Current Trends in Pharmacology and Clinical Trials
ISSN: 2642-0848

Academic Strive
Researcher's perception is our reality

Research Article *Volume 4 Issue 1*

Chemical Composition of Essential Oil and Total Alkaloid of Extract from Leaves of Lebanese *Datura Metel* L.

Jaber A[1]*, Dorra Z[1], Harakeh LA[1], Ibrahim G[2] and Cheble E[1]

[1]Faculty of Pharmacy, Lebanese University, Hadath, Lebanon

[2]Faculty of Science, Lebanese University, Hadath, Lebanon

***Corresponding author:** Ali Jaber, Laboratoire de Recherche et Developpement des Medicaments et des Produits Naturels RD-MPN, Faculty of Pharmacy, Lebanese University, Lebanon, Tel: 009613451884; Email: ali.jaber.2@ul.edu.lb

Received Date: February 04, 2021; **Published Date:** February 22, 2021

Abstract

The Essential oil from the leaf of *Datura metel L.*, obtained from south Lebanon, was tested for their chemical compounds. Furthermore, the present study aimed to estimate the total alkaloid contents of seeds, fruits, stems, and leaves extracts, obtained using two different solvent systems. The volatile compounds were extracted using the Clevenger apparatus and analyzed by GC-MS. A total of 22 compounds were separated and 7 compounds of them were identified from the oil. These compounds identified took up 93.43 % of the total peak areas. The result showed the main compounds of the volatile constituents in leaves of *D. metel* were terpenes. Total alkaloids from different parts of the plant ranged from 0.83 to 5.2 %, and methanol/acetonitrile is the best solvent system. Also, the content of alkaloids in fruits was the highest, followed by seeds and leaves, the least were stems. According to the potent activity of the founded compounds, essential oils of *D. Metel* plants examined would be considered as promising holders of bioactive phytochemicals. Further investigations are a must to evaluate the biological activities of this plant.

Keywords: *Datura Metel L*; GC-MS, Essential Oils; Volatile Compounds; Alkaloids

Abbreviations: *D. Metel: Datura Metel*; EOs: Essential Oils; TA: Total alkaloid; GC-MS: Gas Chromatography-Mass Spectrometry; DPPH: 2, 2-diphényl-1-picrylhydrazyle.

Introduction

Although they are known for their potent bioactivity, essential oils (EOs) are often considered as a relic of medieval medical practice by representatives of modern medicine [1]. During recent years, EOs capture the interest of researchers to find an effective treatment for many incurable diseases using natural compounds [2]. For instance, recently a Cretan aromatic plant (*Thymbra capitata* (L.) Cav., *Salvia fruticosa* Mill and *Origanum dictamnus* L.) Essential oil mixture may be useful for the prevention and/or treatment of mild COVID-19 ambulatory patients, awaiting verification through a prospective randomized controlled clinical trial. [3].

Alkaloids, another class of nitrogen-containing secondary metabolites, are known to have wide pharmacological activity and have great potential for the elaborating of new drugs to treat a wide array of diseases. Recently, in silico studies showed an affinity of tested alkaloids for binding to the receptor-binding domain of the SARS-CoV-2 spike protein; hypothetically hamper it from binding to the host cell [4]. Results show that the alkaloids are interesting compounds with potential use as bioactive agents against SARS-CoV-2 [5].

Datura metel (D. Metel) is a wild-growing plant of the Solanaceae family [6]. The name *Datura* is supposed to arise from Sanskrit *Dustura or Dahatura* [7], but it is mostly known as *Angel's and Devil's trumpet, Locoweed, Jimson weed, or Datura* [8]. In Lebanon however, where it is widely distributed in several regions [9], it is known as "Jawz-

meaael". It comprises different types of phytochemicals such as flavonoids and glycosides. Its leaves and seeds are rich in alkaloids, such as atropine, scopolamine, and hyoscyamine [7]. Due to its alkaloid content, this plant was primarily used as an intoxicant and hallucinogen [8-10].

The bioactive components existing in the extracts of the *Datura's plants* have been tested for their anti-microbial and anti-fungal activities. The seed's extract contains various pharmaceutical activities such as having an anesthetic, hallucinogenic, anti-asthmatic, narcotic, bronchodilator, hypnotic, and mydriatic activities [8]. On the other hand, when mixed with mustard oil the leaves of *D. Metel* help to treat skin disorders [11]. Also, the whole plant is well known as an anticholinergic, insecticidal, herbicidal, anti-bacterial, anti-fungal, anti-inflammatory, anti-tumor, and anti-rheumatoid plant [12]. Consequently, the plant is used in different treatment regimes.

