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# High Amounts of *n*-Alkanes in the Composition of *Asphodelus aestivus* Brot. Flower Essential Oil from Cyprus

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**Abstract: There is only a couple of reports indicating essential oil composition of *Asphodelus* species in the literature. However, from the members of this genus many non-volatile secondary metabolites were isolated. In Cyprus, *Asphodelus aestivus* Brot. can be found abundantly in all regions of the island. This plant has various ethnobotanical uses in Cyprus. There is no report on the volatiles nor the essential oil composition of *A. aestivus*. The smell of *A. aestivus* flowers resembles that of a cat pee which caught our attention. Therefore, we have carried out GC, GC/MS analysis of the essential oil (yield: 0.01 v/w) obtained from *Asphodelus aestivus* flowers. Seventeen compounds were identified in the essential oil comprising 96.2% of the oil. The major components of the essential oil were hexadecanoic acid 35.6%, pentacosane 17.4%, tricosane 13.4% and heptacosane 8.4%. In our results, we expected to see sulfur containing cat pee odorants due to the odor of the flower whereas high amounts of *n*-alkanes, saturated fatty acids and minor amounts of acyclic diterpenes were observed.**

**Key words:** Xanthorrhoeaceae, *Asphodelus aestivus*, hexadecanoic acid, pentacosane, tricosane, *n*-alkanes

## 1 Introduction

The genus *Asphodelus* L. (Xanthorrhoeaceae) is represented with four species in Cyprus including *A. aestivus* Brot. (Syn = *A. microcarpus* Viv.; Syn = *A. ramosus* L.)[1)]. Different parts of (roots, tubers) *A. aestivus* is reported to be used (external, oral) for the treatment of headache, toothache, skin and intestinal problems in Cyprus[1, 2)]. Additionally in Cyprus, it is reported to be used for cosmetic (promote growth of hair) purposes[2)]. The starchy tubers of this plant were reported to be used as a food source after being boiled to remove bitter substances[1)]. Previously, *Asphodelus* species were reported to have gastroprotective effect[3)], anti-inflammatory[4)], antifungal[5)], antioxidant[6)], phytotoxic[7)] and insecticidal activities[8)].

Until now from the other species of the genus *Asphodelus*, naphthalene, anthraquinone derivatives[9－13)] and triterpene-diglycoside[14)] type compounds were reported. Additionally, from *A. aestivus* chlorogenic acid, adenosine, tryptophan, phenylalanine, aloe-emodin, aloe-emodin acetate, chrysophanol 1-*O*-gentiobioside, isovitexin, isoorientin, isoorientin 4'-*O*-β-glucopyranoside, 6"-*O*-(malonyl)-isoorientin, 6"-[(S)-3-hydroxy-3-methylglutaroyl]-isoorientin[15)], asphodelin, asphodelin 4'-*O*-β-D glucoside[16)] were isolated.

In the literature, there is only one report regarding the essential oil composition of *Asphodelus* species which reports essential oil composition of *A. microcarpus* flowers comprising of high amounts of germacrene D (78.3%), germacrene B (3.9%), α-elemene (3.8%) and caryophyllene (3.3%)[17)]. Another report on the non-polar fraction of *A. ramosus* flower petals reported high amounts of tricosane (11.9%), docosane (11.0%), tetracosane (8.6%) and pentacosane (7.2%). In this report, non-polar fractions obtained from flower petals mainly composed of *n*-alkanes and their corresponding aldehydes[18)].

In our field studies in Cyprus, we have observed that *A. aestivus* is found abundantly in all regions of the island. This plant contains tubers that are rich in starch[1)]. The tubers of this species could be regarded as good nutrious food source for wild animals (rodents etc.). In our field studies, we have never encountered any disturbance of *A. aestivus* tubers by any wild animals. Interestingly the

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flowers of the *A. aestivus* flowers have a smell resembling of cat pee. The odor of the plant and/or the chemical composition of tubers (anthroquinones, saponins, etc.) could be related with the fact that this plant is not consumed by wild animals. In our field study, we have also observed that the leaves of this plant are not harmed by any herbivore insects. Previously various extracts obtained from the aerial parts of *A. fistulosus* L. were reported to have insecticidal activity which also supports our observation[8]. It is also worthy to mention that *Urginea maritima* (L.) Baker (Liliaceae) is a similar plant to *A. aestivus* which is known to contain scilliroside (bufadienolide glycoside) derivatives in its roots that are poisonous to rodents[19]. In the course of our phytochemical screening of wild edible plants of Cyprus, here we report the essential oil composition of *A. aestivus* from Cyprus for the first time.

## 2 Materials and Methods

### 2.1 Plant Materials

Plant material was collected during the flowering period 19 March 2011 from the vicinity of Buffavento castle in Cyprus. Voucher specimen have been deposited at the Herbarium in the Near East University, Cyprus (Voucher specimen no: 6778). Plant material was identified by Dr. Kaan Polatoglu and Dr. Salih Gucel.

### 2.2 Isolation of the Oil

Flowers (100 g each) of the air dried plant was subjected to hydrodistillation for 3 h, using a Clevenger- type apparatus to produce essential oils. *A. aestivus* afforded yellow colored oil from flowers 0.01 (v/w) yield. The oil was recovered with *n*-hexane and preserved in amber vials under −20℃ until the day they were analyzed.

### 2.3 GC/MS analysis

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60℃ for 10 min and programmed to 220℃ at a rate of 4℃/min, and kept constant at 220℃ for 10 min and then programmed to 240℃ at a rate of 1℃/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250℃. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

### 2.4 GC analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300℃. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of the GC analysis are given in Table 1.

