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# Pharmacological Effects of *Cyperus articulatus* L. Rhizome Essential Oil against Cough, Inflammation, and Nociception in Mouse Models

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**ABSTRACT**

**Background:** *Cyperus articulatus* L. rhizomes are widely used in traditional medicine for different ailments. different studies have reported the pharmacological properties of *C. articulatus* essential oil.

Objectives: To explore the antitussive, anti-inflammatory, and antinociceptive potentials of *C. articulatus* essential oil.

**Methods:** *C. articulatus* essential oil (CAEO) was obtained by hydro-distillation, and Gas Chromatography-Mass Spectrometry was used to analyze its chemical composition. Male and female mice (20-27 g) were used in this study. Oral toxicity test and anti-tussive screening were conducted, nociception and inflammation were induced. The antitussive, anti-inflammatory, anti-nociceptive activities and the central analgesic activity of the plant were evaluated.

**Results:** α-pinene (7.7 %), (-)-β-pinene (7.5 %), neoisolongifolene (6.9%), isopinocarveol (6.7 %), α- copaene (5.2%), (-)-Myrtenol (4.8 %), isolongifolen-5-one (4.1 %), and mustakone (3.8 %) were present in CAEO. The essential oil displayed antibacterial activity against *Staphylococcus aureus* (minimum inhibitory concentration: 0.89 mg/mL). CAEO significantly ($p < 0.01$) suppressed cough bouts and inhibited abdominal pain at the doses administered. Furthermore, the essential oil reduced pain severity at various phases and exhibited central antinociceptive activity after its oral administration.

**Conclusion:** *C. articulatus* essential oil possesses bioactive compounds and demonstrates antitussive, anti-inflammatory, and antinociceptive activity. It can be further developed as a remedy against cough, inflammation, and pain.

**Keywords:** Antitussive; Anti-inflammatory; antinociceptive; *Cyperus articulatus*; essential oil; GC-MS

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# Effets pharmacologiques de l'huile essentielle de rhizome de *Cyperus articulatus* L. contre la toux, l'inflammation et la nociception chez des modèles murins

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**RÉSUMÉ**

**Contexte :** Les rhizomes de *Cyperus articulatus* L. sont largement utilisés en médecine traditionnelle pour traiter diverses affections. Plusieurs études ont fait état des propriétés pharmacologiques de l'huile essentielle de *C. articulatus*.

**Objectifs :** Explorer les potentiels antitussifs, anti-inflammatoires et antinociceptifs de l'huile essentielle de *C. articulatus*.

**Méthodes :** L'huile essentielle de *C. articulatus* (HECA) a été obtenue par hydrodistillation, et sa composition chimique a été analysée par chromatographie en phase gazeuse couplée à la spectrométrie de masse. Des souris mâles et femelles (20-27 g) ont été utilisées dans cette étude. Un test de toxicité par voie orale et un criblage antitussif ont été réalisés, et une nociception et une inflammation ont été induites. Les activités antitussive, anti-inflammatoire, antinociceptive, ainsi que l'activité analgésique centrale de la plante ont été évaluées.

**Résultats :** L'huile essentielle HECA contenait de l'α-pinène (7,7 %), du (-)-β-pinène (7,5 %), du néoisolongifolène (6,9 %), de l'isopinocarvéol (6,7 %), de l'α-copaène (5,2 %), du (-)-myrténol (4,8 %), de l'isolongifolène-5-one (4,1 %) et de la mustakone (3,8 %). Cette huile essentielle a démontré une activité antibactérienne contre *Staphylococcus aureus* (concentration minimale inhibitrice : 0,89 mg/mL). Aux doses administrées, la HECA a significativement ($p < 0,01$) atténué les quintes de toux et inhibé les douleurs abdominales. En outre, l'huile essentielle a réduit l'intensité de la douleur à différentes phases et a exhibé une activité antinociceptive centrale après son administration par voie orale.

**Conclusion :** L'huile essentielle de *C. articulatus* contient des composés bioactifs et présente des propriétés antitussives, anti-inflammatoires et antinociceptives. Elle pourrait faire l'objet de développements supplémentaires en tant que remède contre la toux, l'inflammation et la douleur.

**Mots-clés :** Antitussif ; anti-inflammatoire ; antinociceptif ; *Cyperus articulatus*; huile essentielle ; CG-SM

## INTRODUCTION

*Cyperus articulatus* L. (family: Cyperaceae) is a plant native to the Amazon basin where it has been used as medicine for centuries. It also thrives in tropical and sub-tropical climates in Africa, Asia, northern Australia, and most countries in Central and South America.[1] It is a resilient perennial plant that can reach a height of 1.75 meters. It grows from a rhizome, contributing to its exceptional endurance and longevity. One distinctive feature of this plant is its unique stem structure, which comprises a series of segments or joints, hence its common name, "jointed flat sedge".[2] These joints give the stem a distinctively knobby appearance, making it highly resilient to damage or bending.[3,4]

*Cyperus articulatus* has been used worldwide for the treatment of various ailments, including nausea, vomiting, stomach pains, intestinal gas, headaches, colds, flu, mouth sores, ulcers, and hypertension.[5] It has also been known to possess properties such as analgesic, sedative, antimicrobial, anti-malaria, and mosquito-repellant among other properties. Extracts of the rhizomes are used orally for cough relief in northern Nigeria, Ghana, and KwaZulu-Natal in South Africa while its decoction is drunk in Congo for all respiratory troubles.[6]

A decoction and the methanolic extract of the rhizome of *C. articulatus* have been documented to possess sedative effects and anti-convulsant activity respectively.[7,8] The essential oil of *C. articulatus* rhizome has also been reported to inhibit the growth of Staphylococcus aureus and Aspergillus niger.[9] Cyperus species have been reported to be generally constituted by sesquiterpenoids and traces of monoterpenoids with mustakone as the main marker compound.[10] Other constituents identified in *C. articulatus* rhizome essential oil include; cedrol, guaia- 5-en-11-ol, cyperotundone, sabinene, α-pinene, trans- pinocarveol, cis-carveol, trans-carveol and α-cardinol.[11]

*Cyperus articulatus* essential oil is medicinally valuable, and this necessitates further studies as the basis for its continuous development. It has been widely marketed and used globally, particularly in Asia, South America and Africa for the treatment of respiratory diseases such as cough, tuberculosis, and acute lung inflammation via inhalation.[12] No report was found in the literature on the effect of the essential oil of *C. articulatus* rhizome against cough. Given the traditional uses and pharmacological properties, this study was carried out to explore the antitussive, anti-inflammatory and antinociceptive potentials of CAEO. This is aimed at further expanding the economic value of the plant. Additional investigations carried out were chemical characterization, oral acute toxicity and antimicrobial screening.

## MATERIALS AND METHODS

### Materials

Acetic acid, formalin, and indomethacin were sourced from Sigma Aldrich through a regional representative. Dihydrocodeine (GlaxoSmithKline, UK) was sourced locally.

### Microorganisms

Typed bacterial and fungal cultures were used: *Bacillus subtilis sbsp*. spizenii ATCC 6633, *Staphylococcus aureus* NCTC 6571, Escherichia coli ATCC 11775, Pseudomonas aeruginosa 27853, *Salmonella enterica* subsp. enterica serovar typhymurium ATCC 14025, *Streptococcus pneumonia* ATCC 6305, Proteus mirabilis, ATCC 12453, *Candida albicans* ATCC 10231.

