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Limonene and Fatty Acid Content of the Volatile Oil of *Raphia australis* Fruit Pulp and Their Synergistic *In vivo* Anti-inflammatory Activity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the *in vivo* anti-inflammatory activity of the essential oil and fatty acid compositions of the volatile oil of Raphia *australis* fruit pulp.
Method: The oils of pulp and seed of *Raphia australis* were isolated using Clevenger apparatus and were further subjected to GC-MS analyses. Anti-inflammatory test was carried out on the pulp of *Raphia australis* oils using four groups of rats comprising six rats per group (n=5). Group one were

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administered normal saline (0.09 % v/v NaCl) while group 1, 2 and 3 were administered 40 mg kg-1(volatile oil), 80 mg kg-1 (volatile oil) and 100mg kg-1 aspirin, respectively. Inflammation was induced in all the groups using lipopolysaccharide.

Results: From the GCMS analysis, 20 compounds were detected in the oil of *R. australis* pulp while 19 compounds were shown to be present in the seed oil. The total fatty acid content of R. *australis* seed oil was 38.1 %. The rest were essential oil compounds (21.6 %). There are 6.6% unknown compounds present in the oil obtained from the seed of *R. australis*. The essential oil content of the pulp of *R. australis* was 68.6 %. The pulp contained 16 % of fatty acids with 9.1 % unknown compounds. The limonene content (38.4 %) of *Raphia australis* pulp was found to be about 6 times higher than that of the seed (6.3 %). However, the quantity of hexadecenoic acid obtained from the seed (34.4 %) was more than 3 times higher than the amount found in the pulp (10.8%). Other volatile compounds present in the pulp include 2-pentylfuran (3.2 %), germacrene D (7.1 %), nonanal (3.3 %) alpha cadinene (5.2 %), tertradecanoic acid (2.2 %) while nonanal (8.5 %), eicosane (3.9 %) b-pinene (2.2%), 2 pentyl furan (1.9%) and tetradecanoic acid (1.7 %) constitute the major component of the rest of the volatile oil of the seed of *Raphia australis*. The result of inflammatory activity of the volatile oil of *Raphia australis pulp* using adult male mice showed that 80 mg kg-1 dose of the oil elicited a significant anti-inflammatory effect ($P<0.01$) when compared to 100mg kg^{-1} of aspirin ($P<0.01$). The analgesic activities of aspirin ($P<0.05$) and the 80 mg kg-1 dose ($P<0.05$) of the oil were only significant in the inflammatory phase of the analgesic test.

Conclusion: These results show that the volatile oil of *Raphia australis* has therapeutic potential for use in the management of inflammation-related disorders.

Keywords: Raphia australis pulp; volatile oil; hexadecanoic acid; anti-inflammatory activity; analgesic activity; fatty acids; essential oil.

1. INTRODUCTION

Palm tree is a general word for a set of perennial plants comprising trees from various genera and species including *Elaeis guineensis jacq., Phoenix reclinate jacq., Dypsis canaliculate (jum.) beentje & j.dransf, Raphia farinifera (gaertn.)Hyl., Raphia hookeeri G.Mann & H.Wendl, Raphia vinifera P.Beauv., and Raphia australis Oberm. & Strey. (R. australis)"* (Obermeyer and Strey 1969, Gruca et al. 2014). They are evergreen perennial plants with characteristic long stems. "Palm leaves are larger than those of all other trees and *Raphia* palm leaf is the longest among palm leaves. The *Raphia* palm belongs to the branch of Spermaphytes, sub-branch Angiosperms, class Monocotyledons, super-order Spadiciflores, order Palmae, family Palmaceae, subfamily Lepidocaryoids, genus *Raphia"* (Otedoh 1982).

Raphia Palm can grow as tall as 16 metres. They are unique for their compound leaves. Some species have leaves that grow as long as 25 metres with a width of three metres. The plants are either monocarpic flowering once and dry after the maturation of the seeds or with individual stem drying after fruiting while the root system remains alive and continue to grow new stems. The genus *Raphia* has various species distributed throughout Africa, Central America and South America and comprises about 20 species distributed all over the world, with *R. australis* being the only species indigenous to South Africa (Coates and Keith 2002).

