

## Phytochemical Studies of *Ficus Binnendijkii* Leaf Extracts: Fractionation and Bioactivities of Its Petroleum Ether Extract

Hanaa Mohamed El-Rafie<sup>1\*</sup>, Amany Ameen Sleem<sup>2</sup>

<sup>1</sup>Pharmacognosy Department, <sup>2</sup>Pharmacology Department, National Research Centre, 33 El-Bohouth St. Former El-Tahrir St., P.O. 12622 [ID: 60014618], Dokki, Giza, Egypt.

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### ABSTRACT

*Ficus binnendijkii* is one of the therapeutically active plants belonging to the family Moraceae. This work was carried out to elucidate the phyto-constituents contained in different solvent-based [petroleum ether (PtE), chloroform (ChE) & ethyl acetate (EaE)] extracts from the leaves of *Ficus binnendijkii*. These extracts showed positive results for the presence of carbohydrates, steroids, flavonoids, tannins, proteins and alkaloids. The total ash, acid insoluble ash, water soluble ash, protein percentage and total carbohydrate content of the powdered leaves were (10.18%), (8.2%), (2.5%), (8.2 %) and (20.4%) respectively. Further identification of the chemical composition of PtE fractions (unsaponifiable mater and fatty acid methyl esters) were done using the GC-MS analysis which revealed the identification of forty six compounds in the unsaponifiable fraction constituting 82.54% of the total peak area, the major compounds were  $\beta$ -amyryn (23.52%), 23S-ethylcholest-5-en-3- $\beta$ -ol (12.68%), phytol (7.76%) and moretenol (6.60%), whereas thirteen compound representing 78.28% of the total identified peak area of the fatty acid methyl esters fraction the major compounds were methyl hexadecanoate (31.24%), methyl-9,12-octadecadienoate (15.52%) and methyl tetradecanoate (7.62%). This study aimed also to evaluate the analgesic, antipyretic and anti-inflammatory activities of PtE using acetic acid-induced writhing test, Brewer's yeast induced pyrexia and carrageen hind paw oedema models in rats, respectively. The administration of mice with 50 and 100 mg/kg body weight of PtE reduced pain, fever and inflammation in a dose dependent manner.

**Keywords:** *Ficus binnendijkii*, petroleum ether extract, analgesic, antipyretic, anti-inflammatory.

### INTRODUCTION

In the last decade, there has been revived interest in alternative therapies and the therapeutic utilization of natural products and a growing interest has been directed to search for phytochemicals of native and naturalized plants for pharmaceutical and nutritional purposes<sup>1,2</sup>. Pain is a sensorial modality within which numerous cases represent the sole symptom for the diagnosis of many diseases. It frequently has a protective function all through history and man has used various therapies for the pain management. Medicinal plants are highlighted because of their wide use and fewer side effects. Inflammation additionally has become the focal point of global scientific research owing to its implication in virtually all human and animal ailments. As a consequence of unfavorable impacts like gastric lesions brought about by nonsteroidal anti-inflammatory drugs [NSAIDs], tolerance, and reliance impelled by opiates, the employment of these drugs as anti-inflammatory and analgesic agents has not been effective in all cases<sup>3</sup>. Thusly, new anti-inflammatory and analgesic drugs diminishing these side effects are being scrutinized as substitutes to NSAIDs and opiates. Attention is being directed to the investigation of the efficiency of plant-based drugs utilized in the traditional medicine as they are low-cost and have few side effects<sup>4,6</sup>. *Ficus binnendijkii*, known as *Ficus* Amstel Queen, is a species in the genus

*Ficus* which contains about 850 species of woody trees, shrubs, vines, epiphytes and hemiepiphytes of the family Moraceae. The latter, referred to as fig trees, constitutes an imperative group of trees with massive medicinal value. It is a smallish tree with firm, narrow leaves, not simply recognized as a fig tree unless you recognized its leaf-buds and milky latex. This is a patented, man-made hybrid, *Ficus binnendijkii* 'Alii' synonym is *Ficus longifolia*. This plant is employed for many purposes, embracing Topiary art and indoor environments. It also thought of as top 30 plants to detox our home. This plant is reproduced through the vegetative method<sup>7</sup>. A variety of *Ficus* species are indigenous to Egypt, like *Ficus pseudosycomorus* Decene, *Ficus salicifolia* Vahl, and *Ficus sycomorus* Linn. Other species are latterly introduced, such as *Ficus benjamina* Linn, *Ficus glomerata* Roxb and *Ficus binnendijkii*<sup>8,9</sup>. *Ficus* plants were recorded to be used in folk medicine as antidiabetic and hypotensive, also used as a mild laxative, antirheumatic, galactagogue, digestive and as anthelmintic against intestinal parasites<sup>10-12</sup>. The chemical review on genus *Ficus*, discloses the presence of various chemical classes of bioactive compounds like sterols, coumarins and/or furanocoumarin, chromone, triterpenes, glycosides, isoflavones and lignans<sup>13-18</sup>. The scarcity of scientific data to support the claims created in ancient literature with *binnendijkii* species incited the goals of this work, to assess

