



## Research article

## Fatty acids, triterpenes and cycloalkanes in ficus seed oils

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## ABSTRACT

The compositions of the seed oils obtained by hexane extraction of three members of the *Ficus* genus of the Moraceae plant family was determined, namely *Ficus nota*, *Ficus septica*, *Ficus ulmifolia*. Linolenic acid is the most prominent fatty acid in the seed oils followed by linoleic acid, with these two fatty acid comprising about 75% of the fatty acids in the oils. The high level of polyunsaturated fatty acids coincides with high levels of these acids predominating among the fatty acids observed in various plant parts of other *Ficus* species. Besides the fatty acids, a variety of phytochemicals also found in other plant parts of *Ficus* species were observed in the seed oils, including squalene, pentacyclic triterpenes such as  $\alpha$ -amyrin,  $\beta$ -amyrin and lupeol, as well as sterols such as cholesterol and  $\gamma$ -sitosterol, the former at unusually elevated levels. The levels of these phytochemicals vary from species to species and location of harvest, with *F. ulmifolia* showing by far the highest level of these materials and with  $\alpha$ -amyrin,  $\beta$ -amyrin and lupeol being the most common, their amounts exceeding those of fatty acids for samples from one specific location. Surprisingly, low levels of macrocyclic alkanes in the range of C<sub>24</sub>-C<sub>30</sub> were identified.

## 1. Introduction

The most important information regarding seed or other triacylglycerol-based oils of plants is their fatty acid composition as this feature largely determines properties and possible applications. While the fatty compositions of many plant oils have been determined, little to no information appears to be available on the composition of seed oils of the *Ficus* genus (fig trees) of the Moraceae plant family. Moreover, it appears that little to no information is available on the fatty acid composition of plant oils of the Moraceae family. Accordingly, there also appear to be virtually no studies on the properties or possible utilization of these oils.

The ca. 800 *Ficus* species, the largest genus in the Moraceae family, occur globally throughout the tropics with different subgenera occurring in different parts of the world (Berg, 1989; Harrison, 2005). The Asian-Australasian region exhibits the richest and most diverse *Ficus* flora (Berg, 1989) which is found in lowland tropical rainforests (Harrison, 2005) providing nourishment (figs) to a large number of vertebrates (Harrison, 2005). Almost all *Ficus* species exude latex as a defensive mechanism (Lasky et al., 2014). *Ficus nota* (subgenus *Sycomorus*) occurs in Malaysia Sabah, Sarawak and The Philippines (USNPGS, 2017). *Ficus septica* (subgenus *Sycomorus*) is found naturally mainly on the islands of Eastern Asia (Indonesia, Japan, Malaysia,

Papua New Guinea, Philippines, Solomon Islands, Taiwan) and neighboring Pacific areas (Australia, Vanuatu) (USNPGS, 2009a). *Ficus ulmifolia* (subgenus *Sycidium*) occurs naturally in The Philippines (USNPGS, 2009b) and is a vulnerable species on the Red List (IUCN). Some *Ficus* species are native to or have been introduced in parts of the United States and the Caribbean area (NRCS; DiTomaso and Kyser, 2013).

While the seed oils of *Ficus* (and other Moraceae) species have not been studied, compositional analyses of various plant parts of *F. awkeotsang* Makino revealed that the neutral lipids of achene oil bodies were highly unsaturated (62.65%  $\alpha$ -linolenic acid, 18.24% linoleic acid, and 10.62% oleic acid) (Chua et al., 2008). On the other hand, various phytochemicals, including sterols and pentacyclic triterpenes, have been observed in different plant parts of *Ficus* species. These include 3b-acetyl urs-14:15-en-16-one and lanosterol-11-one acetate isolated together with five other triterpenes (3b-acetyl-22,23,24,25,26,27-hexanordamaraman-20-one, 24-methylenecycloartenol, sorghumol (isoarborinol), 11a, 12a-oxidotaraxeryl acetate, and urs-9(11):12-dien-3b-ol acetate) in the bark of *F. fistulosa* (Tuyen et al., 1998), the dichloromethane extract of air-dried leaves of *F. pseudopalma* and *F. ulmifolia* exhibiting squalene, polyprenol, amyirin acetates and others (Ragasa et al., 2009), dichloromethane extracts of air-dried twigs of *F. ampelas* containing ursolic and oleanolic acids (Ragasa et al.,

