Study of Some Boswellia Carterii Contents and Effect of their Extracts as Antioxidants and Antibacterial

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Abstract

Boswellia carterii leaves are one of the most popular herbal medicines. The purpose of this study was to use Boswellia carterii leaf extracts to find out how many free amino acids are in a sample. Phenolic compounds, particularly flavonoids, were estimated in the three types of extracts using the high-performance liquid chromatography device (HPLC) and many other important biologically active factors due to their benefits to human health and antioxidant and antibacterial effects. Aqueous extracts, ethanol and ethyl acetate showed five types of amino acids, the highest of which was Glutamic acid. In addition to containing good numbers and quantities of phenolic compounds, many of which contain multiple biological properties such as antioxidant and antibacterial, this research has been carried out. An antioxidant analysis showed that ethanol extract showed more antioxidant properties than aqueous extract and ethyl acetate extract. However, compared to vitamin C measurement the three extract sections were able to inhibit a good ratio of 1, 1- diphenyl 2-picrylhydrazyl free radical (DPPH) with a good excellent ratio balance. The maximum activity of DPPH root rickets for vitamin C (38.5) ppm, ethanol extract (69.5) ppm, ethyl acetate extract(115) ppm and aqueous extraction (125) ppm were concentrated. The antibacterial activity showed a maximum inhibition area of 24 mm for Staphylococcus aureus as Shigella flexneri at a concentration of 500 ppm .

Keywords*:* *Boswellia carterii, Amino acids, Phenolic compounds, Antioxidants, Antibacterial.*

INTRODUCTION

The Boswellia genus is made up of around 20 species; however, this number is in dispute.1 Boswellia species are well known for producing frankincense, an aromatic oleogum resin Frankincense has been used in traditional medicine and cultural and religious ceremonies for thousands of years. More recently essential oils derived from frankincense resins have become[1] Among the various species of Boswellia, only a few are traded in a significant amount: B. sacra, B. carteri, B. frereana, B. papyrifera, and B. serrata. The highest quality frankincense resins are produced by B. carteri trees [2] Studies have also demonstrated that these

extracts can improve blood flow, lower the risk of clotting, support capillary walls and their flexibility, and protect neurons from damage that could result from oxygen deprivation[3] Boswellia carterii leaves contain large amounts of protein, lipids, carbohydrates, vitamins, organic acids, polyphenols, beta carotene, flavonoids, and terpenoids [4]. In addition to the well-known terpene lactones and flavonol glycosides, amino acids from Ginkgo Biloba leaves are frequently used in meals, drinks, and medications [5]. Chemical compounds known as amino acids (AAs) have both amino and acid groups[6]. The fundamental building ingredients that support life are amino acids. It is well known how they contribute to the production of protein synthesis, this metabolic rewiring and support for numerous immune cell functions depend on crucial amino acid metabolism. [7][8] .Many plants contain a broad collection of secondary metabolites called flavonoids. It has a flavone structure, in which the C ring of the pyran ring (which may have hydroxyl, methoxy, methyl, or isoamyl substituents) connects the two benzene rings (A and B rings)[9] Plants receive their color, tastes, and medicinal effects from flavonoids [10] The main sources of flavonoids are fruits and vegetables [11]. Plants are shielded from harmful environmental factors by flavonoids [12], which also contain biological features like increased blood circulation, lowered cholesterol, UV protection, angiogenesis suppression, antibacterial, and anti-inflammatory Activities[9] They have consequently attracted a lot of interest. To determine if they can aid in treating a variety of acute and chronic human illnesses, they have been put to the test in numerous epidemiological and experimental research [14]. In vitro and animal studies have demonstrated the anti-inflammatory and immunomodulatory activities of flavonoids [15] as well as their powerful anticancer properties [14]. [16] [17]. The oxidative effects of free radicals and other oxidants in biological systems can be mitigated by antioxidants [18] Based on their existence in the human body, antioxidants are classified as endogenous or exogenous[19][20] Due to their special chemical and biological characteristics, they are advantageous to a variety of human products [21]. Because they can shield the body against oxidative damage to biological macromolecules including lipid, protein, and nucleic acid, which is predominantly brought on by secondary metabolism, the hunt for new and natural antioxidants from dietary plants is gaining traction[22].