R. australis is called unVuma by the Zulu speaking tribes in South Africa while it is called 'Kosi palm' by the Afrikaans. Unlike West African palm trees, *R. australis* is not cultivated for palm wine making. *R. australis* fruits grow in bunches, and each fruit varies in size depending on the species and maturity stage. The fruit has a hard pulp covered with hard interlocking scales (Obermeyer and Strey 1969) Fig. 1.

The different parts of various species in the Raphia genus have been used in fish harvesting (Cletus et al. 2013) and traditionally to manage a wide variety of metabolic disorders such as sickle cell anaemia (Ibegbulem et al. 2011) alcoholic intoxication (Enomfon and Itoro 2004) filariasis, hyperglycaemia (Mpiana et al. 2013).

Inflammation is the human body immune response to perceived injury caused by microbial infections, endogenous and exogenous chemicals or physical irritants (Guyton and Hall 2011). The five markers of inflammation are pain, swelling, redness, increased temperature, and loss of function (Kockerling et al. 2013).

Fig. 1. Images of *R. australis* fruit used for this work

Man's attempts to treat inflammation predates Hippocrates's (460-370 BC) use of willow bark to treat this ubiquitously linked immune disorder (Silva 1994, Cavaillon 2021). The desire to synthesise an anti-inflammatory drug with milder side effects, higher potency and efficacy inspired medicinal chemists towards the semi synthesis of aspirin from salicin in 1897 (Desborough and Keeling 2017). Other non-steroidal anti-inflammatory drugs (NSAIDs) were later synthesised including antipyrine, phenacetin, phenylbutazone, fenamates, indomethacin and naproxen. (Sir Vave 2000). John Vane reported that anti-inflammatory drugs in this category exhibit their pharmacological activities by inhibiting the cyclooxygenase enzyme (Vane 1971). As a result of their effort to circumvent the adverse effects of the existing NSAIDs, Peedleman and his research group were able to unveil the two isoforms of the cyclooxygenase enzyme known as COX-1 AND COX-2 (Fu et al. 1990). Hoping to eliminate the unwanted side effects, medicinal chemists successfully synthesized selective COX-2 inhibitors ('coxibs') including rofecoxib, celecoxib and etoricoxib (Mitchell et al. 1993, Cairns 2007). Disappointingly, soon after the long-awaited arrival of this new class of active pharmaceutical ingredients, it was observed that COX-2 inhibitors also caused gastrointestinal bleeding, albeit to a lesser degree. Furthermore, the use of selective COX- 2 inhibitors was reported to be attributed to high risks of cardiac complications. The discovery of the third isoform of cyclooxygenase enzyme known as COX-3 (a splice variant of COX-1 isoform) in 2002 finally ended the inquiries on the mechanism of action of paracetamol which had baffled the scientific community for more than a century. Research revealed that paracetamol preferentially inhibits the newly discovered isoform (COX-3) over COX-1 and -2 (Chandrasekharam et al. 20020).

While the development of the various NSAIDs was ongoing, medicinal chemists had been developing various anti-inflammatory agents from the endogenous steroidal hormones since Philip Hench's observed that arthritis patients who later developed jaundice or got pregnant experienced relief from arthritis-related pains. Steroid hormones were found to be responsible for the observed relief experienced by the arthritis patients. This interesting observation prompted the isolation of 6 steroidal hormones by Kendall, labelled compound A to F, from bovine adrenal cortex. Kendal later achieved an economically feasible and less laborious synthesis of cortisone from ox bile. Since the isolation of the steroidal hormones, a plethora of corticosteroids including cortisone, dexamethasone, prednisolone, betamethasone, meloxicam and deflazacort have been synthesized, all with their associated side effects such as diabetes (steroid-induced diabetes), skin hypertrophy, adrenal suppression, glaucoma, osteoporosis and hypertension (Chandrasekharam et al. 2002, Kendall 2002, Sinniah et al. 2021).

Due to the side effects associated with all these classes of drugs, there has been a lot of research work to obtain natural products compounds which retain potency with mild to no side effects. Since antiquity, both essential oils and many volatile compounds from various plants have been used to manage inflammatory disorders. To date and to the best of our knowledge, there is no literature report detailing the anti-inflammatory activity of the oil of *R. australis* pulp. This paper therefore investigates the chemical constituents of the volatile oil of *R. australis* pulp and their synergistic anti-inflammatory property.

