

Original Research Article

**Nutrients and Constituents Relevant to Antioxidant,
Antimicrobial and Anti-Breast Cancer Properties of *Salvia
officinalis* L.**

Running Title: Constituents Relevant to Sage Properties

ABSTRACT

Objective: Comprehensive assessment of chemical composition of sage leaves (*Salvia officinalis* L.) to mark constituents responsible for antioxidant, antimicrobial and anticancer properties.

Methods: Amino acids, fatty acids and phenolic compounds content were assessed via High Performance Liquid Chromatography (HPLC). Antioxidant activity was characterized using, α -diphenyl- β -picrylhydrazyl (DPPH) and reducing power methods. The antimicrobial was explored against nine pathogenic strains. Anticancer activity was determined against MDA-MB-231 cell line.

Results: Sage considered a good source of calcium, iron, zinc, vitamins B6 and B12. HPLC analysis showed good content of essential amino acids, lysine, phenylalanine and leucine, polyunsaturated fatty acids, high concentrations of quercetin and cinnamic. It interpreted its high antioxidant powers. In addition, to remarkable antimicrobial potentials that could be beneficial in food preservation and pharmacologically as anti-inflammatory. Sage performed anti-proliferative activity against MDA-MB-231 cell line with IC_{50} of 300 μ g/mL.

Conclusion: Obtained results emphasis sage leaves content of variable nutrients and active compounds that reflected on its vast nutritional and pharmacological potentials such as; antioxidant, antimicrobial and anti-breast cancer



24 **Key words:** Sage (*Salvia officinalis*); HPLC; Antioxidant potentials; Antimicrobial
25 properties; MDA-MB-231 cell line.

26 1. INTRODUCTION

27 One of the fastest growing areas of research is exploring natural plant products as an option
28 to find new chemicals as anticancer agents (Gautam et al., 2014). Among the alternative
29 traditional approaches, various biologically active plant products classified as alkaloids,
30 saponins, triterpenes, and polyphenols have shown promising anticancer properties in both *in*
31 *vitro* and *in vivo* based on anti-proliferative activity on tumor cells and antioxidant
32 scavenging activity (Xavier et al., 2009). In addition, they found to possess antimicrobial,
33 anti-mutagenic, anti-inflammatory, and anti-cholinesterase properties. Currently, the demand
34 for these plants and their derivatives has increased in food and pharmaceutical industries
35 because they are recognized as safe products (Yadav et al., 2017).

36 *Salvia* “sage” are derived from the Latin *salvere* (to save), referring to the healing properties,
37 and in the Levant it’s called Maramia (Hamidpour et al., 2014). *Salvia* has a long history of
38 uses in culinary, medicinal, nutritional and pharmacological purposes. Studies highlighted the
39 effect of *Salvia* in preventing and controlling various diseases naturally in a safe manner.

40 Sage was reported to be rich in several bioactive compounds, which in most of cases seem to
41 be responsible for the pharmacological properties of these plants, including cytotoxicity
42 against human cancer cells (Xavier and Pereira-Wilson, 2015). German Commission E (a
43 governmental regulatory agency) has accepted the use of *S. officinalis* for a number of
44 medical applications (Ghorbani and Esmailizadeh, 2017).

45 The objective of the present study was the assessment of nutritional and chemical
46 compositions of sage leaves (*Salvia officinalis*) in order to mark nutrients and constituents
47 responsible for its antioxidant, antimicrobial and anticancer properties against human breast
48 cancer MDA-MB-231 cell line.

49 2. MATERIAL AND METHODS

50 2.1. Plant material and preparation

51 Common sage leaves (*Salvia officinalis* L.) was obtained from a reliable spice dealer,
52 Alexandria, Egypt. To prepare sage aqueous extract (SAE), dried grounded leaves was
53 extracted in Milli-Q water with ratio (1:20 w/v), stirred for 3 h at temperature (60°C) and
54 centrifuged (Pro-Centrifuge, Centurion Science Limited, UK) for 20 min at 5500 rpm. The
55 aqueous extract was filtered, freezed and then dried using vacuum freeze-dryer (Model FDF
56 0350, Korea) according to Garcia (Garcia et al., 2016) with modifications.

57 The plant leaves has been checked with

58 <http://www.theplantlist.org/tpl1.1/search?q=salvia+officinalis>

59 2.2. Proximate analysis

60 2.2.1. Nutrients content

61 Percent of moisture and ash content was determined as described by (AOAC, 1990). The
62 protein content was determined by Kjeldahl method as described by (AOAC, 1990), and
63 calculated according to equation; Protein= N x 6.25 to convert nitrogen content into protein
64 content.

65 The crude fat content was determined using an Ankom^{XT10} fat extractor (Ankom Technology
66 Corp., Macedon, NY). Crude Fat contained within a food or feed sample can be calculated
67 using the following formula:

$$\% \text{ Crude fat} = \frac{100 \times (W2 - W3)}{W1}$$

68 Where: W1 = Original weight of sample, W2 = Weight of pre-dried sample and filter bag,
69 W3 = Weight of dried sample and filter bag after extraction.

70 Crude fibre content was determined using Crude Fiber Analysis in Feeds - Filter Bag
71 Technique A2000 - AOCS Approved Procedure Ba 6a-05 (Ankom Technology Corp.,
72 Macedon, NY). Crude fibre percent according to the formula:

$$\%Crude\ fibre = \frac{W3 - W1}{W2} \times 100$$

73 Where: W1 = Weight of pre-dried filter bag, W2 = Original weight of sample, W3 = Weight
74 of filter bag contains organic residue remaining after digesting <https://www.ankom.com>
75 Total carbohydrate by difference was calculated by the following formula;

76 $100 - (\text{weight in g [protein + fat + water + ash] in 100 g of food})$ (WHO/FAO, 2002)

77 It should be clear that carbohydrate estimated in this fashion includes fibre.

78 **2.2.2. Electrolytes and minerals concentrations**

79 Concentrations of electrolytes; sodium and potassium and minerals; zinc, iron, magnesium
80 and calcium were determined using Atomic Absorption Spectrometry analytical technique
81 according to (Beaty and Kerber, 1993). Concentration of phosphorus was determined using
82 simplified colorimetric ammonium paramolybdate-vanadate method (yellow method), the
83 colour intensity was measured at wave length 420 nm (T80 UV/VIS Spectrophotometer, PG
84 Instruments Ltd) according to (Olsen and Sommers, 1982). Phosphorus concentrations were
85 calculated using the formula;

$$\%P = P\ from\ st.\ curve \times \frac{V1}{W\ sample} \times \frac{1}{V2} \div 10\ 000$$

86 Where: V1: Volume of stock standard solution of sample (50 mL), V2: Volume of stock
87 standard solution of sample used in measurements (5mL), W_{sample}: Weight of sample ($\cong$ 0.5
88 gm).