MATERIAL AND METHODS

Sample Collection:

Boswelliacarterii leaves were collected from Sulaymaniyah governorate, Iraq, in September 2022. The leaves were then washed with deionized water, dried in shade for several days at room temperature, and ground as a powder The conventional Soxhlet extraction method was applied to samples according to [24] for obtaining aqueous and alcohol and ethyl acetate extract.

Determination of Amino Acids and Phenols concentrations:

All of the reference materials were made up entirely (99%) of Samarra Pharmaceuticals using an HPLC system (Sykamn s3210, Germany) and a C18 column (4.6mm 150 mm, 5m). Mobile phase (acetonitrile: H2O) (5: 95) for amino acids, the flow rate of 2 mL/min, and the detection system using fluorescence (340nm-450nm) are the corresponding values [25]. For the following parameters: flow rate (1.2 mL/min), detection fluorescence system

(260nm-310nm) and phenols mobile phase (H2O: acetonitrile)(70:30) respectively [26]

Antibacterial Activity studies:

Many microorganisms were employed to create bacteria; bacteria from selected microorganisms were tested for antibacterial activity using a spreading tablet; and Boswellia extracts were tested for antimicrobial activity using a well-chosen deployment method. [27][28] According to the McFarland method, samples were loaded on cellulose tablets in diameter (5mm) bacterial insulation was developed, and 0.5 was transported 1 ml of bacterial and bacterial filaments equivalent to (1.5 108) cells/ml to the dish that contains the center of Müller Hanton's den. The study included testing in-kind water, alcohol and ethyl acetate extracts from the Boswellia plant in inhibiting the growth of bacteria causing diseases positive for Kram dye outside the living body until the implant is absorbed, disseminate, and let the dish at room temperature for 15–20 minutes. At temperature of 37 C, bacteria are grown on plates, extract-loaded tablets, and other media. For each type of bacteria utilized, the diameters of the tablet regions around the chips are measured for a whole day. Determination of free radical scavenging activity

The DPPH was used to assess the free radical scavenging capacity of Boswellia carterii preparations. The extract was assessed at various concentrations (12.5, 25, 50, 100, and 200 g/mL). An addition of a (150L) methanol solution comprising varying quantities of sample solution was made to 3 mL of daily generated methanol and DPPH solution The sample's scavenging activity was determined by comparing its absorbance to that of the DPPH reference solution The following formula was used to evaluate the radical scavenging activity percentage of the DPPH: % Antiradical activity percentage equals (**A**o – **A**C) /**A**o× **100** Where Ao is the absorbance of the control and Ac is the absorbance of the sample or standard in the present of the control. The results of three independent trials were averaged and expressed as a percentage of mean radical scavenging activity [29].

RESULTS AND DISCUSSION

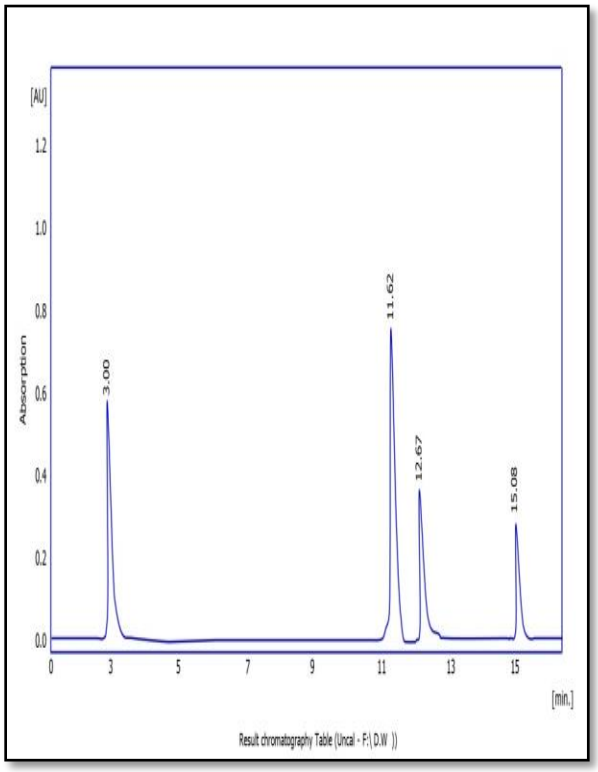
The results revealed that B. carterii leaf extracts were high in free amino acids, especially those considered essential, such as (Arginine, Valine, and Glycine,), in addition to (Cysteine, Alanine, and Glutamic acid)The total amount of amino acids in the sample ranged from (10.25 to 14.00) µg /gm in aqueous extract, while it was from (13.59 to 16.28) µg /gm in the alcoholic extract, and in the ethylacetate extract it was from(8.22 to 15.98) µg /gm according to the Table (1) and Fig.(1)

