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Original Article

# Spectral analysis and antibacterial effect of cold methanolic extract of *Artemisia absinthium* L.

## Análise espectral e efeito antibacteriano do extrato metanólico frio de *Artemisia absinthium* L.

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**Abstract**

The study aimed to determine the bioactive components and antibacterial activities of cold methanolic extract leaves (CMMEL) of *Artemisia absinthium* L. CMMEL was tested for phytochemicals, GC-MS analyses was performed to identify the bioactive components, and anti-bacterial properties. The phytochemical analysis of CMMEL revealed the presence of carbohydrates, steroids, saponins, and amino acids. GC-MS analysis of CMMEL of *A. absinthium* L. revealed several unique bioactive compounds, including margaspidin, stigmasterol, octadecanoic acid, hexadecanoic, corymbolone, and bicyclo [2.2.1] heptan-2. The antibacterial spectrum of CMMEL can be sequenced as *Streptococcus pyogenes* (8.83 ± 1.8 mm) > *Escherichia coli* (7.6 ± 0.6 mm) > *Bacillus subtilis* (6.6 ± 1.6 mm) > *Klebsiella pneumoniae* (6.5 ± 0.3 mm) > *Pseudomonas aeruginosa* (6.1 ± 1.1 mm) > *Staphylococcus aureus* (5.23 ± 0.8 mm). Although the CMMEL of *A. absinthium* L. showed the presence of many bioactive compounds but did not substantially inhibit the growth of Gram-positive or Gram-negative bacteria, according to the findings.

**Keywords:** herbal extracts, *Artemisia absinthium*, maceration extractions, phytoconstituents, antibacterial activity.

**Resumo**

O estudo teve como objetivo determinar os componentes bioativos e atividades antibacterianas de folhas de extrato metanólico frio (CMMEL) de *Artemisia absinthium* L. CMMEL foi testado para fitoquímicos, análises GC-MS foram realizadas para identificar os componentes bioativos e propriedades antibacterianas. A análise fitoquímica da CMMEL revelou a presença de carboidratos, esteroides, saponinas e aminoácidos. A análise GC-MS de CMMEL de *A. absinthium* L. revelou vários compostos bioativos exclusivos, incluindo margaspidina, estigmasterol, ácido octadecanoico, hexadecanoico, corimbolona e biciclo [2.2.1] heptan-2. O espectro antibacteriano de CMMEL pode ser sequenciado como *Streptococcus pyogenes* (8,83 ± 1,8 mm) > *Escherichia coli* (7,6 ± 0,6 mm) > *Bacillus subtilis* (6,6 ± 1,6 mm) > *Klebsiella pneumoniae* (6,5 ± 0,3 mm) > *Pseudomonas aeruginosa* (6,1 ± 1,1 mm) > *Staphylococcus aureus* (5,23 ± 0,8 mm). Embora o CMMEL de *A. absinthium* L. tenha mostrado a presença de muitos compostos bioativos, mas não inibiu substancialmente o crescimento de bactérias gram-positivas ou gram-negativas, de acordo com os achados.

**Palavras-chave:** extratos de ervas, artemísia absinto, extrações de maceração, fitoconstituintes, atividade antibacteriana.

## 1. Introduction

The genus Artemisia contains several important medicinal plants that have sparked interest in phytochemical research to understand their medicinal properties better, as well as in the production of aromatic oils (Sultan et al., 2020; Liang et al., 2018; Guetat et al., 2017; Ornano et al., 2016b; Ornano et al., 2016a; Abolaji et al., 2014; Petretto et al., 2013). *A. absinthium* L. (Asteraceae), commonly known as wormwood. *A. absinthium* L. (Asteraceae's aerial parts), have been reported for the presence of fixed oil, which has medicinal values (Mohammed et al., 2016). The plant *A. absinthium* L. has traditionally been used in the Arabic system of medicine and is widespread in Saudi Arabia (Nigam et al., 2019). *A. absinthium* L. is a medicinal remedy used in the Arabic region, Europe, North Africa, and Asia (Pan et al., 2013). Absinthe has been reported as a major compound in *A. absinthium* L. (Farukh et al., 2012). Based on several studies on the medicinal value of *A. absinthium* L. for various ailments (Bora and Sharma, 2011) and this research was performed to demonstrate the phytochemical composition and antibacterial potency of cold methanolic extract of leaves (CMMEL) of *A. absinthium* L. of Saudi Arabia, as a continuation of previous work (Sultan et al., 2020).

