ORIGINAL PAPER

Nig. J. Pharm. Res. 2017, 13 (2) pp 137-146
ISSN 0189-8434 **Available online at http://www.nigjpharmres.com**

Phytochemical, Analgesic and Anti-Inflammatory Studies of the Methanol Leaf Extract of *Commiphora Mollis* (Oliv.) Engl. (Burseraceae)

*H. S. HASSAN[1A, C-F], J. YAU[2A,C], J. ABUBAKAR[1B,C] and A. A. AHMADU[3E,F]

[1]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria-Nigeria
[2]Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria-Nigeria
[3]Department of Pharmaceutical and Medicinal Chemistry, Kaduna State University, Kaduna-Nigeria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Introduction: *Commiphora mollis* is used in African traditional medicine as analgesic, anti-inflammatory, anti-bacterial, anti-fungi, anti-oxidant as well as cytotoxic and cytostatic agent.

Objective: To carry out phytochemical, analgesic and anti-inflammatory studies on the methanol leaf extract of *Commiphora mollis*.

Methods: The powdered leaf material of *Commiphoral mollis* was extracted with methanol and partitioned using hexane, chloroform and ethylacetate. The methanol leaf extract was subjected to preliminary phytochemical screening while the fractions were studied using column chromatography for the isolation of compounds. The structure of the isolated compound was established by spectral analysis using UV, IR, 1D and 2D NMR spectroscopy. The methanol leaf extract was also subjected to acute toxicity, analgesic and anti-inflammatory studies using animal models.

Results: The preliminary phytochemical screening of the methanol leaf extract revealed the presence of terpenes, flavonoids, tannins, saponins and carbohydrates. The column chromatography of the chloroform and ethylacetate fractions led to the isolation of a curcuminiod compound. The results of intrapretoneal LD_{50} were 1385.64 mg/kg in mice and 3807.8 mg/kg in rats. The analgesic studies were carried out at doses of 75, 150 and 300 mg/kg body weight i.p. using acetic acid-induced writhing and thermally-induced pain in mice. The extract significantly $P<0.05$ inhibited the acetic acid-induced writhing in mice with highest protection (89.40%) produced at the dose (300 mg/kg) which compare well with the standard drug piroxicam (10 mg/kg). The extract also significantly ($P<0.05$) increased the reaction time in a dose dependant manner to the thermal stimulus. The extract at 150 mg/kg body weight i.p showed 2.5 ± 0.43 seconds reaction time which was greater than the standard drug pentazocine at 10mg/kg (2.17 ± 0.31 seconds). The carrageenan-induced paw oedema test was employed in investigating the anti-inflammatory activity in rats at doses of 150, 300 and 600 mg/kg body weight i.p. The extract inhibited hind paw oedema at all doses tested. The standard drug ketoprofen produces (91.30%) inhibition which is greater than that of the test groups (40, 50 and 60% respectively).

Conclusion: The results indicated that the methanol leaf extract possess significant dose dependant analgesic activity ($P < 0.05$) and significant ($P < 0.05$) anti-inflammatory activity. Thus, the traditional use of the plant for treatment of pain and inflammation is justified in this study.

Keywords: Curcuminoid, *Commiphora mollis*, Analgesic, Anti-inflammatory, Phytochemical constituents.

INTRODUCTION

Plants are valuable sources of food and medicines for the prevention and cure of diseases. They are also important for the maintenance of good health. *Commiphora mollis* belongs to the family Burseraceae, commonly called "Corkwood" and *Dashi* in Hausa. *Commiphora mollis* is employed in traditional medicine throughout savanna region of Central and West Africa as therapeutic agent for the treatment of inflammation, dry cough, typhoid fever, wound healing, cancer, ulcer and rheumatoid arthritis. *Commiphora mollis* has been reported to have antibacterial, antifungal, cytotoxic, cytostatic and antioxidant activities (Paraskeva *et al*., 2008). The resin is applied topically to aid wound healing and has been reported to have anti-bacterial, anti-fungal, anti-inflammatory, antioxidant activity as well as cytotoxic and cytostatic (David *et al*., 2010; Kumari *et al*., 2011). Terpenoids especially the sesquiterpeniods and triterpenoids are the most abundant constituents in *Commiphora* species (Hanus *et al*., 2005; Shen *et al*., 2008).

Pain is one of the most common complain for which patients seek medical consultation in various health care delivery systems worldwide. Majority of medical conditions associated with humans are usually accompanied with pains which can be mild, moderate or severe. Examples of such disease conditions include cancer, migraine, sickle cell anemia, burns and surgery, which can interfere with individual quality of life and general functioning (Linton *et al.*, 1980). Pain relievers are often used as adjuncts in the therapeutic management of several diseased conditions. Unfortunately the uses of commonly available analgesic drugs are associated with side effect such as renal, gastrointestinal (GIT) and liver disorder (Linton *et al.*, 1980). Currently available analgesic drugs such as NSAIDs are not useful in all cases due to their side effect profile. Opiod analgesics such as morphine has strong addictive potential and other side effects including respiratory depression, drowsiness, decreased gastro-intestinal motility, nausea and several alterations of endocrine and autonomic nervous system while NSAIDs are well known for their ability to produce gastrointestinal bleeding, ulceration etc (Almeida *et al*., 2001; Mate *et al*., 2008). Therefore, the search for new analgesic drugs with promising pharmacological action and reduced side effects has become an urgent necessity.

