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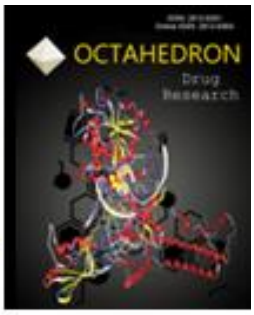

Gas Chromatography-Based Chemical Investigation of *Ficus pandurata* Fruits´ Lipoidal Matter

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ABSTRACT

Ficus pandurata Hance, (Moraceae) is a medium size tree native to tropical Africa, Mediterranean region, India, Saudi Arabia, China, and Japan. Particularly, the fruit of this plant is known to contain large amounts of triterpenes and sterols. A chemical analysis using gas chromatography is done on the ethanolic extract of *Ficus pandurata* Fruits' saponified and unsaponified fractions. However, saponified fraction showed eleven fatty acids methyl esters, in which hexadecanoic acid and octadecanoic acid were the two fatty acids found to be the most prevalent, accounting for around 34% and 14% of the overall composition of the saponified fraction, respectively. Twenty compounds were also found in the unsaponifiable fraction, accounting for 95.72% of the total unsaponifiable content. Phytosterols was the most prevalent identified class, accounting for around 55% of the total unsaponifiable content. Three major phytosterols, i.e., sitosterol, stigmasterol, and campesterol, were found, accounting for 31.47%, 18.22%, and 5.25% of the total unsaponifiable content, respectively. Triterpenes and deoxygenated hydrocarbons were detected in lower percentage in unsaponifiable fraction.

1. Introduction

The utilization of medicinal plants as sources of active compounds for the treatment of human diseases with significant therapeutic potential is well-known [1]. Genus *Ficus* (Moraceae), a large genus of trees or shrubs, comprises more than 800 species, which are extensively cultivated for their ornamental leaves and edible fruits [2, 3]. *Ficus* plants are distributed in tropical and subtropical regions [4] and are known to be abundant sources of triterpenes and sterols [4-7]. The fruits also rich in flavonoids specially of flavones and catechins/procyanidins types [8]. Ayurvedic and traditional Chinese medicine (TCM) use *Ficus* species extensively to treat various ailments such as inflammation, diabetes, tumor, and malaria [9]. In Egypt, *Ficus* species are widely distributed in streets, gardens, parks, and outside canal banks [10]. *Ficus carica* L. and *Ficus sycomorus* L. are the two most popular fruits consumed by Egyptians, and traditional Egyptian medicine uses *Ficus* species to treat

respiratory disorders, skin diseases, anti-diabetic, hypotensive, and anti-cough applications [11-13]. The wood of *Ficus* species contains large quantities of latex, which is a source of rubber, representing one of the most significant economic uses of *Ficus* in Egypt [12]. *Ficus pandurata* Hance, commonly known as Xiao xianggou in Lishui District (Zhejiang, China), is used in TCM to treat gout, arthritis, hyperuricemia, and indigestion [14, 15]. Furthermore, it has been elucidated that *F. pandurate* exerts an inhibitory impact on ulcerative colitis, as well as colitis-associated secondary liver damage, through augmentation of antioxidative activity [16]. In context, *F. pandurate* has demonstrated a hepatoprotective effect against acute alcohol-induced liver damage. This observed effect is discernibly attributed to the attenuation of oxidative stress, inflammatory responses, and apoptosis [17]. However, there is limited information on the phytochemical composition and biological activities of *F. pandurata* [18-21]. In the previous phytochemical studies of *F. pandurata*, sterols and triterpenes were isolated and identified utilizing different spectroscopic techniques [22-24]. Therefore, the purpose of this study is to identify the fatty acids and sterols, i.e., saponifiable, and unsaponifiable matter, respectively, in Egyptian *pandurata* Hance fruits, using GC-MS.

2. Material and Methods

2.1. Plant material

Fruits of *F. pandurata* were collected in May 2013 from authorized trees growing in the garden of the Faculty of Pharmacy, Assiut University. The plant was taxonomically identified by Prof. Dr. Salah EL-Nagar (Professor of Botany, Department of Botany, Faculty of Science, Assiut University, Egypt). A voucher specimen (registration code FPF- 2013) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.