Table 1: Phytochemical constituents detected in successive extracts of the *Ficus binnendijkii* leaves

Phytochemical constituents	PtE	ChE	EaE
Carbohydrates	-	-	+
Fats/Oils	+	+	-
Saponins	-	-	-
Terpenoids	+	+	-
Steroids	+	+	-
Flavenoids	-	-	+
Phenolics/Tannins	-	-	+
Glycosids	-	+	-
Proteins/Amino acids	-	-	+
Alkaloids	-	+	-
Anthraquinones	-	-	-

+ = Present; - = Absent; PtE = Petroleum ether extract; ChE = Chloroform extract; EaE = Ethyl acetate extract

the phytochemical compounds, pharmacopoeial constants and percentage of the extractive yield of the *Ficus binnendijkii* leaves successive extracts. Further GC/MS analysis of petroleum ether fractions (unsaponifiable matter and fatty acid methyl esters) and evaluating its analgesic, antipyretic and anti-inflammatory activities. This work reported on the phytochemical screening and biological activities of the extracts from the leaves of *Ficus binnendijkii* cultivated in Egypt. Further GC/MS analysis of petroleum ether extract (PtE) and appraising its anti-inflammatory, analgesic and antipyretic activities were also reported.

## MATERIAL AND METHODS

### Plant material

*Ficus binnendijkii* leaves, collected from the Giza Zoo Garden, Cairo, Egypt, in April 2015, and identified by Mrs. Tereez Labib, director of the Orman Botanical Garden and consultant of Plant Taxonomy at the Ministry of Agriculture, were thoroughly washed with tap water for about 20 minutes until the foreign material and soil particles were totally removed and after that dried in air under the shade at room temperature. The dried plant leaves were finely powdered using an electric grinder and kept in a bag of cotton fabric for extraction.

### Extraction

Extraction of the powdered leaves was carried out as follows: 100g of the plant leaves was consecutively extracted with solvents of increasing polarity in the order: petroleum ether, chloroform and ethyl acetate respectively until complete extraction in a Soxhlet apparatus. Each

extract was independently evaporated in a rotary evaporator, weighed and subjected to phytochemical screening for the identification of the various phytoconstituents. The % extractive yield was calculated by formula as listed underneath:

$$\% \text{ Extractive yield (w/w)} = \frac{\text{weight of dried extract}}{\text{weight of dried leaves}} \times 100$$

### Phytochemical screening

The successive extracts of *Ficus binnendijkii* leaves, petroleum ether, chloroform and ethyl acetate were separately subjected to qualitative chemical analyses to detect the presence of various phytoconstituents<sup>19-21</sup>.

### GC-MS Analysis

Quantitative determination for the phytoconstituents of the leaves petroleum ether extract (PtE) was further analyzed by GC/MS capillary column of fused silica (5% phenyl methyl polysiloxane), 30m length, 0.25mm I.D. and 0.25  $\mu$ m thickness, DB-5, carrier gas helium at 13 psi; oven temperature 50-280°C, chart speed 0.5 cm/min; ion source temperature 220°C; ionization voltage 70eV; accelerated voltage 2000 v; volume injected 1  $\mu$ l. The results are listed in Figures 1&2 and Tables 4&5. The identification of the compounds was accomplished by comparing their retention times and mass spectral data with those of the library (Wiley Int. USA), NIST (Nat. Inst. St. Technol., USA) and / or published data<sup>22</sup>.

### Experimental animal

In this study, Albino mice of 25-30g body weight and adult male albino rats of Sprague Dawley strain of 130-150g b. weight were obtained from the animal house, National Research Center, Egypt. Handling of animals was complied with the ethical guidelines of Medical Ethical Committee of National Research Centre in Egypt (Approval no: 12035). The animals were kept under the same hygienic conditions and on a standard laboratory diet consisting of a mineral mixture (4%), vitamin mixture (1%), corn oil (10%), sucrose (20%), casein 95% pure (10.5%), starch (54.3%) and cellulose (0.2%).