This study was aimed at carrying out the phytochemical screening and the evaluation of analgesic and anti-inflammatory properties of the methanol leaf extract of *Commiphora mollis* using animal models.

MATERIALS AND METHODS

Solvents of analytical grade obtained from Sigma-Aldrich, Germany, were used. Chromatographic materials used include pre-coated TLC plates, Silica gel (60-120 mesh size), Sephadex LH 20 (Sigma), Chromatographic column (100 x 4 cm). Thermoelectro UV machine of the Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria was used for UV-spectroscopy. Shimadzu FTIR 8400S of the National Research Institute for Chemical Technology, Zaria was used for infrared spectroscopy. Bruker Avance III NMR spectrometer (600MHz) of the School of Chemistry, University of Kwa-Zulu Natal, Durban, South Africa was used for 1D and 2D NMR spectroscopy. The melting point of the isolated compound was determined using Gallenkamp melting point apparatus at the Department of Pharmaceutical and Medicinal Chemistry, ABU, Zaria.

Collection and identification of plant material

The leaves of *Commiphora mollis* were collected from Bassawa in Zaria, it was identified and authenticated at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria by Mallam Musa. A sample of the plant was deposited with a voucher number 331 assigned. The leaves were air dried under shade and pulverized using mortar and pestle.

Extraction and partitioning

The powdered leaf (300 g) was extracted using 75% methanol by maceration for 5 days. The extract was concentrated using Rotary evaporator. Fifty gram of the methanol leaf extract was suspended in 200 mL of distilled water and filtered using a filter paper to obtain water soluble and water insoluble parts. The water insoluble part was partitioned with hexane, chloroform and ethyl acetate to give the hexane, chloroform and ethyl acetate fractions respectively.

Column chromatography of combined chloroform and ethylacetate partitioned fractions

The chloroform and ethyl acetate water insoluble fractions were merged due to similar TLC profile using hexane:ethylacetate (9:1) and 5.0 g was subjected to column chromatography. The column was packed using wet slurry method with hexane using 100g of silica gel. The sample was previously adsorbed on silica gel and loaded on the packed column. The column was eluted by gradient elution with hexane 100% followed by 95:5 hexane - ethyl acetate, 90:10 hexane - ethyl acetate, 85:15 hexane -

ethyl acetate, 80:20 hexane - ethyl acetate, 75:25 hexane - ethyl acetate, 70:30 hexane - ethyl acetate, 65:35 hexane - ethyl acetate, 60:40 hexane - ethyl acetate and finally the column was eluted with methanol. This gave a total of 76 column fractions which were pooled together based on similarity in their TLC profile to give 6 major fractions coded F1 to F6.

Gel filtration chromatography

Fraction F4 which has a TLC profile of four spots (1 major and 3 minor) was obtained from column fractions 55-61 with eluting solvent system of hexane:ethylacetate (60:40), was further subjected to gel filtration chromatography using Sephadex LH-20. This lead to 20 fractions which was pooled together to give 5 column fractions coded S1 to S5. Fraction S5 showing 2 spots was subjected to another gel filtration chromatography and it gave 5 column fractions coded A to E. Fractions D and E on evaporation gave rise to compound J2. The isolated compound was subjected to spectroscopic analysis including 1 and 2D NMR, UV and IR. The compound was also subjected to melting point determination.

Preliminary phytochemical screening of the methanol leaf extract.

The preliminary phytochemical screening of the methanol leaf extract was carried out using standard techniques for the detection of plant secondary metabolites as described by Sofowora (1982), Trease and Evans (1996) and Silva *et al*. (1998).

Animals

Swiss albino mice and rats of either sex (weighing 18-25 g) for the mice and (180-200 g) for the rats were obtained from the Animal House of Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were kept in standard animal cages at room temperature and provided with standard laboratory diet and water. The study was conducted in accordance with the rules and ethics governing the use of laboratory animals in Ahmadu Bello University, Zaria-Nigeria.

Drugs and dosage

The following chemicals and drug were used, carrageenan (Sigma-Aldrich), acetic acid (Ranbaxy laboratory Ltd, Punjab), Ketoprofen (Lek, Slovania), Pentazocine, Piroxicam, Methanol leaf extract of *Commiphora mollis* (75, 150, and 300 mg/kg) for the mice, and (150, 300 and 600 mg/kg) for the rats. All test solutions were administered intraperitonially (i.p).