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## 2. Experimental

### 2.1. Plant material and processing

Fresh *A. absinthium* L. was obtained from a local market in the Jazan region of Saudi Arabia, packed and immediately transported to the laboratory. All the adhesive impurities were removed by extensive washing with tap water. A sample of the plant was registered and deposited by a taxonomist at the Herbarium of Jazan University (JAZUH 1620). The plants were further rinsed with Millipore water and left to dry for 30 min. Next, the dried plant material was spread over a thin sheet of polyethylene and ground in a ventilated room. Powdered plant material were collected and stored properly for further analysis.

### 2.2. Extraction procedure

The bioactive components of the plant were extracted by cold methanol maceration. Fresh leaves of *A. absinthium* L. (25 g) was soaked in 50 mL methanol. Due to the increasing fragrance emitted daily, the soaking process was extended up to 10 d, which was observed to be at its maximum on the 8$^{th}$ day and sustained until the 10$^{th}$ day. The macerated liquid was centrifuged using a Sigma table-top centrifuge at 2000 × g for 10 min. The supernatant solution was passed through filter paper (Whatman, no. 1), and the resulting extract was air-dried at 25 °C. Using GC-MS analysis, the phytochemical constituents in the air-dried samples were analysed and used for further antibacterial studies.

### 2.3. Phytochemical tests

The air-dried extracts were subjected to qualitative testing in accordance with the standard protocol for the identification of various phytochemical components (Moni et al., 2018).

### 2.4. GC-MS analysis

The presence of essential bioactive components in CMMEL of *A. absinthium* L. was evaluated using GC-MS (Thermo Scientific GC-MS-AS 3000 autosampler - ISQ detector). The powdered sample was diluted in methanol, and 2 µL of the solution was injected into a TR 5MS capillary column for partial separation of the bioactive components. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. Mass spectrophotometry was performed, and spectral analysis was performed using Xcalibur software. The mass spectra were interpreted using the NIST and MAINLIB software libraries.

### 2.5. Antibacterial studies

#### 2.5.1. Type of bacteria and cultures used

*Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were the bacteria used in this research. A 24 h culture was prepared and standardized using a nutrient broth gradient dilution of $10^{-1}$ to $10^{-7}$. The colony forming unit in 1 mL (CFU/mL) was used to calculate the viability of the bacterial culture.

#### 2.5.2. Antibacterial susceptibility study

Bacterial subcultures were created from the stock culture, and the culture was ready to be tested for antibacterial activity after 24 hours of incubation. Agar well diffusion method was employed for sample analytes and disc diffusion method was used for the standard ciprofloxacin disc (5 µg/disc). The agar plates were inoculated by immersing a sterile cotton swab into the standardized (CFU/mL) culture individually with different organisms and spreading a quantity of microbial inoculum over the MH agar plate surfaces with the spinning of the petri dish to assure an equal distribution of the liquid culture. After allowing the plates to dry for 10 min, the sample analytes were administered; and holes were punched in inoculated MH agar plates using a standard sterile stainless-steel borer. The plates were incubated for 24 hours at 37° C, and the antibacterial spectrum was assessed by the formation of inhibitory zones around the wells. The Kirby–Bauer method was used with the regular ciprofloxacin disc (5µg/disc). The method was conducted as mentioned previously in the agar well diffusion technique by placing the ciprofloxacin disc (5µg/disc) on the agar surface rather than punching the well. Plates were incubated at 37° C for 24 h, and the spectrum of activity was quantified by the inhibition zone around the disc.

### 2.6. Statistical analysis

Statistical analysis was performed by using Graph pad Prism software (Version 8.3.1), USA through one-way analysis of variance (ANOVA), followed by Dunnett multiple comparison test as a post-hoc test.