### Collection of plant material and essential oil extraction

Dried rhizomes of *C. articulatus* L. (black variety) were collected in November 2022. The rhizomes were identified and authenticated, and a voucher specimen (NIPRD/H/6915) was prepared and deposited for reference in NIPRD herbarium. The essential oil from the rhizomes of *C. articulatus* was obtained by hydro-distillation. Briefly, *C. articulatus* rhizomes (3 kg) were crushed into coarse powder and transferred into a round bottom flask. Distilled water (10 L) was added into the round bottom flask and the plant material was subjected to hydro-distillation using a Clevenger apparatus. The plant material was extracted for 2 h after which *C. articulatus* essential oil (CEAO) was collected into a clean, air-tight, glass sample bottle. This was properly sealed and stored frozen in a Thermo Scientific HERAfreeze ultra-low-temperature freezer at -80$^{o}$C.

### Chemical characterization

Chemical characterization of CAEO was done by Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS analysis was carried out on a Hewlett Packard 5890 chromatograph equipped with an FID detector and coupled with a mass spectrometer of the Hewlett Packard 5971 type operating in the EI mode at 70 eV. Capillary column DB-5 (30 m x 0.25 mm internal diameter; film thickness 0.25 µm) was used. The DB-5 column operating conditions were as follows: from 50$^{o}$C (5 min), 50$^{o}$C to 250$^{o}$C at the rate of 2$^{o}$C/min. The injector and detector temperature were set at 250$^{o}$C and 300$^{o}$C,

respectively. The carrier gas was helium at a flow rate of 1.50 mL/min. Samples (0.2 µL) of non-diluted essential oil were injected for analysis. Data was acquired on Agilent Mass Hunter software and compounds were identified based on information in the National Institute of Standards and Technology standard reference library.

**Antimicrobial assay**

***Standardization of inoculum***

A loopful of 24 h-surface growth of each of the culture bacteria was transferred to 0.9 % NaCl solution to obtain a homogenous suspension. This suspension was used for inoculation. Turbidity was adjusted to match that of a 0.5 McFarland standard by visual observation.

***Determination of Minimum Inhibitory Concentration (MIC)***

The essential oil was subjected to antimicrobial susceptibility testing by broth microdilution method, run in duplicates.[13] A 96-microtiter well was prepared by dispensing 50 µL of Muller Hinton broth into wells. Fifty microliters (50 µL) of eight different dilutions of CAEO were added to test wells while control wells contained 1% DMSO in place of CAEO and thus served as organism viability control. To each well containing CAEO dilutions or standard drug, another 50 µL of microorganism suspension was added. The plates were gently shaken for 20 s and then incubated at 37$^{o}$C for 24 h. At the end of the incubation period, tetrazolium dye was added to the well to aid visualization which forms a formazan complex in the presence of the microorganism. Minimum inhibitory concentrations (MIC) were calculated as the lowest concentration showing no turbidity (did not react with the dye after incubation), while turbidity/ violet color was interpreted as visible growth of the microorganism. Ciprofloxacin and terbinafine hydrochloride severed as positive controls for bacteria and fungi respectively. Antimicrobial activity was classified based on MIC as follows; < 0.1 mg/mL: strong activity, 0.1 - 0.5 mg/mL: moderate, 0.5 - 1 mg/mL: weak, > 1000 µg/mL: inactive.[14]

**Animals**

Ethical approval for the study was obtained from the Animal Care and Ethics Committee, NIPRD (Number: NIPRD:05.03.05 - 42). The study followed the institutional and the Animal Research Reporting of In Vivo Experiments (ARRIVE) Guidelines for conducting and reporting animal research.[15] Male and female (nulliparous and non-pregnant) BALB/c mice (4-6 weeks old), weighing 20-27 µg, were obtained from the Animal Facility Center of the National Institute for Pharmaceutical Research and Development, Abuja. The mice were housed by sex in plastic cages with sawdust bedding, in groups of five per cage. The mice were housed under regulated environmental conditions of temperature (22-24$^{o}$C) and a 12-hour light/dark cycle, maintained using an automatic timer switch to control the lighting system. Fresh water and standard rodent food were provided ad libitum daily.

**Oral acute toxicity study**

A limit test was conducted following the Organization for Economic Co-operation and Development (OECD) guidelines on oral acute toxicity testing.[16] Male and female mice were collected and fasted overnight. 3 male mice and 3 female mice were weighed and coded and then placed in individual cages. The mice received CAEO at 2000 mg/kg each while two additional mice (one male, one female) received a volume of the vehicle based on the body weight and served as controls. After extract administration, food was withheld for a further 1-2 h. The mice were observed for signs of toxicity closely at 15 min, 30 min, 1, 2, 4, and 8 h, and then once daily for 14 days. Observations were recorded per animal based on the Hippocratic screening table for drug materials.[17] Parameters that were observed in each mouse include general signs and symptoms of central nervous stimulation or depression, and mortality.

**Anti-tussive activity study**

The ammonia vapor-induced model was employed for anti-tussive screening.[18] Thirty (30) mice were randomly allotted into five groups of six mice per group. Group 1 was treated orally (p.o.) with the vehicle, Tween 80 in distilled water (1 %v/v); Groups 2 - 4 were treated orally with freshly prepared CAEO (100, 300, and 900 mg/kg, p.o.); while Group 5 received 30 mg/kg dihydrocodeine to serve as the standard control. An hour after the treatment, each mouse was placed in a 10×10×10 cm glass chamber and exposed to 0.3 mL of 25 % ammonia vapor for 1 min, then transferred to a 1000 mL beaker. The number of cough bouts exhibited by each mouse within 5 min was counted. The criteria used to define cough in mice were opening of the mouth with contraction of the thoracic and abdominal muscles and jerking of the front body. The antitussive activity was assessed as a percentage of inhibition of the number of cough bouts relative to the control group as follows:

Inhibition (%) = [(Co-Ct)/Co×100%] ..... (1)

Where Co: the number of cough bouts of control, Ct: the number of cough bouts of the treatment group

**Evaluation of anti-inflammatory and anti-nociceptive activity**

***Peripheral analgesic activity (acetic acid-induced writhing test)***

This test was performed as described previously, by inducing abdominal writhing via intraperitoneal administration of dilute acetic acid (0.65 %v/v).[19] Forty mice were divided into five groups of eight mice each (n = 8). The negative control group was orally treated with vehicle, the positive control group with indomethacin (5 mg/kg), and other groups were treated with 100 mg/kg, 300 mg/kg, and 900 mg/kg of CAEO respectively. Sixty minutes after treatment, the groups received an intraperitoneal injection of acetic acid (0.65 %, v/v in saline, 10 mL/ kg), and then, the number of contortions was counted for 30 minutes.