89 **2.2.3. B Vitamins content**

90 Concentration of some B Vitamins; Niacin Vitamin B3, Pyridoxine Vitamin B6 and Vitamin
91 B12 was determined quantitatively via High Performance Liquid Chromatography (HPLC) at
92 Food Safety and Quality Control Lab (FSQCLab), Faculty of Agriculture, Cairo University,
93 Egypt, according to (Agilent Application Note, Publication number 5989-9313EN 2008).
94 Agilent 1260 infinity HPLC Series (Agilent, USA), equipped with Quaternary pump, a

95 Kinetex XB-C18 column 100 mm X 4.6 mm (Phenomenex, USA), operated at 35°C. The
96 separation was achieved using a binary linear elution gradient with (A) 25 mM NaH₂PO₄ pH
97 2.5 (v/v), (B) methanol. The injected volume was 20 µL. VWD detector was used for at 220
98 nm for vitamins B3, B6 and B12.

99 **2.3. High Performance Liquid Chromatography (HPLC) of main components**

100 For determining amino acid content, acid hydrolysis was carried out according to Csomos
101 (Csomos and Simon-Sarkadi, 2002). Free amino acids extraction was carried out according to
102 Emam (Emam, 2011). Amino acid analysis was carried out using Automatic Amino Acid
103 Analyzer (AAA 400 INGOS Ltd). amino acids required pattern values are according to Food
104 and Agriculture Organization (FAO) (FAO, 1985), while Amino Acid Score (AAS%) or
105 Chemical Score (CS) was calculated as “Percentage of adequacy” as follows;

$$\text{Amino Acid Score} = \frac{\text{mg of amino acid in 1 g test protein}}{\text{mg of amino acid in requirement pattern}} \times 100$$

106 Fatty acids was determined via saponified and unsaponified fat separation according to
107 AOAC (AOAC, 2005).

108 Phenolic compounds of sage leaves was identified according to Croci (Croci et al., 2009),
109 using the following standards; gallic acid, caffeic acid, coumaric, syringic acid, vanillin,
110 cinnamic acid, pyrogallol, catechin, quercetin and rutin.

111 **2.3. Pharmacological potentials**

112 **2.3.1. Antioxidant activity**

113 Phytochemical screening analysis for SAE were; Wagner’s method, Fehling’s test, foam test
114 and Salkowki’s test and were performed according to Wadood (Wadood et al., 2013).

115 Total phenolic content assay was performed on SAE according to Singleton (Singleton et al.,
116 1999) using Folin-Ciocalteu reagent. The total phenolic content was expressed as gallic acid
117 equivalents (GAE mg/g). All measurements were performed in triplicates.

118 Total flavonoid contents of SAE was determined using colorimetric method as described by
 119 Sakanaka (Sakanaka et al., 2005). The results were expressed as mg/g of sample. All samples
 120 were performed in triplicate.

121 Antioxidant scavenging activity of SAE was determined using two different methods; DPPH
 122 (2,2-diphenyl-1-picrylhydrazyl) assay that was performed as described by Brand-Williams
 123 (Brand-Williams et al., 1995), and reducing power assay as described by Ferreira (Ferreira et
 124 al., 2007).

125 2.3.2. Antimicrobial screening

126 Antimicrobial potentials of SAE was screened against nine pathogenic strains using agar well
 127 diffusion assay as described by Hamad (Hamad et al., 2017). The nine tested pathogenic
 128 organisms were; five Gram-positive strains; *Staph. aureus* NCTC10788, *Staph. pyogenes*
 129 EMCC1772, *St. mutants* EMCC1815 , *B. subtilis* DB100 and *Clostridium* *botulinum*
 130 ATCC3584, three Gram-negative strains; *E. coli* BA12296, *E. coli* ATCC25922 and *K.*
 131 *pneumonia* ATCC12296 and one yeast strain; *Candida albicans* ATCCMYA2876, and the
 132 minimum inhibitory concentration (MIC) was assessed within the concentrations of 75, 50,
 133 25, 12.5 and 6.25 mg/ mL of sage extract.

134 2.3.3. Cytotoxicity and anticancer assay

135 This test was used to evaluate cytotoxicity. Membrane of living cells collect neutral red,
 136 therefore after subsequent lysis, absorbance can be used as a measure of cell viability
 137 (Borenfreund and Puerner, 1985). The stock solution of NR (3.3 g/l) was diluted to 1/100 in
 138 the cell culture medium and the extracting solution consisted of 50% (v/v) ethanol in Milli-Q
 139 water with 1% (v/v) acetic acid. MDA-MB-231 Triple negative breast cancer cells were
 140 grown for 48h in presence of SAE, (50–600µg) after incubation 150 µL of freshly prepared
 141 neutral red solution pre-warmed to 37 °C was added to each well and all plates were
 142 incubated at 37 °C for additional 4 h. The cells were washed twice with PBS, 150µl of destain

solution were added to each well and plates were shaken for 15 min. The absorbance was determined at 540 nm using a Microplate Elisa Reader while, light microscope (Olympus IX70-58F2 Multi-parameter Fluorescence Microscope) was used for monitoring the morphological changes in treated MDA-MB-231 cells after 48 h of incubation.

2.13. Statistical analysis

Statistical analysis was performed using Analytical Software SPSS[®] 13.0 (Statistical Package for the Social Sciences) (2005).

