Yield, characterization and possible exploitation of *Cannabis Sativa* L. roots grown under aeroponics cultivation.

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Abstract

*Cannabis Sativa* L. has been used for a long time to obtain food, fiber and as a medicinal and psychoactive plant. Today the nutraceutical potential of *C. Sativa* is being increasingly reappraised; however, *C. Sativa* roots remain poorly studied, despite citations in the scientific literature. In this direction, we identified and quantified the presence of valuable bioactives (namely β-sitosterol, stigmasterol, campesterol, friedelin and epi-friedelanol) in the root extracts of *C. Sativa*, a finding which might pave the way to the exploitation of the therapeutic potential of *C. Sativa* in all its parts. To facilitate roots harvesting and processing, aeroponic (AP) and aeroponic elicited cultures (AEP), have been set up and compared to soil-cultivated plant (SP): interestingly a considerable overgrowth of the plants - particularly of roots - and a significant increase (up to 20 fold in the case of β-sitosterol) in the total content of the above roots' bioactive molecules have been observed in AP and AEP.

In conclusion aeroponics, an easy, standardised, free of contaminant cultivation technique, allows an ease harvesting/processing of roots along with a greater production of their secondary bioactive metabolites which could be utilized in the formulation of health promoting and health care products.

**Key words:** *Cannabis Sativa* L.; Aeroponics; Roots; Campesterol; Stigmasterol; β-Sitosterol; Epi-friedelanol; Friedelin.
**Introduction**

Modern nutrition increasingly involves the consumption of functional foods and nutraceuticals that are useful for health maintenance, and for the prevention and treatment of some diseases [1]. Functional foods are solid and/or liquid foods, processed or not, which in addition to nourishing contain also biologically active compounds associated with health benefits. Nutraceuticals - a technical evolution of functional foods - are preparations such as tablets, syrups, powder, etc. that contain active ingredients mostly extracted from plant foods (botanicals); nutraceuticals must provide clinically proven health benefits as well as for the prevention and management and treatment of some chronic diseases [2]. In parallel, the use of health promoting products containing the above bioactives for non-nutritional purposes (skin care, inhalers for respiratory tract, rhinological and otological formulations, eyedrops) is on the rise [3].

*Cannabis Sativa* L. is a dioecious annual herbaceous plant, also defined as a big grass, native to Central Asia and belonging to the botanical family of Cannabaceae. For centuries, it has been essential for humans to obtain food, fiber and as a medicinal and psychoactive plant. Today the nutraceutical potential of *C. Sativa* is being increasingly reappraised [4].

Two subspecies can be identified within *C. Sativa*: *C. Sativa* subsp. *indica*, that contains more than 20% of the psychoactive compound D9-tetrahydrocannabinol (THC) in the resin produced by the female buds, and *C. Sativa* subsp. *Sativa* which contains numerous bioactive molecules in all parts of the plant but shows a much lower content of psychoactive molecules, particularly THC, which must not exceed 0.2% [5]. The subspecies *Sativa* is now commonly and legally cultivated in many European countries for the production of cannabidiol/cannabidiolic acid (CBDs) - the most abundant non-psychoactive cannabinoids - as well as of seeds which represent an excellent source of nutrients such as lipids, proteins, carbohydrates, minerals, vitamins, amino acids and essential fatty acids as well as insoluble fiber; edible oil and flour for human use can be obtained from *C. Sativa* seeds [6]. It has been shown that even the sprouts obtained from the germination of *C. Sativa* seeds represent an interesting example of functional food because sprouts of three/five days are rich in compounds with anti-inflammatory activity such as the prenylflavonoids cannaflavins A and B [5].
Interestingly, in the ‘70s of the last century, Slatkin et al. (1971) [7] and Sethi et al. (1977) [8], demonstrated the presence of triterpenoids and sterols in the root extracts of mature *C. Sativa* plants. More recently, Jin Dan et al. (2020) [9] have completely profiled the groups of secondary metabolites in the individual parts of the plant, highlighting the presence of sterols (β-sitosterol, stigmasterol, campesterol) and triterpenoids (friedelin and epi-friedelanol) in mature *C. Sativa* roots, paving the way to the reappraisal and implementation of the therapeutic potential of *C. Sativa* in all its parts.