Acute toxicity study (LD_{50})

The intraperitoneal LD_{50} of the extract in mice and rats was conducted according to the method reported by Lorke (1983). The study was carried out in two phase, at first phase, nine (9) mice were divided into three groups each containing 3 animals and were treated with the methanol leaf extract of the plant at doses of 10, 100 and 1000 mg/kg body weight i. p and observed for signs of toxicity and death after 24 hours. In the second phase, 4 groups each containing one mouse were injected with 4 more specific dose; 1200, 1600, 2900, and 5000 mg/kg respectively based on the result of the first phase. They were observed for signs and symptoms of toxicity and death after 24 hours. The LD_{50} was determined by calculating the geometric means of the lowest dose that caused death and the highest dose for which the animal survived. The same procedure was also used in rats.

Acetic acid-induced writhing test in mice

Thirty mice of either sex weighing between 18 to 28 g were used for this experiment. The mice were divided into 5 groups each containing 6 mice. The control group received normal saline (10 mL/kg i.p) the test groups were treated with 75, 150 and 300 mg/kg i.p of the extract, while the fifth group received pentazocine at the dose of 10 mg/kg i.p; after 30 minutes of drug administration, the mice were treated with 0.6% acetic acid at 10 mg/kg weight i.p (Koster *et al.,* 1956). Five minutes after acetic acid injection, mice were placed in individual cage and number of abdominal constriction or writhing was counted for each mouse for a period of 10 minutes after 5 minute latency and the percentage inhibition of writhing was calculated using the following formula.

% inhibition =

$$\frac{\text{mean number of writhing (control)} - \text{mean number of writhing (test)}}{\text{mean number of writhing (control)}} X100$$

Thermally-induced pain test in mice (Hot plate test)

Thirty mice of either sex weighing between 18 to 28 g were used for this experiment. The paws of the mice are very sensitive to temperature of 50℃ which are not damaging to the skin. The animals were placed on Eddy's hot plate kept at a temperature of 55 ± 0.5℃ and a cut off period of 15 sec (Franzotti *et al*., 2000) was observed to avoid damage to the paw. Reaction time was recorded when animals licked their hind paws, or jumped at, 0, 30, 60 and 90 minutes after i.p administration of the test drug (Eddy and Leimback, 1953). The animals of the test groups received extract at the dose of 75, 150 and 300

mg/kg, respectively. The positive control group was treated with piroxicam 10 mg/kg.

Carrageenan-induced paw oedema test in rats

Thirty rats of either sex weighing between 150 to 180 g were used for this experiment. The rats were divided into five groups each containing 6 rats, acute inflammation was induced by injecting 0.1 mL of 1% Carrageenan into sub plantar surface of rat hind paw (Winter *et al.*, 1962). The methanol leaf extract (150, 300 and 600 mg/kg), normal saline (10 mL/kg) and ketoprofen (10 mg/kg) as positive control were administrated 30 mins before carrageenan injection. The paw volume was measured at 0, 1, 2, 3, 4 and 5 h, using vennier caliper to determine the diameter of oedema. The difference between the readings at time 0 h and different time interval was taken as the thickness of oedema.

Statistical analysis

The results were expressed as Mean±SEM and the mean values of the control groups were compared with the mean values of the treated groups using one way ANOVA followed by posthoc Dunnets t-test for multiple comparison. The results obtained were considered statistically significant at ($P < 0.05$).

RESULTS

Compound J2 (7.5 mg) is as an orange amorphous solid with a single spot on TLC using hexane:ethylacetate (6:4) as solvent system with R_f value of 0.75. The melting point was determined to be 195 to 198° C.

The result of the U.V spectroscopy of J2 showed λ_{max} (MeOH) at 307.00, 272.00, and 264.00. The result of the IR spectroscopy of J2 shows bands at (3853.90 cm^{-1}), (3737.20 cm^{-1}), (3439.19 cm^{-1}) phenolic OH stretching, (1701.27 cm^{-1}) C=O α–β conjugated stretching, (1527.67 cm^{-1}) C=C stretching of aromatic ring. The proton NMR spectrum of J2 revealed 5 aromatic proton resonances (J values):- [7.52 d (8.4), 7.14 d (7.9), 6.85 m (8.2), 6.85 m (8.2), 7.24 s]; phenolic hydroxyl protons at 4.55 s; a methoxy proton signal 3. 93 s; 2 olefinic protons [6.64 d (16.9); 7.60 d (15.8)] and a methylene proton signal at 1.31 s. The ^{13}C-NMR spectrum of J2 revealed a total of 18 carbon signals; 14 unsaturated carbons signals (159.65, 149.1, 148.1, 140.77, 140.49, 129.75, 127.24, 126.66, 122.72, 120.8, 120.6, 115.5, 115.22, 110.44), 2 carbonyl carbons (183.5, 183.4), a methoxy (55.1) and a methylene (29.3) carbon signals. The DEPT spectrum of J2 revealed 9 methine carbon signals and a methyl carbon signal. The COSY spectrum of J2 showed correlations between the protons at δ_H 7.60 ppm and 6.64 ppm, 7.52 ppm and 6.85 ppm and also between protons at δ_H 7.14 ppm and 6.85 ppm. The HSQC spectrum of J2 showed the following H//C correlations: 7.60//140.78, 140.49; 7.52//129.76; 7.24//110.45; 7.14//122.73; 6.85//115.22, 115.53; 6.64//120.63, 120.69; 3.93//55.13 while the HMBC spectrum showed the following major H//C correlations: 7.60//183.52, 129.76, 122.73, 110.45; 7.52//159.65, 140.76, 140.49, 129.76; 7.24//149.07, 140.76, 140.49, 122.73; 7.14//149.07, 140.76, 140.49, 110.45; 6.85//159.65, 148.07, 127.24, 115.53, 115.23; 6.64//183.52, 1272.4, 126.66; 3.93//148.07 as summarized in Table 1 with comparism to the spectra data of Demethoxycurcumin (DMC) as reported by Almeida *et al*. (2005).