2. MATERIALS AND METHODS

2.1 Plant Collection

The fresh fruits of *R. australis* were collected from Mr. Bruce Hooper of KwaZulu-Natal and transferred in jute bags to Organic Chemistry Research Laboratory, Walter Sisulu University Mthatha, within 48 hours. Authentication of *R. australis* was done by Dr. K. L. Immelman of Department Biological and Environmental Sciences, Walter Sisulu University, Mthatha. The seed of *R. australis* was manually separated from the pulp and both were air-dried at room temperature and then transferred into a grinder for size reduction.

2.2 Animal Handling

Mice (20-30g) were obtained from the South African Vaccine Initiative, Johannesburg and kept at Animal Holding Facility at Walter Sisulu University, NMD campus, Mthatha. They were acclimatized to the laboratory environment for 1 week, maintained under 12 h light/dark cycle at temperature of 22 ± 2 ℃ and housed (5 animals per cage) in a Plexiglas cage with wood shavings as beddings. The animals were fed with standard laboratory food for rodents and water was provided freely except during the experiment. This study was approved by the Department of Higher Education, WSU and Ethical Clearance Approval obtained from Walter Sisulu University Ethics Committee with Reference No. DVC (AA&R) DRD/SREC: FNS 01/02/2017.

2.3 Isolation of Volatile oils from the Pulp and Seed of Raphia Australis

Volatile oil extraction was done on the dried *R. australis* pulp and seed according to British Pharmacopeia method (1980). 600 g of the dried sample was transferred into a round-bottomed flask. Distilled water was then added until the sample was covered. The flask was placed on a heating mantle connected to a condenser which was fitted into a recuperating container containing methanol and water. This served as a coolant. The mixture was brought to boil and then the heating mantle temperature was set at 70 ^{0}C for 4 hours. The oil extracted was collected over hexane in the Clevenger apparatus. The extracted oil was stored in an air-tight sample vial and kept in a refrigerator at a temperature of 4 ^{0}C until the time for analysis. The aqueous supernatant of the flask's content was poured into a beaker and concentrated at 40 ^{0}C.

2.3.1 GC/ GC-MS analysis

The oil was analysed by GC and GC/MS. The GC analysis was carried out on a Perkin Elmer 8500 gas chromatograph with a FID detector with a SGE BP X5 column that is 30m in length with a thickness of 0.25 µm and a diameter of 0.25mm ID. The operating conditions are as follows: carrier gas, nitrogen with a flow rate of 3.0 ml min^{-1}; column temperature, 60-275 ^{o}C at 4 ^{o}C min^{-1}; injector and detector temperature, 250 ^{o}C; volume injected 0.1µl of the oil; split ratio, 1:100.

Gas Chromatography / mass spectrometer (GC/MS) of the volatile oil was carried out using an Agilent Gas Chromatography 890 equipped with a capillary column (Agilent 190915 30 m X 0.25 µm calibrated) attached with an Agilent mass spectrometer system (5975C VL MSD with triple axis Detector). The oven temperature was programmed from 50^{0}C – 280^{0}C helium was used as the carrier gas at a flow rate of 1mlmin^{-1}. The solution was manually injected into the GC/MS. The chemical composition of the volatile oil of the dried *Raphia* pulp was determined according to their retention time and spectrometric libraries (WILEY NIST) Equation

2.3.2 Identification of compounds

Compounds in the essential oil were identified by matching their retention indices and mass spectra with the ones recorded in NIST 11 library and by comparing retention indices and mass spectra with literature values (Adams., 2012; Joulain and Koenig, 1998).

2.4 Biological Studies

2.4.1 Acute toxicity test

Acute toxicity of the volatile oils and ethanol extracts of the pulp and seed of *R. australis* was assessed in mice using oral route (p.o) according to Lorke's method (Lorke 1983). Each oil extract was tested for acute toxicity (LD50) effect orally using 13 animals each. The procedure was divided into two phases, phase I used 3 animals per dose of 10, 100 and 1000 mg/kg. Phase II used one animal per dose levels of 1000, 1600, 2900 and 5000 mg/kg. Each animal after treatment was observed for a period of one h initially to check for immediate effect and then for up to 24 h after for mortality. Animal that survived for more than 24 h is scored no mortality. Animal that survived beyond 24 h is assumed to have LD50 of 5000 mg/kg. The LD_{50} of the infusion

extract was estimated as the geometric mean of the lowest dose causing death and the highest dose causing no death according to the formula below:

$$LD_{50} = \sqrt{A \times B}$$

A is the maximum dose producing 0% death and B is the dose that produces 100% death (Lorke,1983). From the result of LD_{50}, the working doses was chosen such that the highest working dose is below half of the LD_{50} according to the equation: Working dose $\leq$ ½ (LD_{50}).