3. RESULTS

3.1. Proximate composition

Table (1) illustrated proximate composition of sage leaves (*Salvia officinalis*) including, nutrients, minerals and B vitamins. Proximate nutrient composition of sage (*Salvia officinalis*), indicated that sage is a high-carbohydrate high-fiber nutrient source plant. Another advantage is the relatively high protein (17g/100 g) with low-fat content (3.46 g/100g). High ash content indicated sage richness in minerals. The sage showed remarkable concentration of iron (84.037 mg/100gm), calcium that comes in second rank of minerals' concentration (894.273 mg/100g) and zinc that was (5.472 mg/100g). On the other hand, sage showed poor content of sodium and potassium (19.893, 34.605 mg/100g). Vitamin B concentrations of sage were (3.258, 1.545, 0.329 mg/100g) of vitamins B3, B6 and B12 respectively.

3.2. HPLC analysis

3.2.1. Amino acids profile

Amino acid content of sage (*Salvia officinalis*) are represented in (Table 2). Obtained results showed that the main essential amino acid (EAA) in sage was lysine with content of (10.44 g/100g). Sage also provided considerable content of phenylalanine and leucine (0.74 and 0.45 g/ 100g respectively), limiting content of valine, threonine, histidine and isoleucine. On the



other hand, sage showed its richness in non-essential amino acids such as; glycine, glutamic, alanine, and aspartic, 1.02, 0.83, 0.75 and 0.53 g/ 100g respectively.

3.2.2. Fatty acids content

Fatty acids (FA) profile of sage (*Salvia officinalis*) was exhibited in (Table 3). Sage showed USFAs/ SFAs ratio of 0.32 due to the superiority of SFAs. The highest content of SFAs belonged to tridecylic (C13:0) (11.33 g/100g of total FA), followed by equal amounts of lauric (C12:0) and myristic (C14:0) (7.6 g/100g of total FA). PUFAs 18:3 ω -3, C18:2 ω -6 and C18:1 ω -9 was present with values of (6.46, 4.40 and 3.13 g/100g of total FA respectively).

3.2.3. Phenolic compounds profile

Table (4) demonstrated phenolic compounds concentrations of sage aqueous extracts. Within the ten used phenolic compound standards, three compounds were not detected in SAE; gallic acid, catechin and pyrogallol. Quercetin and cinnamic acid showed the highest concentrations (604.7575 and 390.3971 μ g/mL respectively) representing (0.6756 and 0.4361%) of sage TPC (Table 5). The rest of tested phenolic compounds standards; caffeic acid, syringic acid, coumaric acid, vanillin and rutin were existed in little concentrations (0.6132, 0.5134, 0.5134, 0.0856 and 0.0766 μ g/mL respectively).

3.3. Pharmacological potentials

3.3.1. Phenolic and flavonoids content and antioxidant powers

Qualitative screening of sage phytochemicals results showed presence of a wide range of constituents include, carbohydrate (reducing sugars), volatile oil, phenolic compounds, flavonoids/ flavones, tannins, steroids, terpenoids and amino acids which are mostly responsible for antioxidant, antimicrobial and cytotoxic activities.

Table (5) illustrated phenolic, flavonoid content and antioxidant scavenging potentials of sage aqueous extract. Sage showed a total phenolic content of 88.57 ± 3.14 mg GAE/ g, while total

flavonoids was 37.13 ± 1.83 mg catechol equivalent/ g. Antioxidant and scavenging potentials of aqueous extract of sage; DPPH results represented with inhibitory concentration at which 50% of DPPH radicals are scavenged (IC_{50} mg/mL) and reducing power represented with effective concentration at which the absorbance is 0.5 (EC_{50} mg/mL). Sage leaves water extract showed IC_{50} and EC_{50} values (27.52 and 239.52 mg/ mL respectively).

3.3.2. Antimicrobial activity

Table (6) presented inhibition zone diameter in mm of different concentrations of sage (*Salvia officinalis*) aqueous extract and Minimal Inhibitory Concentration (MIC) for each strain. Results revealed that sage leaves extract owned antimicrobial activity against all tested pathogenic strains with varied MICs. Best antimicrobial effect of sage extract was against *St. aureus* NCTC10788 with inhibition zone diameter started from 23 mm with concentration of 75mg/mL, and ended with 6 mm with concentration of 6.25 mg/mL, which was the lowest MIC amongst the five Gram-positive strains. On the other hand, Sage showed MIC of 5 mg/mL against the other four Gram-positive strains with inhibition zone diameters started from 18.5 - 22.5 mm with concentration of 75mg/mL. Antifungal activity of sage aqueous extract was obvious against *Candida albicans* ATCCMYA2876 with MIC of 6.25 mg/ mL, the inhibition zone diameter reached 25 mm with concentration of 75 mg/mL.

3.3.3. Cytotoxicity and anticancer potentials

Figure (1) showed anti-proliferative activity of sage (*Salvia officinalis*) aqueous extract (SAE) against MDA-MB-231 cell line via neutral red assay (IC_{50} of 300 μ g/mL). Sage clearly affected cell viability as revealed by neutral red assay with an IC_{50} of 300 μ g. The effects of sage on cell viability was studied using neutral red assays on MDA-MB-231 cell line. Figure 2 (a and b), illustrated morphological changes in MDA-MB-231 Triple negative breast cancer cell following treatment with sage aqueous extract (SAE) with concentration of 300 μ g/ mL for 48 h. Incubation of breast cancer cells with sage resulted in morphological changes that

218 shown to be induced by SAE, in addition to decrease of neutral red uptake in a concentration-
219 dependent manner 

220 4. DISCUSSION

221 Plant-fiber-enriched foods are reported to improve glucose and lipid metabolism, they lower
222 serum cholesterol concentrations in patients with diabetes, in addition to multiple effects on
223 different cardiovascular risk factors (De Natale et al., 2009). The electrolytes results

224 emphasized that sage contains 3 times more calcium and 5.5 times more zinc than milk.