## 3. Results and Discussion

In this study, many compounds were identified in the CMMEL of *A. absinthium* L., an aromatic, small perennial shrub. The qualitative phytochemical analysis of CMMEL of *A. absinthium* L. is depicted in Table 1. The qualitative analysis showed the presence of carbohydrates, steroids, saponins, and amino acids. Figure 1 represents GC-MS chromatogram of CMMEL of *A. absinthium* L. depicts various unique bioactive constituents. The bioactive compounds, their molecular formula, and retention

**Table 1.** Phytochemical analysis of methanolic cold maceration extraction of *Artemisia absinthium* L.

| Phytochemicals | Observations |
|---|---|
| Carbohydrate | Present |
| Proteins | Absent |
| Alkaloids | Absent |
| Tannins | Absent |
| Steroids | Present |
| Saponins | Present |
| Amino acids | Present |
| Flavonoids | Absent |

time were summarized in Table 2, and the structure of bioactive compounds was illustrated in Figure 2. In this study margaspidin a phloroglucinol derivative was exhibited a maximum retention time 65.63 min. Margaspidin, a phloroglucinol derivative, has been detected as a major bioactive compound in this study. An earlier study reported that margaspidin showed anti-cancer properties *in vitro* (Kapadia et al., 1996). Stigmasterol is an unsaturated phytosterol indicating a good retention time of 59.12 min. Stigmasterol has been shown to have a significant anti-osteoarthritic effect via inhibition of pro-inflammatory mediators (Gabay et al., 2010). Recently, stigmasterol has been reported to play

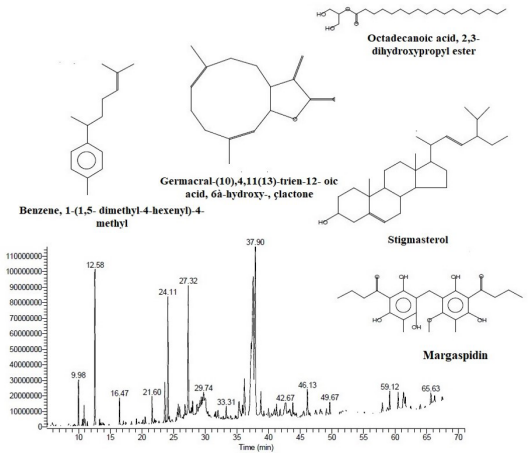


**Figure 1.** GC-MS chromatogram of cold methanolic extract of the leaves of *Artemisia absinthium* L.

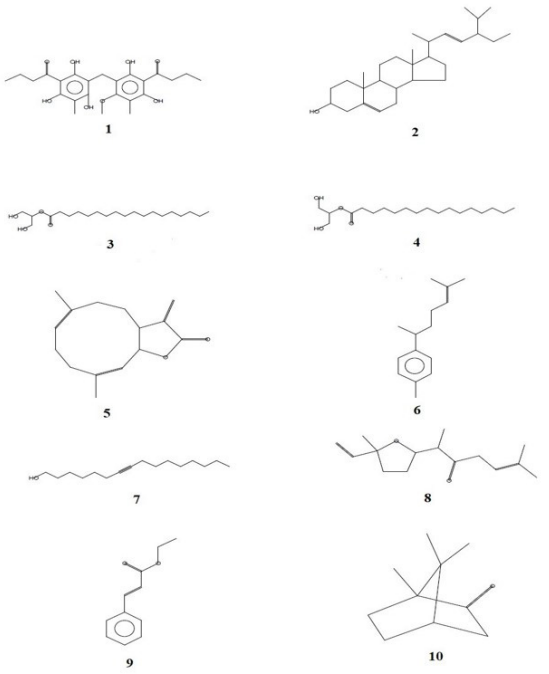


**Figure 2.** GC-MS detection of bioactive compounds of cold methanolic extract of the leaves of *Artemisia absinthium* L. (1) Margaspidin; (2) Stigmasterol; (3) Octadecanoic acid, 2,3-dihydroxypropyl ester; (4) Hexadecanoic acid, 2,3-dihydroxypropyl ester; (5) Germacra1(10),4,11(13)-trien-12- oic acid, 6à-hydroxy-, çlactone, (E,E); (6) Benzene, 1-(1,5- dimethyl-4-hexenyl)-4-methyl; (7) 7-Hexadecyn-1-ol; (8) 5-Hepten-3-one, 2-(5-ethenyltetrahydro-5- methyl-2-furanyl)-6- methyl- (9) 2-Propenoic acid, 3- phenyl-, ethyl ester (10) Bicyclo[2.2.1]heptan-2- one, 1,7,7-trimethyl-, (1R)-.