Table 1 Essential oil composition of *Asphodelus aestivus* flower oil from Cyprus.

| No | RRI | Compound | % |
|---|---|---|---|
| 1 | 1400 | Tetradecane | tr |
| 2 | 1500 | Pentadecane | 0.3 |
| 3 | 1600 | Hexadecane | 0.5 |
| 4 | 1700 | Heptadecane | 0.6 |
| 5 | 1900 | Nonadecane | 1.0 |
| 6 | 2000 | Eicosane | 0.6 |
| 7 | 2100 | Heneicosane | 4.5 |
| 8 | 2131 | Hexahydrofarnesyl acetone | 1.7 |
| 9 | 2200 | Docosane | 1.3 |
| 10 | 2300 | Tricosane | 13.4 |
| 11 | 2400 | Tetracosane | 3.0 |
| 12 | 2500 | Pentacosane | 17.4 |
| 13 | 2600 | Hexacosane | 2.0 |
| 14 | 2622 | Phytol | 4.5 |
| 15 | 2670 | Tetradecanoic acid (= *Myristic acid*) | 1.4 |
| 16 | 2700 | Heptacosane | 8.4 |
| 17 | 2931 | Hexadecanoic acid | 35.6 |
| | | **Total** | 96.2 |

RRI: Relative retention indices calculated against *n*-alkanes, Relative percentage "%" calculated from FID data, tr: Trace (< 0.1 %).

### 2.5 Identification of Essential Oil Components

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library)[20, 21] and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data[22], was used for the identification.

## 3 Results and Discussion

Flower essential oil of *A. aestivus* was analysed with GC, GC/MS and seventeen compounds were identified comprising 96.2% of the oil. The yield of the oil was 0.01 v/w which is very low. The major components of the oil were hexadecanoic acid 35.6%, pentacosane 17.4%, tricosane 13.4% and heptacosane 8.4%. The essential oil was mainly composed of *n*-alkanes which corresponds 53.0% of the whole oil. Additionally second largest group was saturated fatty acids 37.0% of the whole oil. The flower oil of *A. aestivus* also contains hexahydrofarnesyl acetone (1.7%) and acyclic diterpene (phytol 4.5%) in minor quantities. Unlike the previous report on *A. microcarpus* from Algeria which contain monocyclic sesquiterpene germacrene D comprising the main portion of the oil[17], sesquiterpene type compounds were not present in the essential oil from Cyprus. Additionally, the oil from Algeria also contained acyclic monoterpenes, bicyclic and tricyclic sesquiterpenes in very small quantities[17] which lacked completely in the oil from Cyprus. Another report on the GC/MS analysis of the non-polar extract obtained by cyclohexane fractionation from *A. ramosus* petals[18] showed similarities with the composition of *A. aestivus* flower oil from Cyprus. The cyclohexane fraction of *A. ramosus* petals contained high amounts of *n*-alkanes[18] similar to the oil from Cyprus. However, the saturated fatty acids were in very low amounts[18] unlike the oil from Cyprus. The sesquiterpene and diterpene type compounds were completely missing but aldehydes and ketones of corresponding *n*-alkanes are also present in the non-polar extract of *A. ramosus*[18]. The essential oil composition we report here is more similar to the non-polar extract of *A. ramosus* flowers previously reported[18]. The floral records also indicate that *A. ramosus* is the synonym name of *A. aestivus*[23] which could explain the similarities. However another floristic record indicates that *A. microcarpus* is also the synonym name of *A. aestivus*[1]. Therefore further studies should still be conducted from the samples of *A. aestivus* from other locations where they are available in order to prove further similarities, dissimilarities of *A. aestivus*, *A. ramosus* and *A. microcarpus*.

The flower oil from *A. aestivus* contains *n*-alkanes from tetradecane to heptacosane missing only octadecane. The smell of the flowers resemble the odor of cat urine after they were cut from the plant, however, in the essential oil we have not observed any compounds related to cat pee odor namely: 3-Mercapto-3-methylbutan-1-ol, 4-methoxy-2-methylbutane-2-thiol, 4-mercapto-4-methylpentan-2-one[24]. Additionally the flower essential oil obtained does not produce cat odor sensation. Probably during the drying process or in the distillation of the flowers, cat pee odorants or substances that could produce this odor were lost because of their high volatility. According to the previous reports *A. aestivus* extracts produced considerable insecticidal activity[8] and our field observations also prompted us to think that other volatile substances do exist in this plant which we were not able to determine and which we think that are related to both insecticidal activity and cat pee odor. Therefore, we plan to study this plant using more plant material and alternative methods to obtain volatiles to find substances both responsible for cat odor and insecticidal activity.

## 4 Conclusion

The essential oil of *A. aestivus* flowers from Cyprus contains high amounts of *n*-alkanes unlike the previously reported *A. microcarpus* (Syn = *A. aestivus*) from Algeria[17] but similar to apolar extract of flowers of previously reported *A. ramosus* (Syn = *A. aestivus*) from Greece[18]. There are very few reports on the essential oil or volatile composition of *Asphodeleus* species therefore it is difficult to produce a comment on the chemo-systematic position of this species according to current findings and the existing reports. Therefore further studies are required in order to define the similarities and dissimilarities of the essential oils of *A. aestivus*, *A. ramosus* and *A. microcarpus* from other locations. We believe the results obtained from this research will stimulate further research on the chemistry of *Asphodelus* species.

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