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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 µg/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.

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

1. INTRODUCTION

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

Salvia "sage" are derived from the Latin salvere (to save), referring to the healing properties, and in the Levant it's called Maramia (Hamidpour et al., 2014). *Salvia* has a long history of uses in culinary, medicinal, nutritional and pharmacological purposes. Studies highlighted the effect of *Salvia* in preventing and controlling various diseases naturally in a safe manner. Sage was reported to be rich in several bioactive compounds, which in most of cases seem to be responsible for the pharmacological properties of these plants, including cytotoxicity against human cancer cells (Xavier and Pereira-Wilson, 2015). German Commission E (a governmental regulatory agency) has accepted the use of *S. officinalis* for a number of medical applications (Ghorbani and Esmaeilizadeh, 2017).

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

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2. MATERIAL AND METHODS

2.1. Plant material and preparation

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

The plant leaves has been checked with

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

2.2.Proximate analysis

2.2.1. Nutrients content

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

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

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

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

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

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$$\%Crude\ fibre = \frac{W3 - W1}{W2} X100$$

Where: W1 = Weight of pre-dried filter bag, W2 = Original weight of sample, W3 = Weight of filter bag contains organic residue remaining after digesting https://www.ankom.com

Total carbohydrate by difference was calculated by the following formula;

100 – (weight in g [protein + fat + water + ash] in 100 g of food) (WHO/FAO, 2002)

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

2.2.2. Electrolytes and minerals concentrations

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

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

Where: V1: Volume of stock standard solution of sample (50 mL), V2: Volume of stock standard solution of sample used in measurements (5mL), W*sample*: Weight of sample (≅0.5 gm).

2.2.3. B Vitamins content

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

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Kinetex XB-C18 column 100 mm X 4.6 mm (Phenomenex, USA), operated at 35°C. The separation was achieved using a binary linear elution gradient with (A) 25 mM NaH_2PO_4 pH 2.5 (v/v), (B) methanol. The injected volume was 20 µL. VWD detector was used for at 220 nm for vitamins B3, B6 and B12.

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

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

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

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

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

2.3. Pharmacological potentials

2.3.1. Antioxidant activity

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

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

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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 cytoxicity. 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

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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

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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 µg/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 µg/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

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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 12.5 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 300µg/mL). Sage clearly affected cell viability as revealed by neutral red assay with an IC_{50} of 300µg. 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 µg/ mL for 48 h. Incubation of breast cancer cells with sage resulted in morphological changes that

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

4. DISCUSSION

Plant-fiber-enriched foods are reported to improve glucose and lipid metabolism, they lower serum cholesterol concentrations in patients with diabetes, in addition to multiple effects on different cardiovascular risk factors (De Natale et al., 2009). The electrolytes results emphasized that sage contains 3 times more calcium and 5.5 times more zinc than milk. USDA (USDA, 2016) reported different values for sage mineral except for zinc and sodium content. Alterations in essential trace elements like Fe, Zn and Ca may play an important role in the pathogenesis of complex diseases including cancers. Dietary calcium intake found to be associated with a reduced incidence of cancer, while iron, is involved in many processes including DNA, RNA and protein synthesis, and Zn may represent an independent risk factor for cancer and therefore a possible target for prevention (Al Faris and Ahmad, 2011; Singh et al., 2016). Obtained vitamin results indicated that sage could be considered a good source of vitamins B6 and B12 and encourage suggestions of using as food supplement especially for vegetarians and elderly. What is most important that, Vitamin B6 and B12 was reported to sensitize a large panel of cancer cell lines to apoptosis (Pandey et al., 2013; Saghiri et al., 2017). Obtained values of B vitamins fluctuated between agreement and disagreement with (Lovik et al., 2009; USDA, 2016). Nutritional profile may aid to explain the current increased demand on Salvia species for nutritional and pharmacological industries (Yadav et al., 2017). Many biological functions requiring lysine; include synthesis of connective tissues and carnitine, conversion of fatty acids to energy that support healthy growth in children and maintenance of healthy immune function especially in the management of Herpes simplex virus (HSV) infections. Lysine also found to enhance calcium absorption preventing Osteoporosis. On food technology wise; lysine is involved in the browning reaction applied

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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

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

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

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

Alterations of the MDA-MB-231 cell surface or of the sensitive lysosomal membrane lead to lysosomal fragility and other changes that gradually become irreversible. The main advantage of neutral red assay is that it detects only viable cells (Babich and Borenfreund, 1987). Previous researches reported the antitumor properties of sage extract which probably in correlation to its strong antioxidant activity as in other plant extracts (Garcia et al., 2016; Rupasinghe et al., 2016). Sage bioactive compounds, Linalyl acetate (Ly), Terpeniol (Te) and Camphor (Ca), has been reported to have synergistic effect on two human colon cancer cell lines HCT-116 (p53 (+/+) and p53 (-/-)). Apoptosis by Ly + Te + Ca, in p53 (+/+) cells (Itani et al., 2008). Despite progress in cancer research, the biological active components and antitumor mechanisms have not yet been exactly determined, but mitochondrial membrane

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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 µg/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.