### Drugs

Indomethacin and Carrageenan were kindly supplied from Sigma Company.

### Toxicological studies

Determination of the LD<sub>50</sub> of the petroleum ether extract was carried out according to Karber procedure<sup>23</sup>.

### Analgesic test

The peripheral analgesic activity of PtE was evaluated in male albino mice (20-25g) using the modified acetic acid-induced writhing test<sup>24</sup>. In the writhing test, male mice (n=6) were orally administered PtE (50,100mg/kg), before 1h of intraperitoneal injection of acetic acid (0.6%, 0.2ml/mice), each mouse was then placed in an indri clear plastic observation chamber and total number of writhing reflexes/30min was counted for each mouse.

### Antipyretic test

Table 2: Pharmacopoeial constants of the leaves of *Ficus binnendijkii*

Sample	Total ash	Acid insoluble ash	Water soluble ash	Moisture content	% of protein content	% of total carbohydrates
% Yield	10.18	8.2	2.5	7.82	8.2	20.4

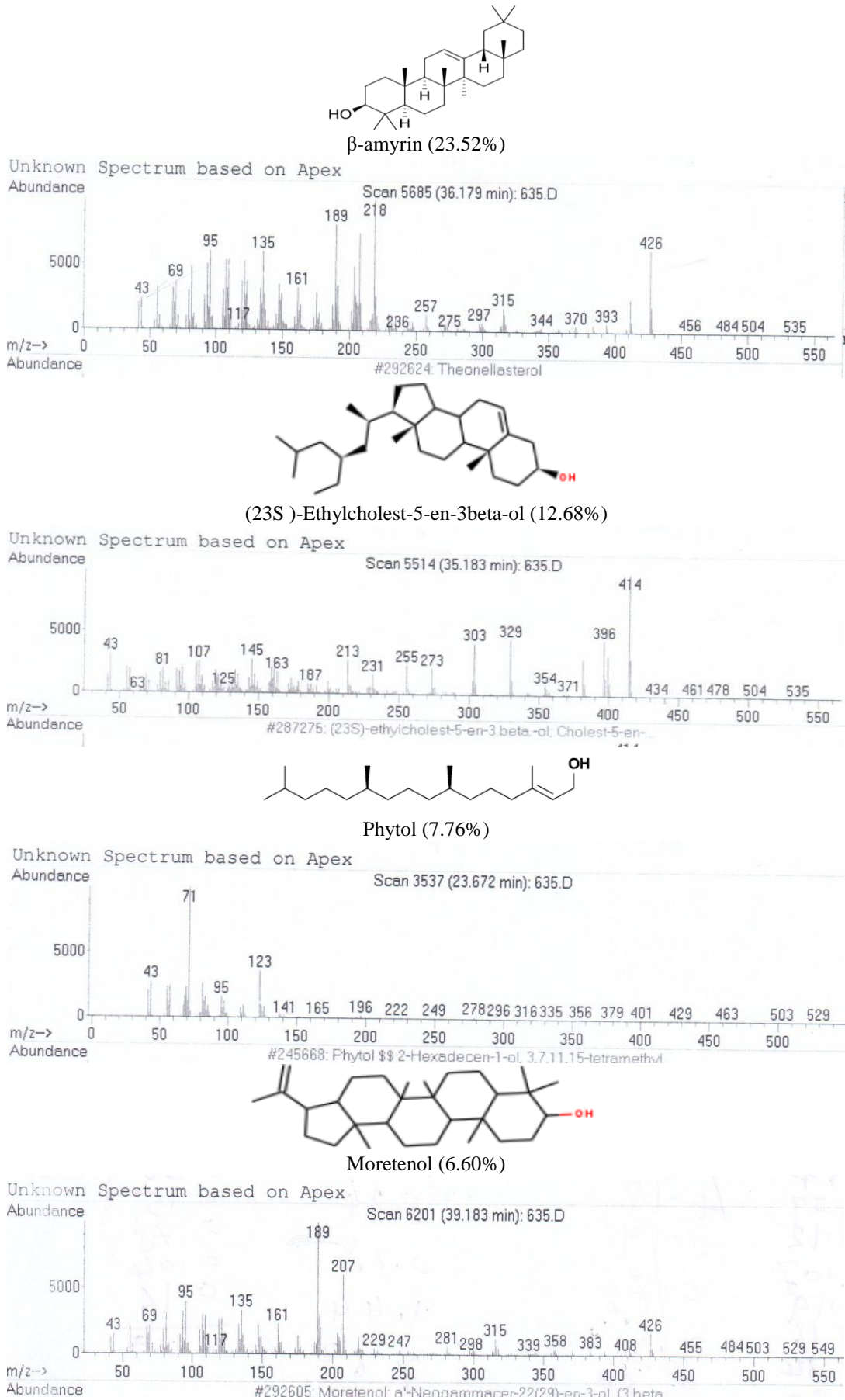


Figure 1: Chemical structure of major identified compounds in the unsaponifiable fraction