**Table 2.** GC-MS detection of bioactive compounds of CMMEL of *Artemisia absinthium* L.

| Sample ID | Bioactive compound | Molecular formula | Retention time (Minutes) | Molecular weight |
|---|---|---|---|---|
| 1 | Margaspidin | $C_{24}H_{30}O_8$ | 65.63 | 446 |
| 2 | Stigmasterol | $C_{29}H_{48}O$ | 59.12 | 412 |
| 3 | Octadecanoic acid, 2,3- dihydroxypropyl ester | $C_{21}H_{42}O_4$ | 49.67 | 358 |
| 4 | Hexadecanoic acid, 2,3- dihydroxypropyl ester | $C_{19}H_{38}O_4$ | 46.13 | 330 |
| 5 | Germacral-(10),4,11(13)-trien-12- oic acid, 6à-hydroxy-, çlactone, (E,E) | $C_{15}H_{20}O_2$ | 38.79 | 236 |
| 6 | Benzene, 1-(1,5- dimethyl-4-hexenyl)-4-methyl | $C_{15}H_{22}$ | 33.31 | 201 |
| 7 | 7-Hexadecyn-1-ol | $C_{16}H_{30}O$ | 27.32 | 238 |
| 8 | 5-Hepten-3-one, 2-(5-ethenyltetrahydro-5- methyl-2-furanyl)-6-methyl-, [2S- [2à(R*),5à]]- | $C_{15}H_{24}O_2$ | 24.11 | 236 |
| 9 | 2-Propenoic acid, 3- phenyl-, ethyl ester | $C_{11}H_{12}O_2$ | 21.60 | 176 |
| 10 | Bicyclo[2.2.1]heptan-2- one, 1,7,7-trimethyl-, (1R)- | $C_{10}H_{16}O$ | 12.58 | 142 |

**Table 3.** The antibacterial studies of CMMEL of *Artemisia absinthium* L.

| Bacterial organisms | Concentration (CFU/mL) | Zone of inhibition (mm) | |
|---|---|---|---|
| | | CMMEL* | Ciprofloxacin disc (5μg/disc) |
| *Staphylococcus aureus* | $2 \times 10^{-6}$ | 5.23 ± 0.8 | 27.3 ± 0.8 |
| *Streptococcus pyogenes* | $3 \times 10^{-7}$ | 8.83 ± 1.8 | 26.6 ± 0.8 |
| *Bacillus subtilis* | $3 \times 10^{-7}$ | 6.6 ± 1.6 | 28.6 ± 2.1 |
| *Escherichia coli* | $2 \times 10^{-7}$ | 7.6 ± 0.6 | 31.8 ± 2.2 |
| *Klebsiella pneumoniae* | $3\times 10^{-5}$ | 6.5 ± 0.3 | 28.6 ± 2 |
| *Pseudomonas aeruginosa* | $4 \times 10^{-6}$ | 6.1 ± 1.1 | 27 ± 0.9 |

Each value is the mean of $n$=3 batches with standard deviation by performing Dunnett multiple comparison test, (post hoc test). All the values of CMMEL were significantly lesser than standard ciprofloxacin disc at $p < 0.05$. CFU: colony forming unit.*CMMEL: Cold methanolic maceration extract of the leaves of *Artemisia abysinthium* L.