Table 1: NMR spectral data of J2 (CD_3OD, 600MHz) and DMC

No.	DMC	δ_H [mult., *J* (Hz)]	δ_C (mult.)	HMBC
1	100.9	1.31	29.30 (CH_2)	-
2, 2’	183.2/183.1	-	183.52, 183.46 (C)	-
3, 3’	121.1/120.8	6.64 d (16.9)	120.69, 120.63 (CH)	2’, 5’, 5
4, 4’	140.7/140.4	7.60 d (15.8)	140.49, 140.78 (CH)	2, 6’, 6
5, 5’	126.4/125.8	-	126.66, 127.24 (C)	-
6	111.2	7.52 d (8.4)	129.76 (CH)	8,10
6’	130.4	7.14 d (7.9)	122.73 (CH)	8’, 4’, 10’
7	148.0	6.85 m (8.2)	115.22 (CH)	8, 9, 9’, 5’
7’	115.7	6.85 m (8.2)	115.53 (CH)	8, 9, 9’, 5’
8, 8’	149.8/159.8	-	159.65 (C)	-
9, 9’	115.9/115.7	-	148.07 (C)	-
10	123.2	-	149.07 (C)	-
10’	123.1	7.24 s	110.4 (CH)	-
H-OCH_3	55.7	3.93	55.13 (CH_3)	9’

Result of Preliminary Phytochemical Screening
The preliminary phytochemical screening of methanol extract revealed the presence of saponins, flavonoids, tannins, terpeniods and carbohydrates as shown in Table 2.

Table 2: Result of phytochemical screening

Constituent	Test	Methanol extract
Carbohydrates	Molisch's Test	+
Saponins	Froth Test	+
Flavonoids	Shinoda Test	+
	Soduim hydroxide test	+
	Ferric chloride Test	+
Alkaloids	Dragendorff's Test	-
	Meyer's Test	-
	Wagner's Test	-
Steroids/Terpenoids	Salkowski's Test	+
Tannins	Lead sub acetate Test	+

Key: + Present
- Absent

Result of Acute Toxicity Study of Methanol Extract
The intraperitoneal mean lethal dose LD_{50} was found to be 3807.8 mg/kg in rats, and 1385.64 mg/kg in mice.

Acetic acid-induced Writhing Test in Mice
The methanol extract significantly ($P < 0.05$) attenuated the acetic acid induced writhing in mice with highest protection (89.40%) produced at the highest dose (300 mg/kg), The standard drug piroxicam afforded (86.90%) protection at 10 mg/kg

Table 3: Effect of methanol extract on acetic acid-induced writhing test in mice

Treatment	Dose mg/kg	Number of Abdominal Constriction Mean ± SEM	% Inhibition Abdominal Constriction
N/Saline	10ml/kg	55.5 ± 10.40	0
Extract	75	5.7 ± 1.80**	82.9
Extract	150	7.3 ± 1.5**	86.3
Extract	300	9.2 ± 2.10***	89.4
Piroxicam	10	7.0 ± 1.5**	86.9

The data were analysed using one way ANOVA followed by Dunnets test $P < 0.01$ and 0.05 respectively. $N = 6$.

Thermally-induced Pain Test in Mice (Hot Plate Test)

The methanol extract significantly (P<0.05) increased the reaction time in a dose dependant manner to the thermal stimulus. At 30 min, the dose of 75 and 150 mg/kg showed slight increase in reaction time. But at 60 and 90 mins, the dose of 150 mg/kg gave a significant increase in reaction time. The effect of methanol extract at 150 mg/kg body weight i.p was greater than that of standard drug pentazocine at 10 mg/kg.

Table 4: Effect methanol extract on thermally-induced pain test in mice

Treatment	Dose mg/kg	Mean pain latency (sec) Mean ± SEM		
		30 mins	60 mins	90 mins
N/Saline(ml/kg)	10	1.0 ± 0.00	1.33 ± 0.21	1.33 ± 0.21
Extract	75	1.5 ± 0.34	1.5 ± 0.22	1.33 ± 0.21
Extract	150	1.5 ± 0.34	2.2 ± 0.31*	2.50 ± 0.43*
Extract	300	1.5 ± 0.22	2.0 ± 0.26	2.0 ± 0.26
Pentazocine	10	1.33 ± 0.79	1.83 ± 0.17	2.17 ± 0.31*

Data were analyzed using one way ANOVA followed by Dunnets test. * represents the level of significant at P < 0.05 N = 6, f = 10.

