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Phenolic Compounds And Biological Activities Of Salvia Officinalis And Moringa Oleifera Leaves

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Abstract
In this study, the phenolic content and biological activity of Salvia officinalis and Moringa oleifera leaves, were compared. Spectrophotometric methods are used to determine total phenolic content, total flavonoids, and 1,1-diphenyl-2-picrylhydrazyl (DPPH). The methanolic extracts' antibacterial activity against three bacterial strains (Escherichia coli, Staphylococcus aureus, and Salmonella enterica) and Candida albicans as yeast was tested using the agar-well diffusion method. High Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD) was performed to determine the specific phenolic compounds by comparing the retention times of phenolic compounds in samples to 19 standard compounds. Salvia officinalis and Moringa oleifera had total phenolic contents of 166.574 and 96.066 mg GAE/g, total flavonoids of 127.573 and 139.591 mg GAE/g, and DPPH radical scavenging activity of 70.445 and 61.392, respectively. Salvia officinalis exhibited the highest antimicrobial action against Candida albicans, while antibacterial activity was equal in the two samples. The HPLC results revealed variances in phenolic content. Salvia officinalis exhibited high composition levels. The main compounds discovered in the Salvia officinalis and Moringa oleifera samples were rosmarinic acid and gallic acid, respectively. All results are compared to previous studies.
Key words: *Salvia officinalis, Moringa oleifera, total phenolic content, total flavonoids, DPPH and HPLC-DAD.*

INTRODUCTION

Because of their uses in medicine, nutrition, beverages, insect repellents, fragrances, flavourings, cosmetics, and dyes, medicinal and aromatic plants are becoming increasingly in demand in many countries [1]. Significant economic benefits might result from the variety of these plants as well as their vital use as a source of natural goods and raw minerals. These plants produce numerous secondary metabolites. Among these metabolites, essential oils are volatile substances with strong added value and olfactory activity. [2]

Common sage (*Salvia spp*., with the most common species being *Salvia officinalis*) is a valuable medicinal and aromatic plant because of its bioactive components, which are secondary products of metabolism. These components mostly include phenolics, terpenoids, polyphenols, and flavonoids. Many studies have identified their critical role in combating oxidative stress in cells and organisms, as well as their anticancer, antibacterial, and anti-inflammatory properties [3]. Sage has been the focus of extensive research in recent decades due to its ability to extract flavonoids and phenolics from the plant. Therefore, food formulations are increasingly using sage [4], [5]. When Lima *et al*. [6] looked into the antioxidant activity of Salvia tea in vivo, they found that the antioxidant status of the liver improved after 14 days of tea consumption. *Salvia officinalis's* aqueous extract possesses fungicidal, antiviral, antibacterial, and antioxidant properties [7], [8].

Moringa oleifera has gained popularity due to its dense nutritional content and its role in addressing malnutrition. In addition to providing a range of nutrients, like proteins, fibers, minerals, and a complete amino acid profile, the plant is abundant in phytochemicals such as flavonoids, isothiocyanates, phenolic acids, and tannins. It contains 13 species of trees and shrubs that are indigenous to Africa and India and flourish in numerous tropical and arid areas [9, 10]. Numerous studies highlight the potential of bioactive compounds from Moringa trees in creating new functional foods, cosmetics, and medicines [11]. Flavonoids, including quercetin and kaempferol, are among the remarkable natural antioxidants found in *M. oleifera*, according to Yameogo *et al*. [12]. According to Sidduraju and Becker [13], the antioxidant profiles of *M. oleifera* extracts from various agroclimatic zones varied. Ascorbate (vitamin C), phenolics, α-tocopherol, and β-carotene are some of the compounds that have dry weights between 70 and 100µmol/g, 74 and 210µmol/g, 0.7 and 1.1 mmol/g, and 1.1 and 2.8µmol/g, respectively. When compared to other fruits and vegetables, the antioxidant content of the leaves is noticeably higher. For example, carrots have 1.8 µmol/g of beta-carotene, while strawberries have 190 µmol/g of gallic acid. Hot peppers have 110 µmol/g of ascorbate, while soybeans have 1.8 µmol/g of tocopherol [14]. Several investigations have validated the bactericidal qualities of *M. oleifera* leaves. One study that looked at leaf extracts made with different solvents [15] found that the ether extract of *M. oleifera* leaves was the most effective against Proteus mirabilis, a bacterium that can cause pelvic infections. Researchers have also studied the antibacterial

