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Lemon Oil Enhances the Anti-Rheumatic Activity of Woody Essential Oils in Formaldehyde-Induced Arthritis in Wistar Rats

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Abstract: Rheumatoid Arthritis (RA) is a major challenge, especially in the elderly. Many orthodox drugs have been helpful in managing the disease; however, deleterious side effects have been reported. Essential oils have been shown to exhibit anti-arthritic properties. This study highlights and compares the chemical composition and anti-arthritic activities of essential oils from *Citrus limon*, *Zingiber officinale*, and *Curcuma longa*. Twenty-seven *Wister rats* of both sexes were divided into 9 groups of three animals each. Acute non-immunological arthritis was induced by a sub-plantar injection of 0.1 mL formaldehyde (2% v/v). The animals were treated topically with essential oils extracted by hydro-distillation and analyzed by gas chromatography. Measurement of paw volumes was done daily for 10 days using the volume displacement method. Ginger oil and turmeric oil achieved a 100% reduction in paw volume from the 8th day ($p<0.05$). The coconut oil as carrier for the essential oils exhibited sub-optimal reductions when administered alone as a control. Combinations of the oils showed an antagonistic effect in the ginger/turmeric oil blend, while the essential blends of turmeric/lemon and ginger/lemon oil performed better than the individual oils. The findings from this study showed that the woody essential oils (ginger and turmeric) exhibited the greatest anti-arthritic activity of all the oil treatment groups. Terpenes like ar-turmerone, turmerone, zingiberene, and D-limonene may have been responsible for the activities observed with the turmeric, ginger, and lemon essential oils, respectively. Lemon, ginger, and turmeric essential oils are effective for the management of rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease whose hallmark feature is a persistent symmetric polyarthritis (synovitis) that affects the hands and feet. Any joint lined by a synovial membrane may be involved, but extra-articular involvement of organs such as the skin, heart, lungs, and eyes can be significant (1). RA is a significant medical condition in Nigeria. It predominantly affects females. Nigerians who suffer from RA have predominantly high titers of Rheumatoid Factor (RF) and anti-cyclic citrullinated Proteins Erythrocyte Sedimentation Rate (anti-CCP ESR); also, to a lesser extent, C- Reactive Protein (CRP) are usually elevated while disease activity is usually high at presentation (2). There has been an increase in global age-standardized prevalence and incidence rates of rheumatoid arthritis, according to the results of a systematic analysis published in the Annals of the Rheumatic Diseases. The analysis also indicated that rising prevalence and incidence rates could contribute to the increased global burden of RA (3). RA patients use a variety of treatment categories to create a multimodal treatment plan which targets inflammation reduction and alleviates pain and stiffness. In addition to natural home remedies, prescription medications, exercise regimens, physical and occupational therapy, and dietary adjustments, many patients also use essential oils to supplement their RA treatment plans (4).

Several therapeutic approaches have been employed including the use of Disease Modifying Anti-Rheumatic Drugs (DMARDS) and Non-steroidal anti-inflammatory drugs (NSAIDS). Unfortunately, the use of NSAIDs cannot block the development and progress of rheumatoid arthritis (5), and DMARDs have been impeded by their potential of long-term side effects, toxicity and immunosuppression (6). Yao et al. (7) reported that searching for new therapeutic drugs with greater efficiency and lower toxicity from a natural source is important.

Essential oils are derived and produced from the oils of certain natural herbs, flowers, fruits, and other plants. They characteristically emit a strong, soothing fragrance or have a strong, sweet, or spicy taste. More than 90% of the essential oils of medicinal plants consist of monoterpenoids (8). The beneficial effects of ginger to reduce pain associated with RA by inhibiting prostaglandin and leukotriene biosynthesis have been documented (9).