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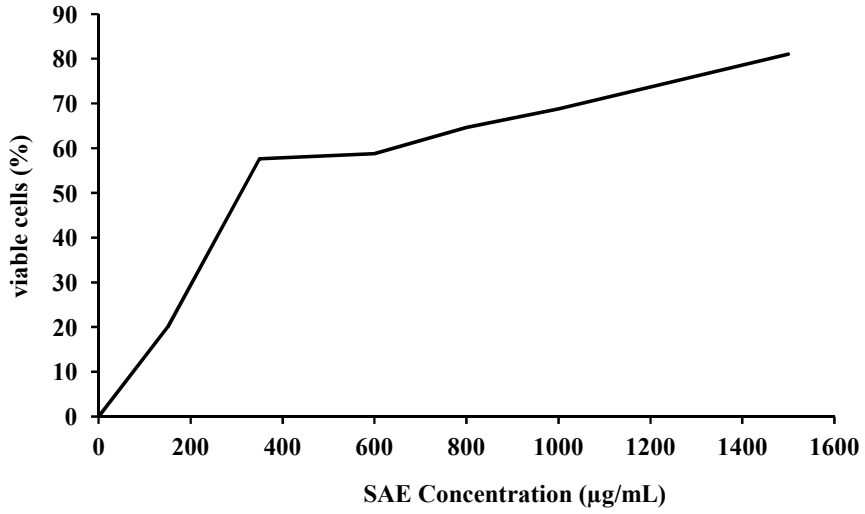

Figure 1. Anti-proliferative activity of sage (*Salvia officinalis*) aqueous extract (SAE) against MDA-MB-231 cell line via neutral red assay (IC_{50} of 300µg/mL)

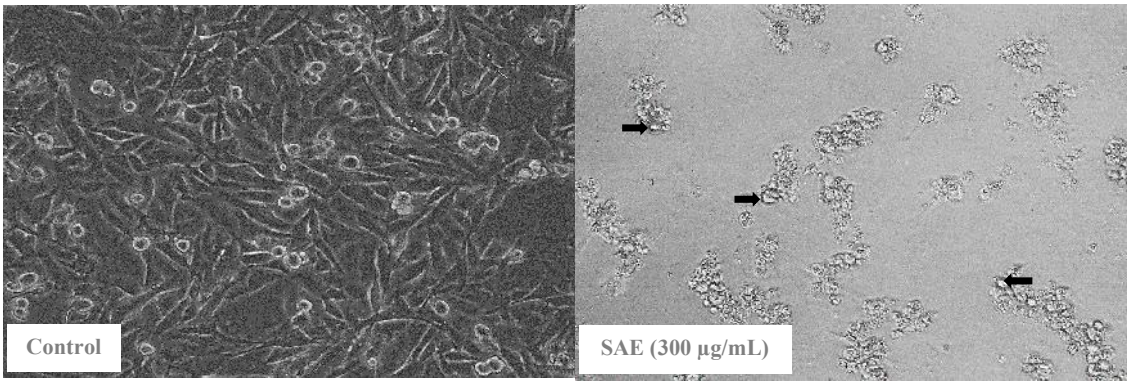

Figure 2. Morphological changes in MDA-MB-231 Triple negative breast cancer cell following treatment with sage aqueous extract (SAE) with concentration of 300 µg/ mL for 48 h.

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.

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

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

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

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

Amino Acid	Symbol	Content (g)
Essential amino acids		
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	-	-
Non-essential amino acids		
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

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Data represented are per 100g of sample

Table (3): Fatty acid profile of sage (*Salvia officinalis*)

Fatty acid	Symbol	g/100g
SFAs		
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
USFAs		
MUSFAs		
Oleic	C18:1 ω-9	3.13
PUSFAs		
Linoleic	C18:2 ω-6	4.40
α-Linolenic	C18:3 ω-3	6.46
%of total fat		13.99
USFAs/ SFAs		0.32
ω6/ ω3 ratio		0.68

SFAs; Saturated fatty acids, USFAs; Unsaturated fatty acids, MUFAs; Monounsaturated fatty acids, PUFAs; Polyunsaturated fatty acids

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

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507 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

515 Table (6): Inhibition zone diameter in mm of different concentrations of sage (*Salvia*

516 *officinalis*) aqueous extract and MIC for each strain

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