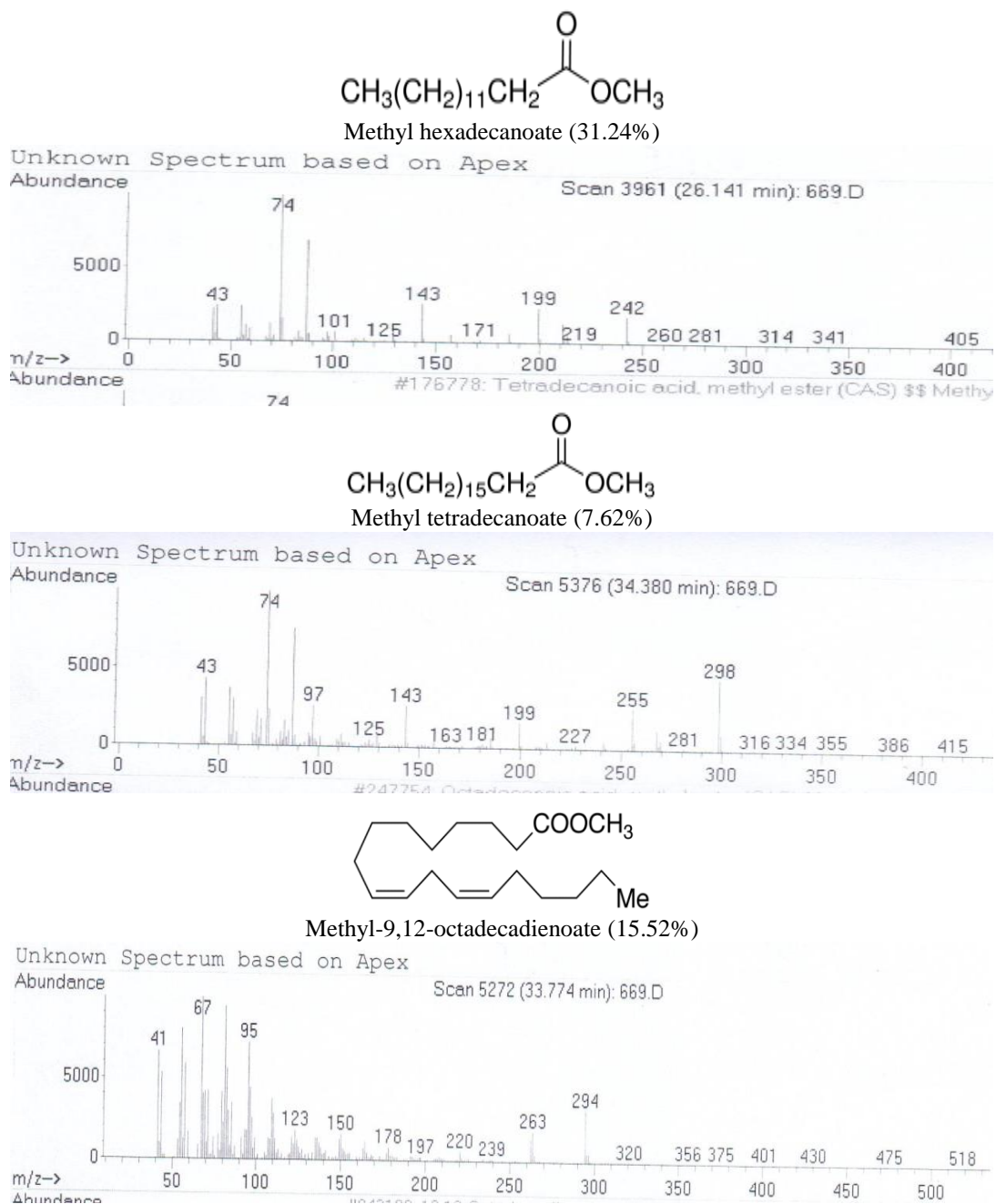


Figure 2: Chemical structure of major identified compounds in the fatty acid methyl ester fraction