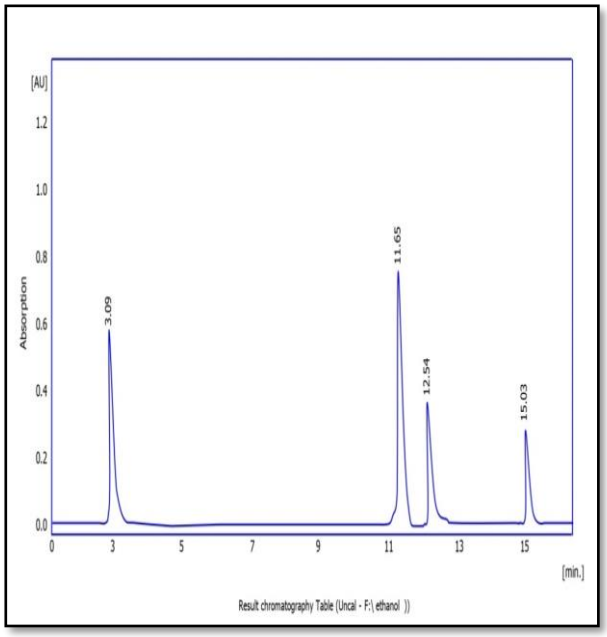
Table 1: Concentration of amino acids in the aqueous, alcohol extracts and Ethyl acetate extract of the Boswellia carterii

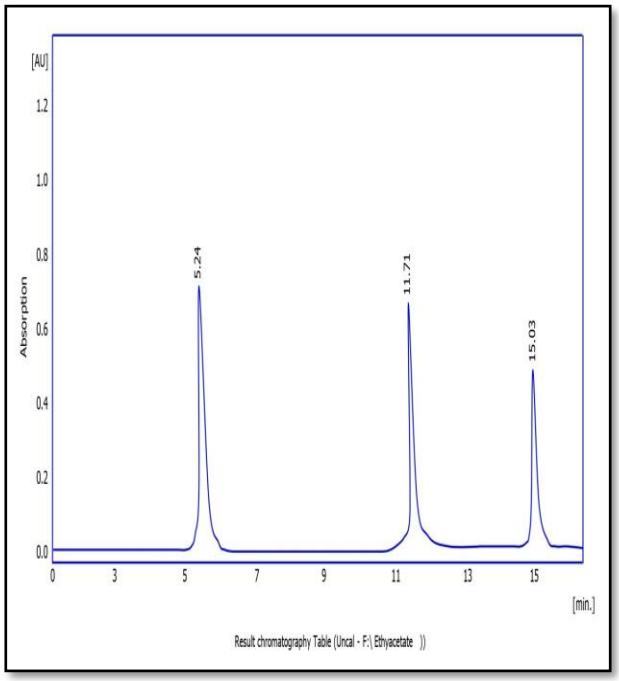
Aqueous extract			Alcoholic extract			Ethyl acetate extract
Amino acid	**Retain. Time [min]**	**Amount [µg / gm]**	**Retain. Time [min]**	**Amount [µg /gm]**	**Retain. Time [min]**	**Amount [µg /gm]**
Alanine	3.00	11.25	3.09	13.59	-	-
Arginine			-	-	5.24	8.22

Cysteine	-	-	-	-	-	-
Glutamic acid	11.62	14.00	11.65	16.28	11.71	15.98
Glycine	12.67	12.59	12.54	14.11	-	-
Proline	15.08	10.25	15.03	13.69	15.03	10.46

Figure 1: HPLC analysis for(A) Amino acid in the aqueous extract(B) Amino acid in ethanol extract (C) Amino acid in ethyl acetate extract