2.2. Preparation of samples

2.2.1 Preparation of the ethanolic extract

The air-dried powdered fruits of *F. pandurata* (350 g) was extracted by maceration with 90% ethanol (1x3), and then concentrated under reduced pressure to give (25.50 g) viscous residue of the ethanol extract. Then, the ethanol extract (25.50 g) was suspended in 700 ml of 70% methanol, and then transferred to a separating funnel and partitioned with successive portions of *n*-hexane (500 ml each). The combined *n*-hexane fractions were concentrated under reduced pressure to yield (7.5 g). The remaining mother liquor was concentrated under reduced pressure and suspended in 500 ml of distilled water, also transferred to a separating funnel and partitioned with successive portions of dichloromethane (500 ml each), ethyl acetate (500 ml each). These fractions were concentrated under reduced pressure to afford 3.2 g, 7.7 g and mother liqour, respectively.

2.2.2. Preparation of the Unsaponifiable Matter

Saponification of the samples was carried out by the method of Kovacs and others (1979) [25]. The obtained dried *n*-hexane extract (5 g) was saponified in a round-bottom flask containing 25 mL 50% KOH and 100 mL 95% EtOH. The mixture was refluxed for 2 h with moderate stirring, utilizing a heating mantle and a magnetic stirrer. After two hours, the mixture was cooled to room temperature. A major part of the alcohol present was distilled off and the aqueous liquid was diluted with water then extracted with several portions of *n*-hexane till exhaustion. The *n*-hexane extract was washed with water until the washings became free from any alkalinity, the *n*-hexane extract was dehydrated over anhydrous sodium sulphate and then the *n*-hexane was distilled off under reduced pressure and kept for investigation of the unsaponifiable matter [26].

2.2.3. Preparation of the Fatty Acids

The alkaline aqueous solution (soap) that remained after removal of the unsaponifiable matter was acidified with sulphuric acid (20 %) and the liberated fatty acids were extracted with *n*-hexane. The *n*-hexane extract was washed with distilled water, dried over anhydrous sodium sulphate, then concentrated under reduced pressure and kept for investigation of the fatty acids [26].

2.2.4. Preparation of the Fatty Acid methyl esters

The residue obtained after solvent evaporation was subjected to methylation by mixing with anhydrous K_2CO_3 (2.09 g) and $(CH_3)_2SO_4$ (5 ml) in dry acetone and refluxed for 4 hr. after filtration. The filtrate was concentrated to remove acetone, diluted with water, and extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried over anhydrous sodium sulphate, and then concentrated to yield an oily residue kept for further investigation [27].

2.3. GC-MS analysis

The preparation of the samples was carried out by the method of Davenport J and others (1971) [28]. The sample was resuspended in 500 µl pyridine containing [*N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) + 1% TMS (Tetramethylsilane)] and transferred to a 200 µl glass insert

and analysed using a Thermo Trace GC coupled to a Voyager MS equipment (Thermo Electron, San Jose, USA) operating in EI mode at 70 eV and scanning from m/z 50–650. Samples were injected at a 1:1 split ratio, and the inlet and transfer line were held at 280 °C. Separation was achieved with a temperature program of 80 °C for 2 min, then ramped at 5 °C min^{-1} to 315 °C and held for 12 min, a 60 m DB-5MS column (J&W Scientific, 0.25 mm ID, 0.25 μm film thickness) and a constant flow of 1.0 ml min^{-1}. MS interface and ion source were set at 280 and 180 °C, respectively. Compounds were identified by comparing their mass spectra with those available in several databases, including Wiley library 09, NIST, Mainlib and Replib.

3. Results and discussion

3.1. GC-MS analysis of saponifiable matter

Eleven fatty acids methyl esters were identified in *F. pandurata* fruits´ saponifiable fraction, representing 67.71% of the total saponifiable content (**Table 1**, **Figure 1, 3**). Hexadecanoic acid and octadecanoic acid were the most abundant fatty acids identified, making up approximately 34% and 14% of the total composition, respectively. The fatty acids tridecanoic acid, eicosanoic acid, and heptadecanoic acid were present in moderate amounts, ranging from 3.76% to 4.75%. The remaining fatty acids were found in minor quantities, each accounting for less than 1% of the total saponifiable matter composition.