a role in lipid metabolism and to have a more potent cholesterol-lowering effect (Feng et al., 2018). In the present study, octadecanoic acid- 2,3- dihydroxypropyl ester and hexadecanoic acid- 2- hydroxy-1- (hydroxymethyl) ethyl ester, were identified in the CMMEL of *A. absinthium* L., exhibited unique retention time. Octadecanoic acid, 2,3-dihydroxypropyl ester is a carboxylic ester derivative of fatty acid showed a good retention time of 49.67 min followed by hexadecanoic acid- 2- hydroxy-1- (hydroxymethyl) ethyl ester is otherwise called 2-Palmitoylglycerol which is a derivative of hexadonic acid exhibited the retention time of 46.31 min. It has been reported that octadecanoic acid- 2, 3-dihydroxypropyl ester exerts numerous pharmacological effects such as antioxidant, hepatoprotective, anti-eczemic, hypocholesterolemic, and antihistaminic properties (Fadeyi et al., 2015). Hexadecanoic acid- 2- hydroxy-1- (hydroxymethyl) ethyl ester possesses antioxidant, hypocholesterolemic, nematocidal and anti-androgenic properties (Tulika and Mala, 2017). Germacral-(10),4,11(13)-trien-12-oic acid, is a sesquiterpenoid called germacrene A acid was showing 38.79 min. Corymbolone is a eudesmane sesquiterpenoid identified in the CMMEL of *A. absinthium* L., exhibiting a retention time of 37.90 min. Interestingly benzene was present in CMMEL that showed a retention time of 33. 31 min. 7-Hexadecyn-1-ol is otherwise called as 7-hexadecenol have been observed in CMMEL of *A. absinthium* L. with a retention time of 27.32 min. 5-Hepten-3-one and 2-Propenoic acid, 3- phenyl-, ethyl ester exhibited fair retention times of 24.11 and 21.60 min, respectively. Bicyclo[2.2.1]heptan-2- one, 1,7,7-trimethyl-, the least retention time among the various bioactive compounds present in CMMEL of *A. absinthium* L. 12.58 min. The presence of terpineol in the essential oil of *A. absinthium* L. has been reported (Mohammed, 2022; Pan et al., 2013).

Bicyclo[2.2.1]heptan-2-one was present in the CMMEL of *A. absinthium* L. and reported as a good fragrance agent (Krishnamoorthy et al., 2018). Similar results have been obtained in studies on the chemical composition of *Artemisia vulgaris* L. (Kumar et al., 2015). In 2013, germacrene was reported for antibacterial effect (Mora et al., 2013). Corymbolone have been reported as an antifungal agent (Shareef et al., 2016). Many compounds were recognized in the CMMEL of *A. absinthium* L. but they did not produce significant antibacterial effects against the selected human pathogenic bacteria even though terpineol possesses antimicrobial properties. Table 3 is the representation and self-exemplary of the antibacterial spectrum of CMMEL of *A. absinthium* L. The study suggested the mild antibacterial effect showed by CMMEL of *A. absinthium* L., which had a significantly lesser effect than standard ciprofloxacin disc against screened a set of Gram-positive and Gram-negative bacteria. The present study results suggest that CMMEL of *A. absinthium* L. contains a lesser spectrum antibacterial component. However, further studies are under process to isolate the compound and to analyze the pharmaceutical significance.

## 4. Conclusion

The natural world provides a rich diversity of plants, many of which have significant potential in the pharmaceutical industry. The Kingdom of Saudi Arabia is home to a variety of rare plants that have been included into traditional local treatments. The study demonstrated that the leaves part of *A absinthium* L. contain many bioactive molecules but have less antibacterial potential.

## References

ABOLAJI, A.O., ETENG, M.U., EBONG, P.E., DAR, A., FAROMBI, E.O. and CHOUDHARY, M.I., 2014. Artemisia annua as a possible contraceptive agent: a clue from mammalian rat model. *Natural Product Research*, vol. 28, no. 24, pp. 2342-2346. http://dx.doi.org/10.1080/14786419.2014.936016. PMid:25022560.

BORA, K.S. and SHARMA, A., 2011. The genus Artemisia: a comprehensive review. *Pharmaceutical Biology*, vol. 49, no. 1, pp. 101-109. http://dx.doi.org/10.3109/13880209.2010.497815. PMid:20681755.