2.4.2 Anti-inflammatory test

The anti-inflammatory activity of the volatile oil of *R. australis* pulp was evaluated using lipopolysaccharide-induced oedema model (Cartorce and Gervekian 2016). In this test, four groups of five rats per group were used. Four groups of rats were randomly allocated, group 1 was the negative control (5% Tween 80), groups II and III were administered volatile oil (40 and 80 mg/kg respectively) and group IV was the positive control (aspirin 100 mg/kg). All treatments were by the oral route and pretreatment was 1 h prior to lipopolysaccharide injection. Paw sizes were measured at time 0, 1, 2, 3, 4 and 5 h post-lipopolysaccharide injection. Baseline paw size was measured before and after 1, 2, 3, 4 and 5 hours post injection of lipopolysaccaride using a Vernier Calipers (Hunskaar and Hole 1987).

2.4.3 Analgesic (formalin-induced paw licking) test

The method of (Hunskaar and Hole 1987 as modified by Hajhashemi *et al.* was used for the analgesic bioassay (Hajhashem et al. 2003). Four groups of mice consisting of 5 mice each were randomly selected. Group 1 (control) was orally administered 5% Tween 80 normal saline. Groups 2 and 3 were orally treated with the volatile oil (40 and 80 mg/kg respectively). Groups 4 mice were treated with diclofenac (100 mg/kg, p.o.) 1 h prior to administration of 0.02 ml of 2.5% formalin into the sub-planter space of the right hind paw and the duration of paw licking was determined 0-5 minutes (1st Phase or neurogenic phase) and 20-25 minutes (2nd phase or inflammatory phase) after formalin administration. The 1st phase is regarded as the neurogenic mechanism and the 2nd phase is the inflammatory mechanism. The number of paw licks in the first and second phases were recorded for each animal.

2.4.4 Analysis of results

All results were presented as Mean ± SEM and further analysed with one way analysis of variance (ANOVA), followed by Dunnet's post hoc test, and values were considered significant at $p<0.05$. GraphPad Version 3.0 and GraphPad Prism Version 5 copyright © 2013 by GraphPad Software Inc. USA were used for analyzing the results.

3. RESULTS AND DISCUSSION

3.1 Extraction of the Volatile Oils of the Pulp and Seed of R. Australis

A light greenish yellow oil was obtained from the extraction of *R. australis* pulp while the seed gave a cloudy yellow oil (Fig. 2). The higher percentage yield of the volatile oil of the pulp shows that it has a higher volatile oil content as shown in Table 1.

The oil of *R australis* pulp has a higher percentage yield when compared to the percentage yield of the oil from the seed as shown in the Table 1.

Fig. 2. Images of the oils of *R. australis* pulp and seed

Table 1. Percentage yield of the oils of the pulp and seed of *R. australis*

Plant part	Mass of sample (g)	Mass of oil obtained (mg)	Percentage yield
Raphia australis dried fruit pulp	600	500	0.083
Raphia australis dried fruit seed	600	400	0.067

3.2 GCMS of the Oils of the Pulp and Seed of R. Australis

Twenty compounds accounting for 97.2% of the total oil composition were obtained from the GCMS analysis of *R. australis* pulp oil. Sixteen compounds (87.9%) were fully identified using literature search and software thereby leaving only four unknown compounds (9.1%) (Table 2). 71.9 % of the total oil extracted were essential oil compounds.

The essential oil of the pulp was characterized by monoterpenes which accounts for 38.4% of the total oil composition. Limonene was identified as the major component, and the only monoterpene present in the oil. This was closely followed by sesquiterpenes with percentage composition 16.3%, fatty acids (16.2%) and oxygenated monoterpenes (9.5%).

Lauric acid, also known as dodecanoic acid, is a middle chain saturated fatty acid. It is found in many vegetable fats, particularly in coconut and palm kernel oils, and it is known to be used by man for medicinal purposes. Lauric acid is used for treating viral infections including influenza (the flu), swine flu, avian flu, the common cold, fever blisters, cold sores, and genital herpes caused by herpes simplex virus (HSV), genital warts caused by human papillomavirus (HPV), cardiovascular diseases, inflammatory disorders and HIV/AIDS. It is also used for preventing the transmission of HIV from mothers to children (Gogneni et al. 2015).