The genus *Curcuma longa* or turmeric is best known for being an essential source of coloring and flavoring agents in Asian cuisines, traditional medicines, spices, dyes, perfumes, cosmetics, and ornamental plants (10). Turmeric is credited with hot potency and anti-inflammatory action with specific lipoxygenase- and COX-2-inhibiting properties. Rheumatic complaints are often connected with inflammatory changes in joints. It cures the etiological factors and pathological inflammation changes (11, 12). Curcuminoids inhibit lipoxygenase, cycloxygenase, phospholipases, leukotrienes, prostaglandins, thromboxane, nitric oxide elastase, hyaluronidase, collagenase, monocyte chemoattractant protein-1, interferon inducible protein, Tumor necrosis factor (TNF) and interleukin-12 (13). Other Curcuma sesquiterpenes like zingerone suppress nuclear factor NF-kB activation in aged rat and is beneficial for suppressing both oxidative stress and age-related inflammation through a modulation of several key proinflammatory genes and transcription factors (14).

Lemon essential oil is extracted from *Citrus limon*, of the Rutaceae family and is also known as cedro oil (terpeneless oil) (15). A study published in 2014 demonstrated that lemon extract showed the maximal prevention of articular cartilage degeneration, inflammatory cells' infiltration in joint cavity, synovial hyperplasia and pannus formation in arthritis mice (16). Several mechanisms explaining the anti-inflammatory activity of lemon have been described, including: (a) anti-oxidative and radical scavenging activities, (b) regulation of cellular activities of inflammation-related cells, (c) modulation of the activities of arachidonic acid metabolism enzymes (phospholipase A2, cyclooxygenase lipoxygenase) and nitric oxide synthase, (d) modulation of the production of other pro-inflammatory molecules, (e) modulation of pro-inflammatory gene expression (17), possibly causing a decrease in ESR and serum CRP levels.

Ginger (*Zingiber officinale*) is an herbaceous and perennial plant, from the Zingiberaceae family, whose rhizome is widely used in the food and pharmaceutical industry (18). It contains phenolic compounds like gingerols, shogaols, and paradols (19) and others like quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione (20, 21). In addition, there are several terpene components in ginger, such as β-bisabolene, α-curcumene, zingiberene, α-farnesene, and β-sesquiphellandrene, which are considered the main constituents of ginger essential oils (22). A comparison of ginger with ibuprofen showed similar anti-inflammatory activity (23, 24).

Several studies have highlighted the importance of these essential oils and their components in managing inflammation and pain associated with rheumatoid arthritis. However, none of the studies has explored combining them to leverage their unique properties. The study aimed to explore the effect of combining these essential oils for enhanced anti-inflammatory activity. In the study, coconut oil (utilized as a carrier oil to reduce the phototoxicity and irritant effects of applying the essential oils directly to the skin) was also tested to see if it had any anti-inflammatory effect when utilized as a carrier oil for these essential oils. Furthermore, the ability of lemon oil to provide analgesia was seen as an added advantage of the combination since sufferers of the disease, rheumatoid arthritis, usually need analgesic and anti-inflammatory medications. The study evaluated the anti-inflammatory effects of single and coadministration of lemon, ginger, and turmeric oil. Essential oils were obtained by hydrodistillation, and a formaldehyde-induced rat model was used to study this anti-inflammatory effect.

Experimental Section

Plant Materials

The fresh rhizomes of ginger plant (*Zingiber officinale*), turmeric plant (*Curcuma longa*), and fresh lemon (*Citrus limonum*) were obtained from a local herbalist outlet in Awka, Anambra state. The plant samples were identified by Dr. Innocent Mary of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Chemicals

Formaldehyde 40% was obtained from CIL chemicals, Nigeria. Dilution was done to a 2% v/v concentration.