In our previous work, leaf extracts of *D. metel* were tested for phytochemical and antioxidant activities, and the study revealed that the methanolic extracts exhibit the presence of secondary metabolites. The free radical scavenging methods DPPH and reducing power assay results prove a marked antioxidant activity of the extract [13]. On the other hand, there are few reports about the volatile components of *D. metel* EO's. Consequently, the present study was carried out to investigate, for the first time, the total alkaloids contents in different plant parts and the chemical composition of essential oil from the leaves of *D. Metel L*. from the south of Lebanon.

Materials and Methods

Chemicals and Instruments

All of the chemicals used were of analytical grade. Methanol, ethanol, and acetonitrile were purchased from BDH (England). The water used in all procedures was an ultrapure one, obtained from a water purification system (TKA MICROMED, Germany). Hexane (HPLC grade) was purchased from Sigma Aldrich (USA). Samples were weighed using a RADWAG XA 82/220/2X laboratory balance. The dried leaves were grinded using a POLYMIX grind mill.

Plant Materials: Collection, Identification, and Preparation

Datura metel materials were harvested in late January – mid-February period of the year 2018, from Blida (33°08′0″N 35°31′0″E, 630 meters above sea level), South of Lebanon, and were identified by Prof. Jean HABIB, Professor of Pharmacognosy in the Faculty of Pharmacy at the Lebanese University. The samples were transported to the laboratory and kept at room temperature until processing. The harvested plant materials initially underwent natural drying (in the shade, at room temperature) for four weeks, followed by segregating the leaves from the dry plant material. Then the leaves were grinded to fine powder, using a manual grinder. Finally, the grinded materials were stored in a well-sealed container for future use.

Determination of the Total Alkaloids (Ta) Content

The total alkaloid content was determined gravimetrically, after extraction, by a selective protocol [14]. For this purpose, 15 g of lone Daturas' grinded leaves were macerated for 24 hr in methanol/acetonitrile or ethanol/acetonitrile (80/20, v/v), followed by filtering the supernatant on filter papers, then drying them under reduced pressure at 40°C. The dried extract was then washed by 25 mL of H_2SO_4 (0.2 M) and (2 x 25 mL) of $CHCl_3$. The mixture was then shaken in a separatory funnel and the upper aqueous phase was collected. The combined aqueous layers were alkalinized by NH_4OH (25 %) till pH=9-10 is reached. This was followed by extraction of the aqueous layer by (2 x 25 mL) of $CHCl_3$. The collected lower organic layers were then combined and dried by Na_2SO_4, and later concentrated under reduced pressure at 40°C. The percentage of TA content was calculated using equation (1) mentioned in 2.5.

Essential Oils (Eo) Content

Foremost, 100 g of *D. Metel* leaves were chopped into small pieces then carefully introduced into a 1000 mL round bottom flask containing 500 mL of distilled water. The hydro-distillation was carried out in a Clevenger-type distillation unit designed according to the British Pharmacopoeia specification [15]. After 3 hrs of distillation, the EO was collected in the receiver arm. The oils were sealed and kept in dark glass vials in the refrigerator at 4°C for further analysis. The percentage of EO content was calculated using equation (1) mentioned 2.5.

Determination of Percentage Yield (%)

The extraction yield was calculated according to the following equation (1):

$$\text{Yield}\,\% = \frac{W_2}{W_1} X100 \quad (1)$$

Where W_1 is the dry weight of the used matter and W_2 is the weight of collected extract after evaporation of the solvent.

Gc-Ms Analysis

One microliter of Datura EO's sample was diluted (1:100) with hexane and injected into the gas chromatographymass spectrometry (GC-MS) system. GC SHIMADZU QP2010 system was used to analyze the volatile compounds in the propolis extracts (without derivatization). DB-5MS (5 %

diphenyl / 95 % dimethylpolysiloxan) capillary column having (30 m length, 0.25 i.d., film thickness 0.28 µm) and helium as the carrier gas was used for compound separation. The oven temperature was programmed from 65°C (2 min initial time) increased to 300°C at 10°C/min (isothermal for the final time) and the MS was operated in the electron impact mode at 70 eV ion source energy. The injection volume was 1 µL and a total run of one hour is performed. Data receipt and processing were performed using Shimadzu GC-MS solution software. The compounds were identified based on a comparison of their mass spectra with data in NIST (National Institute of Standards and Technologies, Mass Spectra Libraries).