FADEYI, O.E., OLATUNJI, G.A. and OGUNDELE, V.A., 2015. Isolation and characterization of the chemical constituents of Anacardium occidentale cracked bark. *Natural Products Chemistry & Research*, vol. 3, no. 5, p. 1000192. http://dx.doi.org/10.4172/2329-6836.1000192.

FENG, S., DAI, Z., LIU, A.B., HUANG, J., NARSIPUR, N., GUO, G., KONG, B., REUHL, K., LU, W., LUO, Z. and YANG, C.S., 2018. Intake of stigmasterol and β-sitosterol alters lipid metabolism and alleviates NAFLD in mice fed a high-fat Western-style diet.

*Biochimica et Biophysica Acta. Molecular and Cell Biology of Lipids*, vol. 1863, no. 10, pp. 1274-1284. http://dx.doi.org/10.1016/j.bbalip.2018.08.004. PMid:30305244.

GABAY, O., SANCHEZ, C., SALVAT, C., CHEVY, F., BRETON, M., NOURISSAT, G., WOLF, C., JACQUES, C. and BERENBAUM, F., 2010. Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. *Osteoarthritis and Cartilage*, vol. 18, no. 1, pp. 106-116. http://dx.doi.org/10.1016/j.joca.2009.08.019. PMid:19786147.

GUETAT, A., AL-GHAMDI, F.A. and OSMAN, A.K., 2017. The genus Artemisia L. in the northern region of Saudi Arabia: essential oil variability and antibacterial activities. *Natural Product Research*, vol. 31, no. 5, pp. 598-603. http://dx.doi.org/10.1080/14786419.2016.1207071. PMid:27546287.

KAPADIA, G.J., TOKUDA, H., KONOSHIMA, T., TAKASAKI, M., TAKAYASU, J. and NISHINO, H., 1996. Anti-tumor promoting activity of Dryopteris phlorophenone derivatives. *Cancer Letters*, vol. 105, no. 2, pp. 161-165. http://dx.doi.org/10.1016/0304-3835(96)04275-9. PMid:8697439.

KRISHNAMOORTHY, C., UMA, S.K., JADHAV, P.P., GHAZAL, K. and CHIDAMBARAM, R., 2018. Gas chromatography/mass spectroscopy analysis of phytochemicals present in orange peel powder and in bread prepared using it. *Asian Journal of Chemistry*, vol. 30, no. 7, pp. 1599-1602. http://dx.doi.org/10.14233/ajchem.2018.21268.

LIANG, J.-Y., GUO, S.-S., ZHANG, W.-J., GENG, Z.-F., DENG, Z.-W., DU, S.-S. and ZHANG, J., 2018. Fumigant and repellent activities of essential oil extracted from *Artemisia dubia* and its main compounds against two stored product pests. *Natural Product Research*, vol. 32, no. 10, pp. 1234-1238. http://dx.doi.org/10.1080/14786419.2017.1331227. PMid:28532258.

MOHAMMED, H.A., 2022. Phytochemical analysis, antioxidant potential, and cytotoxicity evaluation of traditionally used *Artemisia absinthium* L. (Wormwood) growing in the central region of Saudi Arabia. *Plants*, vol. 11, no. 8, p. 1028. http://dx.doi.org/10.3390/plants11081028. PMid:35448756.

MOHAMMED, M.S., SIVAKUMAR, S.M., AAMENA, J., FOZIYAH, Z., FARAH, I., BAGUL, U.S., SANOBAR, S., TARIQUE, A., RAHIMULLAH, S., ABDUL, H.S.M., MOHAMED, E.E., BARIK, B.B. and MOHAMMAD, F.A., 2016. Therapeutic potential of chitosan nanoparticles as antibiotic delivery system: challenges to treat multiple drug resistance. *Asian Journal of Pharmaceutics*, vol. 10, pp. S61-S66.

MONI, S.S., ALAM, M.F., SAFHI, M.M., JABEEN, A., SANOBAR, S., SIDDIQUI, R. and MOOCHIKKAL, R., 2018. Potency of nano-antibacterial formulation from *Sargassum binderi* against selected human pathogenic bacteria. *Brazilian Journal of Pharmaceutical Sciences*, vol. 54, no. 4, p. e17811. http://dx.doi.org/10.1590/s2175-97902018000417811.