**Formalin-induced nociception and inflammation test**

Thirty mice were divided into five groups of six mice each and were treated with vehicle (group I), 100 mg/kg, 300 mg/kg, and 900 mg/kg CAEO (groups II - IV) and dihydrocodeine 30 mg/kg (group V). Sixty minutes after treatment, the mice received 20 µL of intra-plantar formalin injection 1 % (v/v) in the right hind paw. Thereafter, the mice were placed individually in acrylic chambers and observed for grading of pain severity. Pain severity was graded as follows: 0 = normal resting of weight on the injected paw, 1= light placing of the paw on the floor, 2= lifting of the injected paw, and 3= continuous licking or biting of the injected paw. The observation was performed in two phases, the first being 0-5 minutes immediately after formalin injection, and the second phase at 15-30 min.[20]

***Central analgesic activity (Hot plate test)***

Forty mice were submitted to a baseline test with the hot plate maintained at 55± 0.5ºC. Mice that reacted to thermal stimulus after 10 s exposure were excluded from the test to ensure response homogeneity of 0 - 10 s. After this, thirty mice were selected and randomized into five groups with six mice in each group (n=6) and treated orally according to groups, with the vehicle, dihydrocodeine (30 mg/kg), 100 mg/kg, 300 mg/kg, or 900 mg/kg of CAEO respectively. After 1 h, treated mice were placed individually on the hot plate and the response time to thermal pain was recorded. Responses to the thermal pain were observed as the act of lifting or licking the paws or jumping. This was repeated at 90- and 120-min post-treatment. A cut-off time for exposure to thermal stimuli was set at 25 s, after which the animal was removed to avoid plantar tissue damage.[21]

**Statistical analysis**

Data was analyzed using GraphPad Prism v.6.01. Results were expressed as mean ± SEM, and by graphical representation. Parametric One-way ANOVA was used to compare the means between groups, followed by Dunnet's test for multiple comparisons. Results with $p<0.05$ were considered statistically significant.

**RESULTS**

**Yield and composition of *C. articulatus* essential oil**

The calculated yield of CAEO relative to powdered plant material was 0.046 ± 0.77 %. Oxygenated monoterpenes and monoterpene hydrocarbons were the prevalent constituents in the oil, followed by sesquiterpene hydrocarbons and oxygenated sesquiterpenes. Fifty-five peaks were identified in the essential oil (Fig. 1). Major compounds identified were α-pinene (7.7 %), (-)-β-pinene (7.5 %), neoisolongifolene (6.9 %), isopinocarveol (6.7 %), α- copaene (5.2 %), (-)-Myrtenol (4.8 %), isolongifolen-5-one (4.1 %), and mustakone (3.8 %) (Table 1). GC-MS analysis revealed two distinct peaks (number 34 and 35) with identical molecular weights of 220.53 at retention times 12.9 min and 13 min, suggesting the presence of isomeric compounds. Similarly, compounds at peaks 52 and 55 with are likely isomers, with overlapping fragmentation patterns.