225 USDA (USDA, 2016) reported different values for sage mineral except for zinc and sodium
226 content. Alterations in essential trace elements like Fe, Zn and Ca may play an important role
227 in the pathogenesis of complex diseases including cancers. Dietary calcium intake found to
228 be associated with a reduced incidence of cancer, while iron, is involved in many processes
229 including DNA, RNA and protein synthesis, and Zn may represent an independent risk factor
230 for cancer and therefore a possible target for prevention (Al Faris and Ahmad, 2011; Singh et
231 al., 2016). Obtained vitamin results indicated that sage could be considered a good source of
232 vitamins B6 and B12 and encourage suggestions of using as food supplement especially for
233 vegetarians and elderly. What is most important that, Vitamin B6 and B12 was reported to
234 sensitize a large panel of cancer cell lines to apoptosis (Pandey et al., 2013; Saghiri et al.,
235 2017). Obtained values of B vitamins fluctuated between agreement and disagreement with
236 (Lovik et al., 2009; USDA, 2016). Nutritional profile may aid to explain the current increased
237 demand on Salvia species for nutritional and pharmacological industries (Yadav et al., 2017).

238 Many biological functions requiring lysine; include synthesis of connective tissues and
239 carnitine, conversion of fatty acids to energy that support healthy growth in children and
240 maintenance of healthy immune function especially in the management of Herpes simplex
241 virus (HSV) infection  Lysine also found to enhance calcium absorption preventing
242 Osteoporosis. On food technology wise; lysine is involved in the browning reaction applied

243 to foods such as pastries and desserts (Braun and Cohen, 2014) through obtained results, it
 244 is recommended to mix sage with foods rich in limited EAA. Apart from amino acid,
 245 starvation diets theories - that stimulates autophagy in cancer- which is complex and likely
 246 context-dependent, the maintenance of the intracellular level of amino acids is crucial for
 247 cellular homeostasis. Deficiency or imbalance of essential amino acids in the diet was
 248 reported to produce profound depression in the immune resistance of the host animal to
 249 tumors (Lashinger et al., 2016; Saito et al., 2018).

250 Increased intake of lauric, myristic or palmitic acid was reported to raise serum total, LDL
 251 and HDL cholesterol levels, and lowered triglyceride levels, while lauric acid alone reduced
 252 the total cholesterol to HDL cholesterol and LDL cholesterol to HDL cholesterol ratios
 253 (Mensink, 2016). polyunsaturated fatty acids (PUFAs) was reported to exert antineoplastic
 254 activity by inducing apoptotic cell death in human cancer cells through increasing the
 255 sensitivity of tumor cells to conventional therapies (D'Eliseo and Velotti, 2016). In this
 256 context, USDA (USDA, 2016) reported vitiated values about sage. Reported laboratory
 257 studies indicated clear role for omega-3 PUFAs in preventing cancer development at various
 258 stages (de Aguiar Pastore Silva et al., 2015; Eltweri et al., 2017). Generally, it is difficult to
 259 match results of nutrients and constituents with earlier researchers since *Salvia* species have
 260 various compositions depending on the genetic, climatic, seasonal, and environmental factors
 261 (Hamidpour et al., 2014).

262 Caffeic acid and its derivatives including rosmarinic acid was reported to be the most
 263 representative phenolic compounds in *Lamiaceae*, *S. officinalis* extracts (Olah et al., 2004).
 264 Anticancer activities of cinnamic acid derivatives include induction of apoptosis by
 265 irreversible DNA damage leading to cell death was reported (Niero and Machado-Santelli,
 266 2013). Since phenolic compounds are the key for several therapeutic properties, quantitative
 267 identification of sage leaves phenolic compounds indicated pharmacological potentials to be

268 useful as a source for novel applications in functional food and pharmaceutical industries
269 (Bahadori et al., 2017).

270 Earlier studies on sage reported the presence of obtained phytochemicals classes of active
271 compounds (Ghorbani and Esmailizadeh, 2017; Hamidpour et al., 2014). Obtained results of
272 phenolic and flavonoid content were in context with Özcan (Özcan and Özkan, 2015), while
273 it disagreed with Gird and Pop (Gird et al., 2014; Pop et al., 2014). Sage was reported to
274 possess the greatest antioxidant capacity amongst fresh herbs, due to its phenolic and
275 flavonoids content which related for multiple health benefits, including reduction in cancer
276 risk and modification of tumor behavior (Kaefer and Milner, 2011; Scapin et al., 2016).

277 Activity of sage against filamentous fungi and yeasts such as *Candida albicans* was
278 previously reported by Sirirat and Jelena (Sirirat and Jelena, 2010), and thus may be useful in
279 the treatment of different kinds of fungi in human. Antimicrobial results can nominate sage as
280 a promising natural antimicrobial agent which is in accordance to the results of (Zomorodian
281 et al., 2015). Strong antimicrobial and antioxidant activities encouraged further investigation
282 of anti-breast cancer activities of sage extract.

283 Alterations of the MDA-MB-231 cell surface or of the sensitive lysosomal membrane lead to
284 lysosomal fragility and other changes that gradually become irreversible. The main advantage
285 of neutral red assay is that it detects only viable cells (Babich and Borenfreund, 1987).

286 Previous researches reported the antitumor properties of sage extract which probably in
287 correlation to its strong antioxidant activity as in other plant extracts (Garcia et al., 2016;
288 Rupasinghe et al., 2016). Sage bioactive compounds, Linalyl acetate (Ly), Terpeniol (Te) and
289 Camphor (Ca), has been reported to have synergistic effect on two human colon cancer cell
290 lines HCT-116 (p53 (+/+)) and p53 (-/-)). Apoptosis by Ly + Te + Ca, in p53 (+/+) cells (Itani
291 et al., 2008). Despite progress in cancer research, the biological active components and
292 antitumor mechanisms have not yet been exactly determined, but mitochondrial membrane

potential decay is one of the accompanying processes of the intrinsic apoptotic pathway activation (Jantová et al., 2014). On the other hand, concerns and limitations for the amount and quantity used of sage attributed to the predominant volatile neurotoxic components; camphor, α - and β -thujone, which are most present phytochemicals in *Salvia officinalis* leaves. A daily intake of 5.0 mg/ person is acceptable for a maximum duration of use of 2 weeks, and the overdose is more than 15 g of sage leaves per day (Iatrou et al., 2013). It is noteworthy that sage use during pregnancy and lactation is not recommended (Wharf, 2009). Our findings encourage further studies of sage components as promising antitumor therapy against breast cancer.