Extraction of Essential Oils

Approximately 138.9 g of fresh ginger rhizomes were washed. The weights of the pericarps were obtained

(unpeeled), and size reduction into chunks was done using a kitchen-type knife. Blending was done using about 100 ml of water. The blended plant material was transferred into a 1000 ml round bottomed flask and the volume was made up to 500 ml. The fresh rhizomes were subjected to hydro-distillation for two hours using the clevenger-type apparatus. Extraction was done in batches till all plant materials were exhausted. The essential oil was separated from its hydrosol, filtered and stored at 4°C for further experiments. The entire process was repeated using fresh lemon peels (1874.8 g) and fresh unpeeled turmeric rhizomes (1634.9 g).

Extraction of Carrier Oil (Coconut oil)

Virgin Cocos nucifera was extracted using 304.1 g of coconut meat. The meat was crushed, and the milk extracted using hot water maceration for one hour. The milk was kept in the refrigerator at 4°C for 24 h. Pure colorless oil was collected over the mixture and the bulk liquid was discarded. About 12 mL of pure oil was collected.

The percentage (extraction) yields of ginger, lemon, turmeric, and coconut oils were calculated (see Equation 1) as the ratio of the volume of oil extracted to the mass of the starting plant material and expressed as a percentage (v/w).

Chemical Analysis

The chemical compositions of the oils were analyzed using Gas Chromatography (Agilent technologies 7890A, USA). Helium was used as the carrier gas at the constant flow of 1.2 mL/min and split ratio 1:30. The oven temperature was held at 50°C for 1 min, and then programmed to 280°C at a rate of 5°C /min. Helium flux was 30 mL/min and air flux was 300 mL/min. The injector temperature is 280°C and detector temperature is 300°C. The injection volume was 1 µL.

Evaluation of Anti-Arthritic Activity

All animal experiments were carried out in accordance with the guidelines of the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria and EU directive 2010/63/EU for animal experiments.

Nishat et al.'s (26) method was employed. Briefly, twenty-seven (27) male Wister rats were divided into nine (9) groups of three animals in each group. Measurement of their paw volumes was done using volume displacement. The animal groups and the respective administrations (in parenthesis) are as follows: Group I (Ginger), II (Turmeric), III (Lemon), IV (Ginger and Turmeric), V (Turmeric and Lemon), VI (Ginger and Lemon), VII (Coconut oil),VIII (formaldehyde), IX (Voltaren® cream). The administration was carried out accordingly for 10 days. On day one, 30 min after topical treatment, acute non-immunological arthritis was induced by sub-plantar injection of 0.1 mL formaldehyde (2% v/v) into the right hind paw of all the animals and repeated on day 3. Treatment continued till the 10th day for all experimental groups. Arthritis was assessed by measuring the mean changes in paw volume over a period of 10 days using Equation 1.

$$I = 1 - \frac{\Delta V_{treated}}{\Delta V_{untreated}} \times 100\% \quad \textbf{Equation 1}$$

Where I = inhibition of paw oedema, ΔVuntreated = mean change in paw volume of untreated animals, and ΔVtreated = mean change in paw volume of treated animals.

Statistical Analysis

The recorded data were subjected to paired T-test to check for statistical differences in the mean paw sizes of the rats across different groups. Correlation analyses (ANOVA) were carried out to assess if there is a relationship between the anti-arthritic reductions in the different treatment classes being tested. All the statistical analyses were computed with IBM SPSS statistics software version 23. A p-value<0.05 was considered as the significant cutoff.

Results

Yield of Essential Oils

Essential oils can be defined as any volatile fragranced nonpolar organic plant extract that is responsible for a plants characteristic odor and they are reported to have therapeutic uses (27). The yield of essential oils from plants varies widely, and the broad range is 0.05 – 18.0% (28).

The results in Table 1 show that lemon oil had the lowest percentage yield of all three essential oils. However, coconut oil had the highest yield (being a fixed oil).

Table 1. Percentage yield of essential oils obtained.