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2014), and  $\alpha$ -amyrin, oleanolic acid, ursolic acid (Santiago et al., 2014) as well as lupeol (Santiago and Mayor, 2014) in the ethanolic leaf extract of *F. pseudopalma*. These terpene constituents of *Ficus* are of interest in various biomedical applications (Vázquez et al., 2012; Tsai et al., 2016). While such triterpenes are generally considered rare, some authors have suggested that they are more common in plants than conventionally assumed, having been overlooked by usual analytical methods (Shan et al., 2015). The natural occurrence of compounds with the  $\beta$ -amyrin frame has been reviewed and they have been shown to be widely distributed (Boar and Allen, 1973). Other rare compounds have been detected in *Ficus* species, for example, diarylbutanoids in *F. nota* (Latayada et al., 2017). The analysis of these and other phytochemicals in vegetable oils using chromatographic methods has been discussed in the literature (Abidi, 2001; Xu et al., 2018).

In the present work, the seed oils of three species of the *Ficus* genus, *F. nota*, *F. septica* and *F. ulmifolia*, as obtained by hexane extraction were investigated for their composition. To the best of our knowledge and as mentioned above, no prior information of any kind on these oils is available. No information is available either on the seeds containing these oils as well as oil content and other information. We note that besides fatty acids, especially in *Ficus ulmifolia* oil, numerous phytochemicals are present which have been reported in previous literature in other *Ficus* plant parts and that, as a surprising result, minor amounts of cycloalkanes were observed. To the best of our knowledge, therefore this is also the first report on a seed oil containing significant amounts of various triterpenes and cycloalkanes.

## 2. Experimental

*Ficus nota*, *Ficus septica* and *Ficus ulmifolia* seeds were harvested at the Laguna Campus of De La Salle University (DLSU) in Biñan, Laguna, the Philippines (14.26° N, 121.04° E) and this location will be referred to as L1 in the further text. Additionally, two batches of *Ficus ulmifolia* seed were collected at Kalayaan, Laguna, the Philippines (14.32° N, 121.48° E) and this location will be referred to as L2 in the further text. The L1 location is a man-made forest which was originally a grassland area and sugarcane plantation and was only revegetated with tree species in the last 15 years while the L2 collection site is a secondary growth forest with relatively diverse vegetation. L1 samples were collected at the onset of dry season (April 2017) while the L2 samples were obtained during the rainy season (January 2017 and August 2017; these differences will be indicated as L2a and L2b, respectively) thus the amount of available water for plant uptake is expectedly higher at the L2 site. Voucher specimens of all the *Ficus* species collected were deposited at the DLSU Herbarium located at Science and Technology Research Center in DLSU-Manila. *Ficus* fruits are usually small and green when they emerge from the branches but usually turn from red to dark violet after two weeks which indicates maturity. If not harvested, the fruits detach and will fall to the ground. *Ficus* species analyzed in this study were handpicked from hanging branches. Collected fruits were first air-dried for five (5) days and then seeds were manually extracted using a stainless steel knife and adhering seeds on the pericarp were scraped off using stainless steel scissors.

The seeds were Soxhlet-extracted with hexane for 24 h. Oil content as determined by weighing the seeds before and after Soxhlet extraction was 4.7% for the *Ficus nota* seeds, 9.5% for the *F. septica* seeds, and 14% for the *F. ulmifolia* seeds. The acid values are relatively high, for example, the acid value of one *F. ulmifolia* seed oil sample was determined as 13.5%.