Although historically *C. Sativa* roots were widely used as medicines to treat inflammatory conditions, joint pain, gout and more, the therapeutic potential of *C. Sativa* roots has been largely ignored in modern times. Indeed, few studies have examined the composition of *C. Sativa* roots and their medical potential [10], and even less are the studies in which alternative methods are considered for the production of *C. Sativa* roots or to increase their content of biological active molecules.

The relative scarcity of similar studies likely depends on the fact that the research on *C. Sativa* is mainly focused on the most widespread cannabinoids that are not significantly present in the roots [10].

With the aim of filling this gap, in the present work *C. Sativa* var. *Kompolti* - a legal variety routinely used also for food production purposes - has been cultivated through aeroponics [11], a method allowing the plants to grow in a highly controlled manner, free of contaminants and suspended in an environment devoid of soil or other support means. This system has been selected among others as it theoretically allows a greater production of roots as compared to traditional cultivation in soil, with the additional advantage that there is no need for time consuming and expensive rinsing procedures to isolate and process the roots.

As a consequence, the aeroponics culture should provide a greater production of *C. Sativa* roots secondary bioactive metabolites.

Here we have compared morphological features of plants grown in aeroponics (AP) or in soil (SP), as well as characterized and compared the secondary metabolites contained in their roots, with the aim of determining the yield of relevant compounds which could be included as active ingredients in the formulation of health promoting and health care products.
Materials and methods

Chemicals and reagents

Extraction solvents (analytical grade), cholesterol, β-sitosterol, stigmasterol, campesterol, and friedelin were obtained from Sigma-Aldrich (St. Louis, MO).

Plant material and cultures

*C. Sativa* Kompolti seeds were supplied by Appennino Farm, Gaggio Montano, Bologna (Italy), lot B30756201900001. The seeds were germinated in filter paper wetted with distilled water - 10 seeds in glass Petri dish 14 cm diameter - in the dark, at a constant temperature of 25 °C. After 4 days the rooted seeds were transferred into plastic pots, with a diameter of 5.5 cm and a height of 6.0 cm containing a mixture of 50% peat and 50% vermiculite wetted with Hoagland's half strength nutrient solution up to when the seedlings were not ready for transplanting. The pots (one rooted seed per pot) were placed in a climatic cell with a photoperiod of 18 hours (lamp and conditions as below) until the first two true leaves are fully developed. At this point, five *C. Sativa* seedlings of uniform size, length of the first two true leaves equal to about 3.0 cm, were selected each time. The base of the stem of each seedling was fixed with a sponge, placed in a reticulated pot for aeroponics (diameter 6.0 cm and height 6.0 cm) and this placed on the hole of the lid of a tub for aeroponic cultivation in black PVC (50.0 cm x 50.0 cm x 34.0 cm height) capable of hosting five plants each time at a distance of fifteen cm from each other. Complete Haogland nutrient solution was sprayed at the roots of the plants for a duration of fifteen minutes every hour, and recovered via a closed circuit. Electrical Conductivity (EC) and pH of the nutrient solution were controlled at 0.6-0.7 ms/cm and 6.0, respectively. The aeroponics culture system was maintained in a climatic cell with photoperiod as above with high pressure sodium lamps (Sonlight AGRO 250W grown+bloom). Photosynthetic photon flux density (PPFD) at the plant canopy was about 150 μmol/m² • s. Temperatures during the periods of light and dark were maintained at 27 ± 1 °C and 22 ± 1 °C.
respectively. Relative humidity was 65 ± 5% and mean CO$_2$ concentration was 670 ± 30 μmol/mol.