Oils	Mass of plant material (g)	Vol. of oil extracted (ml)	Percentage yield (%)
Lemon	1874.8	10	0.53
Turmeric	1634.9	7	0.43
Ginger	138.9	4	2.89
Coconut	304.1	12	3.95

Table 2. Relative percentage of constituents of essential oils from *C. limon*, *Z. officinale*, *C. longa*, and *C. nucifera*.

No	Constituent	RT	Rel %			
			L	G	T	C
1	β-Myrcene	6.1	1.5		*	*
2	D-Limonene	6.9	90.7		1.8	*
3	Camphene	5.3	*	1.0	*	*
4	Sabinene	6.9	*	1.9	*	*
5	Eucalyptol	6.9	*	1.3	*	*
6	Citral B	11.1	*	3.2	*	*
7	Citral A	11.6	*	5.6	*	*
8	Curcumene	15.4	*	9.0	*	*
9	Zingiberene	15.6	*	5.4	1.8	*
10	Naphthalene.	15.7	*	2.5	*	*
11	α-Farnesene	15.8	*	3.0	*	*
12	beta-Bisabolene	15.8	*	4.9	*	*
13	Cyclohexene	16.1	*	6.8	*	*
14	β-farnesene	17.6	*	1.3	*	*
15	Zingiberenol	17.9	*	1.5	*	*
16	2,3-dihydrofarnesol	18.9	*	1.0	*	*
17	Geranyllinalool	9.1	*	1.9	*	*
18	Cis- citral	20.2	*	3.4	*	*
19	iso-Bornyl methacrylates	20.5	*	9.1	*	*
20	cis-Verbenol	21.6	*	1.1	*	*
21	2-Dodecen-1-yl(-)succinic anhydride	21.9	*	2.6	*	*
22	α-Phellandrene	6.4	*	*	1.1	*
23	α -Curcumene	15.4	*	*	1.5	*
24	Cyclohexene	16.1	*	*	1.8	*
25	Neophyl chloride	17.1	*	*	1.1	*
26	α -(Z,E)- Farnesene	17.9	*	*	1.3	*
27	Ar-tumerone	18.5	*	*	35.5	*
28	Tumerone	18.5	*	*	24.6	*
29	Curlone	18.9	*	*	14.9	*
30	3-Decen-5-one	23.1	*	*	1.1	*
31	1-Propene,1-methylthio-2-trifluoromethyl-1,3,3,3-tetrafluoro-	10.7	*	*	*	2.0
32	Poly(ethylene glycol) diamine	11.8	*	*	*	7.1
33	2,4-Decadienal, (E,E)-	12.3	*	*	*	17.3
34	Sulfonium, dimethyl-, cyanonitromethylide	13.1	*	*	*	9.8
35	Tetrahydro-4H-pyran-4-ol	14.9	*	*	*	6.1
36	dl-Allo-cystathionine	15.4	*	*	*	1.1
37	Methyl 8-methyl-nonanoates	15.6	*	*	*	5.2
38	Butyl vinyl carbinol	16.7	*	*	*	10.3
39	Methyl tetradecanoate	19.1	*	*	*	1.0
40	Hexadecanoic acid	21.9	*	*	*	10.1
41	Cyclohexane	22.6	*	*	*	1.4
42	13-Hexyloxacyclotridec-10-en-2-one	23.9	*	*	*	2.5
43	Lineoleic acid	24.2	*	*	*	1.6
44	Octadecanoate	24.2	*	*	*	7.8
45	Heptadecanoic acid	24.6	*	*	*	2.2
Total compounds identified			2	18	11	15

Key: L: Lemon. G: Ginger, T: Turmeric, C: Coconut, RT: Retention Time, Rel%: Relative Percentage.

Chemical Composition of Oils

The hydrodistillation of Citrus limon peels gave a colorless oil with a pleasant odor and yield of 0.53% (v/w) based on the fresh weight. Table 2 demonstrates the list of compounds whose GC/MS concentration is not less than 0.1% of the total peak concentration. Lemon oil's major constituents were D-limonene (90.7%) and other constituents in trace amounts. Regarding phytochemical classification, the essential oil comprised monoterpenoids, sesquiterpenoids, and other organic compounds in trace amounts.