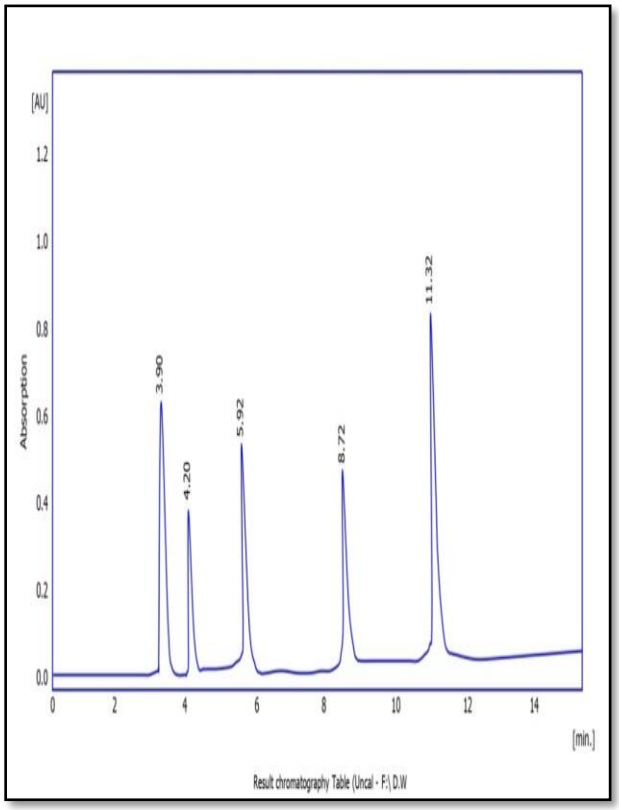


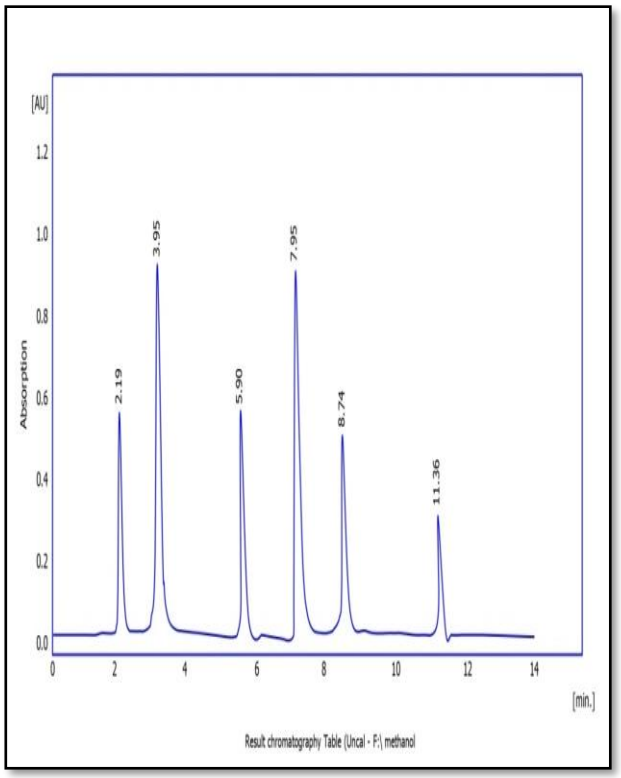
Amino acids serve as vital structural constituents and a source of energy. An amino group, a carboxylic acid, and a unique carbon structure are all present in every amino acid. Twenty amino acids are required by humans; the majority of them can be produced naturally, but nine of them are "essential," meaning they must be ingested The components of proteins that are either ketogenic, glycogenic, or both, recycle as needed, and serve as the building blocks for intermediates in the Krebs cycle and other metabolites, making amino acids essential energy sources [30] Amino acids (AA) are used of product protein and a variety of low-molecular-weight molecules with physiological important. Nutritionally, amino acids are essential for maximal growth breastfeeding and good health, well-being, and reproduction [31] In the human body, specific amino acids can be changed into other amino acids, proteins, glucose, fatty acids, or ketones [32]. Neurotransmitters, or chemical messengers in the nervous system, are Alanine play a direct role in gluconeogenesis; transamination [34]. precursors to nucleic acids, which are parts of DNA [33]. Alanine has a direct function in gluconeogenesis; transamination [34] Arginine contains antioxidants, hormone secretion, ammonia detoxification, gene expression, and immunological function-regulating capabilities [35] Cysteine is a disulfide bond in proteins that allows sulfur to be transferred [36]. Glutamine: The production of arginine connects the urea and Krebs cycles. Isoleucine Synthesis of glutamine and alanine; regulation of protein synthesis and gene expression; activator of glutamate.; production of tyrosine; neurological development [37]. Tyrosine is the source of dopamine, epinephrine, norepinephrine, and thyroxin [38] The HPLC analysis of Boswellia carterii Extracts looked for important antioxidant phenolic compounds in this study We utilized several a variety of standard solutions for phenols. The results demonstrated that B.carterii intriguing focus extracts because it contains a high number of phenol which each compound was calculated by comparing the area of the pick of the standard substance with the size of the picked choice for the desired combination and according to the subsequent equation: C(sample) = [A (sample) ×A (standard)] /C average In the aqueous extract, the concentration of (Glumamic acid) was the highest and had the best separation of the peak at retention time (11.62min) according to the peak of standard substances, and the presence of all phenolic compounds at range retention time (2-11min), Figure (2) and table (2). In the alcohol extract, the concentration of (Glumamic acid) too was the highest and had the best separation of the peak at retention time (11.65 min) according to the rise of standard substances, and the presence of all phenolic compounds at the range retention time (2-11min), Figure (2) and table (2).In the ethylacetate extract, the concentration of (Glumamic acid)) was the highest and had the best separation of the peak at retention time (11.71min) according to the peak of standard substances, and the presence of all phenolic compounds at range retention time (2-11min), Figure (2) and table (2).