4. Conclusion

In conclusion, *Salvia officinalis* possessed strong antioxidant, antimicrobial and anti-breast cancer activities due to its rich combination of nutrients and constituents. Sage considered a good source of calcium, iron, zinc, vitamins B6 and B12, essential amino acids, lysine, phenylalanine and leucine and polyunsaturated fatty acids (PUFAs) ω -3, ω -6 and ω -9, which all reported to have a role as anti-cancer in different mechanisms. Presence of a wide range of constituents include, volatile oil, flavonoids/ flavones, tannins, steroids, terpenoids combined with significant amounts of phenolic compounds, especially quercetin and cinnamic, interpreted its high antioxidant powers. In addition, to remarkable antimicrobial potentials that showed against all nine tested pathogens that could be beneficial in food preservation and pharmacologically as anti-inflammatory. SAE performed anti-proliferative activity against MDA-MB-231 cell line with IC_{50} of 300 μ g/mL, which encourage further studies on sage components as promising antitumor therapy. Sage leaves possessed variable nutrients and active compounds that reflected on its vast nutritional and pharmacological potentials.

318 **References**

- 319 Agilent_Technologies (2008). Agilent Application Note, Publication number 5989-9313EN.
- 320 de Aguiar Pastore Silva, J., Emilia de Souza Fabre, M., and Waitzberg, D.L. (2015). Omega-
- 321 3 supplements for patients in chemotherapy and/or radiotherapy: A systematic review. Clin.
- 322 Nutr. 34, 359–366.
- 323 AOAC (1990). AOAC: Official Methods of Analysis (Association of Official Analytical
- 324 Chemists. INC., Arligton, Virginia, USA.).
- 325 AOAC (2005). AOAC: Official Methods of Analysis (AOAC INTERNATIONAL,
- 326 Gaithersburg, Maryland 20877-2417, USA).
- 327 Babich, H., and Borenfreund, E. (1987). Structure-activity relationship (SAR) models
- 328 established in vitro with the neutral red cytotoxicity assay. Toxicol. Vit. 1, 3–9.
- 329 Bahadori, M.B., Asghari, B., Dinparast, L., Zengin, G., Sarikurkcu, C., Abbas-Mohammadi,
- 330 M., and Bahadori, S. (2017). Salvia nemorosa: A novel source of bioactive agents with
- 331 functional connections. LWT - Food Sci. Technol. 75, 42–50.
- 332 Beaty, R.D., and Kerber, J.D. (1993). Concepts, Instrumentation and Techniques in Atomic
- 333 Absorption Spectrophotometry (The Perkin-Elmer Corporation, Norwalk, CT, U.S.A.).
- 334 Borenfreund, E., and Puerner, J.A. (1985). Toxicity determined in vitro by morphological
- 335 alterations and neutral red absorption. Toxicol. Lett. 24, 119–124.
- 336 Brand-Williams, Cuvelier, M.E., and Berset, C. (1995). Use of a Free Radical Method to
- 337 Evaluate Antioxidant Activity. Food Sci. Technol. 28, 25–30.
- 338 Braun, L., and Cohen, M. (2014). Herbs & natural supplements, Volume 2. An evidence-
- 339 based guide. (Churchill Livingstone Australia, Elsevier).
- 340 Croci, A.N., Cioroiu, B., and Lazar, D. (2009). HPLC evaluation of phenolic and
- 341 polyphenolic acids from propolis. Farmacia LVII, 52–57.
- 342 Csomos, E., and Simon-Sarkadi, L. (2002). DETERMINATION OF BIOLOGICALLY

343 ACTIVE COMPOUNDS. 46, 73–81.

344 D’Eliseo, D., and Velotti, F. (2016). Omega-3 Fatty Acids and Cancer Cell Cytotoxicity:
 345 Implications for Multi-Targeted Cancer Therapy.

346 Eltweri, A.M., Thomas, A.L., Metcalfe, M., Calder, P.C., Dennison, A.R., and Bowrey, D.J.
 347 (2017). Potential applications of fish oils rich in omega-3 polyunsaturated fatty acids in the
 348 management of gastrointestinal cancer. *Clin. Nutr.* 36, 65–78.

349 Emam, S.S. (2011). Bioactive constituents of *Atriplex halimus* plant Shalabia. *J. Nat. Prod.* -
 350 India 4, 25–41.

351 FAO (1985). Food and Agriculture Organization (FAO) (Food and Agriculture Organization
 352 of the United States/ World Health Organization/ United Nations University, Energy and
 353 Protein requirements. Report of a joint FAO/WHO/UNU meeting. World Health
 354 Organization, Geneva).

355 Al Faris, N.A., and Ahmad, D. (2011). Distribution of trace elements like calcium, copper,
 356 iron and zinc in serum samples of colon cancer - A case control study. *J. King Saud Univ.* -
 357 Sci. 23, 337–340.

358 Ferreira, I.C.F.R., Baptista, P., Vilas-Boas, M., and Barros, L. (2007). Free-radical
 359 scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal:
 360 Individual cap and stipe activity. *Food Chem.* 100, 1511–1516.

361 Garcia, C.S.C., Menti, C., Lambert, A.P.F., Barcellos, T., Moura, S., Calloni, C., Branco,
 362 C.S., Salvador, M., Roesch-Ely, M., and Henriques, J.A.P. (2016). Pharmacological
 363 perspectives from brazilian *Salvia officinalis* (Lamiaceae): Antioxidant, and antitumor in
 364 mammalian cells. *An. Acad. Bras. Cienc.* 88, 281–292.

365 Gautam, N., Mantha, A.K., Mittal, S., Gautam, N., Mantha, A.K., and Mittal, S. (2014).
 366 Essential Oils and Their Constituents as Anticancer Agents: A Mechanistic View, Essential
 367 Oils and Their Constituents as Anticancer Agents: A Mechanistic View. *BioMed Res. Int.*

368 BioMed Res. Int. 2014, 2014, e154106.

369 Ghorbani, A., and Esmailizadeh, M. (2017). Pharmacological properties of *Salvia officinalis*
370 and its components. *J. Tradit. Complement. Med.* 8–15.