Unsaturated fatty acids amounted about 0.72% of the total saponifiable matter, represented only in two chromatographic peaks both were identified as Octadecenoic acid and its isomer. whereas the saturated fatty acids amounted about 67% of the total saponifiable matter. The fatty acid composition observed in *F. pandurata* fruits was consistent with previously published findings for Fig (*Ficus carica*, var. Mission) fruits [27].

3.2. GC-MS analysis of unsaponifiable matter

The study also focused on analyzing the unsaponifiable fraction of *F. pandurata* fruits, and a total of twenty compounds were identified, which accounted for 95.72% of the total unsaponifiable content. The identified compounds belonged to three classes: phytosterols, triterpenes, and hydrocarbons.

Among the identified compounds, phytosterols were the most abundant class, constituting approximately 55% of the total unsaponifiable content. The specific phytosterols identified were *β*-sitosterol, stigmasterol, and campesterol, which accounted for 31.47%, 18.22%, and 5.25% of the total unsaponifiable content, respectively. These findings were consistent with the content of these phytosterols in Fig (*Ficus carica*, var. Mission) fruits. Triterpenes, on the other hand, were represented by only two chromatographic peaks, namely *α*-amyrin and *β*-amyrin, which accounted for 4.85% and 3.57% of the total unsaponifiable content, respectively. It seems that the identified compounds in the unsaponifiable fraction of *F. pandurata* fruits align with previous studies on Fig fruits (*Ficus carica*, var. Mission), suggesting similarities in the composition of phytosterols and triterpenes between the two species [27]. Deoxygenated hydrocarbons, i.e., alkanes and alkenes, amount to about 22.58% of the total unsaponifiable content. Tetracosane was the highest identified alkane amounting to 5.51%, whereas nonadecene was the highest identified alkene amounting 8.57% of the total unsaponifiable content. The identified oxygenated compounds were only one monoacylglycerol, i.e., monomyristin and three unsaturated fatty acids alcohols, amounting about 9.78% of the total unsaponifiable matter (**Table 2 Figure 2, 4)**. 1-Octadecanol was the highest identified fatty acid alcohol amounting 6.73% of the total unsaponifiable matter.

4. Conclusion

The study conducted a thorough analysis of the phytochemical composition of *Ficus pandurata* Hance fruits lipoidal matter, shedding light on the saponifiable and unsaponifiable fractions present in these fruits. The findings have provided valuable insights into the chemical constituents of *F. pandurata* and their potential implications for medicinal and nutritional applications. The saponified fraction, which accounts for 67.71% of the total saponifiable composition, was found to be composed of eleven fatty acids methyl esters. Hexadecanoic acid and octadecanoic acid were the most common fatty acids discovered, with tridecanoic acid, eicosanoic acid, and heptadecanoic acid present in minor amounts. Furthermore, 0.72% of the total saponifiable matter consisted of unsaturated fatty acids. In addition, deoxygenated hydrocarbons and triterpenes were also identified in smaller amounts in the unsaponified fraction, at 22.58% and 8.42%, respectively, whereas phytosterols were the most prevalent class, yielding about 55% of the unsaponified content. The most prevalent phytosterols were campesterol, stigmasterol, and *β*-sitosterol. However, tetracosane was the highest recognized alkane, accounting for 5.51% of the total deoxygenated unsaponifiable content, whereas nonadecene was the highest identified alkene, accounting for 8.57%. However, there were only two chromatographic peaks for triterpenes, namely *α*-amyrin and *β*-amyrin. The

study contributes to the growing body of knowledge surrounding the chemical composition of *F. pandurata* and its potential applications in healthcare and nutrition. Future studies may explore the anti-inflammatory, antioxidant, and other health-related properties of these compounds to validate the traditional medicinal uses of *F. pandurata*.

Ethical consideration: All the participants in this study gave their informed permission.

Conflicts of Interest

No conflicts of interest are disclosed.

Table 1. Fatty acid composition of *Ficus pandurate* fruits analyzed by GC-MS.