Statistical Analysis

All the determinations were conducted three times (n = 3); the statistical mean was calculated with ± SD using Excel 2013 (Microsoft Corporation, Redmond, WA, USA). The statistical analysis was performed using Statistical Package for the Social Sciences program version 23 (SPSS Inc, Chicago, USA), and P-value less than 0.05 was considered statistically significant.

Results and Discussion

Total Alkaloids

The alkaloid content was determined gravimetrically [16]. TA content (Figure 1) of *D. Metel* was found to range between 5.21.34 % (w/w) using methanol/acetonitrile solvent, and between 3.260.827 % (w/w) using ethanol/acetonitrile. TA content was higher than those cited in the literature [17] but comparable to that obtained with *D. metel* in Nigeria [18]. Those alkaloids can be used as an immensely powerful mindaltering drug [19]. It is known that the extraction solvent affects the extraction yield and biological activity [20]. Even it was in a study of the EOs, Zhen-guo's, et al. [21] research concluded that there exist great differences in constituents of volatile oil in plant and fruit of D. stramonium and should be treated differently in use. As result, methanol/acetonitrile is more efficient than ethanol/acetonitrile for the extraction of alkaloids. Besides, the content of alkaloids in fruits was the highest, followed by seeds and leaves, the least were stems.

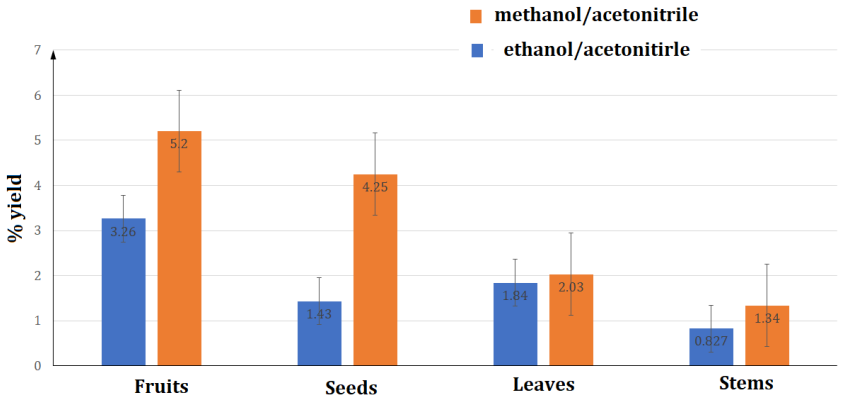

Figure 1: The total alkaloid of *D. Metel* different parts using two different solvents. Values are means ± SD of three replicates.

The mean comparison of the triplicate measurements was performed, and Table 1 below shows the significant differences between the various means.

Plant Part	Yield by solvent		SEM
	Ethanol/Acetonitrile	Methanol/Acetonitrile	
Fruits	0.0326[a,1]	0.0520[b,1]	0.006
Seeds	0.0143[a,2]	0.0425[b,1]	0.006
Leaves	0.01842	0.02032	0.006
Stems	0.00827	0.01342	0.002
SEM	0.003	0.0049	

a-b Means in a row with different alphabetical superscripts are significantly different (P < 0.05)
1-2Means in a column with different numerical superscripts are significantly different (P < 0.05)
SEM = Standard Error of Mean

Table 1: Mean comparison of the different measurements.

The results show that methanol/acetonitrile solvent is by far better than the ethanol/acetonitrile, comparing the means horizontally. Since as we can see from the above table, we are always obtaining higher yields via methanol/acetonitrile solvent. The fruit is the plant part that contains a higher concentration of Alkaloids (higher yield) comparing the means vertically. So, it is better to use methanol/acetonitrile as a solvent for a significantly higher yield, and the parts containing the highest Alkaloid content was the fruit.