371 Gîrd, C.E., Nencu, I., Costea, T., Duțu, L.E., Popescu, M.L., and Ciupitu, N. (2014).
372 Quantitative analysis of phenolic compounds from *Salvia officinalis* L. leaves. *Farmacia* 62,
373 649–657.

374 Hamad, G.M., Darwish, A.M.G., Abu-Serie, M.M., and El Sohaimy, S.A. (2017).
375 Antimicrobial, Antioxidant and Anti-inflammatory Characteristics of Combination (*Cassia*
376 *fistula* and *Ocimum basilicum*) Extract as Natural Preservative to Control & Prevent
377 Food Contamination. *J. Food Nutr. Res.* 5, 771–780.

378 Hamidpour, M., Hamidpour, R., Hamidpour, S., and Shahlari, M. (2014). Chemistry,
379 Pharmacology, and Medicinal Property of Sage (*Salvia*) to Prevent and Cure Illnesses such as
380 Obesity, Diabetes, Depression, Dementia, Lupus, Autism, Heart Disease, and Cancer. *J.*
381 *Tradit. Complement. Med.* 4, 82–88.

382 Iatrou, G., Lamari, F., Dimitrellos, G., and Tsakiri, M. (2013). Catalogue of aromatic and
383 medicinal plants (European Territorial Cooperation Programme Greece - Italy. Eptalofos, S
384 A, Athens, Greece.).

385 Itani, W.S., El-Banna, S.H., Hassan, S.B., Larsson, R.L., Bazarbachi, A., and Gali-Muhtasib,
386 H.U. (2008). Anti colon cancer components from lebanese sage (*Salvia libanotica*) essential
387 oil: Mechanistic basis. *Cancer Biol. Ther.* 7, 1765–1773.

388 Jantová, S., Hudec, R., Sekretár, S., Kučerák, J., and Melušová, M. (2014). *Salvia officinalis*
389 L. extract and its new food antioxidant formulations induce apoptosis through
390 mitochondrial/caspase pathway in leukemia L1210 cells. *Interdiscip. Toxicol.* 7, 146–153.

391 Kaefer, C.M., and Milner, J.A. (2011). Herbs and Spices in Cancer Prevention and
392 Treatment. *Herb. Med. Biomol. Clin. Asp.* 19, chapter 16.

393 Lashinger, L.M., O’Flanagan, C.H., Dunlap, S.M., Rasmussen, A.J., Sweeney, S., Guo, J.Y.,
394 Lodi, A., Tiziani, S., White, E., and Hursting, S.D. (2016). Starving cancer from the outside
395 and inside: separate and combined effects of calorie restriction and autophagy inhibition on
396 Ras-driven tumors. *Cancer Metab.* 4, 18.

397 Lovik, M., Marchelli, R., Martin, A., and Moseley, B. (2009). Scientific Opinion of the Panel
398 on Dietetic Products Nutrition and Allergies on a request from the European Commission on
399 the safety of ‘Chia seed (*Salvia hispanica*) and ground whole Chia seed’ as a food ingredient.
400 *EFSA J.* 996, 1–26.

401 Mensink, R.P. (2016). Effects of saturated fatty acids on serum lipids and lipoproteins: a
402 systematic review and regression analysis.

403 De Natale, C., Annuzzi, G., Bozzetto, L., Mazzarella, R., Costabile, G., Ciano, O., Riccardi,
404 G., and Rivellese, A.A. (2009). High-Carbohydrate / High-Fiber Diet Versus Carbohydrate
405 Diet on Postprandial Lipids in Type 2 Diabetic Patients. *Diabetis Care* 32, 2168–2173.

406 Niero, E.L. de O., and Machado-Santelli, G. (2013). Cinnamic acid induces apoptotic cell
407 death and cytoskeleton disruption in human melanoma cells. *J. Exp. Clin. Cancer Res.* 32, 31.

408 Olah, N.K., Hanganu, D., Oprean, R., Mogoşan, C., Dubei, N., and Gocan, S. (2004).
409 Selective extraction of caffeic acid derivatives from *Orthosiphon stamineus* Benth.
410 (Lamiaceae) leaves. *J. Planar Chromatogr. - Mod. TLC* 17, 18–21.

411 Olsen, S.R., and Sommers, L.E. (1982). Phosphorus. In: Page A.L., et al (eds), *Methods of*
412 *Soil Analysis.* (Part 2, 2nd edn, Agron Monogr 9.ASA and ASSA, Madison WI), pp. 403–
413 430.

414 Özcan, M.M., and Özkan, G. (2015). Determination of antioxidant activity and total phenol
415 contents of two *Salvia* extracts. *Indian J. Tradit. Knowl.* 14, 226–230.

416 Pandey, S., Garg, P., Lim, K.T., Kim, J., Choung, Y.H., Choi, Y.J., Choung, P.H., Cho, C.S.,
417 and Chung, J.H. (2013). The efficiency of membrane transport of vitamin B6 coupled to

418 poly(ester amine) gene transporter and transfection in cancer cells. *Biomaterials* 34, 3716–
419 3728.

420 Pop, A. V, Tofana, M., Socaci, S., Varban, D., Nagy, M., Bors, M., and Sfechis, S. (2014).
421 Evaluation of antioxidant activity and phenolic content in different *Salvia officinalis* L.
422 extracts. *Bull. UASVM Food Sci. Technol.* 71, 195–199.

423 Rupasinghe, V., Jiang, Y., and Zhang, L. (2016). The anticancer properties of phytochemical
424 extracts from *Salvia* plants. *Bot. Targets Ther.* Volume 6, 25.

425 Saghiri, M.A., Asatourian, A., Ershadifar, S., Moghadam, M.M., and Sheibani, N. (2017).
426 Vitamins and regulation of angiogenesis: [A, B1, B2, B3, B6, B9, B12, C, D, E, K]. *J. Funct.*
427 *Foods* 38, 180–196.

428 Saito, Y., Moriya, S., Kazama, H., Hirasawa, K., Miyahara, K., Kokuba, H., Hino, H.,
429 Kikuchi, H., Takano, N., Hiramoto, M., et al. (2018). Amino acid starvation culture condition
430 sensitizes EGFR-expressing cancer cell lines to gefitinib-mediated cytotoxicity by inducing
431 atypical necroptosis. *Int. J. Oncol.* 1165–1177.