properties of M. *oleifera* leaves against different bacterial species [16]. According to Chuang *et al.* [17], M. oleifera leaf essential oils and basic extracts have antifungal efficacy against Microsporium canis, Trichophyton rubrum, T. mentagrophytes, and Epidermophyton floccosum. Patel *et al.* [18] found that ethanolic and aqueous extracts of M. *oleifera* leaves were effective against certain yeasts, except for Candida albicans. Economic and environmental issues regarding Saudi Arabian medicinal plants, which have the potential to greatly advance the creation of new uses for natural goods, served as a driving force behind our study. The current study aims to explore the phenolic components and biological activities of the leaves of the *Salvia officinalis* and *Moringa oleifera*.

MATERIALS AND METHODS

samples

Dry leaves of *Salvia officinalis* and *Moringa oleifera* from south Saudi Arabia (abha) was obtained from local markets in Qassim region, Saudi Arabia. After collecting, we use an electric grinder to reduce the material into a fine powder, which we then store clean until needed.

Determination of total phenolic Content (TPC)

The total phenolic content (TPC) of *Salvia officinalis* and *Moringa* oleifera leaf extracts were quantified calorimetrically with the Folin-Ciocalteau reagent, following the methodology outlined by Mythili *et al.* [19]. We dissolved 1.0 gram of each sample in 20 cc of 98% methanol. We filtered the methanol extract using Whatman No. 1 (Grade 589/2) filter paper. Folin-Ciocalteu reagent (1 ml diluted with distilled water at a ratio of 1:10) was mixed with 1 ml of the extract sample for three minutes. Then, 3 ml of 2% sodium carbonate (1 M) was added. After maintaining the mixture at room temperature for 15 minutes, we quantified the polyphenols using an automated UV-VIS spectrophotometer (JASCO, Corporation Model V-730, S.N. A112961798, Tokyo, Japan) at 765 nm, based on a gallic acid calibration curve (0–100 mg/l). The blank was made using the identical process, substituting 20 µl of clean water for the extract.

The results are expressed as equivalents to Gallic acid (mg GAE/ 0.1 g sample). Equivalent Gallic acid content in the test samples was determined using the standard linear equation (Y=1.0752X+0.0002; R2= 0.9999).

Determination of total Flavonoids Content (TFC)

Ebrahimzadeh et al. [20] and Nabavi *et al.* [21] outlined the methodology for assessing the total flavonoid content (TFC) of *Salvia officinalis* and *Moringa oleifera* leaves. We dissolved 1.0 gram of each sample in 20 cc of 98% methanol. We filtered the methanol extract using Whatman No. 1 (Grade 589/2) filter paper. We combined a 1 ml sample with 1.5 ml of methanol, 0.1 ml of aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. They thereafter remained at room temperature for 10 minutes. We recorded the absorbance of the combination at 415 nm using a UV/visible spectrophotometer (JASCO, Corporation Model V-730, S.N. A112961798, Tokyo, Japan). We quantified the quercetin concentration in the test samples using the standard linear equation (A=0.022X+0.006; R2= 0.999).

Determination of DPPH radical scavenging activity

Burits and Bucar [22] developed a technique to evaluate the radical scavenging activity of the investigated chemical. We dissolved 1.0 grams of each sample extract (*Salvia officinalis* and *Moringa oleifera* leaves) in 20 ml of 98% methanol. We filtered the methanol extract using Whatman No. 1 (Grade 589/2) filter paper. We added one millilitre of the sample to the DPPH reaction solution (1 millilitre) at a concentration of 0.2 mM. After vigorously agitating the mixture and allowing it to stand at room temperature for 30 minutes, we measured the absorbance of the solution using a spectrophotometric device (JASCO, Corporation Model V-730, S.N. A112961798, Tokyo, Japan) at 517 nm.% DPPH radical scavenging activity = (Ac–As) / Ac × 100 A is the absorbance of the sample; Ac is the absorbance of the control without the sample.

Antimicrobial Activity

Tested Microorganisms:

We have used three strains of bacteria to assess antibacterial activity of the obtained methanolic extracts of *Salvia officinalis* and *Moringa Oleifera* Leaves one Gram positive pathogenic bacteria; *Staphylococcus aureus* ATCC 13565-, and two-Gram negative bacteria; *Escherichia coli* O157-H7 ATCC 51659, *Salmonella enterica* and *Candida albicans* ATCC 10231 as yeast. The strains were grown on nutrient agar dishes at 37° C for 24 hr and kept in a refrigerator at 4° C until use .