Essential Oil

The essential oils were obtained by hydro-distillation of the air-dried, pulverized leaves of *D. metel.* The hydro-distillation method offered a 0.35 % yield in EOs calculated on a dry weight basis (equation 1). The obtained oils have a light yellow color and a significant aromatic odor. Studies on the EOs from *D. metel* are very limited except for a single study on the cytotoxic activity [22]. *D. stramonium*, per contra, have been reported to display allelopathic, antibacterial, insecticidal, and antifungal effects [23]. Despite the rare studies on the EO's of Datura families, our results were consistent with this obtained during the study of *D. stramonium* in the work of Aboluwodi A, et al. [23].

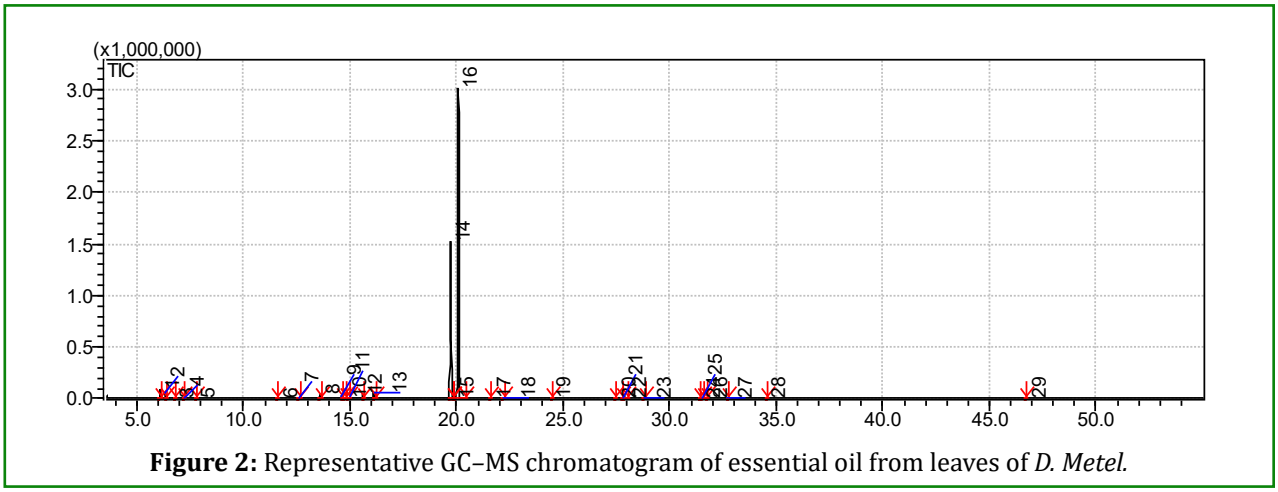

Figure 2: Representative GC–MS chromatogram of essential oil from leaves of *D. Metel.*

Compound	RT (min)	MW (g.mol-1)	Formula	% Area	Family
3-pentene-2-one	12.6	84	C_5H_8O	0.12	Alkane
camphor	13.583	152	$C_{10}H_{16}O$	0.63	Cyclic Monoterpene
carvomenthol	14.992	154	$C_{10}H_{18}O$	0.51	Terpenene
α-terpineol	15.617	154	$C_{10}H_{18}O$	0.65	Monoterpene -Alcohol
D-verbenone	16.125	150	$C_{10}H_{14}O$	1.04	Terpene
carvacrol	19.717	150	$C_{10}H_{14}O$	30.15	Monoterpene
thymol	20.083	150	$C_{10}H_{14}O$	60.33	Monoterpene

Table 2: GC-MS analysis of *D. Metel* leaves essential oil.

The EO of *D. Metel* was analyzed by GC–MS (Figure 2) to detect volatile, small, and non-thermolabile metabolites. The Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library. According to the % area obtained (Table 2), the main compounds of this oil are the natural terpenoid thymol and its phenol isomer carvacrol and also in small quantities D-verbenone (1.04 %), α-terpineol (0.65 %), camphor (0.63 %), carvomenthol (0.51 %) and 3pentene-2-one (0.12 %).