The antipyretic activity of PtE was evaluated using male albino rats of 100g body weight by a modified method<sup>25</sup>. According to this method, selected animals were healthy and normal rectal temperature of each rat was checked by using a digital thermometer. Pyrexia was induced in all rats by intramuscular injection of 1ml/100g body weight injection of 44% yeast suspension. The site of injection was then massaged to spread the suspension beneath the skin. After 18h of the rectal temperature of each rat was recorded for all groups [group I received saline (1 ml/kg), group II received PtE (100 mg/kg), while group three received 20mg/kg of paracetamol as a standard drug] to serve as the baseline of elevated body, to which the

Table 3: percentage of the extractive yield of the *Ficus binnendijkii* leaves

Solvent	Petroleum ether	Chloroform	Ethyl acetate
Extractive yield	9.32	3.11	5.42

antipyretic effect will be compared. One and two hours later, other records of vaginal temperature were determined.

*Anti-inflammatory test*

The anti-inflammatory activity of PtE was evaluated with carrageenan-induced rat paw edema model [Winter *et al.*,

Table 4: GC/MS analysis of the unsaponifiable fraction of the petroleum ether extract of *Ficus binnendijkii* leave

S. NO	Name of compound	Molecular formula	Molecular weight	Retention time/min	Base peak	Area %
1	Butylated hydroxytoluene	C <sub>15</sub> H <sub>24</sub> O	220.35	15.89	205	1.56
2	5-Phenyldecane	C <sub>16</sub> H <sub>26</sub>	218.37	16.21	91	0.11
3	Hexadecane	C <sub>16</sub> H <sub>34</sub>	226.44	17.07	57	0.14
4	2-Phenyldecane	C <sub>16</sub> H <sub>26</sub>	218.37	17.16	105	0.22
5	5-Phenylundecane	C <sub>17</sub> H <sub>28</sub>	232.40	17.63	91	0.47
6	4-Phenylundecane	C <sub>17</sub> H <sub>28</sub>	232.40	17.78	91	0.38
7	3-Phenylundecane	C <sub>17</sub> H <sub>28</sub>	232.40	18.07	91	0.38
8	2-Phenylundecane	C <sub>17</sub> H <sub>28</sub>	232.40	18.59	105	0.47
9	Octadecene	C <sub>18</sub> H <sub>36</sub>	252.47	18.90	55	0.66
10	5-Phenyldodecane	C <sub>18</sub> H <sub>30</sub>	246.43	19	91	0.21
11	4-Phenyldodecane	C <sub>18</sub> H <sub>30</sub>	246.43	19.16	91	0.14
12	3-Phenyldodecane	C <sub>18</sub> H <sub>30</sub>	246.43	19.45	91	0.28
13	octadecane	C <sub>18</sub> H <sub>38</sub>	254.49	19.81	43	0.29
14	2-Phenyldodecane	C <sub>18</sub> H <sub>30</sub>	246.43	19.96	105	0.37
15	6-Phenyltridecane	C <sub>19</sub> H <sub>32</sub>	260.45	20.22	91	0.41
16	5-Phenyltridecane	C <sub>19</sub> H <sub>32</sub>	260.45	20.31	91	0.21
17	6,10,14-Trimethyl-2-pentadecanone	C <sub>18</sub> H <sub>36</sub> O	268.47	20.44	43	5.58
18	Z-5-Nonadecene	C <sub>19</sub> H <sub>38</sub>	266.50	20.90	83	0.15
19	Nonadecane	C <sub>19</sub> H <sub>40</sub>	268.52	21.07	57	0.12
20	Eicosene	C <sub>20</sub> H <sub>40</sub>	280.53	21.14	55	0.78
21	Eicosane	C <sub>20</sub> H <sub>42</sub>	282.54	21.31	57	1.07
22	Isophytol	C <sub>20</sub> H <sub>40</sub> O	296.53	21.70	71	0.12
23	Docosane	C <sub>22</sub> H <sub>46</sub>	310.60	22.28	57	0.19
24	Tricosene	C <sub>23</sub> H <sub>46</sub>	322.61	23.32	83	0.04
25	Tricosane	C <sub>23</sub> H <sub>48</sub>	324.62	23.45	57	0.16
26	Phytol	C <sub>20</sub> H <sub>40</sub> O	296.53	23.67	71	7.76
27	Tetracosane	C <sub>24</sub> H <sub>50</sub>	338.65	24.55	57	0.34
28	4,8,12,16-Tetramethylhepta-decan-4-olide	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	324.54	26.29	99	4.60
29	Pentacosene	C <sub>25</sub> H <sub>50</sub>	350.66	27.47	55	0.16
30	Pentacosane	C <sub>25</sub> H <sub>52</sub>	352.68	27.62	57	0.20
31	Hexacosane	C <sub>26</sub> H <sub>54</sub>	366.70	28.57	57	0.15
32	Heptacosane	C <sub>27</sub> H <sub>56</sub>	380.73	29.48	57	0.35
33	Octacosane	C <sub>28</sub> H <sub>58</sub>	394.76	30.36	57	0.43
34	Squalene	C <sub>30</sub> H <sub>50</sub>	410.71	30.69	69	0.14
35	Nonacosane	C <sub>29</sub> H <sub>60</sub>	408.78	31.23	57	3.99
35	5,6alpha-epoxy-5alpha-Cholestan-3alpha-ol	C <sub>27</sub> H <sub>46</sub> O <sub>2</sub>	402.65	31.78	402	0.69
36	Triacontane	C <sub>30</sub> H <sub>62</sub>	422.81	32.03	57	0.31
37	β-Tocopherol	C <sub>28</sub> H <sub>48</sub> O <sub>2</sub>	416.67	32.53	416	0.40
38	Hentriacontane	C <sub>31</sub> H <sub>64</sub>	436.83	32.86	57	4.39
39	Vitamin E	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430.70	33.30	430	0.86
40	Dotriacontane	C <sub>32</sub> H <sub>66</sub>	450.86	33.66	57	0.23
41	Campesterol	C <sub>28</sub> H <sub>48</sub> O	400.68	34.24	400	1.18
42	Tritriacontane	C <sub>33</sub> H <sub>68</sub>	464.89	34.59	57	1.94
43	[23S]-Ethyl Cholest-5-en-3beta-ol	C <sub>29</sub> H <sub>50</sub> O	414.70	35.18	414	12.68
44	β-amyrin	C <sub>30</sub> H <sub>50</sub> O	426.71	35.60	218	23.52
45	24-Methylenecycloartanol	C <sub>31</sub> H <sub>52</sub> O	440.74	36.73	207	1.10
46	Moretenol	C <sub>30</sub> H <sub>50</sub> O	426.72	39.18	189	6.60
47	Total identified compounds	82.5%				
48	Unidentified compounds	17.46%				