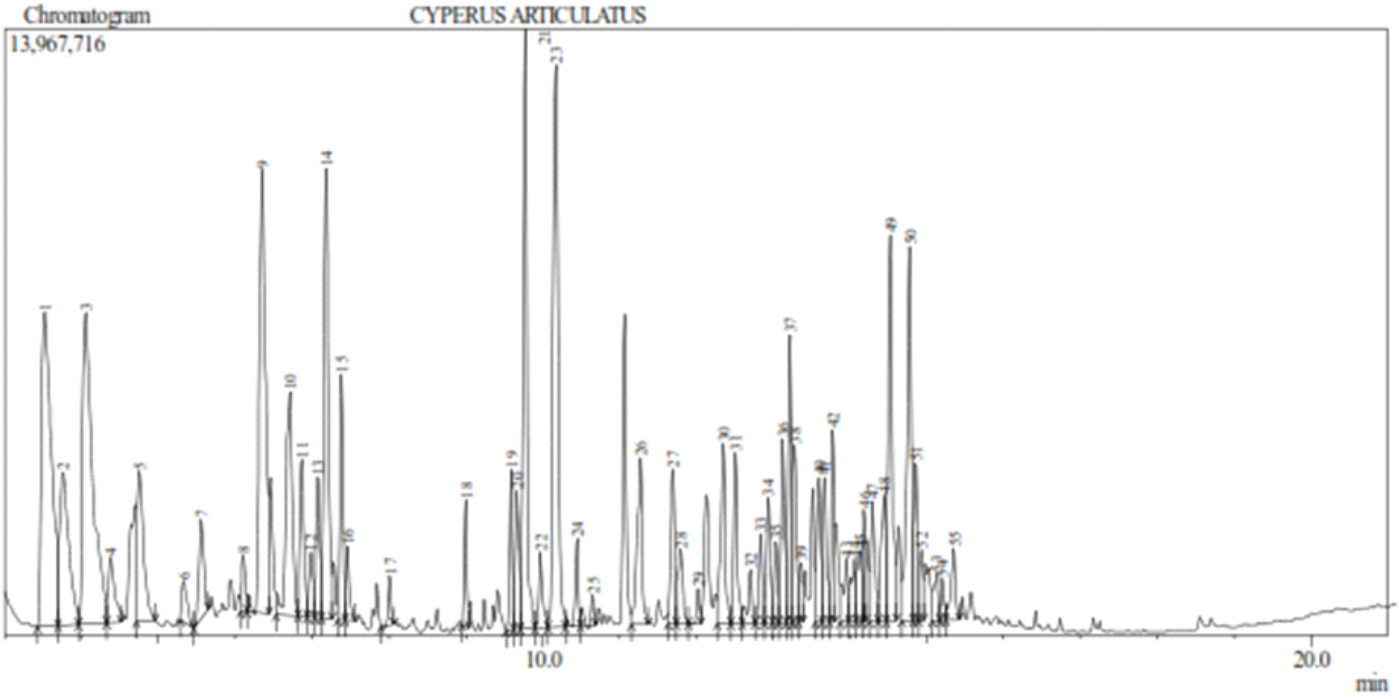



**Fig. 1: GC-MS Chromatogram of *C. articulatus* essential oil**

**Table 1: Chemical composition of *C. articulatus* essential oil**

| Peak | Rt* | Area% | Name | |
|---|---|---|---|---|
| 1 | 3.5 | 7.7 | α-pinene | |
| 2 | 3.8 | 3.3 | Thuja-2,4(10)-diene | |
| 3 | 4.1 | 7.5 | (-)-β-Pinene | |
| 4 | 4.4 | 1.0 | Carveol | |
| 5 | 4.8 | 2.2 | Eucalyptol | |
| 6 | 5.3 | 0.5 | Artemiseole | |
| 7 | 5.6 | 1.2 | 3-Cyclohexen-1-ol,5-methylene-6-(1-methylethenyl)-acetate | |
| 8 | 6.1 | 0.5 | 3-Cyclopentene-1-acetaldehyde,2,2,3-trimethyl- | |
| 9 | 6.4 | 6.7 | Isopinocarveol | |
| 10 | 6.7 | 3.4 | Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl- | |
| 11 | 6.9 | 1.5 | Citronellal | |
| 12 | 6.9 | 0.7 | Thymol | |
| 13 | 7.1 | 1.3 | trans-β-Terpinylbutanoate | |
| 14 | 7.2 | 4.8 | (-)-Myrtenol | |
| 15 | 7.4 | 1.9 | D-Verbenone | |
| 16 | 7.5 | 0.6 | trans-3(10)-caren-2-ol | |
| 17 | 8.0 | 0.3 | Dihydrojasmone | |
| 18 | 8.9 | 0.8 | Ionone | |
| 19 | 9.6 | 1.1 | Cycloisolongifolene, 8,9-dehydro- | |
| 20 | 9.7 | 1.4 | (+)-Cyclosativene | |
| 21 | 9.8 | 5.2 | α-copaene | |
| 22 | 9.9 | 0.7 | β-humulene | |
| 23 | 10.2 | 6.9 | Neoisolongifolene | |
| 24 | 10.4 | 0.7 | β-vatirenene | |
| 25 | 10.6 | 0.3 | Aromadendrene, dehydro- | |
| 26 | 11.3 | 1.9 | Isoledene | |
| 27 | 11.7 | 1.5 | Aromadendrene, dehydro- | |
| 28 | 11.8 | 0.9 | α-bulnesene | |
| 29 | 12.0 | 0.3 | Lanceol, cis | |
| 30 | 12.4 | 1.9 | Spathulenol | |
| 31 | 12.5 | 1.5 | α-Calacorene | |
| 32 | 12.7 | 0.5 | Caryophyllene oxide | |
| 33 | 12.8 | 0.7 | Cadala-1(10),3,8-triene | |
| 34 | 12.9 | 1.2 | Diepicedrene-1-oxide | |
| 35 | 13.0 | 0.7 | Diepicedrene-1-oxide | |
| 36 | 13.1 | 1.6 | Longifolenaldehyde | |
| 37 | 13.2 | 2.3 | Caryophyllene oxide | |
| 38 | 13.3 | 1.5 | β-Cedren-9-α-ol | |
| 39 | 13.4 | 0.5 | Alloaromadendrene oxide | |
| 40 | 13.6 | 1.4 | Ledene alcohol | |
| 41 | 13.7 | 1.3 | cis- α -Copaene-8-ol | 41 |
| 42 | 13.8 | 1.5 | Neoisolongifolene,8,9-epoxy- | 42 |
| 43 | 13.9 | 0.7 | α-Farnesene | |
| 44 | 14.1 | 0.8 | Cubenol | |
| 45 | 14.1 | 0.7 | (+)-Longicamphenylone | |
| 46 | 14.2 | 0.7 | (-)-Globulol | |
| 47 | 14.3 | 1.2 | cis-Thujopsene | |
| 48 | 14.4 | 1.5 | Cadalene | |
| 49 | 14.5 | 3.8 | Mustakone | |
| 50 | 14.8 | 4.1 | Isolongifolen-5-one | |
| 51 | 14.9 | 1.1 | Aristolone | |
| 52 | 14.9 | 0.6 | 9H-Cycloisolongifolene,8-oxo- | |
| 53 | 15.1 | 0.5 | γ-gurjunenepoxide | |
| 54 | 15.2 | 0.3 | (-)-Isolongifolol acetate | |
| 55 | 15.4 | 0.8 | 9H Cycloisolongifolene,8 oxo | |

Rt: retention time, M: molecular weight

**Antimicrobial activity**

The essential oil displayed weak activity against *S. aureus* with an MIC of 0.89 mg/mL, it was found to be inactive (MIC > 1 mg/mL) against other organisms (Table 2).

**Table 2: Minimum Inhibitory Concentrations (MIC) of extracts**

| Test organisms | Minimum Inhibitory Concentrations (MIC, mg/mL) | |
|---|---|---|
| | ***C. articulatus* essential oil** | **Control*** |
| *B. subtilis* | 28.50 | 0.004 |
| *S. aureus* | 0.89 | 0.004 |
| *E. coli* | 7.13 | 0.004 |
| *P. aeruginosa* | 1.78 | 0.004 |
| *C. albicans* | 14.25 | 0.016 |
| *S. enterica* | 28.5 | 0.004 |
| *S. pneumonia* | 3.56 | 0.004 |
| *P. mirabilis* | 7.13 | 0.004 |

*Controls- Ciprofloxacin (bacteria), terbinafine

**Acute oral toxicity**

Oral administration of CAEO at a dose of 2000 mg/kg did not cause changes in behavioral patterns or mortality of the animals, however, minimal changes in general CNS stimulating activity were recorded. One of the animals treated with CAEO had two episodes of loose stooling within 15-30 minutes after administration but no mortality was observed in CAEO-treated groups within the 14 day-observation period.

**Anti-tussive activity**

Pre-treatment with CAEO resulted in a dose-dependent suppression of cough reflex in treated mice (Fig. 2). Results indicate that the group treated with 900 mg/kg CAEO showed significant ($p < 0.01$) decrease in cough bouts, and this was comparatively more significant than the effect produced by dihydrocodeine ($p < 0.05$).

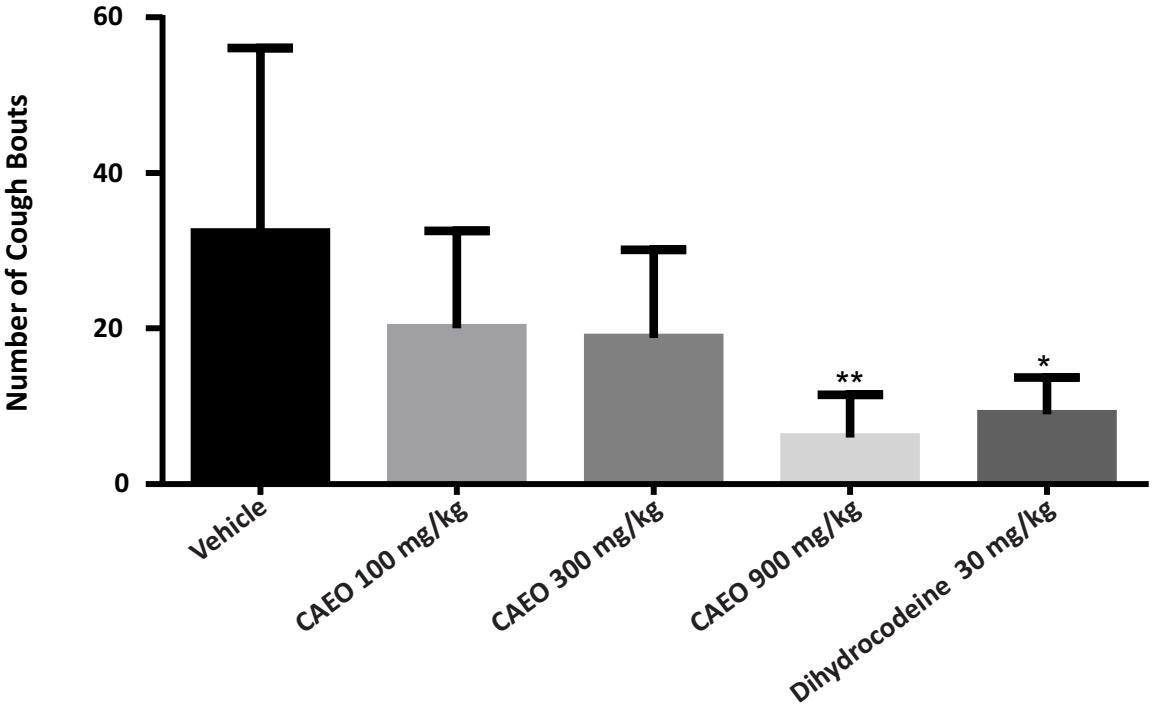



**Fig. 2 Effect of *C. articulatus* oil on the anti-tussive model induced by ammonia vapor. *p < 0.05 and **p < 0.01**

**Effect on acetic acid-induced writhing**

*C. articulatus* essential oil displayed a strong inhibitory effect on peripheral nociception (Fig. 3). All the treatment groups including the positive control group (indomethacin 5 mg/kg) significantly ($p < 0.0001$) reduced the abdominal contortions relative to the vehicle-treated group.