MORA, F.D., ALPAN, L., TOMMASI, N., MCCRACKEN, V.J. and NIETO, M., 2013. New antibacterial germacrene from *Verbesina negrensis. Planta Medica*, vol. 79, no. 8, pp. 707-710. http://dx.doi.org/10.1055/s-0032-1328542. PMid:23670623.

NIGAM, M., ATANASSOVA, M., MISHRA, A.P., PEZZANI, R., DEVKOTA, H.P., PLYGUN, S., SALEHI, B., SETZER, W.N. and SHARIFI-RAD, J., 2019. Bioactive compounds and health benefits of Artemisia species. *Natural Product Communications*, vol. 14, no. 7, pp. 1-17. http://dx.doi.org/10.1177/1934578X19850354.

ORNANO, L., VENDITTI, A., BALLERO, M., SANNA, C., DONNO, Y., QUASSINTI, L., BRAMUCCI, M., VITALI, L.A., PETRELLI, D., TIRILLINI, B., PAPA, F., MAGGI, F. and BIANCO, A., 2016a. Essential oil composition and biological activity from *Artemisia caerulescens* subsp. densiflora (Viv.) Gamisans ex Kerguélen & Lambinon (Asteraceae), an endemic species in the habitat of La Maddalena Archipelago. *Natural Product Research*, vol. 30, no. 16, pp. 1802-1809. http://dx.doi.org/10.1080/14786419.2015.1079190. PMid:26343516.

ORNANO, L., VENDITTI, A., DONNO, Y., SANNA, C., BALLERO, M. and BIANCO, A., 2016b. Phytochemical analysis of non-volatile fraction of *Artemisia caerulescens* subsp. densiflora (Viv.) (Asteraceae), an endemic species of La Maddalena Archipelago (Sardinia–Italy). *Natural Product Research*, vol. 30, no. 8, pp. 920-925. http://dx.doi.org/10.1080/14786419.2015.1079189. PMid:26327252.

PAN, S.-Y., ZHOU, S.-F., GAO, S.-H., YU, Z.-L., ZHANG, S.-F., TANG, M.-K., SUN, J.-N., MA, D.-L., HAN, Y.-F., FONG, W.-F. and KO, K.-M., 2013. New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, p. 627375. http://dx.doi.org/10.1155/2013/627375. PMid:23634172.

PETRETTO, G.L., CHESSA, M., PIANA, A., MASIA, M.D., FODDAI, M., MANGANO, G., CULEDDU, N., AFIFI, F.U. and PINTORE, G., 2013. Chemical and biological study on the essential oil of *Artemisia caerulescens* L. ssp. densiflora (Viv.). *Natural Product Research*, vol. 27, no. 19, pp. 1709-1715. http://dx.doi.org/10.1080/14786419.2012.749471. PMid:23244627.

SHAREEF, H.K., MUHAMMED, H., HUSSEIN, H. and HAMEED, I., 2016. Antibacterial effect of ginger (Zingiber officinale) roscoe and bioactive chemical analysis using Gas chromatography mass spectrum. *Oriental Journal of Chemistry*, vol. 32, no. 2, pp. 817-837. http://dx.doi.org/10.13005/ojc/320207.

SULTAN, M.H., ZUWAIEL, A.A., MONI, S.S., ALSHAHRANI, S., ALQAHTANI, S.S., MADKHALI, O. and ELMOBARK, M.E., 2020. Bioactive principles and potentiality of hot methanolic extract of the leaves from Artemisia absinthium L "in vitro cytotoxicity against human MCF-7 breast cancer cells, antibacterial study and wound healing activity". *Current Pharmaceutical Biotechnology*, vol. 21, no. 15, pp. 1711-1721. http://dx.doi.org/10.2174/1389201021666200928150519. PMid:32988347.

TULIKA, T. and MALA, A., 2017. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and Eichhornia crassipes (Mart.) solms. *Journal of Pharmacognosy and Phytochemistry*, vol. 6, pp. 195-206.