Peak	R_t (min)	Name of compound	Molecular formula	Molecular weight	Area%
1	18. 96	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	0.68
2	22. 42	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	0.57
3	29. 91	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228	3.87
4	32. 35	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	0.19
5	37.77	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	34.42
6	40. 11	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284	4.75
7	40. 55	Dodecanedioic acid, dimethyl ester	$C_{14}H_{26}O_4$	258	0.21
8	43. 35	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	14.35
9	48. 88	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	4.38
10	49. 51	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	0.53
11	54. 16	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	3.76

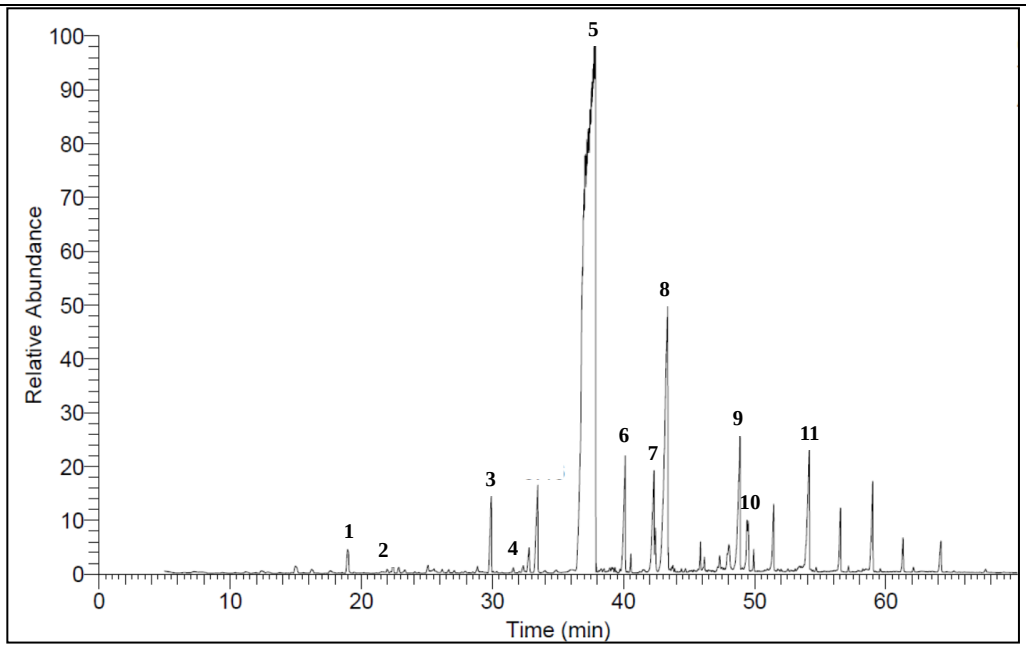

Figure 1: GC-MS trace of *Ficus pandurata* fruits fatty acid content

Table 2. Component of unsaponifiable fraction of *Ficus pandurata* fruits analyzed by GC-MS.

Peak	R_t (min)	Name of compound	Molecular formula	Molecular weight	Area%
1	19. 21	Monomyristin	$C_{17}H_{34}O_4$	302	1.69
2	21. 33	Hexadecane	$C_{16}H_{34}$	226	0.76
3	26. 13	Octadecane	$C_{18}H_{38}$	254	0.77
4	27. 16	Oleyl Alcohol	$C_{18}H_{36}O$	268	0.41
5	30. 43	Eicosane	$C_{20}H_{42}$	282	0.54
6	32. 43	Heneicosane	$C_{21}H_{44}$	296	0.89
7	32. 73	9-Hexadecen-1-ol, (Z)-	$C_{16}H_{32}O$	240	0.95
8	34. 32	Docosane	$C_{22}H_{46}$	310	0.79
9	35. 67	Nonadecene	$C_{19}H_{38}$	266	0.60
10	36. 18	Tetracosane	$C_{24}H_{50}$	338	5.51
11	39. 13	Nonadecene	$C_{19}H_{38}$	266	1.02
12	39. 56	Octacosane	$C_{28}H_{58}$	394	3.13
13	42. 70	Nonadecene	$C_{19}H_{38}$	266	3.12
14	46. 46	1-Octadecanol	$C_{18}H_{38}O$	270	6.73
15	49. 68	Nonadecene	$C_{19}H_{38}$	266	5.45
16	53.35	Campesterol	$C_{28}H_{48}O$	400	5.25
17	18.22	Stigmasterol	$C_{29}H_{48}O$	412	18.22
18	55.84	β-Sitosterol	$C_{29}H_{50}O$	414	31.47
19	56.95	β-amyrin	$C_{30}H_{50}O$	426	3.57
20	60.66	α-Amyrin	$C_{30}H_{50}O$	426	4.85