Carrageenan-induced Paw Oedema in Rats

The methanol extract significantly inhibited hind paw oedema at all doses tested. The extract showed significant increase in percentage inhibition at the time interval of 4 and 5 h at the dose of 600 mg/kg. The extract appeared to be more active when compared to the standard at the time intervals of 4 and 5 h but at 2 and 3h the standard drug was more active than the extract. The standard drug ketoprofen produced 91.30% inhibition which is greater than that of test groups (40.0%, 50.0% and 60.0%, respectively).

Table 5: Effect of methanol extract on carrageenan-induced inflammation in rats

Treatment Dose mg/kg	Mean for diameter (cm) Mean ± SEM				
	1hr	2hr	3hr	4hr	5hr
N/Saline 10 (ml/kg)	0.19 ± 0.01	0.25 ± 0.03	0.23 ± 0.02	0.20 ± 0.3	0.20 ± 0.02
Extract 150	0.16 ± 0.12 (15.8%)	0.19 ± 0.02 (24.0%)	0.16 ± 0.20* (30.4%)	0.13 ± 0.02* (35.0%)	0.12 ± 0.02* (40.0%)
Extract 300	0.11 ± 0.01** (42.1%)	0.16 ± 0.3* (36.0%)	0.14 ± 0.2* (39.1%)	0.12 ± 0.2** (40.0%)	0.10± 0.02* (50.0%)
Extract 600	0.10± 0.01 ** (47.4%)	0.15 ± 0.01* (40.0%)	0.14 ± 0.02* (39.10%)	0.09 ± 0.02** (55.00%)	0.08 ± 0.01** (60.00%)
Ketoprofen 10	0.08 ± 0.02*** (57.90%)	0. 07± 0.03*** (72.00%)	0.09 ± 0.03*** (91.30%)	0.10 ± 0.02** (50.00%)	0.09 ± 0.03** (55.00%)

Data were analyzed using one way ANOVA followed by Dunnets test. Values in parenthesis present percentage inhibition of inflammation *P < 0.05,**P < 0.01,***P < 0.001, n = 6.

DISCUSSION

The column chromatographic separation of the combined chloroform and ethyl-acetate fractions of water insoluble methanol extract followed by purification with gel filtration using sephadex LH - 20 led to the isolation of compound J2. The presence of three λ_{max} at 264 nm, 272 nm and 307 nm in the UV spectrum is an indication of conjugation in the compound. The broad band absorption at 3853 cm^{-1}, 3737 cm^{-1}, 3439 $cm^{-1}$and 2934 cm^{-1} observed in the IR spectrum are characteristics of OH stretching. 1701 cm^{-1} is characteristic of C=O, α - β conjugated stretching and absorption at 1527.67 mc^{-1} C=C is due to stretching of aromatic rings (David, 2005).

The proton NMR spectrum of compound J2 revealed the presence of signals for two pairs of ortho coupled protons at 6.85ppm (2H, m, J = 8.2Hz, H-7'and 7), 7.52ppm (1H, d, J = 8.4Hz, H-6) and 7.14 (1H, d, J = 7.9Hz, H-6'), a singlet signal was also observed at 7.24 (1H, s, H-10'). These signals were assigned to two benzene rings in similar environment, one of which is tri-substituted while the other is di-substituted. The proton NMR spectrum of J2 also

revealed two signals at 7.60 (2H, d, J = 15.8Hz, H-4', 4) and 6.64 (2H, t, J = 16.9Hz, H- 3', 3). By taking into account the large J values, these signals were assigned to two olefinic systems (each consisting of 2 carbon atoms). These proton NMR assignments were further supported by the COSY correlations between the protons of H-6'//H-7', H-6//H-7 and H-4, 4'//H-3, 3'. The J values of the olefinic protons suggest trans-coupling. The proton NMR of J2 further revealed signal for methoxy protons at 3.93 ppm (3H, -OCH_3) and methylene at 1.31 ppm (2H, s, H-1).

The carbon-13 NMR spectrum of J2 revealed a total of 18 carbon signals. They were assigned to two ketonic carbons at δc 183.46 and 183.52; 14 unsaturated carbons at δc 110.45, 115.23, 115.53, 120.63, 120.90, 122.73, 126.66, 127.24, 129.76, 140.49, 140.78, 148.07, 149.07 and 159.65; a methoxy carbon at 55.13 and a methylene carbon at δc 29.30ppm (Etexebarria *et al*., 2004).