The fatty acid compositions of all samples were analyzed as fatty acid methyl esters and picolinyl (nicotinyl; 3-pyridylcarbonyl) esters with the latter prepared as described in the literature (Christie, 2018). Gas chromatography (GC) with a flame ionization detector (FID) for quantitation purposes as well as gas-chromatography-mass spectrometry (GC-MS) for unambiguous identification and verification were employed. Picolinyl esters were analyzed only by GC-MS. Most

components and their retention times were verified against authentic samples of fatty acid methyl esters obtained from Nu-Chek Prep (Elysian, MN), and plant sterols from Matreya (State College, PA), pentacyclic triterpenes purchased from Sigma-Aldrich (St. Louis, MO) with the exception of lupeol from ChromaDex, Inc. (Irvine, CA).

The instrumentation used was for GC-FID a Perkin-Elmer (Norwalk, CT) Clarus 580 gas chromatograph and for GC-MS an Agilent Technologies (Santa Clara, CA, USA) 6890N GC with an Agilent Technologies 5973N mass selective detector employing electron ionization (70 eV). Both gas chromatographs were equipped with a DB-88 ((88% cyanopropyl) methylarylpolydimethylsiloxane) column (30 m  $\times$  0.25 mm ID  $\times$  0.20  $\mu$ m film thickness). The temperature program began at an initial temperature of 100 °C for 15 min, increased to 210 °C at 2 °C/min, then 50 °C/min to 220 °C held for 50 min. The carrier gas was H<sub>2</sub> at 7.1 mL/min. The injector, source (GC-MS) and detector (GC-FID) temperatures were 240 °C, 230 °C, and 280 °C, respectively. Besides verification with authentic samples, peak assignments of the fatty acid components were confirmed by GC-MS evaluation of the picolinyl esters (Christie, 2018) using the same GC-MS instrumentation and conditions.

## 3. Results and discussion

Table 1 gives compositional data on the *Ficus nota* and *Ficus septica* oils with fatty acids being the major constituents. Table 2 provides data for the *Ficus ulmifolia* oil samples studied, for which, with one exception, the various phytochemicals exceed the fatty acids. No comparison data for other *Ficus* seed oils were available as to the best of our knowledge the composition of these seed oils has not yet been studied. This observation can be expanded to the seed oils of the members of the Moraceae family in general. Fig. 1 shows GC-MS traces of the three seed oils investigated here together with assignments of some salient peaks. For the purposes of further discussion, the classes of compounds identified in the seed oils of the *Ficus* species studied here will be divided into three groups, fatty acids, phytochemicals and cycloalkanes.

**Fatty acids.** Fatty acids were identified in the range of C<sub>14</sub>-C<sub>24</sub>. Saturated fatty acids with C<sub>14</sub>, C<sub>17</sub>, and C<sub>24</sub> were present only in minor to trace amounts, however. The most prominent fatty acids in the seed oils of the *Ficus* species investigated here are linoleic and linolenic acids

**Table 1**

Fatty acid profiles of *Ficus nota* and *Ficus septica*, from Location L1 (see experimental section) seed oils as determined in the present work. Minor amounts of phytochemicals found in *F. nota* and *F. septica* not individually quantified.

Fatty Acid/Other	<i>Ficus nota</i>	<i>Ficus septica</i>
16:0	8.4	4.7
18:0	6	5.0
18:1 $\Delta$ 9	11.3	5.1
18:1 $\Delta$ 11	0.6	0.4
18:2	28.4	34.8
18:3	25.0	48.1
20:0	0.3	0.3
22:0	1.1	0.2
24:0	0.5	0.1
Squalene	1.0	0.2
Sitosterol	3.2	1.1
Cycloalkanes <sup>b)</sup>	5.0	< 1

a) Systematic (trivial names) of compounds in this table: 16:0 = methyl hexadecanoate (methyl palmitate); 16:1 = methyl 9(Z)-hexadecenoate (methyl palmitoleate); 18:0 = methyl octadecanoate (methyl stearate); 18:1  $\Delta$ 9 = methyl 9(Z)-octadecenoate (methyl oleate); 18:1  $\Delta$ 11 methyl 9(Z)-octadecenoate (methyl asclepate); 18:2 = methyl 9(Z),12(Z)-octadecadienoate (methyl linoleate); 18:3 = methyl 9(Z),12(Z),15(Z)-octadecadienoate (methyl linolenate); 20:0 = methyl eicosanoate (methyl arachidate); 22:0 = methyl docosanoate (methyl behenate), 24:0 methyl tetracosanoate (methyl lignocerate).

b) Combined for even-numbered cycloalkanes C<sub>24</sub> – C<sub>30</sub>.