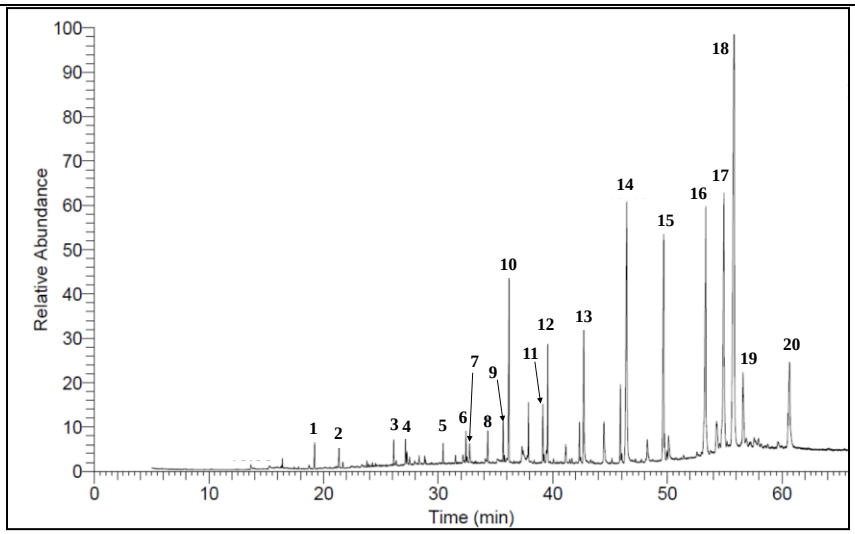

Figure 2 GC-MS trace of *Ficus pandurata* fruits unsaponifiable matter content.

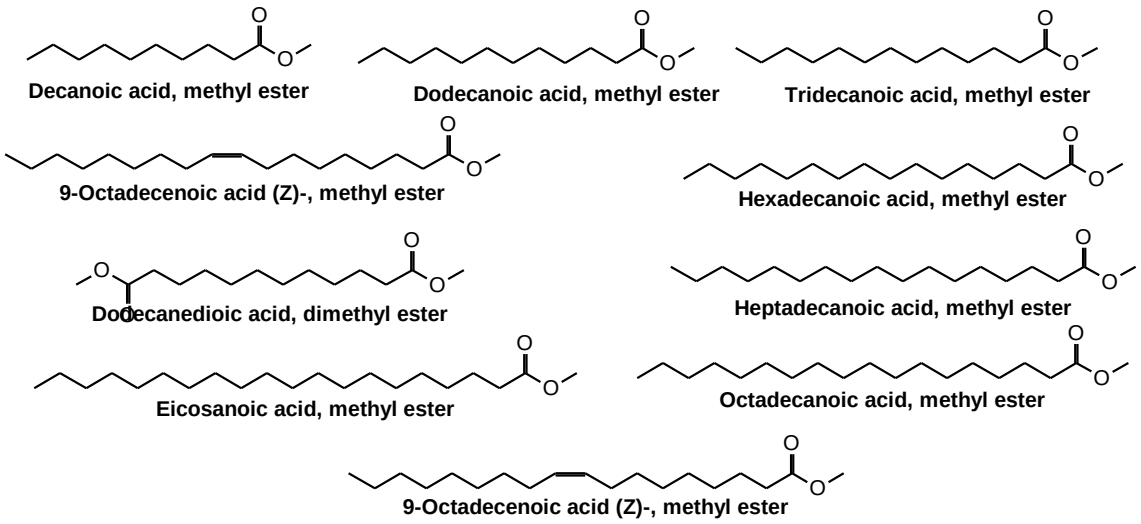

Figure 3 Structure of *Ficus pandurata* fruits fatty acid content.