Taking into account the proton NMR of J2, 4 of the 14 unsaturated carbon signals were assigned to two olefinic systems. The remaining signals were assigned to two benzene rings. The connectivity of these various fragments was established by the 2D NMR data from the HSQC and HMBC spectra. The two olefinic systems were connected to two carbonyl carbons based on ^2J and ^3J correlation of H-3', H-4'/C-2' and H-3, H-4/C-2 respectively. These established two 3- carbon atoms systems which were connected to two aromatic rings based on HMBC correlation of H-4'/C-6', 10'; H-4/C-6 on the one hand and H-3'/C-5'; H-3/C-5 on the other hand. The methoxy carbon was attached to C-9' based on HMBC correlation of H-1''/C-9. Two cinnamaldehyde-like fragments are thus established which were joined together by the methylene carbon. Although no HMBC correlation was observed between the methylene protons and the carbonyl carbon atoms in the structure, the most probable accommodation for the methylene group observed in the NMR spectrum of J2 is to connect the two cinnmaldehyde-like fragments (Macomber, 1998; Payton *et al*., 2007). These analyses of the NMR data of J2 therefore resulted in a curcuminoid structure as shown below.

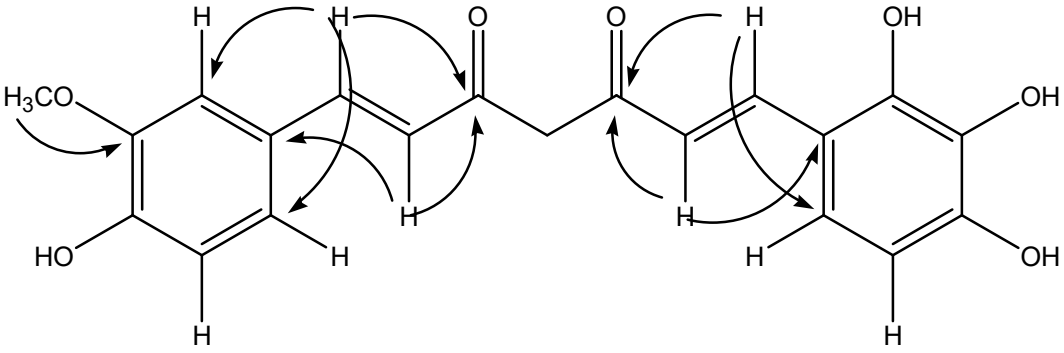
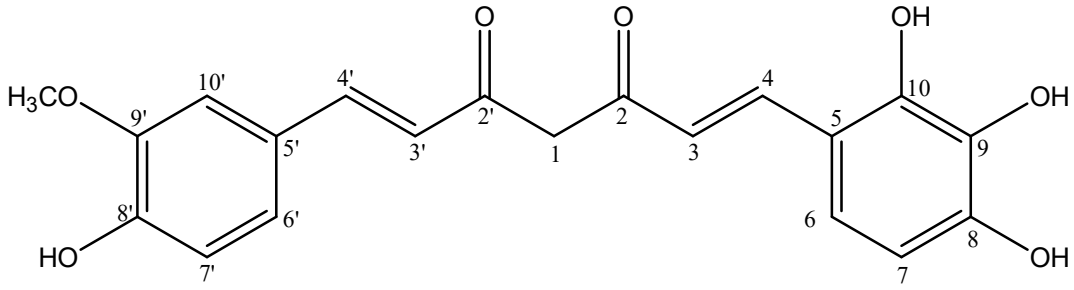


Figure 1: Major HMBC correlations of J2



1-(4-Hydroxy-3-methoxy-phenyl)-7-(2,3,4-trihydroxy-phenyl)-hepta-1,6-diene-3,5-dione

Compound J2 is a derivative of curcumin which is a naturally occurring phenolic compound present in turmeric (*Curcuma longa* L. family Zingiberaceae) and has been shown to possesses various biological properties such as anti-inflammatory, antioxidant properties, antimicrobial, cytotoxic and antirheumatic activities (Abas *et al*., 2006). Curcumin has also been tested for its chemopreventive properties in skin, fore stomach and colon chemically induced carcinogenesis (Kawamori *et al*., 1999).

The result of preliminary phytochemical screening of the methanol leaves extracts revealed the presences of flavonoids, saponins, terpenes, tannins and carbohydrates. The intraperitoneal mean lethal dose LD_{50} was found to be 3807.8 mg/kg in rats and 1385.64 mg/kg in mice. This shows that the methanol leaf extract is moderately not toxic when administered intraperitonally. The acetic acid induced writhing test also called abdominal constriction response is a sensitive procedure use in evaluating peripherally acting analgesic (Gene *et al.*, 1998). The test is very sensitive and able to detect anti – nociceptive effects of compounds at dose levels that may appear inactive in other methods like tail flick test (Collier *et al*., 1968; Bentley *et al*., 1981). Increased level of prostanoids, particularly PGE2 and PGF2a (Derardt *et al*., 1980) as well as lipoxygenase products (Levini *et al*., 1984; Dhara *et al*., 2000) have been found in the peritoneal fluid after intraperitoneal injection of acetic acid. The analgesic effect of the extract may therefore be due either to its action on visceral receptors sensitive to acetic acid, to inhibition of the product of algogenic substances or the inhibition at the central level of the transmission of painful messages. In general, acetic acid causes pain by liberating endogenous substances such as serotonin, histamine and prostaglandins (PGs) which stimulate nerve ending. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response (Derardt *et al.,* 1980).