For the traditional cultivation in pots, the seedlings with two real leaves (about 3 cm long) were placed in a plastic pot (diameter 30.0 cm and height 30.0 cm), containing a mixture as above (50% vermiculite and 50% peat), 3 plants for pot, spaced 15 cm from each other and watered periodically with the whole Hoagland nutrient solution. The pots were placed in a climatic cell with the conditions described for aeroponics and at the same time as this.

Three culture systems of *C. Sativa* Kompolti have been set up: SP (Soil Plant) carried out only for the vegetative phase; AP (Aeroponic Plant) carried out only for the vegetative phase; AEP (Aeroponic elicited Plant) carried out only for the vegetative phase supplemented with the elicitor technique with salicylic acid. AEP were obtained by adding salicylic (25 μM final concentration) acid to the nutrient solution of one-week old plants, according to Ze-Bo Liu et al. [12].

**Biomass production**

Five plants from each culture system were harvested three times after 8 weeks of culture. It should be noted that the plants were kept in vegetative phase maintaining the photoperiod constant (18 h). Under these conditions plants could not flower because flowering requires a gradual reduction of the photoperiod (from 18 to 12 h). The root systems were carefully washed with tap water and dried and with absorbent paper to remove excess water and their fresh weights immediately recorded. The other parameters taken into consideration were: dry weight of the roots (g); fresh and dry weight of the aerial parts (g); height of the aerial parts (cm); diameter of the stems (mm) and surface of the collected leaves at half height (cm).
Extraction of dry C. Sativa roots

Powdered C. Sativa roots (300.0 mg) and 100 µL of the IS solution (cholesterol, 1.128 mg/mL in ethyl acetate) was extracted in ethyl acetate (40 mL) under magnetic stirring for 1.5 h at room temperature, followed by centrifugation at 5000 rpm for 8 min. The supernatant was collected in a flask and the residue was extracted once again in the same manner. The collected organic phases were washed with water (2 x 10 mL) and brine (10 mL) then dried (Na₂SO₄ anhydrous), filtered, and evaporated to dryness in vacuo at 30 °C. The residue was dissolved in 10 mL of ethyl acetate and kept at 4 °C until GC-MS and GC-FID analyses.

Gas Chromatography (GC-MS, GC-FID)

GC-MS analyses were carried out using a Trace GC Ultra gas chromatograph coupled to an ion-trap mass spectrometer (ITMS) detector Polaris Q (Thermo Fisher Scientific, Italy) and equipped with a split-splitless injector. The column was a 30 m x 0.25 mm i.d., 0.1 µm film thickness, fused silica SLB-5ms (Supelco, Sigma-Aldrich, Italy). The initial oven temperature was 240 °C programmed to 280 °C at 2 °C/min and kept at 280 °C for 5 min, the temperature was then raised to 310 °C at a rate of 10 °C/min and maintained at this temperature for 7 min. Samples (1 µL) were injected in the split (1:10) mode. Injector, transfer line and ion source were set at 280, 280 and 200 °C, respectively. Helium was used as carrier gas at a flow of 1 mL min⁻¹. The mass spectra were recorded in electron ionization (EI) mode at 70 eV electron energy with a mass range from m/z 50 to 650 and a scan rate of 0.8 scan/sec. Identification of metabolites was carried out by comparison of the spectral data and retention times with those of standards or to the spectra from the NIST02 spectral library.

A Fisons GC 8000 series gas chromatograph, equipped with a flame ionization detector and a split/splitless injector (Fisons Instruments, Milan, Italy), was used for the quantitation of secondary metabolites. The separation was carried out with a fused silica capillary column DB-5MS UI 30m x 0.250mm x 0.25µm film thickness (Agilent, J&W, Italy). The initial oven temperature was 240 °C programmed to 280 °C at 2 °C/min and kept at 280 °C for 10 min, the temperature was then raised to 310 °C at a rate of 10 °C/min and maintained at this temperature for 15 min. Samples (1 µL) were
injected in the split (1:10) mode. Injector and detector were set at 280 °C. Hydrogen was used as carrier gas at a flow of 1.8 mL/min. Peak areas were integrated using a Varian Galaxie Workstation (Agilent Technologies, Italy).