The other fatty acid components were identified as nonanoic acid (pelargonic acid), Tetradecanoic acid and hexadecanoic acid (palmitic acid). Pellagonic acid occurs naturally as esters in the oil of pelargonium (Franck et al. 2013). Tetradecanoic acid and hexadecanoic acid are saturated fatty acids which were reported to be potent antifungal and antibacterial agents.

Table 2. Chemical composition of the oil of *R. australis* pulp

S/N	RT	KI values	% Composition	Names of compounds	CAS Number
1	6.8334	993	3.2	2-pentylfuran	003777-69-3
2	7.7908	1033	38.4	Limonene	000138-86-3
3	9.684	1100	3.0	Linalool	000078-70-6
4	9.7644	1104	3.3	Nonanal	000124-19-6
5.	11.3476		1.5	Unknown	
6.	14.054		1.2	Unknown	
7.	15.947	1377	1.1	copaene	003856-25-5
8.	16.6588		5.0	Unknown	
9.	17.9157	1467	2.9	δ -humelene	001896-62-4
10	18.5041	1487	7.1	germacrene D	023986-74-5
11	18.702	1572	1.3	caryophyllene oxide	001139-30-6
12	18.785	1580	2.0	dodecanoic acid	000143-07-7
13	18.8196	1607	2.0	humulene epoxide	019888-34-7
14	19.1887	1636	5.2	δ cadinene	000483-76-1
15	22.1893	1640	1.4	t- cadinol	005937-11-1
16	22.4300	1676	3.0	δ -cadinol	000481-34-5
17	28.9629	1720	2.2	tetradecanoic acid	000544-63-8
18	24.4036		1.4	Unknown	
19	27.0887	1972	10.8	hexadecanoic acid	000057-10-3
20	28.7788	2173	1.0	Octadecadienoic acid (linoleic acid)	000060-33-3
		Total % = 97.0			

Hexadecanoic acid, also known as palmitic acid, is an organic acid commonly found within the Aracaceae family of plants. Individuals living with obesity often have a high serum concentration of palmitic acid. Its high concentration in obese patient activates the toll-like receptor (TLR) signalling pathway, especially the TLR4, which is associated with inflammatory disorders such as insulin resistance and non-alcoholic fatty liver syndrome (Kobecki and Bajdak-Rusinek 2019). Conversely, hexadecanoic acid has been reported to inhibit inflammation. According to Vasudevan et al. 2012, the result of an *in vitro* enzyme kinetic study revealed that hexadecenoic acid inhibited inflammation by binding to phospholipase A2 (PLA 2) (Figure 3), thereby preventing the release of arachidonic acid from the cell membrane phospholipids (Vasudevan et al. 2012). In 2019, Malkowski and Smith further revealed that hexadecenoic acid allosterically activates COX-2 more than it inhibits COX-1 (Malkowski and Smith, 2019). Due to the high serum concentration of hexadecanoic acid in obese patients, impaired clinical treatments and amplified COX-2 inhibitors side effects may result.

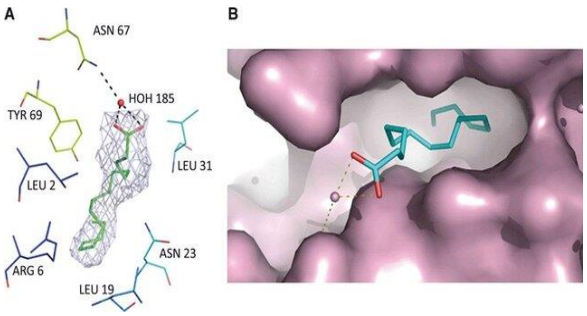

Fig. 3. 2-D (A) and 3-D (B) images of the antagonistic binding of palmitic acid on PLA2