1962]<sup>26</sup>. Male albino rats were used and acute inflammation was produced by sub-plantar injection of 0.1ml of freshly prepared 1% (w/v) carrageenan in normal saline into the right hind paws of rats. Paw volume was measured plethysmometrically using a paw edema calimeter (YLS-7A Shandong Academy of Medical Science device station, Shandong) at 0, 0.5, 1, 2, 3 and, 4h after carrageenin injection. Animals were orally

premedicated with PtE or indomethacin (20mg/kg) before 0.5h of injection. The mean increase in paw volume was measured and inhibitory percentage was calculated. The edema rate of rats was calculated as follows:

$$\text{Edema rate (\%)} = \frac{V_t - V_o}{V_o} \times 100$$

Table 5: GC/MS analysis of the fatty acid methyl esters fraction of the petroleum ether extract of *Ficus binnendijkii* leaves

S No.	Name of compound	Molecular formula	Molecular weight	Area %	Base peak	Retention time/min
1	Butyl-4-oxo-Pentanoate	C <sub>9</sub> H <sub>16</sub> O <sub>3</sub>	172	8.24	75	3.89
2	Methyl decanoate	C <sub>11</sub> H <sub>22</sub> O <sub>2</sub>	186	0.38	74	16.30
3	Methyl dodecanoate	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>	214	1.44	74	21.46
4	Methyl tetradecanoate	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242	7.62	74	26.14
5	Methyl pentadecanoate	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	0.60	74	28.41
6	Methyl hexadecanoate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	31.24	74	30.45
7	Methyl heptadecanoate	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	3.54	74	32.48
8	Methyl-9,12-Octadecadienoate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	15.52	67	33.77
9	Methyl octadecanoate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298	5.48	74	34.38
10	Methyl eicosanoate	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>	326	3.58	74	38
11	Methyl docosanoate methyl	C <sub>23</sub> H <sub>46</sub> O <sub>2</sub>	354	1.40	74	41.30
12	Methyl tetracosanoate	C <sub>25</sub> H <sub>50</sub> O <sub>2</sub>	382	1.76	74	44.38
13	Methyl hexacosanoate	C <sub>27</sub> H <sub>54</sub> O <sub>2</sub>	410	1.76	410	47.26
14	Total identified compounds	78.28%				
15	Unidentified compounds	21.72%				