Quantification of the analytes in the dry *C. Sativa* roots was performed using the internal standard method based on the relative peak area of analyte to IS (cholesterol) from the average of three replicate measurements. When standards were unavailable, the quantification of the target analyte was carried out using the relative response factor of available standards of similar chemical structure.

**Statistics**

Statistical analyses were carried out to compare each root metabolite quantified in AP AEP and SP using Tukey’s multiple comparison tests.

**Results**

**Biomass production**

The growth of *C. Sativa* var. Kompolti plants used in this study was significantly influenced by the two systems tested - i.e. conventional vs aeroponic - with aeroponics promoting a significantly more rapid and intense growth of both aerial parts and root systems (Figure 1; Table 1). On average, after eight weeks of parallel cultivation, the roots of APs showed a 64 and 13 folds higher fresh (FW) and dry weight (DW) as compared to SP, respectively; the aerial parts showed a 39 and 44 folds higher FW and DW; the stems’ average diameter and the mean leaves area increased by 3.89 folds and 8.9 folds, respectively. AP and AEP reached an almost double height (ca. 70 cm) as compared to SP (ca. 30 cm). Finally, the addition of the elicitor salicylic acid to the nutrient sprayed did not result in any significant variation of these parameters in AEP as compared to AP (Table 1, Figure 1).
Figure 1: Plant and roots of *C. Sativa* cultured under soil (A) or aeroponic conditions (B).
Extract characterization

The content of the main roots’ bioactive constituents has been comparatively determined in SP, AP and AEP plants by GC-MS. The main compounds identified were the phytosterols campesterol, stigmasterol and β-sitosterol and the triterpenes epi-friedelanol and friedelin (Figure 2). On a per DW basis (Table 1; Figure 3) the amount of bioactives is higher in SP as compared to both AP and AEP. As to the single constituents, the amount of epi-friedelanol and friedelin was far higher in SP, that of campesterol and stigmasterol was similar in the three types of cultures while β-sitosterol was higher in AP and AEP. On a percent basis (Table 2 and Figure 4), friedelin and epi-friedelanol were the most expressed compounds in SP, while β-sitosterol in AP and AEP; finally, the amount of β-sitosterol and epi-friedelanol in AEP as compared to AP decreased and increased, respectively.

| Culture system | Height (cm) | Roots weight FW/DW (g) | Aerial parts weight FW/DW (g) | Stem average diameter (mm) | Leaves average area (cm²) |
|----------------|-------------|------------------------|------------------------------|---------------------------|--------------------------|
| SP             | 32.0±0.7    | 3.7±0.4/1.2±0.1        | 15.8±0.7/2.9±0.4             | 2.1±0.1                   | 5.2±0.4                  |
| AP             | 70.5±1.8    | 238.7±4.1/15.8±0.5     | 610.9±3.0/129.3±0.7          | 8.3±0.5                   | 48.3±3.7                |
| AEP            | 72.5±1.3    | 246.1±4.3/10.7±0.4     | 656.3±3.1/137.1±1.6          | 8.2±0.4                   | 49.4±3.4                |

Abbreviations: FW = Fresh weight; DW = dried weight; SP = Soil Plant; AP = Aeroponic Plant; AEP = Aeroponic Elicited Plant
Table 2: Content of the main bioactive compounds present in 100.0 mg of dry SP, AP, and AEP roots.