The phytochemical constituents of plants, known to be bioactive ingredients, are of great significance. They are responsible for different medicinal activities such as being antimicrobial, antioxidant, antifungal, anticancer, and antidiabetic [24]. Figure 3 shows the chemical structures of the main compounds of D. metel EO. Thymol and carvacrol, the major of the studied oil its use are known to possess antioxidative, antimicrobial, antitussive, expectorant, antispasmodic, and antibacterial effects [25-28]. The presence of thymol and carvacrol as the main components of the studied oil D. metel from Lebanon and absence of it in previous few works [22,23,29] that dealt with different Datura species is characteristic. The investigation of

the volatile compounds in Aronia berry juice allows the detection of 3-penten-2-one as the most abundant [30]. Since it has a broad spectrum of biological utilization as an antioxidant anticancer anticonvulsant antiulcer antihypertensive antinociceptive, α-terpineol brings a great interest [31]. Likewise, camphor exhibits several biological properties such as antimicrobial, antiviral, and antitussive effects [32]. On the other hand, EOs containing verbenone was reported to have anti-inflammatory effects [33].

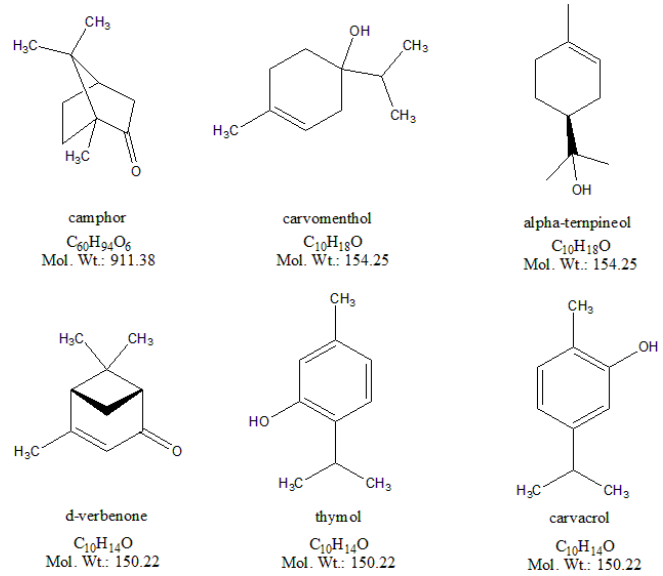

Figure 3: Chemical structures of terpenes founded in *D. Metel* EO's.

Juan Xue, et al. [22] studied the chemical composition and cytotoxicity of the EO from different parts of D. metel. The main components were summarized in Table 3. Clear contrast between these compounds was the main feature of the results. This contrast extends to studies conducted by other researchers, monoterpenoids were found to be the dominant class of compounds [29] in the leaf oil (100 %), flower oil (81.4 %), and fruit oils (58.8 %) of *D. metel.*

Plant parts	Main compounds
Flowers	ketone (23.61%), ethyl palmitate (15.84%).
Leaves	ketone (18.84%), phytol (18.71%)
Petioles	Ketone (39.45%), phytol (31.32%)
Seeds	Palmitic acid (30.60%), ethyl linoleate (21.56%)
Roots	palmitic acid (52.61%)
Stems	palmitic acid (38.38%), ethyl linoleate (17.38%).

Table 3: Main compounds in the EO's of *D. Metel* [22].

While there is an absence of adequate studies related to EO's of *D. metel*, we call for a second widespread species D. stramonium. Previous research in this field shows a great variation in results. While the main components of EO of D. stramonium were sterols and their derivatives in the study of You, et al. [34] and leading to the identification of 44 compounds, representing 88.72% of the total mass, Zhen-guo, et al. [21,35] have identified 58 compounds and cocolactone as the main compounds. Whereas, other works there exist great differences in constituents of volatile oil in plant and fruit of D. stramonium and should be treated differently in use [21]. In the work of Hao-xiang a total of 21 compounds were separated and 18 compounds (91.26% of the total peak areas) were identified from the EO of D. Stramonium [36]. Moreover, citral (26.5%), 4, 8-dimethyl-3,8-dien-2-one (11.2%), sesquirosefuran (11.1%) and geraniol (10.5%) were identified as the major constituents in the seed oil of D. stramonium [23].

All of the above findings, in addition to the results of the conducted previous work [13], confirm the importance of *D. Metel,* and provide insights for further exploitation of this plant for therapeutic purposes.