Limonene (Fig. 4) is a monoterpene with the IUPAC name '1-methyl-4-(methyl-4-(1-methylethenyl) cyclohexene'. This secondary metabolite, which depending on the preponderant isomer, gives the distinguishing aromas to orange and lemon, was reported to exhibit anti-inflammatory and anticancer activities (Miller et al. 2011). It exists in 2 isomeric forms as L- and D-Limonene (Figure 4). Unlike Hexadecanoic acid which, to date, is found to inhibit inflammation via a few pathways, limonene inhibits inflammation through various mechanisms including the suppression of the proinflammatory cytokines (TNF-α and IL-1β) and TLR4/NF-ΚB/AP-1 (Kathem et al. 2014). The result of molecular docking also showed that limonene has the potential to be used for the treatment of Covid 19 inflammatory symptoms and Covid pulmonary fibrous. Although there is a limited literature report on structure-activity relationship (SAR) of limonene as an anti-inflammatory agent, it can be inferred from the work of Sousa and his research group on carvone mechanism of action that the presence of S configuration of the carbon atom at the ipso position to the isopropenyl group and the isopropenyl group itself are the key structural determinants of anti-inflammatory activity of limonene (Jiang et al. 2002, Meeran et al. 2020, Yan et al. 2021). It is therefore safe to suggest that the significant anti-inflammatory activity of the volatile oil of *R. australis* pulp in male mice subjects used in this work was mainly due to the presence of a high concentration of limonene in the oil.

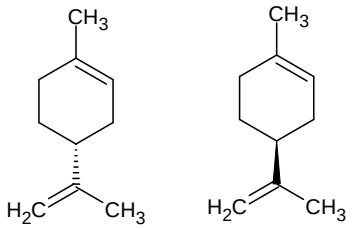

Fig. 4. Structures of the two isomeric forms of limonene

Table 3 displays the chemical constituents of the volatile oil of the seed of *R. australis*. Nineteen compounds (70.2% of the total oil composition) were identified from the GCMS analysis of the seed oil of *R. australis*, of which seven were unidentifiable and twelve compounds were identified accounting for 63.6% of the total oil composition. Fatty acid compounds accounts for the major class of compounds in the seed essential oil (38.1%), followed by oxygenated monoterpene (10.4%) and monoterpenes (8.5%). Interestingly, two aliphatic hydrocarbons (hexadecane and eicosane) totalling 4.6% were identified. The major compounds found in the seed oil were hexadecanoic acid (34.4 %), limonene (6.3 %) and nonanal (8.5 %).

3.3 Acute Toxicity Test

These results obtained in Table 4 below suggest that ethno-medicinal application of this plant's extracts may not pose serious health complications in the short-term period. Further research is important for obtaining the chronic (long term) toxicity profile of this plant using at least two different animal models (species).

Table 3. Chemical composition of the oil of the seed of *Raphia australis*

S/N	RT	% Composition	KI values	Compound name	CAS
1	4.8197	2.2	981	β-pinene	18172-67-3
2	5.5845	1.9	992	furan-2-pentyl	03777-69-3
3	6.9752	6.3	1031	Limonene	00138-86-3
4	10.6176	8.5	1098	Nonanal	00124-19-6
5	20.6891	0.6	1418	Caryophyllene	00087-44-5
6	21.1918	0.5	1480	germacrene D	23986-74-5
7	21.2721	0.5		Unknown	000000000
8	21.3095	0.5		Unknown	000000000
9	21.8604	0.9	1573	Caryophyllene oxide	01139-30-6
10	21.9032	0.7	1600	Hexadecane	00544-76-3
11	22.4969	1.2	1950	Unknown	000000000
12	22.759	1.7	1720	Tetradecanoic acid	00544-63-8
13	22.8606	0.4		Unknown	000000000
14	23.2029	2.0		Unknown	000000000
15	23.2885	1.0		Unknown	000000000
16	23.4008	1.0		Unknown	000000000
17	23.5185	2.0	1953	Palmitoleic acid	00373-49-9
18	23.6094	34.4	1972	n-Hexadecanoic acid	00544-63-8
19	23.679	3.9	2000	Eicosane	00112-95-8
		Total % =	70.2		

Table 4. Acute toxicity profile of the essential oils of the fruit pulp and seed of R. australis in mice

Treatment mg/kg, p.o.	*R. australis* pulp oil Death pattern after 24 h	*R. australis* seed oil Death pattern after 24 h
Phase 1		
10	0/3	0/3
100	0/3	0/3
1000	0/3	0/3
Phase 2		
1000	0/1	0/1
1600	0/1	0/1
2900	0/1	0/1
5000	0/1	0/1
LD_{50}	≥5000 mg/kg	≥5000 mg/kg