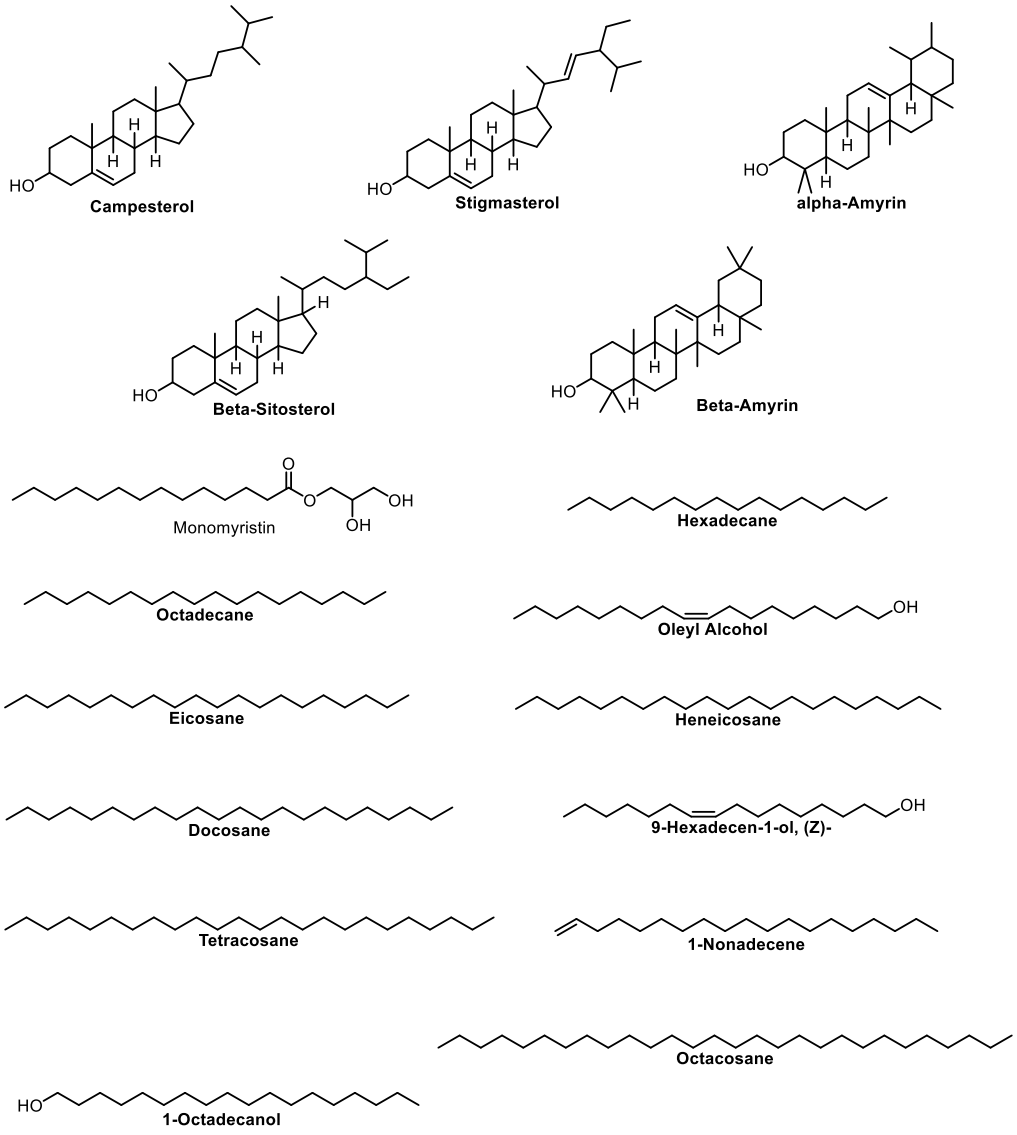

Figure 4 Structure of *Ficus pandurata* fruits unsaponifiable matter content.

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