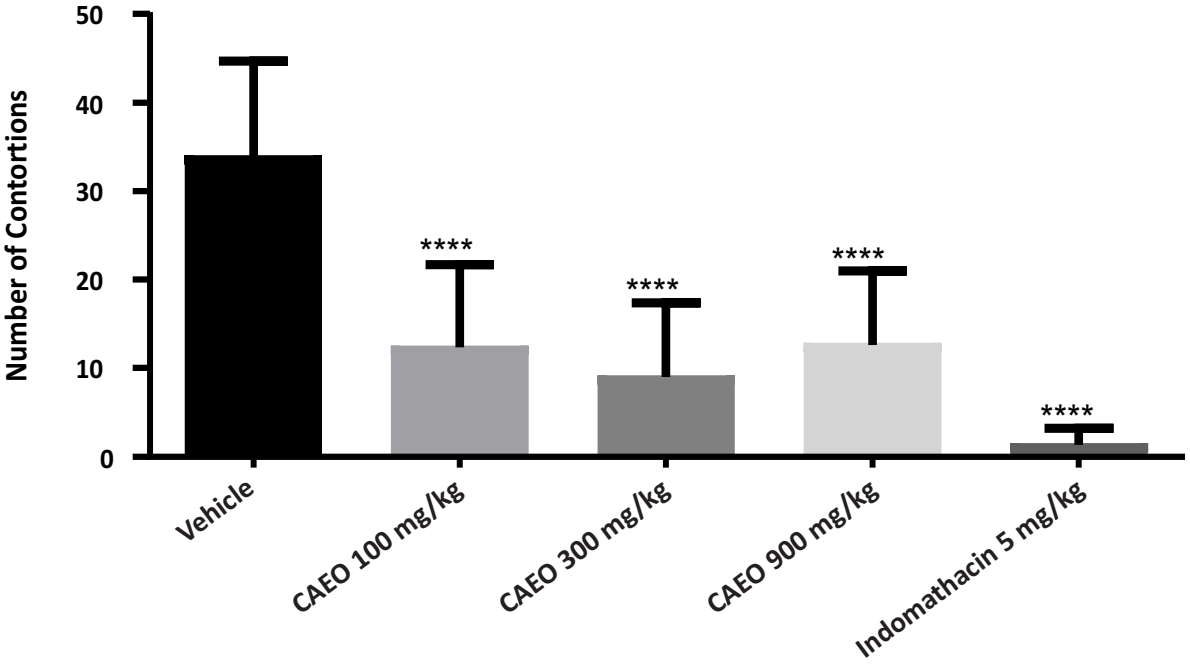



**Fig. 3: Effect of *Cyperus articulatus* essential oil on acetic acid-induced writhing. ****p < 0.0001**

**Activity against formalin-induced pain**

Fig. 4 shows the effect of CAEO on the formalin-induced paw-licking test in the first and second phases of the test. In the first phase, which evaluates neurogenic pain, the essential oil at the doses of 100, 300, and 900 mg/kg showed minimal effect in reducing the pain severity score. In the second phase, which evaluates inflammatory pain, CAEO at doses of 100, 300, and 900 mg/ kg reduced pain severity score but its effect at these doses was statistically insignificant ($p > 0.05$).

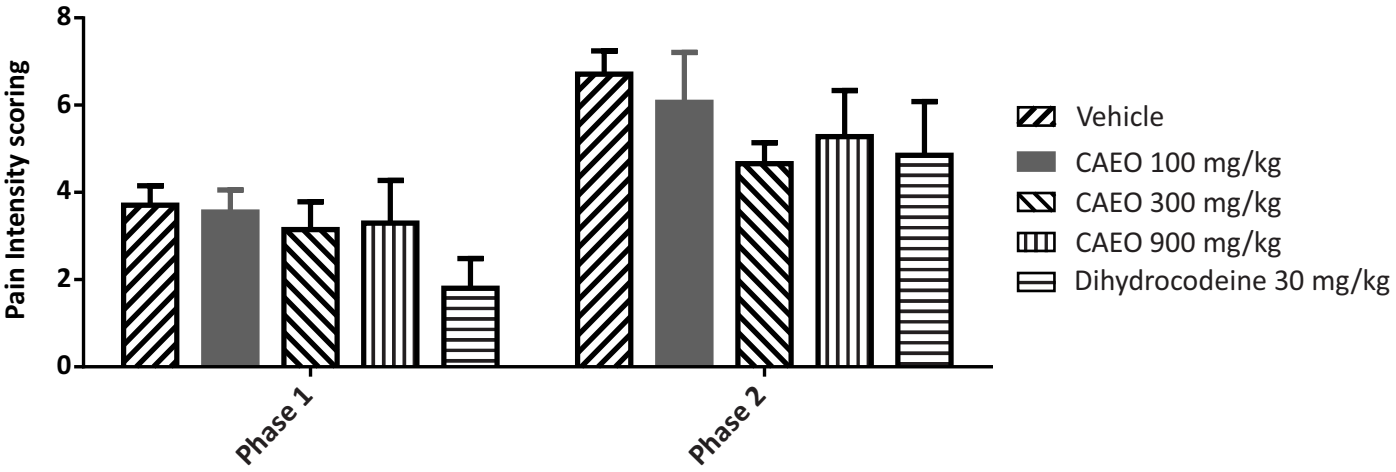



**Fig. 4: Effect of Cyperus articulatus oil on formalin test in mice**

**Analgesic activity in hot plate test**

The results presented in Fig. 5 reveal that there was a statistically significant ($p < 0.05$) increase in pain latency recorded in groups treated with 300 mg/kg and 900 mg/kg doses of CAEO. Although there was no significant difference recorded in any of the groups at 90 min, there was a significant ($p < 0.05, 0.01$) rise in latency time at 120 min post-CAEO administration relative to the vehicle-treated group. This effect was more significant compared to that produced by dihydrocodeine within the same period.