Disc diffusion technique

Antibacterial assay: the assay was performed as recommended in European Committee for Antimicrobial Susceptibility Testing (EUCAST) [23]. Briefly, from the overnight incubated culture, a typical colony was picked and introduced in a 5 ml of tryptone soy broth. The broth culture was incubated at 35oC until visible turbidity reached 0.5 “McFarland" standard solution. Then, nutrient agar plates (25 ml agar / 9 cm plate or equivalent)

were inoculated with sterile cotton swabs in three directions to finally give a semi-confluent growth after overnight incubation. Within 15 minutes, discs with tested substances were applied on the dried surface of the inoculated agar plates. After incubation at 35oC for 20 h, inhibition zone diameters (mm) were recorded

Determination of flavonoids and phenolic acids by HPLC

Sample preparation

One grams of each sample (*Salvia officinalis* and *Moringa oleifera* Leaves) were mixed with 20 ml methanol, sonicated for 15 min and supernatant was filtered through a 0.2 µm Millipore membrane then 1-3 ml was collected in a vial for injection in HPLC

HPLC Conditions:

Agilent 1260 series conducted the HPLC investigation. Zorbax Eclipse C_8 (25 x 0.46 cm, 5 µm) was used as the reverse phase column. The composition of the mobile phase was water (A) and 0.05% trifluoroacetic acid in acetonitrile (B). Table 1 displays the gradient system of the mobile phase, maintaining a constant solvent flow rate of 0.9 ml/min. The photodiode array detector has been monitored at 280 nm. The volume of injections was 5 µl.

Table (1): Mobile phase gradient system

Time(min)	A (%)	B (%)
Initial	82	18
1	82	18
11	75	25
18	60	40
22	82	18
24	82	18

RESULTS AND DISCUSSION

phenolic substances exhibit antioxidant and antibacterial properties.

For millennia, plant extracts have been used to address many illnesses, with their mode of action perhaps linked to the amount of phenolic compounds[24, 25]. Phenolic compounds are the predominant class of phytochemicals, with considerable physiological and morphological significance in plants, along with pronounced antioxidant properties[26]. Numerous studies have associated the antioxidant, anti-inflammatory, anti-cancer, and antibacterial attributes of diverse plants, herbs, and species with their phenolic content. Suaeda fruticosa has anti-inflammatory, antioxidant, and anticancer properties because it has a lot of phenolics (31.8 mg of gallic acid equivalent (GAE) per gramme of dry weight (DW)) [27]. The identification and measurement of phenols from diverse sources is becoming vital due to their potential in disease therapy. *The Salvia officinalis* sample showed a higher level of phenolic compounds, antioxidant and antimicrobial activities than *Moringa Oleifera* as general. The amount of total phenolic contents and total flavonoids contents in *Salvia officinalis* sample were (166.574 mg GAE /g) and (127.573 (mg QE/g) respectively (table 2). This results higher than Mekhaldi Abdelkader *et al* [28] and I. MALIK *et al* [29].

There were 139.591 mg QE/g of total flavonoids and 96.066 mg GAE/g of total phenolics in *Moringa oleifera* (table 2). This was more than what Evi Sulastri *et al* [30], Abdulaziz Rabiu Abdulkadir *et al* [31], El-Fadl, S. A *et al* [32], and Unuigbe, C *et al* [33] found.

The antioxidant activity DPPH of the *Salvia officinalis* sample showed high activity (table 2) when compared to Izabela [Jasicka-Misiak, I *et al* [34]. The activity of *Moringa oleifera* was lower than that of RAMÓN IGNACIO CASTILLO-LÓPEZ *et al* [35] and similar to that of Abdulaziz Rabiu Abdulkadir *et al* [31].

Total phenolics and flavonoids in the samples correlated positively with antioxidant activity (DPPH). A number of authors [36–39] have demonstrated that there is a linear correlation between the content of total phenolic compounds and their antioxidant capacity.

Salvia officinalis and *Moringa oleifera methanolic* extracts (table 3) have shown strong antimicrobial activities against *Candida albicans*.