Conclusion

To the best of our knowledge, we have presented herein the first attempt to study the EO of *"Datura metel"* growing in Lebanon, as well as the determination of the total alkaloids contents in the different parts of the plant. The present study revealed that methanolic/acetonitrile selective extracts of *D. metel* exhibit high efficiency in the extraction of total alkaloids with significant difference ($P<0.05$). The total alkaloids from different parts of the plant was also investigated, it ranged from 0.83 to 5.2 %. Compared with other parts, the content of alkaloids in fruits was the highest, followed by seeds and leaves, the least were stems. Moreover, 22 volatile compounds were detected in the EO, and 7 of them (93.43 % of the total peak areas) were identified. These results suggest that the EO of *D. metel* plants studied is a promising source of bioactive phytochemicals. However, further investigations are a must to continue the identification of unknown compounds as well as to evaluate the biological activities of this plant.

Acknowledgment

The authors would like to thank the Lebanese University, Faculty of Pharmacy, Lebanon for providing all chemicals necessary to carry out this project. The authors would also like to thank the Lebanese Agricultural Research Institute for making possible the GC/MS analysis. A special thanks to Mm. Roula Matta for providing valuable suggestions.

Conflict of Interest

No conflict of interest was declared by the authors.

References

1. Bayala B, Bassole IH, Scifo R, Gnoula C, Morel L, et al. (2014) Anticancer activity of essential oils and their chemical components - a review. Am J Cancer Res 4(6): 591-607.

2. Sylvestre M, Pichette A, Longtin A, Nagau F, Legault J, et al. (2006) Essential oil analysis and anticancer activity of leaf essential oil of Croton flavens L. from Guadeloupe. J Ethnopharmacol 103(1): 99-102.

3. Lionis C, Karakasiliotis I, Petelos E, Linardakis M, Diamantakis A, et al. (2021) A mixture of essential oils from three Cretan Aromatic Plants (thyme, Greek sage and Cretan dittany, CAPeo) inhibits SASR-CoV-2 proliferation: in vitro evidence and a Proof-of-Concept intervention study in mild ambulatory COVID-19-positive patients. medRxiv 2021.01.11.20248947.

4. Fielding BC, Filho CDSMB, Ismail NSM, Sousa DP (2020) Alkaloids: Therapeutic Potential against Human Coronaviruses. Molecules 25(23): 5496.

5. Suryawanshi SS, Patil RS, Jayannache PB, Palled MS, Alegaon SG, et al. (2020) Screening and assessment of selected alkaloids as potential inhibitors of COVID-19 protease enzyme. J Glob Trends Pharm Sci 11(2): 7711-7718.

6. Tijani AA, Eyineyi UG, Ibrahim JA, Okhale SE (2015) Neuro-Toxicological Impacts of Datura Metel Linn. (Family: Solanaceae) Leaves Extract in Mice. J Neurobehav Sci 2(3): 97-101.

7. Deepa M, Sugitha N, Mythili S, Sathiavelu A (2014) Antioxidant activity and Phytochemical Analysis of Datura metel. Int J Drug Dev & Res 6(4): 280-258.

8. Okwu DE, Igara EC (2009) Isolation, characterization and antibacterial activity of alkaloid from Datura metel Linn leaves. Afr J Pharm Pharmacol 3(5): 277-281.

9. Tohme G, Tohme H (2014) Illustrated Flora of Lebanon. National Council for Scientific Research. WorldCat.

10. Sanni DM, Omotoyinbo OV (2016) Inhibitory Effect of Extracts from Datura metel Leaf on Mushroom Tyrosinase. Am J Life Sci 4(2): 47-50.

11. Sayyed A, Shah M (2014) Phytochemistry, pharmacological and traditional uses of Datura stramonium L. review. Journal of pharmacognosie and phytochemistry 2(5): 123-125.

12. Alabri THA, Musalami AHSA, Hossain MA, Weli AM, Al-Riyami Q, et al. (2014) Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of Datura metel L. J King Saud Univ - Sci 26(3): 237-243.

13. Jaber A, Edmond C, Ibrahim G, Lamis AH (2019) Phytochemical study and antioxidant activity of extract from the leaves of lebanese datura metel l. Eur J Pharm Med Res 6(8): 65-71.

14. Bazaoui AEI, Stambouli H, Bellimam MA, Soulaymani A (2009) Determination des alcaloïdes tropaniques des graines du Datura stramonium L. par CPG/SM et CL/SM. Ann Toxicol Anal 21(4): 183-188.