| Compound          | Culture system | SP      | AP      | AEP     |
|-------------------|----------------|---------|---------|---------|
|                   |                | (µg)a   | %       | (µg)a   | %       | (µg)a   | %       |
| Campesterol       |                | 17.8±0.6| 4.8     | 17.2±0.4| 10.9    | 18.5±0.2| 10.0    |
| Stigmasterol      |                | 26.1±0.2| 7.0     | 20.2±0.2| 12.7    | 30.1±1.0| 16.3    |
| β-Sitosterol      |                | 40.4±0.0| 10.8    | 68.7±1.1| 43.3    | 59.0±7.8| 32.0    |
| Epi-Friedelanol   |                | 92.2±0.5| 24.6    | 23.9±0.1| 15.0    | 42.9±1.5| 23.2    |
| Friedelin         |                | 197.5±1.3| 52.8  | 28.8±1.2| 18.1    | 33.9±1.0| 18.5    |
| Total             |                | 374.0±2.6|        | 158.8±1.9|        | 184.3±8.5|        |

Abbreviations: SP = Soil Plant, AP = Aeroponic Plant; AEP = Aeroponic Elicited Plant

aData are expressed as the mean value ± standard deviation; n = 3 repetitions.

Figure 2: Main chemical compounds identified by GC-MS in *C. Sativa* SP (soil), AP (aeroponics) and AEP (aeroponics plus the elicitor salicylic acid) root extracts.
Figure 3: Concentration of the main bioactive constituents of SP, AP and AEP root extracts of *C. Sativa* AEP aeroponic with salicylic acid as elicitor. Data are expressed as μg/100mg DW ± SEM.

Figure 4: Percentage of the main bioactive constituents of the root extracts of SP, AP and AEP *C. Sativa* plants.
Notably, when analyzed on a “per plant” basis the results were markedly different (Figure 5). Indeed, since the biomasses of the plants grown in aeroponics were heavier (13 to 64 folds, roots DW and FW, respectively) both AP and AEP contained significantly higher root bioactives’ amounts (Table 3; Figure 5). The amounts of β-sitosterol from AP (10.86 ± 0.72 mg) and AEP (9.89 ± 2.17 mg) roots were, respectively, 23 and 20 times higher than in SP (0.49 ± 0.05 mg); friedelin, whose concentration on a per weight basis was significantly higher in SP, turns to higher per plant values in AEP and AP (4.55 ± 0.47 mg; 5.67 ± 0.4 mg), respectively than SP (2.37 ± 0.3 mg) (Table 3; Figure 5). Similar proportions have been observed for campesterol, stigmasterol and epi-friedelanol (Table 3).

Figure 5: Total amount of the main bioactive constituents of SP, AP and AEP C. Sativa roots. Data are expressed as mg per plant. * p < .0001 AP vs SP; §§ p < .0001 and § p < .05 AEP vs AP (Tukey’s multiple comparisons test).
Discussion

This study was undertaken for the reason that *C. Sativa* roots - although potentially valuable - are a neglected source of bioactive compounds. The fact that roots lack the most pharmacologically-relevant compounds - namely THC and CBDs - and the relative complexity of root processing probably account for this scarce interest for *C. Sativa* roots. However, unlike SP, roots harvesting/processing from AP is far easier, cheaper and flexible. Indeed AP roots are clean and free from the parasites and contaminants normally present in the soil; more interestingly, as shown in the present study and elsewhere [13,14], the absolute and relative yield of bioactives can be modulated simply varying the composition of the nutrient sprayed onto the roots by adding specific elicitors. Moreover roots of AP may meet the standards of organic cultivations, which today represent a desired and high quality end point.

The chemical characterization of SP, AP and AEP roots indicates that 1) the phytosterols β-sitosterol, campesterol and stigmasterol and the triterpenoids friedelin and epi-friedelanol represent the major components of interest; 2) the relative proportion of these constituents was significantly affected by the cultivation system. Here we demonstrate that aeroponics applied to *C. sativa* var. Kompolti results in a significant modification in the yield of plant biomasses and in the net and relative abundance of root bioactive compounds, as compared to conventional soil cultivation. Indeed the biomass of AP (both aerial parts and roots) after 8 weeks of cultivation
strikingly outpaces that of SP. In particular, the overgrowth of roots was impressive with a 64 and 13 fold increase in FW and DW, respectively, as compared to the roots from SP. The greater increase in FW is likely due to the very high hydration rate attainable under aeroponics.