The antifungal activity of the methanolic extract of *Salvia officinalis* was found to be higher than that of the studies conducted by Dhia Hassawi, & Abeer Kharma [40], Abd-Elmageed, M., & Hussein, B [41], and Bălăşoiu, R. M *et al* [42]. The antibacterial activity against *S. aureus* in the *Salvia officinalis* sample (7.5 mm) was similar to that reported by Arista Wahyu Ningsih *et al* [43]. The antimicrobial activity *of Salvia officinalis* against *E. coli* showed similar findings, as reported by Mosafa, E *et al* [44]. *Salvia officinalis's* sample exhibits higher antibacterial activity against *Salmonella enterica* than the study by Zdolec, N *et al* [45].

The antifungal activity of *Moringa oleifera* methanolic extract was equal to that of Muhammad Maqsood *et al* [46], higher than that of Arista Wahyu Ningsih (2021) [43], and lower than that of Hassan, H. E., & Ahmed, S. H [47]. The antibacterial activity of *Moringa oleifera* against *S. aureus* was lower than that of Arista Wahyu Ningsih1 *et al* [43], but higher than that of Adetitun, D.O *et al* [48] and Rafiq, N[49]. The antibacterial activity of the *Moringa oleifera* sample against *E. coli* was higher than that of Akinyeye, A. J [50]. The activity of the *Moringa oleifera* sample against *Salmonella enterica* was higher than that of Rafiq, N[49] and Adetitun, D.O *et al* [48].

Table (2): phenolic compounds and antioxidant activities of *Salvia officinalis* and *Moringa Oleifera*

Sample	TPC(mg GAE /g)	TFC(mg QE/g)	DPPH radical scavenging %
Salvia officinalis	166.574 ± 10.9^{ab}	127.573 ± 11.5^{b}	70.445 ± 1.8^{b}
Moringa Oleifera	96.066 ± 9.4^{c}	139.591 ± 11.3^{b}	61.392 ± 1.8^{c}

Table (3): Antimicrobial Activity of *Salvia officinalis* and *Moringa Oleifera* methanolic extract:

Extract	Inhibition zone in mm (mean± SE)			
	S. aureus	*E. coli*	*S. enterica*	*C. albicans*
Salvia officinalis	7.50 ± 0.00^{Db}	6.0 ± 0.00^{Dc}	6.0 ± 0.00^{Dc}	27.67 ± 0.34^{Aa}
Moringa Oleifera	6.0 ± 0.00^{Eb}	6.0 ± 0.00^{Db}	6.0 ± 0.00^{Db}	11.33 ± 0.68^{Fa}
Positive control	8.00 ± 0.00^{Cc}	23.67 ± 0.34^{Aa}	13.67 ± 0.68^{Ab}	10.00 ± 1.18^{FGc}

Data expressed as Mean ± SE; A shared Capital letter within the same column or small letter within the same raw means no significance.
C+ (Fungal): Clotrimazole 1 mg/ml; C+ (Bacterial): Amikacin 30 µg/disc

HPLC analysis of Flavonoids and phenolic Acids

The most common method of sample preparation for phenols is extraction using organic solvents, which spectrophotometric and chromatographic methods apply to identify and measure [51, 52]. We used the RP-HPLC/DAD technology to identify the phenolic acids and flavonoids in *Moringa oleifera* and *Salvia officinalis* leaves from Abha.

The phenolic acids and flavonoids found in *Salvia officinalis* and *Moringa oleifera* are presented in Table 4 and Figures 2 and 3, respectively. The identification depends on comparing the retention times (Rt) of the phenolic compounds obtained from the standard mixture chromatogram (Figure 1) with those found in the chromatograms of the samples (Figures 2 and 3). The main compound found in the *Salvia officinalis* sample was rosmarinic acid, which had a high concentration of 128.67 µg/g when compared with *Moringa oleifera* sample, so the results showed that the antioxidant activity and antifungal of *Moringa oleifera* sample was highest, The rosmarinic acid is more prevalent in the Liliaceae family plant species, specifically Rosmarinus officinalis, Perilla, and Salvia [53]. Rosmarinus officinalis essential oil exhibited antifungal and anti-aflatoxigenic activity against A. flavus. Researchers also discovered that Salvia species extract possesses antifungal activity against various Candida species [54–56]. Furthermore, researchers acknowledge that rosmarinic acid exhibits significant antioxidant properties, acting as a scavenger of reactive oxygen species and an inhibitor of lipid peroxidation, with the potential to improve health [57-59]. Additionally, coumaric acid was present in *Salvia officinalis* at a concentration of 11.45 µg/g, other compounds detected in the sample include coffeic acid, gallic acid, chlorogenic acid, elagic acid, vanillin, and ferulic acid. A number of compounds are present in very small quantities: syringic acid, rutin, naringenin, daidzein, querectin, cinamic acid, kaempferol, and hesperetin. These findings agree with the data that was previously documented in Hamrouni-Sellami *et al* [60] and Francik *et al* study [61].