**Table 2**Composition of seed oil of *Ficus ulmifolia* samples studied in the present work.<sup>a)</sup>

Fatty acid/Other	From location L1	From location L2a <sup>b)</sup>	From location L2b <sup>b)</sup>
16:0	6.5	3.4	4.2
18:0	3.8	1.7	2.1
18:1 Δ9	5.3	3.2	3.2
18:1 Δ11	0.4	0.3	0.2
18:2	27.7	9.6	8.2
18:3	52.7	17.5	13.8
20:0	0.2	0.1	0.4
22:0	0.3	2	2.1
24:0	tr <sup>d)</sup>	tr	tr
Squalene	tr	0.4	0.6
Cycloalkanes <sup>c)</sup>	tr	1.5	2.0
Cholesterol	tr	0.9	1.2
Sitosterol	0.5	3.1	3.1
Olean-12-en-3-α-yl acetate;	–	2.7	2.3
Urs-12-en-3-α-yl acetate	–	6.6	6.8
α-Amyrin	tr	15.0	18.8
β-Amyrin	tr	14.7	10.9
Lupeol	tr	8.0	8.5

a) Pentacyclic triterpenes and sterols not necessarily listed in order of elution. See Fig. 1.

b) For differences between L2a and L2b, see experimental section.

c) Value for even-numbered C<sub>24</sub>-C<sub>30</sub> cycloalkanes combined.

d) tr = traces.

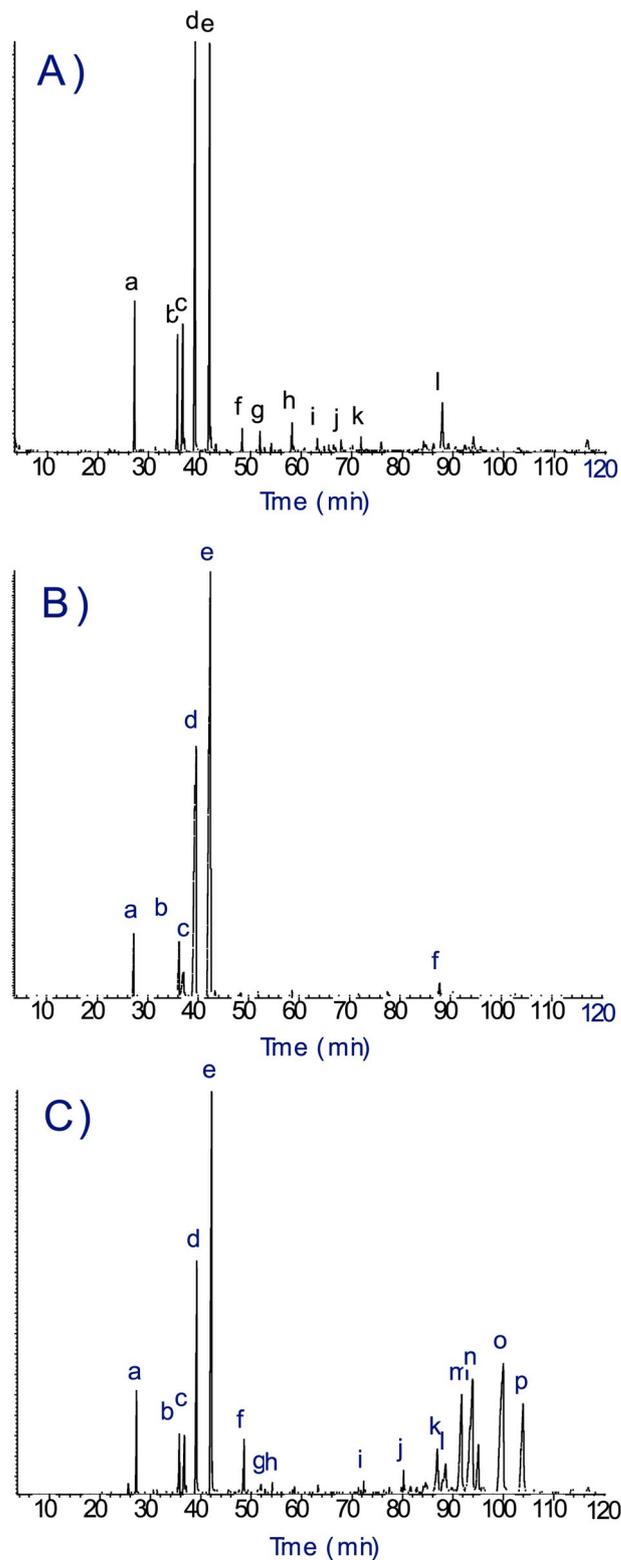
(Tables 1 and 2). Together these two polyunsaturated fatty acids comprise 75% or more of the fatty acids in *Ficus* seed oils. These results coincide with previous reports stating that polyunsaturated fatty acids are the most common fatty acids found in other parts of *Ficus* plants as discussed above (Chua et al., 2008). No fatty acids with less common structural features such as cyclic moieties or unusual double bond locations were identified in any of the oils.