3.4 Anti-Inflammatory and Analgesic Test

A mixture of fatty acids and essential oil compounds in the oil of Raphia pulp (RPEO) displayed substantial anti-inflammatory effect on the lipopolysaccharide (LPS)-induced inflammation model used in this study (Fig. 5). The RPEO and aspirin (standard anti-inflammatory drug) caused insignificant decrease in the oedema size induced by the LPS in rats' paws. After 1-2 h post LPS injection, the oil (40-80 mg/kg) caused significant ($p<0.05$) decrease in oedema sizes compared to the vehicle. However, the RPEO (40-80 mg/kg) and aspirin caused significant ($p<0.01$) reduction in oedema sizes at 4 and 5 hours post LPS injection. Reduction in paw sizes after lipopolysaccharide injection is taken as positive for anti-inflammatory effect of drugs. The results obtained here showed that the RPEO demonstrated significant reduction in paw oedema induced by the LPS in a dose-dependent fashion signifying that this oil possesses anti-inflammatory activity in rats. The presence of lauric acid (2%) and limonene (38.4%) in this oil sample suggests that they play vital roles in contributing to the anti-inflammatory and analgesic activities observed in this study as have been previously reported in literature (Fu et al. 1990, Sinniah et al. 2021).

The effect of the RPEO on formalin-induced paw licking is presented in Fig. 6. The formalin-induced paw licking behaviour in rodents afford evaluations of both analgesic and acute

inflammatory activities of drugs. The oil (40 or 80 mg/kg) and the standard drug (aspirin, 100 mg/kg) caused a non-significant reduction in paw lickings induced by the formalin in the first phase signifying low activity in this model which represent the neurogenic phase. However, *R. australis* pulp oil (RPEO) (80 mg/kg) and aspirin caused significant ($p<0.05$) decrease in the number of paw licks compared to the vehicle suggesting potential acute anti-inflammatory activity in this model.

The potent anti-inflammatory activity of the volatile oil of *R. australis* justifies the use of oils rich in limonene for the management of arthritis in Indian traditional medicine (Vasudevan *et al.*, 2012).

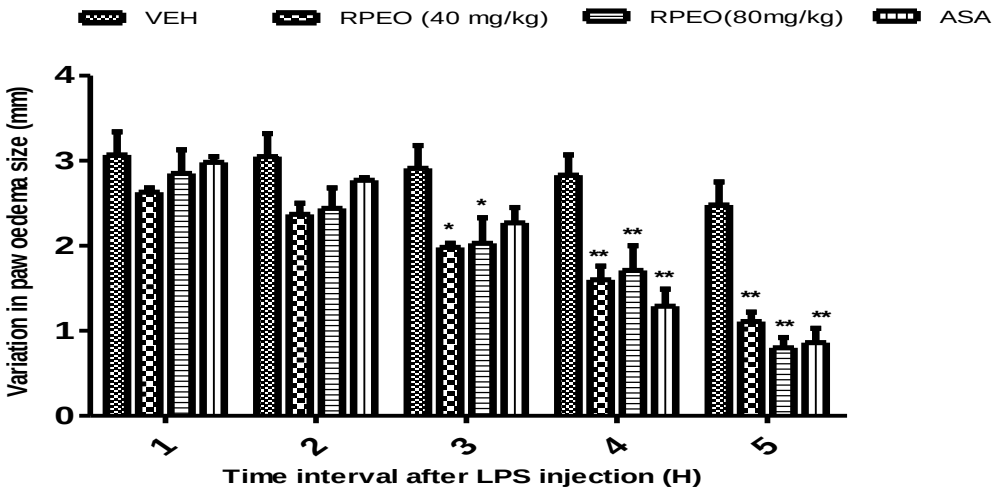

Fig. 5. Effect of *R. australis* pulp essential oil on lipopolysaccharide (LPS)-induced inflammation in rats

VEH, RPEO and ASA represent vehicle (5% Tween 80), Raphia australis essential oil and aspirin (100 mg/kg) respectively.