432 Sakanaka, S., Tachibana, Y., and Okada, Y. (2005). Preparation and antioxidant properties of
433 extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chem.* 9, 569–575.

434 Scapin, G., Schmidt, M.M., Prestes, R.C., and Rosa, C.S. (2016). Phenolics compounds,
435 flavonoids and antioxidant activity of chia seed extracts (*Salvia hispanica*) obtained by
436 different extraction conditions. *Int. Food Res. J.* 23, 2341–2346.

437 Singh, B.P., Dwivedi, S., Dhakad, U., Murthy, R.C., Choubey, V.K., Goel, A., and
438 Sankhwar, S.N. (2016). Status and Interrelationship of Zinc, Copper, Iron, Calcium and
439 Selenium in Prostate Cancer. *Indian J. Clin. Biochem.* 31, 50–56.

440 Singleton, V.L., Orthofer, R., and Lamuela-Raventos, R.M. (1999). Analysis of total phenols
441 and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent.
442 *Oxidants and antioxidants part A.* (Academic Press).

443 Sirirat, D., and Jelena, P. (2010). Bacterial inhibition and antioxidant activity of kefir
444 produced from Thai jasmine rice milk. *Biotechnology* 9, 332–337.

445 USDA (2016). National Nutrient Database for Standard Reference Release 28 slightly revised
446 May , 2016 Full Report (All Nutrients) 12006 , Seeds , chia seeds , dried.

447 Wadood, A., Ghufraan, M., Jamal, S.B., Naeem, M., Khan, A., and Ghaffar, R. (2013).
448 Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. *Biochem.*
449 *Anal. Biochem.* 2, 2–5.

450 Wharf, C. (2009). Community Herbal Monograph on *Salvia Officinalis* L., Folium.
451 Reproduction 1–8.

452 WHO/FAO (2002). Food energy – methods of analysis and conversion. In *Fao Food and*
453 *Nutrition Paper*, p. 93.

454 Xavier, C.P.R., and Pereira-Wilson, C. (2015). Medicinal plants of the genres *Salvia* and
455 *Hypericum* are sources of anticolon cancer compounds: Effects on PI3K/Akt and MAP
456 kinases pathways. *PharmaNutrition* 4, 112–122.

457 Xavier, C.P.R., Lima, C.F., Fernandes-Ferreira, M., and Pereira-Wilson, C. (2009). *Salvia*
458 *fruticosa*, *salvia officinalis*, and rosmarinic acid induce apoptosis and inhibit proliferation of
459 human colorectal cell lines: The role in MAPK/ERK pathway. *Nutr. Cancer* 61, 564–571.

460 Yadav, A., Joshi, A., Kothari, S.L., Kachhwaha, S., and Purohit, S. (2017). Review Article
461 Medicinal, Nutritional and Industrial Applications of *Salvia* species: A Revisit. *Int. J. Pharm.*
462 *Sci. Rev. Res.* 43, 27–37.

463 Zomorodian, K., Ghadiri, P., Saharkhiz, M.J., Moein, M.R., Mehriar, P., Bahrani, F., Golzar,
464 T., Pakshir, K., and Fani, M.M. (2015). Antimicrobial activity of seven essential oils from
465 Iranian aromatic plants against common causes of oral infections. *Jundishapur J. Microbiol.*
466 8, e17766.

467

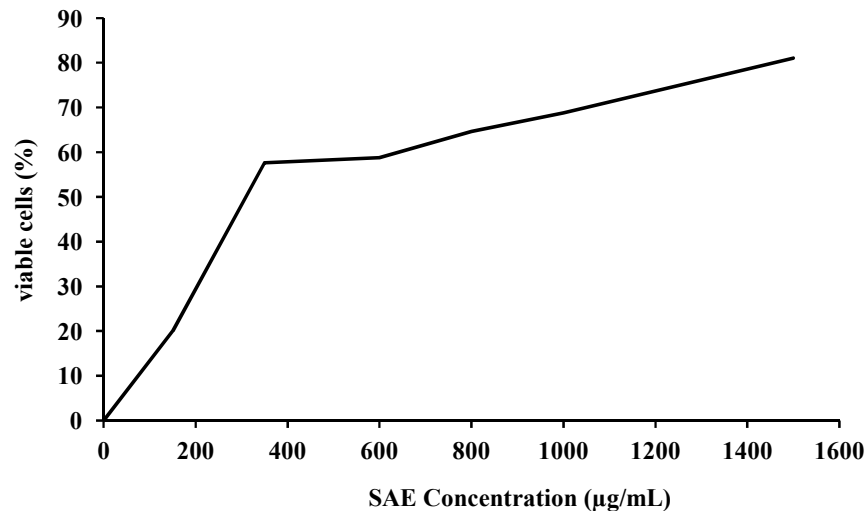


Figure 1. Anti-proliferative activity of sage (*Salvia officinalis*) aqueous extract (SAE) against MDA-MB-231 cell line via neutral red assay (IC₅₀ of 300µg/mL)

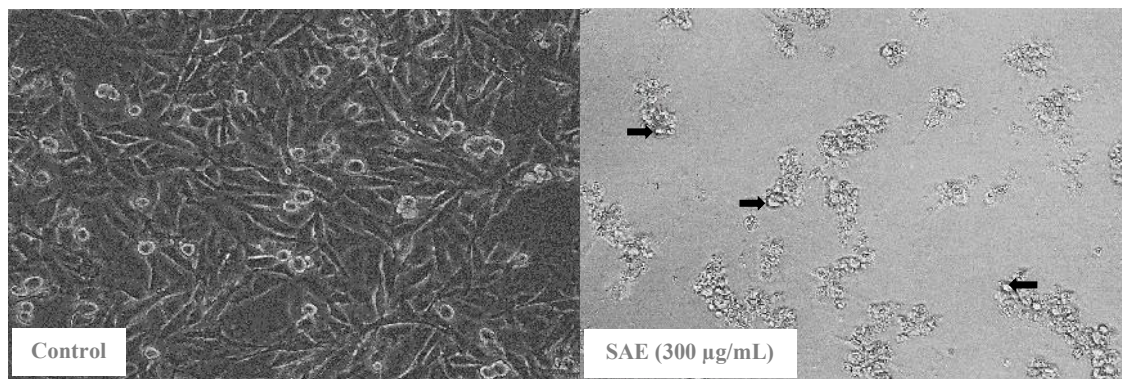


Figure 2. Morphological changes in MDA-MB-231 Triple negative breast cancer cell following treatment with sage aqueous extract (SAE) with concentration of 300 µg/ mL for 48 h.