**Phytochemicals.** The *F. nota*, *F. septica* and *F. ulmifolia* seed oils from the L1 location exhibited only minor to trace amounts of the pentacyclic triterpenes and sterols with sitosterol being the most prominent of these trace compounds. Cholesterol was also observed with the amounts apparently greater than commonly observed in plant oils (Behrman and Gopalan, 2005). On the other hand, the *F. ulmifolia* seed oils from the L2 location were rich in these phytochemicals which exceeded the amount of fatty acids. Cycloalkanes, which in some respect may also be regarded as phytochemicals, were also identified in the oils and are briefly discussed separately below. In the L2 *F. ulmifolia* seed oils, the pentacyclic triterpenes α-amyrin, β-amyrin and lupeol comprised a major part of the seed oil composition. Besides these compounds, olean-12-en-3-α-yl acetate and urs-12-en-3-α-yl acetate were also identified in L2 *F. ulmifolia* seed oils, albeit in lesser amounts and, to the best of our knowledge, have not been reported in seed oils previously. Among sterols, cholesterol was present, slightly more elevated among the L2 *F. ulmifolia* samples. Squalene, which is known to be the precursor of phytochemicals, was also observed in minor to trace amounts in all samples. Tables 1 and 2 and Fig. 1 serve to illustrate these differences. Fig. 2 shows the structures of the major phytochemicals identified.

The difference may be a result of varying soil conditions (organic matter, soil pH, moisture, etc.) but would need to be confirmed by detailed soil and other analyses. Differences in the composition of plant oils depending on soil conditions as well as are well-known and have been observed in various crops (Alam et al., 1996; Anastasi et al., 2010; Chaiyadee et al., 2013; Klein et al., 2017; Li et al., 2017). Overall, the microflora and microfauna present in the soil are expected to be more complex at the L2 location, hence the possibility of higher and diverse amount of phytochemicals present in the tissues of *F. ulmifolia*. Another possibility for their difference is the period (season) of collection. L1 samples were collected at the onset of the dry season (April 2017) while the L2 samples were obtained during the rainy season (January 2017

and August 2017).