The primary compound found in the *Moringa oleifera* sample was gallic acid, which had a high concentration of 51.21 µg/g. Other compounds identified in the sample included vanillin (18.20 µg/g), chlorogenic acid (23.80 µg/g), rosmarinic acid (14.31 µg/g), and rutin (12.13 µg/g), that agree with the study reported by Prabakaran

et al. (2018 [62] saying the main phenolic compounds discovered in MO leaves were kaempferol, myricetin, quercetin, chlorogenic acid, gallic acid, luteolin, vanillin, and rutin. Additionally, we detected trace amounts of catechin, methyl gallate, coffeic acid, syringic acid, elagic acid, ferulic acid, and naringenin. The findings correspond with the data presented in previous studies by Yanqin Zhu *et al* [63, 64]. We found the flavonoid levels in the Moringa oleifera sample to be relatively low compared to Pollini, L *et al* [65] study.
The chromatogram of ***Moringa oleifera*** sample (Fig 3) showed one peak (retention time 21.5 min) with high concentrations, but not detected because haven't standard, and depending on the retention time of the compound , it is likely to be a flavonoid.

Table(4): The phenolic acids and flavonoids of *Salvia officinalis* and *Moringa oleifera*

Flavonoids and phenolic Acids levels (µg/g)		
	Moringa Oleifera	*Salvia officinalis*
Gallic acid	51.21	4.80
Chlorogenic acid	23.80	3.91
Catechin	1.77	0.00
Methyl gallate	2.44	0.00
Coffeic acid	0.61	6.10
Syringic acid	0.04	0.68
Rutin	12.13	0.53
Ellagic acid	3.04	4.66
Coumaric acid	0.00	11.45
Vanillin	18.20	3.26
Ferulic acid	1.23	1.80
Naringenin	1.16	0.14
Rosmarinic acid	14.31	128.67
Daidzein	0.00	0.28
Querectin	0.00	0.45
Cinnamic acid	0.00	0.06
Kaempferol	0.00	0.45
Hesperetin	0.00	0.20