Mentions: Light microscopy observation of SAE-treated MDA-MB-231 Triple negative breast cancer cells revealed morphological changes induced by the extract. As shown on figure 2b, MDA-MB-231 treated cells with concentration of 300 µg/ mL SAE, underwent visible morphological changes after 48 h of treatment, where treated cells appeared smaller and rounded, characteristics of dying cells (Fig.2b, arrows) in addition to decrease of neutral red uptake in a concentration-dependent manner.

489 Table (1): Proximate composition of sage (*Salvia officinalis*)

490

Component	Unit	Content (per 100g)
Nutrients		
Protein	g	17.01±0.64
Crude fat	g	3.46±0.28
Carbohydrate, by difference	g	57.87
Crude fiber	g	35.96±0.46
Moisture	g	9.07±0.30
Ash	g	12.59±0.76
Electrolytes		
Sodium, Na	mg	19.893
Potassium, K	mg	34.605
Minerals		
Calcium, Ca	mg	894.273
Iron, Fe	mg	84.037
Magnesium, Mg	mg	19.601
Zinc, Zn	mg	5.472
Phosphorus, P	mg	Traces
B Vitamins		
Niacin, B3	mg	3.258
Pyridoxine, B6	mg	1.545
Cobalamin, B12	mg	0.329

491 Nutrients data represented are the mean ±standard deviation, n=3

492 Table (2): Amino acids content of sage (*Salvia officinalis*)

493

Amino Acid	Symbol	Content (g)
<i>Essential amino acids</i>		
Histidine	His	0.17
Leucine	Leu	0.45
Isoleucine	Ile	0.13
Lysine	Lys	10.44
Methionine + cystine	Met+ Cys	0.03
Phenylalanine + tyrosin	Phe+ Tyr	0.74
Threonine	Thr	0.17
Valine	Val	0.28
CS	-	-
<i>Non-essential amino acids</i>		
Alanine	Ala	0.75
Aspartic acid	Asp	0.53
Glutamic acid	Glu	0.83
Glycine	Gly	1.02
Proline	Pro	0.01
Serine	Ser	0.25
Tyrosine	Tyr	0.15

494 Data represented are per 100g of sample
 495 Table (3): Fatty acid profile of sage (*Salvia officinalis*)
 496

Fatty acid	Symbol	g/100g
<i>SFAs</i>		
Capric	C10:0	1.28
Undecylic	C11:0	6.07
Lauric	C12:0	7.60
Tridecylic	C13:0	11.33
Myristic	C14:0	7.64
Pentadecylic	C15:0	6.95
Palmitic	C16:0	1.44
Stearic	C18:0	0.93
Arachidic	C20:0	0.54
%of total fat		43.78
<i>USFAs</i>		
<i>MUSFAs</i>		
Oleic	C18:1 ω-9	3.13
<i>PUSFAs</i>		
Linoleic	C18:2 ω-6	4.40
α-Linolenic	C18:3 ω-3	6.46
%of total fat		13.99
<i>USFAs/ SFAs</i>		0.32
ω6/ ω3 ratio		0.68

497
 498 SFAs; Saturated fatty acids, USFAs; Unsaturated fatty acids, MUFAs; Monounsaturated fatty
 499 acids, PUFAs; Polyunsaturated fatty acids
 500

501 Table (4): Phenolic compounds concentrations of sage aqueous extract

Phenolic compound	μg/mL
Caffeic acid	0.6132
Syringic acid	0.5134
Rutin	0.0766
Coumaric acid	0.4653
Vanillin	0.0856
Quercetin	604.7575
Cinnamic acid	390.3971

502
 503
 504
 505
 506

Table (5): Antioxidant and scavenging potentials of sage aqueous extract

Test	Unit	Values
Total phenolic	*mg /g	88.57±3.14
Total flavonoids	**mg /g	37.13±1.83
DPPH	^a mg/mL	27.52±7.63
Reducing power	^b mg/mL	239.52±0.41

508

509 Data represented in means of duplicates ± standard deviation

510 *Total phenolic was expressed as gallic acid equivalents (GAE) mg/ g sample

511 **Total flavonoids was expressed as mg catechol/g sample

512 ^a IC50 (mg/mL): Inhibitory concentration at which 50% of DPPH radicals are scavenged.

513 ^b EC50 (mg/mL): Effective concentration at which the absorbance is 0.5.

514

Table (6): Inhibition zone diameter in mm of different concentrations of sage (*Salvia officinalis*) aqueous extract and MIC for each strain

Pathogenic strain	Inhibition zone diameter (mm)**				
	50*	25*	12.5	6.25*	MIC
Gram-positive bacteria					
<i>Staphylococcus aureus</i> NCTC10788	20.0	18.0	12.0	6.0	6.25
<i>Staphylococcus pyogenes</i> EMCC1772	13.5	10.0	5.5	ND	12.50
<i>Streptococcus mutants</i> EMCC1815	18.0	11.0	7.0	ND	12.50
<i>Bacillus subtilis</i> DB100	19.0	14.5	5.5	ND	12.50
<i>Clostridium botulinum</i> ATCC3584	15.5	11.0	7.5	ND	12.50
Gram-negative bacteria					
<i>Escherichia coli</i> BA12296	18.0	17.0	10.0	5.0	6.25
<i>Escherichia coli</i> ATCC25922	19.0	13.0	5.0	ND	12.50
<i>Klebsiella pneumonia</i> ATCC12296	18.0	13.0	5.0	ND	12.50
Yeast					
<i>Candida albicans</i> ATCCMYA2876	22.0	18.5	8.0	5.0	6.25

517

518 Data represented are average of duplicates, *Concentrations of extract and MIC are in mg/mL

519 **Diameter included 5 mm well diameter, MIC; Minimum Inhibition Concentration