**Cycloalkanes.** Surprisingly, a series of compounds identified as cycloalkanes with rings in the C<sub>24</sub>-C<sub>30</sub> range (also termed macrocyclic alkanes) were detected in the *Ficus* seed oils. The mass spectra agreed well with those shown elsewhere for macrocyclic alkanes (Riache et al., 2014). While only minute traces were identified in *F. septica*, cycloalkanes were more prominent than phytochemicals in *F. nota*,



(caption on next page)

**Fig. 1.** GC-MS traces (total ion chromatograms) of the methylated seed oils with peak assignments denoted by inscribed letters: A) *Ficus nota*: a = methyl palmitate, b = methyl stearate, c = methyl oleate, d = methyl linoleate, e = methyl linolenate, f = methyl docosanoate, g = squalene, h = cyclotetracosane, I = cyclohexacosane, j = cyclooctacosane, k = cyclotriacontane l = sitosterol. B) *Ficus septica*: a = methyl palmitate, b = methyl stearate, c = methyl oleate, d = methyl linoleate, e = methyl linolenate, f = sitosterol. C) *Ficus ulmifolia* (from L2 location): a = methyl palmitate, b = methyl stearate, c = methyl oleate, d = methyl linoleate, e = methyl linolenate, f = methyl docosanoate, g = squalene, h = methyl tetracosanoate, i = cyclotriacontane, j = cholesterol, k = olean-12-en-3- $\alpha$ -yl acetate, l =  $\gamma$ -sitosterol, m = urs-12-en-3- $\alpha$ -yl acetate, n =  $\beta$ -amyrin, o =  $\alpha$ -amyrin, p = lupeol.

although their overall presence is minor compared to the fatty acids in *F. nota*. The amounts of cycloalkanes in *F. ulmifolia* were minor compared to both fatty acids and phytochemicals. Otherwise, cycloalkanes are very rarely observed in plant oils or other plant parts. One report states that macrocyclic alkanes in the C<sub>24</sub>-C<sub>31</sub> region were found in the nonpolar fraction of the hexane extract of black crowberry (*Empetrum nigrum* L. of the Ericaceae (Empetraceae) family) but no relative amounts were given nor the exact plant part discussed (Red'kina et al., 1989). Other previous literature indicates that macrocyclic alkanes were identified at low levels in essential oils of various plants but internal evidence within these papers causes doubts about the evaluation of GC-MS) data presented there and therefore these papers are not discussed. Due to the rarity of cycloalkanes in plant oils, care was taken to exclude external sources as cause for their identification, noting, however, that contamination from external sources by such rare compounds appears highly unlikely. These precautions included running at high concentrations (overload conditions) a common commodity oil (soybean oil) together with the *Ficus* samples and other oils from the Philippines investigated previously to check if such compounds may be present in trace amounts. No cycloalkanes were observed in these other samples. Furthermore, some *Ficus* species, although not the ones in this study, have been observed to be potentially useful for indoor air purification. Specifically, *F. benghalensis* removed xylene and toluene from the air (Kim et al., 2016) and *F. benamina* removed several volatile organic compounds (VOC) also from the air (Song et al., 2011).