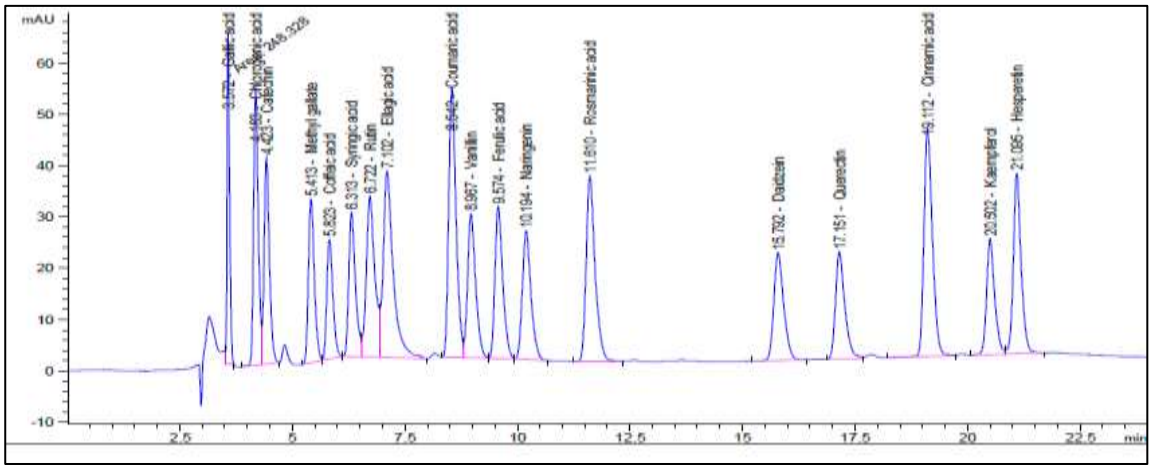

Fig 1: HPLC Chromatogram of Flavonoids and phenolic Acids standards

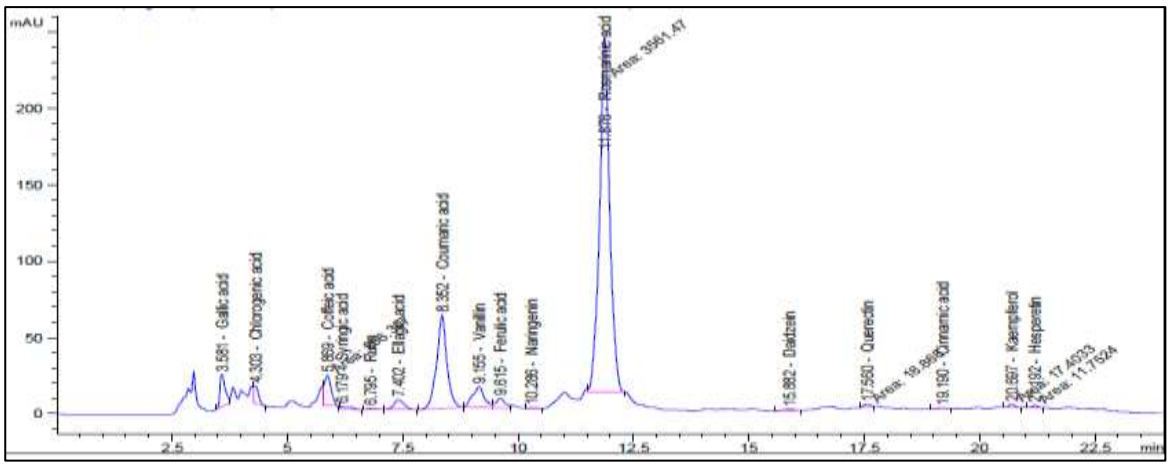

Fig 2: HPLC Chromatogram of Flavonoids and phenolic Acids levels in *Salvia officinalis*

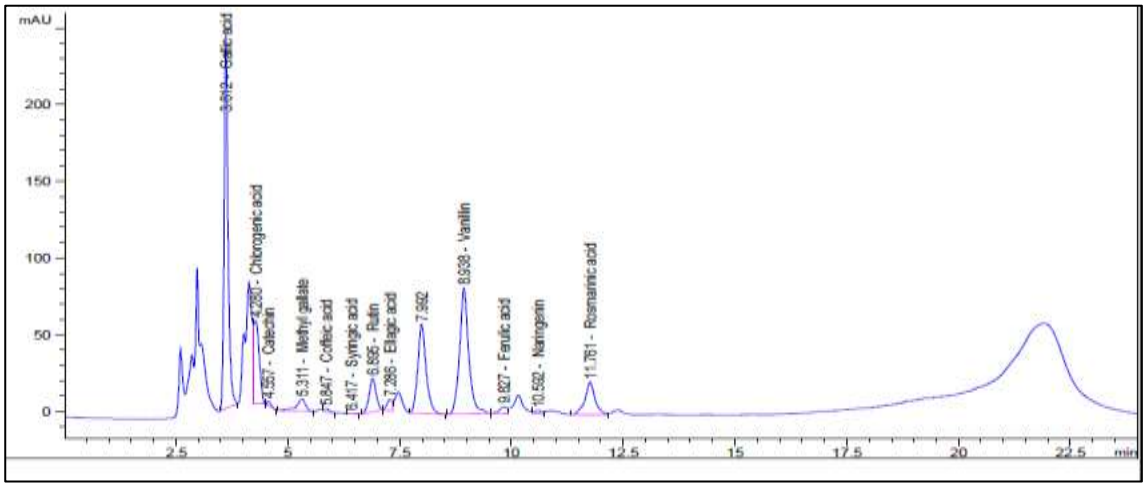

Fig 3: HPLC Chromatogram of Flavonoids and phenolic Acids levels in *Moringa oleifera*

CONCLUSIONS

Methanolic extracts of *Salvia officinalis* leaves grown in Abha, Saudi Arabia, have higher antioxidant, antimicrobial activity, and phenolic substances than *Moringa oleifera*. Total phenolics and flavonoids showed a positive correlation with biological activity. We measured several phenolic acids and flavonoids in both samples using HPLC-DAD. The primary phenolic components in *Salvia officinalis* leaves were rosmarinic acid and coumaric acid, while the main phenolic compounds in *Moringa oleifera* leaves were gallic acid and chlorogenic acid. The results show that methanolic extracts of *Salvia officinalis* and *Moringa oleifera* leaves are good sources of phenolic compounds that are strong antioxidants. These compounds can be found naturally in healthy foods, cosmetics, and can also be used in the pharmaceutical industry.

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