The hydrodistillation of *Z. officinale* gave a pale-yellow oil with a spicy aroma and a yield of 2.89% (v/w) based on the fresh weight. The results demonstrate the list of compounds whose GC/MS concentration is not less than 0.1% of the total peak concentration. Mainly made up of sesquiterpenoids.

The hydro-distillation of turmeric rhizomes gave a bright yellowish oil with a pleasant odor and yield of 0.43% (v/w) based on the fresh weight. Table 2 demonstrates the list of compounds whose GC/MS concentration is not less than 1 % of the total peak concentration. Accordingly, eleven components were identified in the essential oil. The major constituents of *Curcuma longa* oil were characterized as Ar. Turmerone (35.5%) and other constituents in varying amounts. In terms of phytochemical classification, the essential oil comprised 5 sesquiterpenoids (78.3%), three monoterpenoids (7.1%), two esters (8.6%) and other trace organic compounds.

The *Cocos nucifera* extracted was colorless with a fruity aroma and a yield of 3.95% (v/w) based on the fresh weight. The results demonstrate the list of compounds whose GC/MS concentration is not less than 1% of total peak concentration. According to the results, twenty (20) components were identified in coconut oil. Regarding phytochemical classification, the fixed oil yielded largely hydrocarbons (95%), and other trace organic compounds.

Evaluation of Anti-Arthritic Activities

Observations were aimed at looking out for essential oils/oil blends that were able to achieve the same basal foot sizes (at day 10) in their representative classes. A surge in the paw volumes was observed on the fourth day after formaldehyde (2% v/v) injection on day 3. The results showed that only the ginger, turmeric, ginger/lemon, and turmeric/lemon groups achieved this aim. The positive control (Voltaren® cream), lemon and ginger/turmeric groups had similar paw volume reduction activities. However, a significant reduction was not achieved with coconut oil. In the untreated groups, only a small reduction was obtained, after 10 days.

The rationale for using coconut oil was to reduce skin irritations, reduce volatility, and increase absorption (29, 30). This absorption is achieved as carrier oils are composed of molecules that make them closely related to sebum (the skin's natural oil).

Evaluation of Percentage Inhibition

The percentage inhibition measures percentage changes in paw sizes of the animals from different treatment groups over a 10-day period. From the results obtained, it was seen that ginger was able to achieve a 100% reduction in 3 days. The sudden increase on day 4 was because of injection of formaldehyde. Lemon achieved a 100% reduction in paw size on day 1. However, this effect was not sustained. The negative values observed for turmeric and the oil blend groups show that there was a decrease in antagonistic activities at different points of therapy. The positive control (Voltaren® cream) showed unstable reduction patterns throughout the period of study.

Percentage inhibition offers a more corroborative/holistic parameter for measuring the reduction in paw volumes over time. The multiple correlation analysis of the paw volumes obtained when sampled against each group showed a strong positive and significant correlation for the ginger, lemon, and turmeric groups ($p<0.05$). The reverse was the case when they were sampled against coconut and Voltaren® cream groups, which showed a weak positive relationship that was not significant ($p>0.05$).

For the essential oil combinations Ginger and lemon (GL) and turmeric and lemon (TL), multiple correlation analyses (ANOVA) of the paw volumes obtained when sampled against each group showed a strong positive correlation. The reverse was the case when they were sampled against formaldehyde and coconut groups, which showed both weak positive and negative, insignificant relationships.

For the essential oil combination (GT), multiple correlation analysis of the paw volumes obtained when sampled against each group showed a strong positive correlation. The reverse was the case when it was sampled against formaldehyde and coconut groups, which showed both strong and weak positive relationships, respectively, that were not significant.