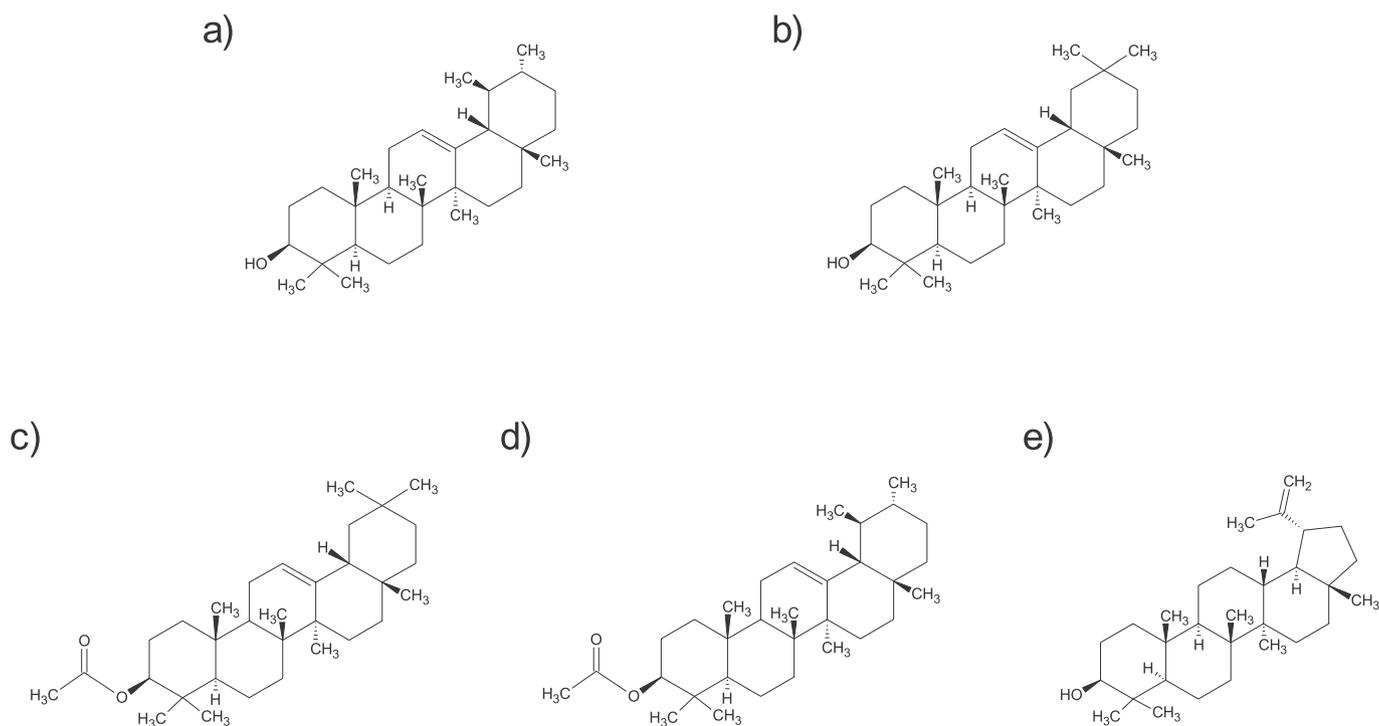
However, any hypothesis that the cycloalkanes came from the air is not supported by the observation that the cycloalkanes from *F. ulmifolia* were observed in location L1 and not location L2 whereas the *F. nota* were obtained from location L1. Also, standard samples of alkanes, alkenes and cycloalkanes additionally indicated that the evaluation as cycloalkanes to the peaks and spectra in question are correct. It may also be noted that besides the phytochemicals found in *Ficus*, other rare species such as the arylbutanoids also mentioned above have been observed so that it appears that these species may be a particularly rich source of unusual phytochemicals.

#### 4. Summary and conclusions

The composition of three *Ficus* seed oils, *F. nota*, *F. septica* and *F. ulmifolia*, was determined. The major fatty acids in the fatty acid profiles are linolenic and linoleic acids. All of the oils as extracted from the seeds contain terpenoids. While only small amounts or traces of the triterpenes were observed in *F. nota* and *F. septica* oils, this observation is especially pronounced with *F. ulmifolia* from the L2 location, in which case nearly 50% of the oil is composed of terpenoids. The presence of terpenoids in the seed oils coincides with previous reports in which terpenoids were shown to be present in other plant parts of *Ficus* species. Surprisingly, cycloalkanes were identified in these oils in minor amounts, which have hardly been ever reported previously in plant oils. Geography, vegetation type and period of collection are among the determining factors that could be responsible for the significant differences in the concentrations of fatty acids and phytochemicals in the samples of *F. ulmifolia* seeds from different locations. A more detailed study on the conditions affecting the composition of the seed oils of *Ficus* species such as studied here is intended. The results also imply that some *Ficus* seed oils may thus be of interest as sources of phytochemicals for biomedical applications.

#### Disclaimer

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not



**Fig. 2.** Structure of triterpenes observed in *Ficus* seed oils. a)  $\alpha$ -amyrin; b)  $\beta$ -amyrin; c) olean-12-en-3- $\alpha$ -yl acetate; d) urs-12-en-3- $\alpha$ -yl acetate; e) lupeol.

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#### CRedit authorship contribution statement

**Gerhard Knothe:** Writing – original draft, Formal analysis. **Luis F. Razon:** Writing – original draft. **Maria Ellenita G. de Castro:** Writing – original draft.

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