This overgrowth has been found to occur also in other plants: Li et al. [15], showed that roots’ DW of two varieties of lettuce grown in aeroponics, was significantly higher than that obtained not only cultivating plants in soil, but also with the hydroponic technique.

Other Authors [16] showed that aeroponics of *Crocus sativus* (saffron) promoted a more robust growth of the root system which, however, was not paralleled by a proportional overgrowth of the aerial parts. This observation suggests that, under aeroponics a larger root system does not necessarily result in a correspondingly greater biomass of the aerial parts. We also found that, although both roots and aerial parts of AP were invariably greater and heavier than SP, aerial parts and roots grew to different extents. The differential overgrowth observed here and elsewhere may be the expression of a plant type-dependent effect of aeroponic cultivation. In fact, both the traditional cultivation method on substrate and the hydroponic one involve a continuous immersion of the roots in nutrients and water. In some cases this can stimulate more efficiently the growth of the aerial part of the plant as compared to the aeroponics method, where the roots are suspended in a chamber, wetted at regular intervals with the nutrient solution and with virtually unlimited access to air O$_2$. The extensive oxygen availability likely represents the most important advantage of the aeroponic culture method over conventional and hydroponic ones [17].

On a pharmaceutical perspective, Hayden et al [18,19], have shown that aeroponics can represent an excellent system for the production of roots from medicinal plants in which these organs are used for the extraction of active molecules such as, for example, burdock (*Arctium lappa*) and ginger (*Zingiber officinale*). Importantly, as compared to other techniques aeroponics allows to obtain perfectly clean root apparatuses which can be immediately harvested, extracted, lyophilized, micronized or subjected to other processing methods. A further advantage of aeroponics is that the culture medium can be easily and precisely enriched with specific elicitors which can further enhance the bioactives’ yield.

Although more appreciated in the market for its content in CBDs from plant’s inflorescences and for the wide utilization of the aerial parts, here we applied and
tested aeroponics to *C. Sativa*, whose roots exhibit a fairly high content in potentially valuable constituents characterized by pharmacological, nutraceutical and cosmeceutical attractive activities. Notably, we show that the yield of these components on a per plant basis was invariably and significantly higher in AP and AEP as compared to SP, and that using salicylate as elicitor [20,21] accumulation of all bioactives could be attained (with the exception of β-sitosterol which only slightly decreased as compared to AP).

Hence these findings could pave the way for a rational implementation of *C. sativa* aeroponic cultivation based on the development of mixtures of specific elicitors to increase and optimize the yield of root bioactive constituents. In this light elicitation may allow to obtain different *C. Sativa* roots’ phytocomplexes with specific attitudes resulting from the relative proportion and peculiar biological features of each component.

As briefly anticipated above, the properties of the bioactives identified in *C. Sativa* roots largely justify their use - either singularly or as phytocomplex - for the preparation of health promoting products. The most abundant components are represented by phytosterols, particularly β-sitosterol. β-Sitosterol is a sterol found in almost all plants. It is one of the main subcomponents of a group of plant sterols known as phytosterols that are very similar in composition to cholesterol. High levels are found in rice bran, wheat germ, corn oil, and soybeans; peanuts and their products such as peanut oil, peanut butter, and peanut flour; *Serenoa repens*, avocados, pumpkin seed, *Pygeum africanum*, and cashew fruit.

The antihypercholesterolemic effect of β-sitosterol has been reported by Cicero et al. [22]; thirtysix human volunteers took 2g/day of β-sitosterol and 8g/day of soy protein for forty days and after this period they showed a significant decrease of LDL (low-density lipoprotein), VLDL (very low density lipoprotein), TG (triglycerides) levels and a significant increase of HDL (high-density lipoprotein) [22]. Studies have suggested that β-sitosterol inhibited proliferation of human prostate cancer cells [23] and growth of tumors derived from PC-3 human prostate cancer cells [24,25].

