C0, the null hypothesis is therefore adopted for E2 and E3.

4. Discussion

The interest in the standardization of medicinal plants for pharmaceutical formulation and pharmacological use is on the rise globally. While various plants with pharmacological effects have been identified, a sizeable amount of these remain in their crude drug forms without research studies aimed at the pharmaceutical formulation of the plants and others remain with undocumented activities. Phytochemical and pharmacological studies of *Cola millenii* report the presence of phytochemicals that possess cardiogenic, anti-hepatotoxic, analgesic, anti-carcinogenic, anti-plasmodic, bactericidal, diuretic, hypotensive, and expectorant properties among others, validating folklore use of the plant in the treatment of a number of diseases especially in monkeys (Borokini, 2012; Okwu and Okwu, 2004; Olaleye, 2007).

Phytochemical assay of *Cola millenii* extract showed a high level of steroids and the presence of saponins, terpenoids, and anthocyanins, in tandem with phytochemical information of the plant, reported in literature (Akinnibosun and Adewunmi, 2019). Reports in literature, of phytochemical analysis of the plant, further identify pharmacologically active phytochemicals like 1,4-benzenediol, alpha-tocospiroA, bis(2-ethyl hexyl) phthalate, stigmasterol, beta-sitosterol, vitamin E, octadecanoic

acid, lup-20(29)-en-3-one and the novel anti-inflammatory and anti-cancer dietary triterpene, lupeol (Akinnibosun and Adewunmi, 2019; Saleem, 2009).

To assay the anti-inflammatory activity of *C. millenii* extract, Enzyme-Linked Immunosorbent Assay (ELISA) was carried out to ensure the anti-inflammatory activity was by a mechanism suitable for application in the treatment of rheumatoid arthritis. The assay method was selected based on its application in the basic understanding of the molecular pharmacology of *C. millenii* extract and its relative cost-effectiveness. Graded concentrations of the plant, 2, 4, 6, and 8% w/v were prepared using absolute methanol as the solvent and assay was done against two chemokines indicated in the pathophysiology of inflammation in rheumatoid arthritis, IL-10 and TNF- α ; and against one enzyme indicated in pain associated with rheumatoid arthritis, COX-2. Colorimetric end-points were derived and spectrophotometric readings were taken as presented in Table 4 as percentage inhibition for the three mediators. It was observed that the plant extract exhibited a dose-dependent anti-inflammatory activity, comparable with the positive control, commercial diclofenac (voltaren®) in addition to exhibiting inhibitory effects on the anti-inflammatory cytokine, IL-10 at levels statistically incomparable with diclofenac. The analgesic activity of the plant extract, as measured by its inhibitory activity against COX-2 was much lower than that of the positive control drug. Based on the results obtained, the extract was formulated as an emulgel using a slightly modified version of the formulation method proposed by Mohamed (2004). Mint oil (5%) was used as the skin permeation enhancer, and carbopol (1%) as the gelling agent. Formulation was prepared with final concentration of extracts, 0.5, 1, and 1.5% w/w of extracts respectively. The emulgels formed were generally glossy, homogenous, and consistent. The emulgels generally had a minty odor, impacted by the mint oil. The minty odor was however masked by the odor of the extract as the concentration of the extract in the formulation increased progressively. The color of the emulgels varied from 'slightly creamy' to 'light brown'. The pH of the formulated emulgels were found to be slightly acidic with the emulgel with the highest extract concentration showing highest acidity at pH 4.34 ± 0.06 and the pH approached neutrality with decreasing extract concentration, suggesting an increase in acidity with concentration of extract in the emulgels. It is preferred that creams, gels, ointments, and other preparations for application to the skin have slightly acidic pH as the pH of the skin surface as proven by past potentiometric measurements is 4.1 - 5.8 on an average (Segger et al., 2008).

The anti-inflammatory activity of the emulgels was measured post-formulation as their inhibitory activities on the chemokines, COX-2, IL-10, and TNF- α , *in-vitro* ELISA technique. A dose-dependent anti-inflammatory activity comparable with diclofenac emulgel at concentrations 1% and 1.5% respectively was exhibited. The formulated emulgels also inhibited the activity of the anti-inflammatory cytokine, IL-10 to extents statistically comparable with diclofenac at 1% and 1.5% concentrations respectively, this significance of this can only be speculated using the pharmacologic profile of diclofenac. These findings further confirm the prospects of *Cola millenii* in the treatment of inflammation associated with rheumatoid arthritis.

5. Conclusion

The *Cola millenii* stem bark ethanol extract obtained showed anti-inflammatory activity comparable with that of the Nonsteroidal anti-inflammatory drug (NSAID), diclofenac. Herbal emulgels were successfully prepared with varying concentrations of the extracts. Post-formulation anti-inflammatory studies of the emulgels formulated also showed a dose-dependent anti-inflammatory activity, comparable with commercial diclofenac emulgel. Overall, the extracts and emulgels from *Cola millenii* gave promising results as herbal formulation intervention in the management of rheumatoid arthritis).

Declaration of Competing Interest

The authors declare no competing interests.

CRediT authorship contribution statement

Adeola T. Kola-Mustapha: Conceptualization, Methodology, Resources, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Fahd A. Khalid-Salako:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization.

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