Table 6: Table 6. Effect of PtE of *Ficus binnendijkii* leaves extract on writhing reflex of mice in the writhing test. When mice were intraperitoneally injected with 0.6% acetic acid (0.2ml/mice), the writhing times were counted immediately for 30min. Data are presented as mean±SD, n=6. P<0.01, significant versus control.

Group	Dose (mg/kg)	Number of abd. constrictions	% Protection
Control	1ml/saline	56.9±1.2	--
PtE	50 mg/kg	37.3±0.9*	34.45
	100 mg/kg	22.8±0.6*	60
Indomethacin	20 mg/kg	17.6±0.5*	69

Where  $V_0$  is the volume before carrageenan injection (ml);  $V_t$  is the volume after carrageenan injection (ml) at different time intervals.

#### Statistical analysis

The data were analyzed using SPSS 13.0 statistical package. Data or multiple comparisons were operated by one-way ANOVA followed by LSD T-test. A value of  $P < 0.05$  was considered statistically significant and all results are presented as mean±SD

## RESULTS AND DISCUSSION

### Phytoconstituents

To explore the significance of any medicinal plant, the initial step is to screen for its phytochemicals, as it gives a broad idea with respect to the nature of compounds existent in the plant. In this study, the various leaf extracts (PtE, ChE and EaE) were preliminarily screened for their phytochemicals. The extracts exhibited positive results for the presence of carbohydrates, steroids, flavonoids, tannins, proteins and alkaloids (Table 1) as proved by previous results for different *Ficus* species<sup>30-32</sup>. The proximate analysis afforded satisfactory results in regard to total ash, acid insoluble ash, water soluble ash, moisture content, percentage of both protein content and total carbohydrates as depicted in Table 2. In Table 3, the extractive value was found to be maximum with PtE (9.32%) as compared with those of ChE (3.11) and EaE (5.42%). Therefore, the PtE extract was chosen for further

study. The highest percentage yield and phytochemically rich extract was PtE therefore its fractions (unsaponifiable mater and fatty acid methyl esters) were subjected to GC/MS analysis which revealed the identification of forty six compounds in the unsaponifiable fraction representing 82.54% of the total peak area, 23.66% of these compounds were terpenes ( $\beta$ -amyryn & squalene), 16.5% were terpenoidal alcohol (isophytol, phytol & moretenol), 14.71% were saturated hydrocarbons (nonacosane, eicosane, hentriacontane and tritriacontane), 1.79% were unsaturated hydrocarbons (octadecene, tricosene, Z-5-nonadecene & pentacosene) and 3.66% were phenyl hydrocarbons (5-phenylundecane, 2-phenylundecane, 2-phenylundecane & 6-phenyltridecane) and other compounds as depicted in Figure 1 and Table 4. In the fatty acid methyl ester fraction, thirteen compounds representing 78.28% of the total peak area were identified the major compounds were methyl hexadecanoate (31.24%), methyl-9,12-octadecadienoate (15.52%) and methyl tetradecanoate (7.62%) as illustrated in Figure 2 and Table 5. These identified compounds were previously reported to have potent analgesic, antipyretic and anti-inflammatory activities<sup>33-35</sup>.

### Analgesic activity

*Ficus* species, including *F. Regligiosa*, *F. glomerata*, *F. bengalensis* and *F. glomerata* exhibited *in vivo* an analgesic activity<sup>36-38</sup>. In this study, the administration of acetic acid to control mice produced 56.9±1.2 writhes

Table 7: Antipyretic activity of PtE of *Ficus binnendijki* leaves and indomethacin drug in male albino rats (n=6)

Group	Dose (mg/kg)	Induced rise in temperature mean ± S.E.	Body temperature change		% of change
			After 1hr	After 2 hrs	
			Mean±S.E.	Mean±S.E.	
Control	1m/saline	38.6±0.3	39.1±0.4	39.2±0.3	1.6
PtE	50mg/kg	38.9±0.2	38.3±0.2*	37.8±0.2*	2.8
	100mg/kg	39.1±0.4	38.1±0.3*	37.2±0.1*	4.9
Indomethacin	20mg/kg	39.2±0.2	37.6±0.08*	36.7±0.04*	6.4