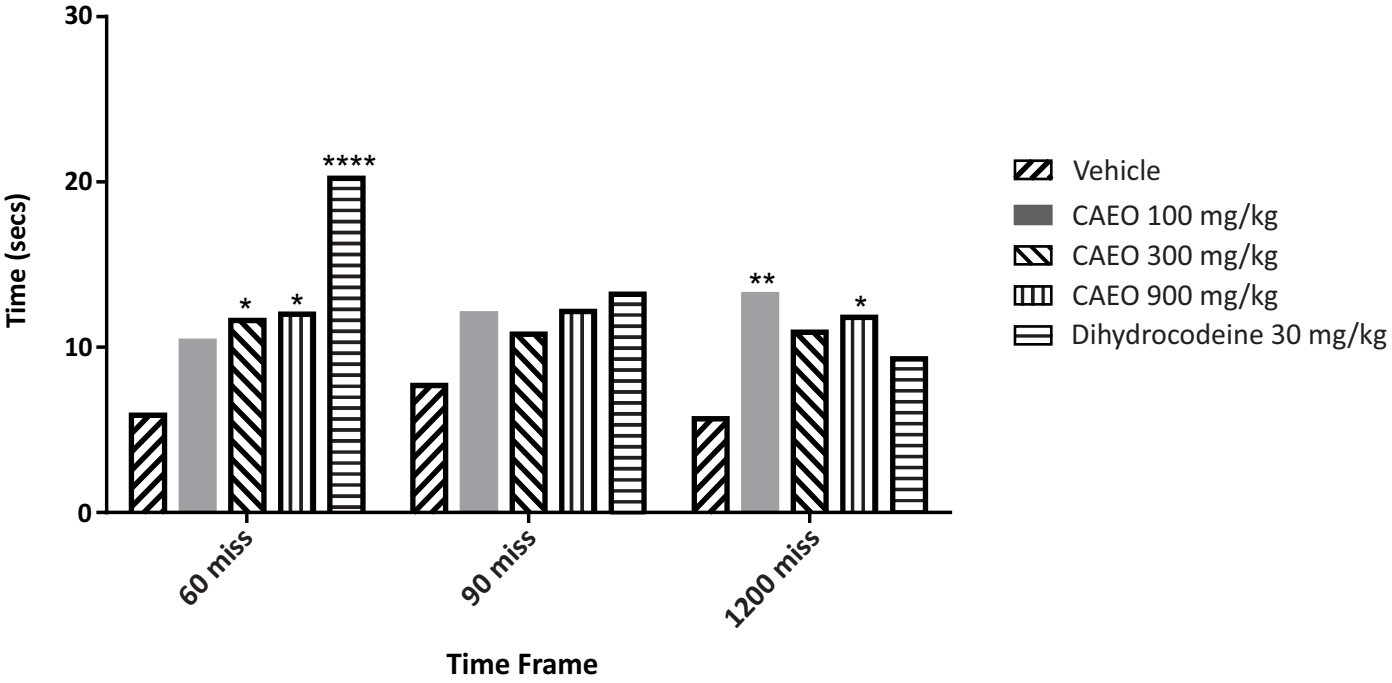



**Fig. 5 Effect of *Cyperus articulatus o*il on the hot plate test in mice. *p < 0.05, **p < 0.01, ****p < 0.0001**

**DISCUSSION**

The use of *C. articulatus* rhizomes spans a range of traditional medicine systems, aimed at addressing an array of ailments. Previous pharmacological studies of *C. articulatus* and its components have reported its anticonvulsant, sedative, antifungal, anti-plasmodial, anti-onchocerciasis, antibacterial, and antioxidant effects.[12] This study focused on further exploring the therapeutic potential of *C. articulatus* essential oil (CAEO) which can ultimately expand its economic value.

Gas chromatography - Mass spectrophotometry analysis revealed that oxygenated monoterpenes and monoterpene hydrocarbons were predominantly present in CAEO, with sesquiterpenes and oxygenated sesquiterpenes identified in relatively smaller quantities. Metabolites like α-Pinene, myrtenol, verbenone, and copaene detected in CAEO have been reported in other studies of C. articulatus essential oil from Nigeria.[22] The essential oil used in this study also showed high similarity in composition (thirteen compounds) with Brazilian *C. articulatus* rhizome essential oil studied by Ferreira *et al*.,[10] including the presence of mustakone which has been reported as a biological marker of C. articulates.[23]

Oxygenated monoterpenes, such as thymol and eucalyptol have anti-microbial[24] and anti-inflammatory effects and have been used in remedies for the treatment of inflammatory conditions.[25] Some monoterpenes, like pinene possess analgesic effects and can ameliorate pain.[26] These bioactive constituents in CAEO may underlie its analgesic and anti-nociceptive effects. Thymol which was detected in CAEO has been found to greatly reduce methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm synthesis and completely cure clinical MRSA biofilm-associated infections when used in combination with vancomycin.[27] CAEO therefore presents a rich source of medicinal compounds for the treatment of infection, pain and inflammation.

*Cyperus articulatus* essential oil showed weak antimicrobial activity against only *S. aureus* in this study and was inactive against other organisms tested, which is corroborated by similar reports.[14,28] The antibacterial activity of *C. articulatus* essential oil has been observed

to be influenced by the type of terpenes extracted by the method used to obtain the essential oil.[14] The antimicrobial activity of essential oils can be elicited through various mechanisms, including disrupting the cell wall or membrane, interfering with protein synthesis, and inhibiting nucleic acid synthesis.[29,30] Essential oils inhibit the growth of drug-resistant strains and microorganisms based on essential oil chemical composition, and type of microorganism.[31] Some antibacterial targets of essential oils include those in the bacterial cell wall and the cell membrane, and other targets mediating ATP production and pH homeostasis.[32] Molecular docking studies of *C. articulatus* essential oil components against *S. aureus* tyrosyl-tRNA synthetase, an enzyme affecting bacterial cell growth revealed that four metabolites, including α-calacorene had comparable docking scores with the standard antibacterial, chloramphenicol.[33] This further strengthens the evidence of this study, and it may be deduced that the presence of α-calacorene in CAEO contributed to its antibacterial effect.

Acute oral administration of CAEO was non-lethal in mice. This finding is supported by a previous report, in which a similar acute dose of 2000 mg/kg administered orally was non-toxic and non-lethal in mice.[10] According to the OECD classification of chemicals, drugs that are classified as category 5 exhibit low acute toxicity and have lethal doses ($LD_{50}$) ranging from 2000 to 5000 mg/kg (OECD Test No. 425, 2022). Thus, CAEO may be regarded as belonging within this category, due to its low acute toxicity. However, repeated dose sub-chronic toxicity of the essential oil is warranted to study its safety following use for extended periods.

The antitussive activity of CAEO may partly be attributed to the presence of eucalyptol and thymol, as these compounds have demonstrated antitussive properties.[34] Furthermore, it may be surmised that CAEO was acting peripherally to suppress the cough center, however, this does not exclude that CAEO may also act centrally to inhibit cough. Antitussive drugs are largely classified into two groups based on their site of action. Peripheral antitussive drugs act outside the central nervous system (CNS) to inhibit cough by suppressing the responsiveness of one or more vagal sensory receptors, while central antitussive drugs act inside the central nervous system to suppress the responsiveness of one or more components of the central reflex pathway for cough.[35-37]

The strong inhibition of peripheral pain by CAEO in acetic acid - induced abdominal contortion indicated that CAEO possesses peripheral analgesic activity. Peripheral pain response involves multiple pathways and mediators, including prostaglandins, transient receptor potential (TRP) channels, opioids, and local anesthetics. One of the key mediators of peripheral pain response is prostaglandins, which are produced by the enzyme cyclooxygenase (COX) in response to tissue damage or inflammation. Prostaglandins sensitize nociceptors (pain receptors) and increase their responsiveness to other pain mediators, such as bradykinin and histamine.[38,39] Another important pathway involved in peripheral pain response is the activation of (TRP) channels on nociceptors. TRP channels respond to various stimuli such as heat, cold, and chemical irritants, and their activation leads to the generation of action potentials and the transmission of pain signals.[40] It can be inferred that CAEO may be targeting these pathways in reducing pain response in the periphery.

The formalin test involved two phases, acute (0-5 mins) and chronic (15-30 mins) phases. The acute phase is characterized by a rapid, intense pain response, mediated by the release of chemical mediators, including histamine, serotonin, and bradykinin, which activate pain-sensing nerve fibers (nociceptors) in the paw. The acute phase is thought to model the initial phase of pain response that occurs in humans after an injury or trauma.[41] The late-onset chronic phase is characterized by a prolonged, persistent pain response, facilitated by the release of inflammatory mediators, such as prostaglandins, cytokines, and leukotrienes, which amplifies the pain signals sent by the nociceptors in the paw. It models the persistent pain that occurs in chronic pain conditions like neuropathic pain and inflammatory pain.[42] The outcome of this test in this study indicated that CAEO inhibits the chronic phase by mitigating the effects produced by cytokines and inflammatory mediators.