For the Coconut oil group, the relationship was positive and non-significant ($p>0.05$). Exceptions were for TL/GL, which was weakly negative, and formaldehyde, which had a strong positive correlation.

For the formaldehyde, multiple correlation analysis of the paw volumes obtained when sampled against

each group showed predominance of a non-significant relationship except for ginger and lemon. A strong positive relationship was observed in all except TL/GL and Voltaren® (which was weakly negative).

Comparison of Mean Changes in Paw Sizes for the Best Treatment Groups

The three best treatment groups (ginger, turmeric, and turmeric/lemon oil) were compared against the arthritic control (formaldehyde). From Figure 1, it was observed that turmeric and ginger (Curcuma species) had same activity from days 9 and 10. The untreated group obviously had the highest paw volume. Therefore, the anti-arthritic efficacy of the oils is in the order of TL blend.

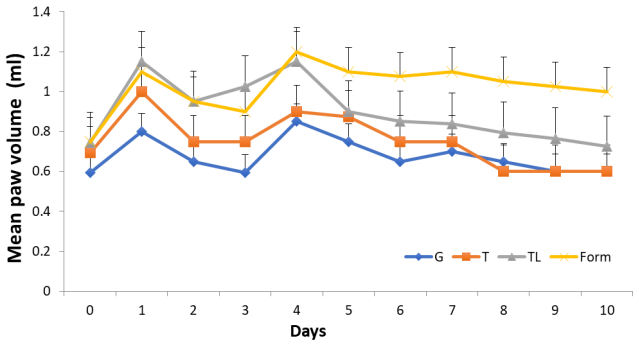


Figure 1. Comparative evaluation of the best treatment groups against the arthritic control. Note: G = Ginger, T = Turmeric, TL = Lemon + Turmeric, Form = Formaldehyde.

Discussion

This study used fresh rhizomes of ginger and turmeric, and turmerones are major components in fresh rhizomes (31). The major volatile singular principle of the rhizome oil was ar-turmerone (Table 2). According to their chemical structure, turmerone and ar-turmerone are ketonic sesquiterpenes of the bisabolane type responsible for turmeric aroma and smell (32) and anti-inflammatory pharmacological activities (33). Hence, this could explain why turmeric oil had more anti-arthritic activity than ginger oil. Furthermore, it has been confirmed in a study by Ramadan et al. (34) that turmeric was more effective in alleviating the inflammatory immune response and oxidant stress in rat models of human rheumatoid arthritis than ginger and indomethacin.

The GC results (Table 2) show that turmeric essential oil is primarily dominated by sesquiterpenoids. The essential oils from leaves and flowers are dominated by monoterpenes, while those from turmeric rhizomes primarily contain sesquiterpenes (35). Essential oils enriched in sesquiterpenes presented the highest anti-inflammatory activity, while those enriched in monoterpenes and oxygenated terpenes showed the highest anti-proliferative characteristics (36). Curcumin is the most active and studied turmeric component, exhibiting its anti-inflammatory activity (37).

However, in addition to curcuminoids, more than 250 mono-, sesqui- di-, and triterpenoids have been identified from curcuma species. These lipophilic compounds have better absorption than curcuminoids and exhibit a wide spectrum of pharmacological properties, which may be as physiologically active, if not more, than curcuminoids (32). The lipophilicity of the predominant sesquiterpenoids in curcuma species explains the reason why ginger and turmeric oils were excellent at reduction of the arthritic rat paws. Previous reports have highlighted the anti-inflammatory effects of fractions enriched in curcumin and tumerones (38, 39).