The ability of β-sitosterol and other phytosterols to inhibit aromatase and 5-alpha-reductase has been well documented, and this inhibitory capacity has been exploited to treat pathologies such as benign prostatic hyperplasia and androgenetic alopecia [26–28]. One of the richest sources of phytosterols, particularly β-sitosterol, is *Serenoa*
*S. repens* whose extracts have been largely studied and used in nutraceutical formulations (Permixon, Calprost, Difaprost) proposed for the adjunct therapy of benign prostatic hyperplasia [25,29,30].

The concentration of β-sitosterol in *S. repens* extract, 0.454±0.018mg/g, dry mass [31], is a hundred times lower than those we found in *C. Sativa* extracts; such a finding would make the use of *C. Sativa* roots extracts for nutraceutical applications as plausible as those from *S. Repens*. As compared to other sources, *C. Sativa* roots have very low content in lipids, proteins and carbohydrates: hence, they could be taken by overweight/obese, diabetic or hypercolestolemic patients without increasing their caloric intake.

Friedelin, a pentacyclic triterpenoid which can be found in many plants, displays a wide spectrum of anti-inflammatory, antipyretic, anticarcinogenic and antitumor effects with low toxicity [32]. Friedelin was reported to promote apoptosis and inhibit the growth of various tumor cell lines including MCF-7 human breast cancer and AML-196 human leukemia cells [33–36]. Friedelin also possesses other remarkable properties such as: *in vivo* anti-inflammatory, analgesic and antipyretic effects in adult Wistar rats [37], mast cell membranes stabilization [38], hypoglycemic effect in diabetic rats [39], gastroprotective and antiulcerogenic activity [40], estrogenic activity [41], antihyperlipidemic and antihypertensive effect [42]. Friedelin shows remarkable antioxidant capacity, comparable to that of BHT or ascorbate [43]. Finally, friedelin, thanks to its antimycobacterial activity, has been proposed as a natural antituberculosis agent [44]. Interestingly, this usage parallels that of *C. Sativa* leaf macerated in warm water and taken as a treatment for tuberculosis by the Bapedi healers of Limpopo Province, South Africa [45].

Another molecule present in significant percentages is epi-friedelanol (Fig.3). This compound is another pentacyclic triterpenoid that shares a large part of the molecular structure with friedelin, with the difference that this latter has a cyclohexanone, while epi-friedelanol a cyclohexanol.

Epi-friedelanol is present in several plants, such as in the root barks of *Ulmus Davidiana* [46], *Cayratia trifolia* [47–49], *Vitis trifolia* [50], *Celtis sinensis* [51], *Mallotus apelta* [52] and *Ulmus pumila* [53]. This triterpenoid has been reported to have
anticancer [50,54], anti-inflammatory [51] and anti-senescence activity [46]. In particular, Yang et al. [46] who found that epi-friedelanol suppresses cellular and replicative senescence in human fibroblasts and human umbilical vein endothelial cells, proposed its use in the formulation of nutraceuticals or cosmeceuticals aimed at modulating tissue aging or aging-associated diseases.
Conclusion

On the whole, the main constituents of *C. Sativa* roots, namely β-sitosterol, friedelin and epi-friedelanol possess converging or complementary biological activities in such a way that their co-presence in *C. Sativa* roots extracts may result in additive or even synergistic effects which could be used as adjunctive treatment in several pathological and physiopathological conditions such as inflammatory states, dyslipidemias, hyperglycaemia, menopausa, skin aging. Hence, the above considerations make *C. Sativa* roots obtained through aeroponic cultivation a valuable material, further enriched by the striking biomass yield, the high bioactive content and the ease of root harvesting/processing characterizing this technique.

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**Conflicts of Interest**

The authors declare no conflict of interest.
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