In the hot plate test, the increase in latency time after administration of CAEO may suggest that it possesses central analgesic activity and may be targeting any of the neurotransmitters and receptors involved in the nociceptive response. Central analgesic drugs act on the central nervous system to relieve pain by acting on opioid receptors.[43,44] Additionally, drugs that increase serotonin levels, such as selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), have been shown to have analgesic effects,45 other systems involved in pain sensitization include the Gamma-aminobutyric acid (GABA) system, and the N-

methyl-D-aspartate (NMDA) receptor system.[46,47] Drugs that modulate the endocannabinoid system and delta-9-tetrahydrocannabinol (THC), have also been shown to have analgesic effects.[48] Although the specific pathway through which CAEO elicited antinociceptive activity was not explored, it may be postulated based on its non-dose dependent activity on pain that CAEO effect on pain is mediated through any of these pathways in a non-specific manner.

## CONCLUSION

This study provides evidence that CAEO is rich in terpenes, including monoterpenes and sesquiterpenes. The essential oil was practically non-toxic following acute oral administration and demonstrated efficacy against some pathogenic microorganisms, chemical irritant-induced cough, and pain of central and peripheral origin. *C. articulatus* essential oil also demonstrates anti-inflammatory and antinociceptive activity, which may involve activity on peripheral and central nociceptors. This supports its use in the management of respiratory troubles.

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## REFERENCES

1. Cyperus articulatus Linn. [family CYPERACEAE] on JSTOR. (n.d.). Retrieved February 15, 2023, from https://plants.jstor.org/stable/10.5555/al.ap.upwta.1_1244
2. Silva ÉB, Barata LE, Arévalo MR, Vieira LQ, Castro W, Ruiz AL, Torre AD, Castro KC, Sartoratto A, Baratto LC, de Santana MB. (2021). Chemical Composition and Antiproliferative Activity of the Ethanolic Extract of *Cyperus articulatus* L. (Cyperaceae). Plants (Basel, Switzerland), 10(10): 2084. https://doi.org/10.3390/plants10102084
3. Taylor L. (1996). Tropical Plant Database. Raintree Nutrition, Incorporated.
4. The Plant List. Cyperus articulatus L. (n.d.). The Plant List. Retrieved from http://www.theplantlist.org/tpl1.1/record/kew-235733
5. Cyperus articulatus Jointed flatsedge, Priprioca, Piripiri PFAF Plant Database. (n.d.). Retrieved March 24, 2023, from https://pfaf.org/user/Plant.aspx?LatinName=Cyperus+articulatus
6. Herrera-Calderon O, Santiváñez-Acosta R, Pari-Olarte B, Enciso-Roca E, Montes VM, Acevedo JL. (2018). Anticonvulsant effect of ethanolic extract of Cyperus articulatus L. leaves on pentylenetetrazol induced seizure in mice. *Journal of Traditional and Complementary Medicine*, 8(1): 95-99.
7. Swain A, Santhoshkannada AN, Prabakaran D, Choudhir G, Puttaswamy H. (2021). Phytochemical and pharmacological exploration of *Cyperus articulatus* as a potential source of nutraceuticals and drug ingredients. *Indian Journal of Pharmaceutical Education and Research*, 55(4): 1084-1095.
8. Nogueira ML, Lima EJ, Adrião AA, Fontes SS, Silva VR, Santos LD, Soares MB, Dias RB, Rocha CA, Costa EV, Silva FM. (2020). *Cyperus articulatus* L.(Cyperaceae) rhizome essential oil causes cell cycle arrest in the G2/M phase and cell death in HepG2 cells and inhibits the development of tumors in a xenograft model. *Molecules*, 25(11): 2687.
9. Yeshi K, Wangchuk P. (2023). Bush medicinal plants of the Australian wet tropics and their biodiscovery potential. In: *Bioprospecting of Tropical Medicinal Plants* (pp. 357-379). Cham: Springer Nature Switzerland.
10. Ferreira JC, Pereira AM, Aranha ES, Moraes CC, de Souza Ferreira B, Sartoratto A, Goes GR, Moraes TM, Moraes WP. (2025). *Cyperus articulatus*: Anti-inflammatory and antinociceptive activity of a medicinal plant from the Amazon. *Journal of Ethnopharmacology*, 337: 118947.
11. Swain A, Duraivadivel P, Choudhir G, Hariprasad P. (2022). Chemical composition and antimicrobial properties of the rhizome essential oil of *Cyperus articulatus* L. grown in Karnataka, India. *Indian Journal of Natural Products and Resources* (IJNPR)[Formerly Natural Product Radiance (NPR)] 13(1):112-118.
12. Machado IR, Mendes KR, Arévalo MR, Kasper AA, Castro KC, de Giorge Cerqueira H, Silva AS, Barata LE. (2020). Medical, therapeutic and pharmaceutical use of *Cyperus articulatus* L.: uma Revisão. *Biodiversidade Brasileira*, 10(3): 11-23.
13. Schön T, Werngren J, Machado D, Borroni E, Wijkander M, Lina G, Mouton J, Matuschek E, Kahlmeter G, Giske C, Santin M. (2020). Antimicrobial susceptibility testing of Mycobacterium tuberculosis complex isolates-the EUCAST broth microdilution reference method for