The terpenoids present in Curcuma species may be equally if not more, biologically active molecules than curcuminoids. They may act alone or possess synergistic activity with curcuminoids (40). This synergistic activity of curcuminoids and terpenoids may have largely contributed to the great interest and focus of chemists and biologists on Curcuma longa spice in their research (41). This again explains why turmeric oil was most effective. However, in this study, there was antagonistic activity in the turmeric and ginger oil blend, and synergism in the turmeric and lemon oil blend. Ginger and turmeric are both rhizomes of the Zingiberaceae family. Both ginger and turmeric have similar patterns of anti-arthritic activity via inhibition of COX-2, lipoxygenase, leukotrienes, prostanglandins, and thromboxanes (9, 13). These enzymes are specific for their inflammatory and pain actions (14).

From the GC results (Table 2), D-Limonene (monoterpene) was the major principal phyto-constituent (90%). Lemon essential oil has a higher potential for pain reduction than anti-inflammation. This could be attributed to the presence of D-Limonene as shown in studies of different models of nociception without opioid receptor stimulation (42). In addition, a study has revealed that monoterpenes are the key holders of analgesic potential in Citrus essential oils, especially D-limonene and linalool (43). This explains why there was no significant reduction in inflammation with lemon oil.

There is a possibility that the synergistic effects observed with the ginger/lemon and turmeric/lemon oil blends as against lemon alone, are due to the combined reaction mechanisms against rheumatoid arthritis, attributable to an abundance of monoterpenes in lemon oil (17, 43), and an abundance of sesquiterpenes in ginger and turmeric oils (9, 35).

The study demonstrates the benefits of combining

these oils, lemon and ginger, and lemon and turmeric, in the management of inflammation of rheumatoid arthritis. This finding is significant because lemon has additional analgesic properties when used alone (42). Combining lemon oil with these other natural anti-inflammatory agents would provide better therapeutic relief for arthritic patients who require both analgesia and antiinflammation in their treatment. Furthermore, using coconut oil as a carrier in delivering these essential oils has the added advantage of preventing the phototoxicity and irritation associated with directly using these oils and contributing to their anti-inflammatory effects. A limitation of this study is that it did not consider the combined effect of blending coconut oil with essential oils (ginger, turmeric, and lemon) to understand the influence of the blends on the overall anti-arthritic study. Some studies have been carried out by the authors (44), though further work still needs to be done. Furthermore, the possibility of the sex of animals affecting the results was not considered, as only male rats were used for this study. Further studies may explore the influence of the sex of animals on response to this therapy. In addition, while the analgesic potential of lemon oil has been previously investigated, a study investigating the analgesic and anti-inflammatory effects of these combinations (lemon and ginger or lemon and turmeric) may yield significant findings on the possibility of utilizing the formulation to achieve analgesia and anti-inflammation, as is obtained with the non-steroidal anti-inflammatory drugs (NSAIDS) used in the symptomatic management of rheumatoid arthritis.

Conclusion

The study revealed that essential oils of ginger, lemon, and turmeric are important anti-arthritic remedies. The presence of monoterpene and sesquiterpene compounds, which are small, non-polar moieties, enable delivery into the skin. From the GC-MS evaluation, the following compounds were most abundant in the essential oils and carrier oil; ar-Turmerone, turmerone and curlone (Turmeric oil); zingiberene (Ginger); D-limonene (Lemon oil) and Hexadecanoic acid (Coconut oil). Previous studies have highlighted the importance of these compounds in reducing inflammation. Coconut oil is used as a carrier oil to mitigate the irritancy and phototoxicity of essential oils. This preliminary study provides a basis for the possible combinations of these essential oils studied in treating inflammatory diseases, especially rheumatoid arthritis. The knowledge garnered will be critical in optimizing and targeting essential oils for delivery via incorporation into suitable topical dosage forms. This could be advantageous in improving sustained anti-arthritic activity. The results also suggest the benefit of blending lemon oil with a woody essential oil obtained from rhizomes of turmeric and ginger for effectiveness in RA therapy.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

Ethics Statement

All animal experiments were carried out following the guidelines of the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria and EU directive 2010/63/EU for animal experiments.

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