Table 2: Concentration of phenolic compounds in the aqueous and alcohol extracts of the Boswellia carterii

phenolic compounds	Result Aqueus Extract			Result in Alcohol Extract			Result in ethyl acetate Extract			Retention Time [Min]
	Area[mV.s]	conc.[µg.mL-1]	Retention	Area[mV.s]	conc.[µg.mL-1]	Retention	Area[mV.s]	conc.[µg.mL-1]	Retention	Standard
Apigenin	9652.58	48.457	11.32	932.02	14.338	11.36	-	-	-	11.38
P-coumaric acid	-	-	3.90	3652.19	36.521	3.95	1625.49	-	-	7.90
Ferulic acid	2305.98	25.036	8.72	1263.58	12.365	8.74	2013.69	21.862	8.72	8.72
Vanillic acid	1365.28	24.191	4.20	-	-	-	-	-	-	4.20
Gallic acid	2698.59	33.726	5.92	2140.88	26.750	5.90	598.28	7.477	5.92	5.91
Qurcetin	-	-	-	2564.85	29.005	2.19	-	24.254	2.15	2.15
Kaempferol	580.44	46.492	3.90		62.917	3.95	-	-	-	3.90

Figure 2: HPLC analysis for(A) Phenolic compounds in the aqueous extract. (B) Phenolic compounds in ethanol extract (C) Phenolic compounds in ethyl acetate extract





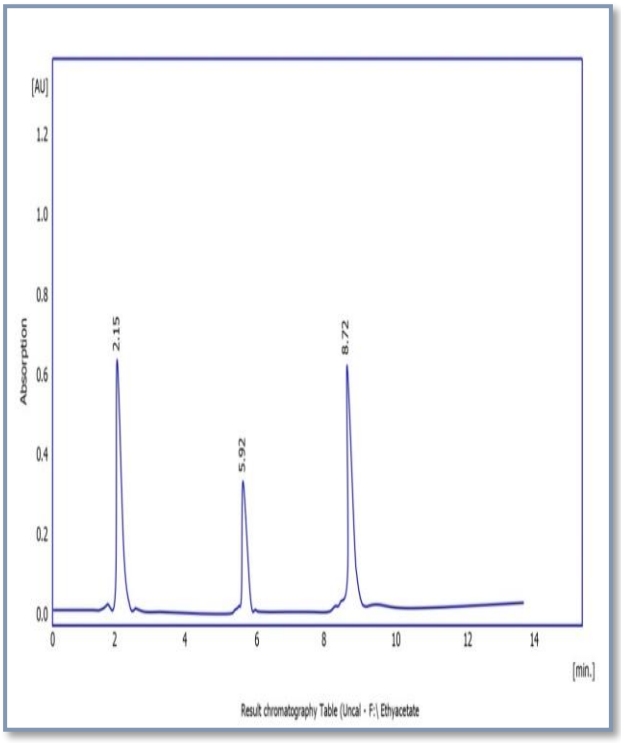
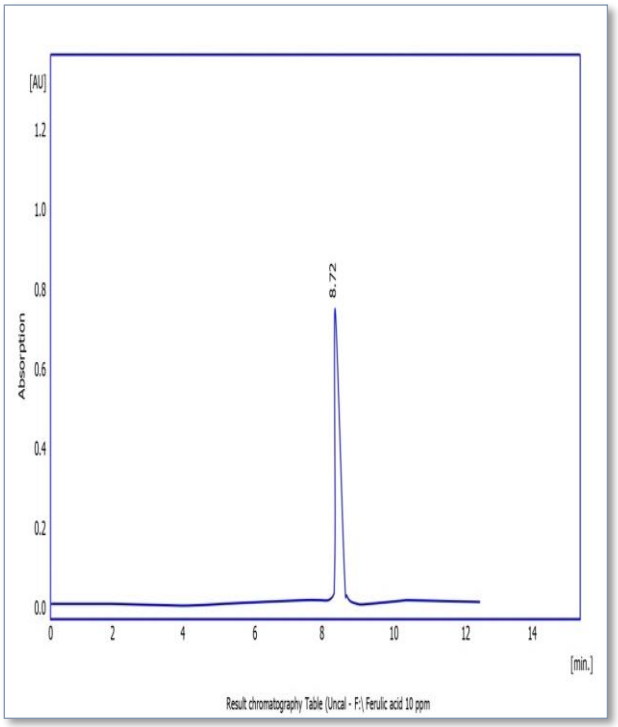