The paws of the mice are very sensitive to temperature which are not damaging to the skin. The hot plate test described by Eddy and Leimbach (1953) evaluates the reaction time of the mice when dropped on heated surface or with a heated stimulus applied on the planter surface of their paws, the response commonly observed are withdrawal and licking of the paws, as well as jumping; many investigators have found it to be suitable for evaluation of centrally acting analgesics (Vogel and Vogel, 1997). Carrageenan-induced paw oedema is a commonly used primary test for the screening of new anti-inflammatory agents and is believed to be biphasic the first phase (1 hr) is due to the release of histamine or serotonin, while the second phase is attributed to the release of prostaglandin and lysosomes in 2-3 hrs (Saha *et al.,* 2007). The extract moderately inhibited carrageenan-induce inflammation in the third hr. The mechanism of action may be due to inhibition of histamine or prostaglandin synthesis. Usually most anti-inflammatory and analgesic drug produced antipyretic action through the inhibition of prostaglandin (Hayare *et al.,* 2000).

CONCLUSION

Column chromatography of the combined chloroform and ethylacetate fractions followed by gel filtration chromatography led to the isolation and characterization of a curcuminiod compound after structural elucidation using spectroscopic data from 1 and 2 D NMR, UV and IR. The analgesic and anti-inflammatory effect of the extract may be due to the presence of flavonoids, tannins, terpenes or saponins contained in the extract. The traditional use of *Commiphora mollis* for management of pain and inflammation has been confirmed by this study.

REFERENCES

Abas, F.; Hui, L.S.; Ahmad, S.; Stanslas, J.; Israf, D.A,; Shaari, K. and Lajis, N.H. (2006). Biological evaluation of curcumin and related diarylheptanoids. Z. Naturforsch. 61c. 625-631

Almeida, R.N., Navarro, D.S. and Filho, J.M.B. (2001). Plants with central analgesic activity Phytomedicine, 88: 310 -322.

Bentley, G.A.; Newton, S.H. and Starr, J. (1981). Evidence of an action of morphine and enkephalins on sensory nerve endings in the mouse peritoneum. Brit. J. Pharmacol. 73: 325-332.

Collier, H.O.J.; Dinnean, L.C.; Johnson, C.A. and Scheider, C. (1968). The abdominal constriction response and its suppression by analgesic drugs in the mouse. Brit. J. Pharmacol. 32: 295-310.

David, G.W. (2005). Infrared Spectrophotometry. In: *Pharmaceutical analysis*, Second edition. Elsevier Ltd. Pp 121 – 125.

David, I., Miri, H., Nechama G.G., and Zohar, A. (2010). Medicinal properties of *Commiphora gileandensis.* African J. Pharm. Pharmacol. 4(8): 516 – 520.

Derardt, R., Jongney, S., Delbalcee, F. and Falhout, M. (1980). Release of protanglandins E and F in an algogenic reactions and its inhibition. Eur. J. Pharmacol. 51: 17-24.

Dhara, A.K., Suba, V., Sen, T., Pal, S. and Chaudhuri, A.K. (2000). Preliminary studies on inflammatory and analgesic activity of methanolic fraction of the root of *Tragia involucrate*. J. Ethnopharmacol. 74: 265-268.

Eddy, N.B. and Leimbach, D. (1953). Synthetic Analgesics II. Dithienylbutenyl and dithienylbutylamines. J. Pharmacol. Exp. Ther. 107:385 – 393.

Edeoga, H.O., Okwu, D.E., Mbaebie, B.O. (2005). Phytochemical constituents of Nigerian medicinal plants. African J. Biotech. 4: 685 – 688.

El-Ashry, E.S., Rashed, N., Salama, O.M., Saleh, A. (2003). Components, therapeutic values and uses of myrrh. Pharmazie, 8: 163–168.

Etexebarria, J., Vicario, J.L., Badia, D, Carrillo, L. (2004). Asymmetric synthesis of beta-amino esters by aza-Michael reaction of alpha, beta-unsaturated amides using (S,S)-(+)-pseudoephedrine as chiral auxillary. The Journal of Organic Chemistry, 69: 2588-2590.

Franzotti, E.M., Santos, C.V.F., Rodrigues, H.M.S.L., Mourao, R.H.V., Andrade, M.R., and Antoniolli, A.R. (2000). Anti – inflammatory, analgesic activity and acute toxicity of *Sida cardiofolia* L. (Malva – branca). J. Ethnopharmacol. (1-2) 72: 273 – 277.

Gene, R.M., Segura, L., Adzet, T., Marin, E., Inglesias, J. (1998). *Heterutheca inuloides* anti-inflammatory and analgesic effect. J. Ethnopharmacol. 60: 157-162.

Hanus, L.O., Rezanka, T., Demvitsky, V.M. and Moussaieff (2005). *Myrrha commiphora.* Journal of Chemistry-Biomedical Papers, 149: 3-28.