MIC determination. *Clinical Microbiology and Infection*, 26(11): 1488-1492.
14. da Silva IC, dos Santos WL, Leal IC, Zoghbi MD, Feirhmann AC, Cabral VF, Macedo EN, Cardozo-Filho L. (2014). Extraction of essential oil from *Cyperus articulatus* L. var. articulatus (priprioca) with pressurized CO2. *The Journal of Supercritical Fluids*, 88: 134-141.
15. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *Journal of Cerebral Blood Flow & Metabolism*, 40(9): 1769-1777.
16. OECD Test No. 425. (2022). Acute Oral Toxicity: Up-and-Down Procedure. https://doi.org/https://doi.org/10.1787/9789264071049-en
17. Cruz LF, de Figueiredo GF, Pedro LP, Amorin YM, Andrade JT, Passos TF, Rodrigues FF, Souza IL, Gonçalves TP, dos Santos Lima LA, Ferreira JM. (2020). Umbelliferone (7-hydroxycoumarin): A non-toxic antidiarrheal and antiulcerogenic coumarin. *Biomedicine & Pharmacotherapy*, 129: 110432.
18. Adejayan AA, Ozolua RI, Uwaya DO, Eze GI, Ezike AC. (2019). Evaluation of the anti-asthmatic and antitussive potentials of methanol leaf extract of Napoleona vogelii in rodents. *Biomedicine & Pharmacotherapy*, 109: 120-126.
19. Conegundes JL, da Silva JM, de Freitas Mendes R, Fernandes MF, Pinto ND, de Almeida MA, Dib PR, de Oliveira Andrade R, Rodrigues MN, Castañon MC, Macedo GC. (2021). Anti-inflammatory and antinociceptive activity of Siparuna guianensis Aublet, an amazonian plant traditionally used by indigenous communities. *Journal of Ethnopharmacology*, 265: 113344.
20. Mirghazanfari SM, Aldein AH, Parviz M, Ahmadi BZ. (2019). Evaluation of Ficus carica Linn fruit extracts on formalin induced pain in the male rat paw. *Annals of Military and Health Sciences Research*, 17(3): 1-6.
21. Deng J, Han J, Chen J, Zhang Y, Huang Q, Wang Y, Qi X, Liu Z, Leung EL, Wang D, Feng Q (2021). Comparison of analgesic activities of aconitine in different mice pain models. *PLoS One*, 16(4): e0249276.
22. Kavaz D, Idris M, Onyebuchi C (2019). Physiochemical characterization, antioxidative, anticancer cells proliferation and food pathogens antibacterial activity of chitosan nanoparticles loaded with Cyperus articulatus rhizome essential oils. *International journal of Biological Macromolecules*, 123: 837-845.
23. Tongnuanchan P, Benjakul S. (2014). Essential oils: extraction, bioactivities, and their uses for food preservation. *Journal of Food Science*, 79(7): R1231-R1249.
24. Martínez-Pabón MC, Ortega-Cuadros M. (2020). Thymol, menthol and eucalyptol as agents for microbiological control in the oral cavity: A scoping review. *Revista Colombiana de Ciencias Químico-Farmacéuticas*, 49(1): 44-69.
25. Eldahshan OA, Barakat A, Efferth T. (2018). The prophylactic effect of a Eugenia aquea extract against oxidative stress and inflammation associated with the development of arthritis in an adjuvant-induced arthritis rat model. Food & Function, 9(12): 6643-6651.
26. Boukhatem MN, Darwish NH, Sudha T, Bahlouli S, Kellou D, Benelmouffok AB, Chader H, Rajabi M, Benali Y, Mousa SA. (2020). In vitro antifungal and topical anti-inflammatory properties of essential oil from wild-growing thymus vulgaris (Lamiaceae) used for medicinal purposes in algeria: A new source of carvacrol. *Scientia Pharmaceutica*, 88(3): 33.
27. Yuan Z, Dai Y, Ouyang P, Rehman T, Hussain S, Zhang T, Yin Z, Fu H, Lin J, He C, Lv C. (2020). Thymol inhibits biofilm formation, eliminates pre-existing biofilms, and enhances clearance of methicillin-resistant Staphylococcus aureus (MRSA) in a mouse peritoneal implant infection model. *Microorganisms*, 8(1): 99.
28. Swain A, Duraivadivel P, Choudhir G, Hariprasad P (2022). Chemical composition and antimicrobial properties of the rhizome essential oil of *Cyperus articulatus* L. grown in Karnataka, India. *Indian Journal of Natural Products and Resources* (IJNPR)[Formerly Natural Product Radiance (NPR)]. 2022 Dec 5;13(1):112-8.
29. Amerikova M, Pencheva El-Tibi I, Maslarska V, Bozhanov S, Tachkov K (2019). Antimicrobial activity, mechanism of action, and methods for stabilisation of defensins as new therapeutic agents. *Biotechnology & Biotechnological Equipment*, 33(1): 671-682.
30. Angane M, Swift S, Huang K, Butts CA, Quek SY. (2022). Essential oils and their major components: an updated review on antimicrobial activities, mechanism of action and their potential application in the food industry. *Foods*, 11(3): 464.
31. Reyes-Jurado F, Navarro-Cruz AR, Ochoa-Velasco CE, Palou E, López-Malo A, Ávila-Sosa R. (2020). Essential oils in vapor phase as alternative antimicrobials: A review. *Critical Reviews in Food Science and Nutrition*, 60(10): 1641-1650.

32. Li H, Zhao L, Dai Q, Mo H, Liu Z, Pu H, Zhu X, Yao L, Xu D, Hu L. (2023). Blended cumin/Zanthoxylum essential oil improve the antibacterial, fresh-keeping performance and flavor of chilled fresh mutton. *Meat Science*, 200: 109173.
33. Swain A, Choudhir G, Prabakaran D, Hariprasad P. (2023). Molecular docking, dynamics simulation and pharmacokinetic studies of *Cyperus articulatus* essential oil metabolites as inhibitors of *Staphylococcus aureus*. *Journal of Biomolecular Structure and Dynamics*, 41(19): 9245-9255.
34. Baky MH, Farag MA, Rasheed DM. (2020). Metabolome-based analysis of herbal cough preparations via headspace solid-phase microextraction GC/MS and multivariate data analyses: A prospect for its essential oil equivalency. *ACS Omega*, 5(48): 31370-31380.
35. Abidi S, Imam S, Tasleem F, Zehra Rizvi SR, Salman S, Gilani U, Mahmood ZA. (2021). Formulation and evaluation of natural antitussive cough syrups. *Pakistan Journal of Pharmaceutical Sciences*, 34(5).
36. Birring S, de Blasio F, Dicpinigaitis PV, Fontana G, Lanata L, Page C, Saibene F, Zanasi A. (2019). Antitussive therapy: A role for levodropropizine. *Pulmonary Pharmacology & Therapeutics*, 56: 79-85.
37. Singh N, Driessen AK, McGovern AE, Moe AA, Farrell MJ, Mazzone SB. (2020). Peripheral and central mechanisms of cough hypersensitivity. *Journal of Thoracic Disease*, 12(9): 5179.
38. Matsuda M, Huh Y, Ji RR. (2019). Roles of inflammation, neurogenic inflammation, and neuroinflammation in pain. *Journal of Anesthesia*, 33(1): 131-139.
39. Yaksh TL, Wallace MS. (2011). Opioids, analgesia, and pain management. *Goodman and Gilman's the pharmacological basis of therapeutics*, 481-526.
40. Niu X, Wang X, Niu B, Meng Y, He H, Wang Y, Li G. (2021). Costunolide loaded in pH-responsive mesoporous silica nanoparticles for increased stability and an enhanced anti-fibrotic effect. *Pharmaceuticals*, 14(10): 951.
41. Mogil JS. (2009). Animal models of pain: progress and challenges. *Nature Reviews Neuroscience*, 10(4): 283-294.
42. Aalidaeijavadi Z, Khodagholi F, Haghparast A. (2026). Potential effects of cannabidiol on formalin-induced inflammatory pain in morphine-dependent rats. *Journal of Psychiatric Research*, 197: 17-25
43. Lisa NP, Millis RM. (2022). Epigenetics and Pain: New Insights to an Old Problem. Cureus, 14(9).
44. Rodriguez-Gaztelumendi A, Spahn V, Labuz D, Machelska H, Stein C. (2018). Analgesic effects of a novel pH-dependent μ-opioid receptor agonist in models of neuropathic and abdominal pain. Pain, 159(11): 2277-2284.
45. Wang L, Tobe J, Au E, Tran C, Jomy J, Oparin Y, Couban RJ, Paul J. (2022). Selective serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors as adjuncts for postoperative pain management: systematic review and meta-analysis of randomised controlled trials. *British Journal of Anaesthesia*, 128(1): 118-134.
46. Liu YJ, Li YL, Fang ZH, Liao HL, Zhang YY, Lin J, Liu F, Shen JF. (2022). NMDARs mediate peripheral and central sensitization contributing to chronic orofacial pain. *Frontiers in Cellular Neuroscience*, 16: 999509.
47. Sivakumar D, Ramli R. (2022). GABAergic signalling in modulation of dental pain. *European Journal of Pharmacology*, 924: 174958.
48. Chen R, Coppes M, Urman RD. (2021). Receptor and molecular targets for the development of novel opioid and non-opioid analgesic therapies. *Pain Physician*, 24(2): 153.