15. (1980) British pharmacopoeia. Her Majesty's Stationery Office, Atlantic House, Holborn Viaduct. London.

16. Rathva B, Patel B, Maurya J, Bera K (2017) Standardization and evaluation of some parameters of adaptogenic polyherbal oral dosage form. Int J Pharm Sci Rev Res 42(1): 1-7.

17. Benitez G, Salas MM, Kamel AV, Jimenez UC, Hernandez J, et al. (2018) The genus *Datura L*. (Solanaceae) in Mexico and Spain – Ethnobotanical perspective at the interface of medical and illicit uses. J Ethnopharmacol 219(7): 133-151.

18. Nuhu H, Ghani A (2002) Alkaloid content of the leaves of three nbigerian datura species. Niger J Nat Prod Med 6(2002): 15-18.

19. Berkov S, Zayed R, Doncheva T (2006) Alkaloid patterns in some varieties of Datura stramonium. Fitoterapia 77(3): 179-182.

20. Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, et al. (2019) Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and In vitro anti-inflammatory activities of Severinia buxifolia. In: J Food Qual 2019: 108178294.

21. Zhen JG (2008) A comparative analysis on chemical components of volatile oil in plant and fruit of Datura stramonium L. Journal of Shangluo University.

22. Xue J, Sun Y, Wei Q, Wang C, Yang B, et al. (2016) Chemical composition and cytotoxicity of the essential oil from different parts of Datura metel L. Nat Prod Res 30(17): 1938-1940.

23. Aboluwodi AS, Avoseh ON, Lawal OA, Giwa AA (2017) Chemical constituents and anti-inflammatory activity of

essential oils of Datura stramonium L. JMPS 5(1): 21-25.

24. Hossain MA, Nagooru MR (2011) Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant Corydyline terminalis L. Kunth. Pharmacogn J 3(24): 25-30.

25. Youdim KA, Dorman HJD, Deans SG (1999) The antioxidant effectiveness of thyme oil, α-tocopherol and ascorbyl palmitate on evening primrose oil oxidation. J Essent Oil Res 11(5): 643-648.

26. Hoferl M, Buchbauer G, Jirovetz L, Schmidt E, Stoyanova A, et al. (2009) Correlation of antimicrobial activities of various essential oils and their main aromatic volatile constituents. J Essent Oil Res 21(5): 459-463.

27. Dorman HJD, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 88(2): 308-316.

28. Barnes J, Anderson LA, Phillipson JD (2007) Herbal Medicines. Pharmaceutical Press.

29. Essien E, Walker TM, Ogunwande LA, Bansal A, Setzer W, et al. (2010) Essential oil composition, cytotoxicity and antimicrobial activities of *Datura metel L*. from Nigeria. Int J Essent Oil Ther 4(2010): 69-72.

30. Kraujalyte V, Leitner E, Venskutonis PR (2013) Characterization of Aronia melanocarpa volatiles by Headspace-Solid-Phase Microextraction (HS-SPME), Simultaneous Distillation/Extraction (SDE), and Gas Chromatography-Olfactometry (GC-O) methods. J Agric Food Chem 61(20): 4728-4736.

31. Khaleel C, Tabanca N, Buchbauer G (2018) α-Terpineol, a natural monoterpene: A review of its biological properties. Open Chem 16(1): 349-361.

32. Chen W, Vermaak I, Viljoen A (2013) Camphor—a fumigant during the black death and a coveted fragrant wood in ancient Egypt and Babylon—A Review. Molecules 18(5): 5434-5454.

33. Kuo CF, Su JD, Chiu CH, Peng CC, Chang CH, et al. (2011) Anti-Inflammatory effects of supercritical carbon dioxide extract and its isolated carnosic acid from Rosmarinus officinalis leaves. J Agric Food Chem 59(8): 3674-3685.

34. You LX, Wang SJ (2011) Chemical composition and allelopathic potential of the essential oil from *Datura Stramonium L*. In: Adv Mater Res 233(235): 2472-2475.

35. Jin Z, Su Z, Ren Y, Li B (2010) Chemical components of volatile oil in Datura stramonium with GC-MS. In: Eur PMC 11: 1905-1908.

36. Yu H, Yu J (2011) Extraction and determination of volatile constituents from Leaves in Datura stramonium. Journal of Mountain Agriculture and Biology.