\* P < 0.01 corresponding induced rise in temp. % of change is calculated as regard the temperature before treatment

Table 8: Effect of PtE extract of *Ficus binnendijkii* leaves on carrageenan-induced rat paw oedema (n=6)

Time (hour)	Zero			1h			2h			3h			4h		
	Group	Paw diameter (mm)	I	II	III	I	II	III	I	II	III	I	II	III	
Control	3.41 ±0.09	4.57 ±0.1*	1.16	34.01	4.68 ±0.1	1.27	37.24	4.77±0.1*	1.36	39.88	4.83±0.1*	1.42	41.16		
PtE 50mg	3.51 ± 0.2	4.45 ±0.1*	0.94	26.78	4.27 ±0.1	0.76	21.65	4.22±0.1*	0.71	20.22	4.18±0.02*	0.67	19.08		
PtE 100mg	3.54 ±0.1	4.42 ±0.04*	0.88	24.85	4.23 ±0.03	0.69	19.49	4.07±0.01	0.53	14.97	3.98±0.06*	0.44	12.42		
Indometacin	3.46 ±0.07	4.19 ±0.08*	0.73	21.09	3.94 ±0.06	0.48	13.87	3.85±0.04	0.39	11.27	3.77±0.01*	0.31	8.95		

PtE= petroleum ether extract, I= Paw diameter (mm), II= Oedema thickness (mm), III= % of change in oedema thickness. The results are expressed as mean±SE. The statistical comparison of the difference between the control group and the treated groups was carried out using two-way ANOVA followed by Dunnett's multiple comparison tests  
\*Significantly different from zero time at p<0.05

within 30-minute inspection period. Pretreatment with the PtE at 50 and 100 mg/kg b.wt. lessened the number of writhes up to 37.3±0.9 (34.45% inhibition) and 22.8±0.6 (60% inhibition), respectively. The standard drug indomethacin lessened the number of writhes to 17.6±0.5 (69% inhibition) at a dose of 20 mg/kg body weight. This implies that PtE considerably inhibited the number of writhing responses in a dose dependent manner within 30min of acetic acid injection. The writhing number of the mice given the high dose of PtE (100mg/kg) was even equivalent to that of the mice received indomethacin as shown in Table 6. The abdominal constriction response is thought to include in part local peritoneal receptors<sup>39</sup>, thus PtE extract of *Ficus binnendijkii* leaves may have interfered with these peritoneal receptors to produce analgesic effect. Acetic acid-induced writhing test has been associated with an increment in the levels of prostaglandins E<sub>2</sub> and F<sub>2α</sub> in peritoneal fluid<sup>40</sup> and lipooxygenases<sup>41</sup>, as well the mechanism of PtE may be related to cyclooxygenases and/or lipooxygenases.

#### Antipyretic activity

Extracts of different organs from *Ficus* species plant displayed prominent antipyretic activity as a result of the impacts of bioactive components in the extracts<sup>27-29</sup>. In this work the PtE greatly ( $p < 0.01$ ) inhibits hyperthermia in yeast induced fevered rats. The inhibition was dose-dependent and remained noteworthy up to 2h of administration. PtE at 100 mg/kg dose imparted the most extreme antipyretic impact and return body temperature to normal levels (37.2±0.1) nearly to standard drug indomethacin 20 mg/kg (36.7±0.04) (Table 7). Inhibition

of prostaglandin synthesis by blocking the cyclooxygenase enzyme activity could be the conceivable mechanism of antipyretic action as that of indomethacin.

#### Anti-inflammatory activity

Carrageenan induced paw edema is a suitable experimental animal model for screening of antiedematous effect of natural product. Our results (Table 8) showed that administration of PtE in a dose of 50 and 100mg/Kg body weight inhibited the edema starting from the first hour by 26.78, 24.85% of change, respectively, and during all periods of experiment till fifth hour by 19.08 and 12.42% of change, respectively, which might be due to the presence of various active constituents in the PtE of *Ficus binnendijkii* leaves. This means that PtE has anti-inflammatory potential in a dose dependent manner as previously mentioned by PtE of other *Ficus* species<sup>42-44</sup>. This impact may probably due to inhibition of the release of serotonin and histamine thereby preventing both inflammation as well as the increased synthesis of prostaglandins in the surroundings of the damaged tissue<sup>45,46</sup>.

#### Conflict of interest statement

We declare that we have no conflict of interest

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