$^{*}P<0.05$, $^{**}P<0.01$ statistically compared to the vehicle

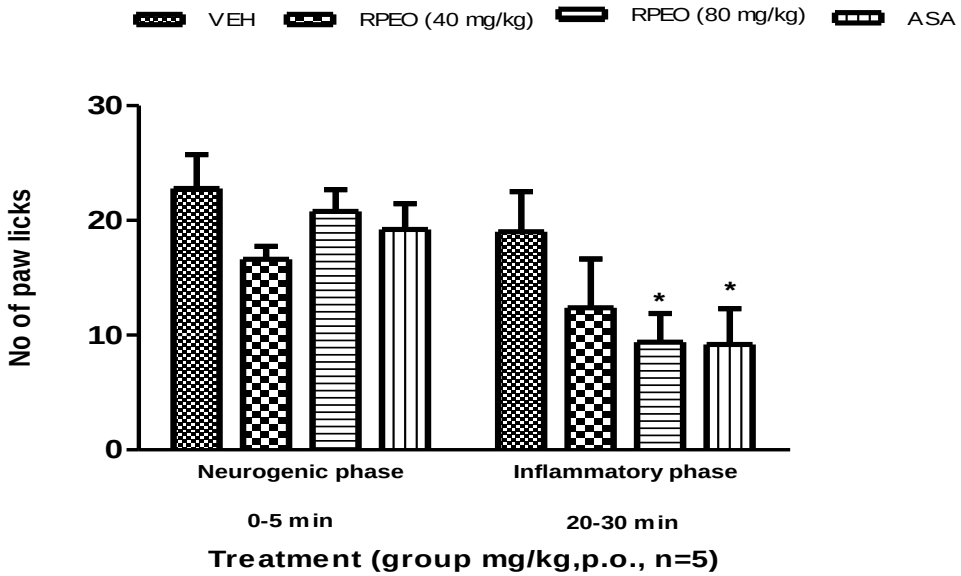

Fig. 6. Effect of *R. australis* pulp essential oil on neurogenic and inflammatory phase in formalin-induced pain model in mice

VEH, RPEO and ASA represent vehicle (normal saline), R. australis essential oil and aspirin (100 mg/kg) respectively.

**p<0.05 statistically significant compared to the vehicle (ANOVA, Dunnett's test)*

4. CONCLUSION

Even though the oils obtained from the seed and the pulp of R. australis both contain a significant quantity of hexadecanoic acid, the concentration of hexadecanoic acid in the seed is about 3 times higher, 34.4 % and 10.8 %, respectively. The oil of *R. australis* pulp (38.4 %) is about 6 times higher in limonene as compared to the seed (6.3 %). While hexadecanoic acid has been reported to be a potent CD4 fusion inhibitor against HIV-1 (Lee et al. 2009). limonene was also reported to inhibits HIV-1 replication (Battinelli et al. 2003). A future investigation which seeks to investigate the degree of synergistic inhibition exhibited by each of the two oils of *R. australis* against HIV-1 is, therefore, deemed necessary. Also, due to the high concentration of saturated fatty acids and limonene in the oil of the pulp of *R. australis*, antifungal, antibacterial and antiviral screening should be carried on the pathogenic strains of these organisms to ascertain its degree of inhibition against various infections (Librán-Pérez et al. 2019, Avrahami and Shai 2004).

Further analyses of the oils are also recommended in future work to determine the identity of the unknown compounds, the relative percentage compositions of the two isomers of limonene present in the oil of *R. australis* pulp, with a view to ascertaining their contributions to the anti-inflammatory response observed in the adult male mice subjects in this work.
The oil of the pulp of *Raphia australis* contains lauric acid, limonene and essential oil compounds which exhibited anti-inflammatory ($P< 0.01$) and analgesic activity ($P<0.05$) which were comparable to that of aspirin when tested on lipopolysaccharide-induced inflamed rat paw and acetic acid-induced paw licking. The anti-inflammatory activity of this volatile oil is suggested to be due to the presence of limonene (38.4 %) in the oil, although the presence of some of the other compounds in the oil also might have contributed to the observed anti-inflammatory activity of the oil.

The results obtained from the anti-inflammatory activity of the oil of *R. australis* pulp hereby justify the use of oils rich in limonene and lauric acid in Indian traditional medicine for the treatment of arthritis, rheumatism and other inflammatory disorders.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ETHICAL APPROVAL

This study was approved by the Department of Higher Education, WSU and Ethical Clearance Approval obtained from Walter Sisulu University Ethics Committee with Reference No. DVC (AA&R) DRD/SREC: FNS 01/02/2017.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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