Hayare, S.W., Chandra, S., Tandan, S.K., Sama, J., Lal J., Telang, A.G. (2000). Analgesic and antipyretic activities of *Dalbergia sissoo leaves.* Indian J. Pharmacol. 32: 357-360.

Kawamori, T.; Lubet, R.; Steele, V.E.; Kelloff, G.J.; Kaskey, R.B.; Roa, C.V. and Reddy, B.S. (1999). Chemopreventive effect of curcumin, a naturally occurring anti-inflammatory agent, during promotion and progression stages of colon cancer. Cancer Research. 59: 597-601

Koster, R., Anderson, M., De beer, E.J. (1959.) Acetic acid for analgesic screening. Federation Proceedings, 18:412.

Koyama, K., Imaizumi, T., Akiba, M., Kinoshita, K., Takahashi, K., Suzuki, A., Yano, S., Horie, S., Watanabe, K. and Naoi, Y. (1997). Antinociceptive components of *Ganoderma lucidium.* Planta Medica, 63: 224-227.

Kumari R.A., Meyyappan A., Nandi D., Agrawalla B.K., Chowdhury A.A., Selvamani P., Latha S., Giri V.S., Mukherjee J., Bandyopadhyay S., Jaisankar P. (2011). Antioxidant and antibacterial activities of bark extracts from *Commiphora berryi* and *Commiphora caudate*. Natural Products Research, 25: 1454–146

Levini, J.D.; Lau, w.; kwait, G. And Goetzl, E.J. (1984). Leukotriene B_4 produces hyperalgesia that is dependent on the polymorphonuclear leukocytes. Science, 225: 743-745.

Linton A.L., Clark W.F. and Driedger A.A. (1980). Acute interstitial nephritis due to drugs, Annual International Medicine, 93: 735-741.

Lorke, D. (1983). A new approach to acute toxicity testing. Archieves of Toxicology, 54:275 – 287.

Macomber, R.S. (1998). *A Complete Introduction of Modern NMR spectroscopy*. Wiley, New York. Pp 216

Mate, G.S., Naikwade, N.S., Chowki, C.S.A.A. and Patil, S.B. (2008). Evaluation of anti – nociceptive activity of *Cissus quardrangularis* on albino mice. International Journal of Green Pharmacy, 2: 118 – 121.

Naik, D.G., Mujumdar, A.M., Wagole, R.J., Kulkarni, D.K. and Kumbhojkar, M.S. (2000). Pharmacological studies of *Sterculia foelida* leaves. Pharmacological Biology, 1(38): 13-17.

Paraskeva, M.P., Vuuren, S.F., Zyl, R.L., Davids, H. and Viljoen, A.M. (2008). The *in vitro* biological activity of selected South African *Commiphora* species. J. Ethnopharmacol. 119: 673–679.

Payton, F., Sandusky, P. and Alworth W.l. (2007). NMR study of the solution structure of curcumin. Journal of Natural Products. 70: 143-146

Saha, A., Mas'ud, M.A., Bachar, S.C., Kundu, J.R., Datta, B.K. and Nahar, L. (2007). The analgesic and anti-inflammatory activities of the extract of *Phyllantus reticulantus.* Pharmaceutical Biology, 45: 335-359.

Shen, T., Wan, W.-Z., Wang, X.-N., Sun, L.-M., Yuan, H.-Q., Wang, X.-L., Ji, M. and Lou, H.-X. (2008). Sesquiterpenoids from the resinous exudates of *Commiphora opobalsamum (Burseraceae)*. Helvetica Chimica Acta, 91: 881–8874.

Silver, G.L., Lee, I. and Duglass, A.K. (1998). Special problem with extraction of plant In: Cannel J. P. R. (eds). *Natural Product Isolation*. Human Publishers, New Jersey, USA, pp. 251 – 293.

Sofowora, A. (1982). *Medicinal Plants and Traditional Medicine in Africa*, 1st edition, John Wiley and Sons, Chichester, pp: 142 – 143.

Trease, K. and Evans, W. C. (1996). *Text Book of Pharmacognosy*, 14th edition, Balliere, Tindall, London, pp 251 – 293.

Vogel, H.G. and Vogel, W.H. (1997). Drug Discovery Evaluation: *Pharmacological Assays* Springer Verlaag, Berling, Heidelberg, pp 204 – 212 and 382 – 388.

Winter, C. A., Riselay, E. A. and Nuss, G.W. (1962). Carrageenan – induced oedema in the hind paw of the rats as an easy for anti – inflammation drugs. Proceeding of the Society for Experimental Biology and Medicine, 111: 544 – 547.

Address for correspondence: H. S. Hassan
Department of Pharmaceutical and Medicinal Chemistry,
Ahmadu Bello University, Zaria-Nigeria
Telephone: +234-8054526909

E-mails: hlmtsdyhassan@gmail.com,

Conflict of Interest: None declared

Received: 7 June, 2017

Accepted: October 10, 2017