Figure 3: HPLC analysis for standards phenolic compound



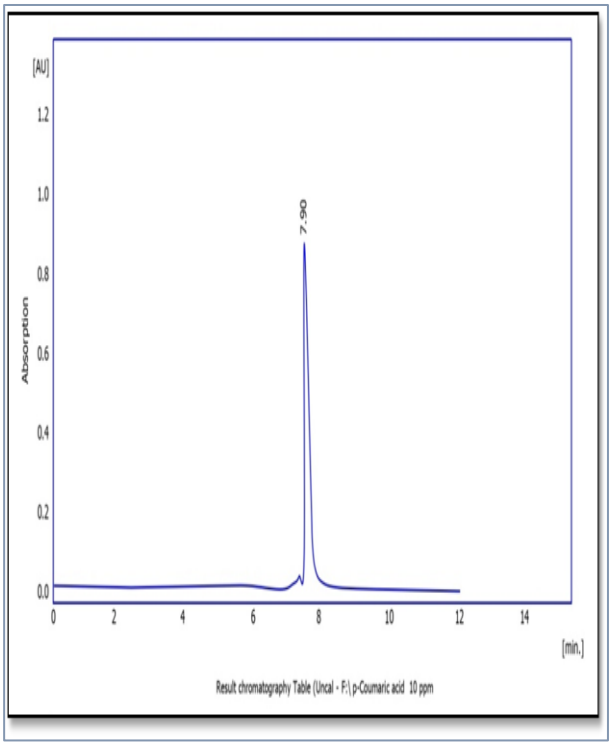
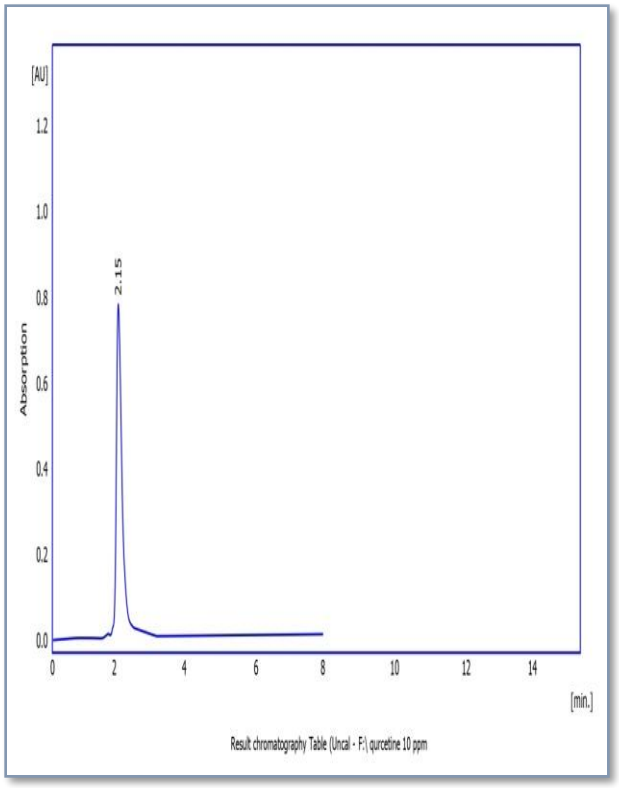
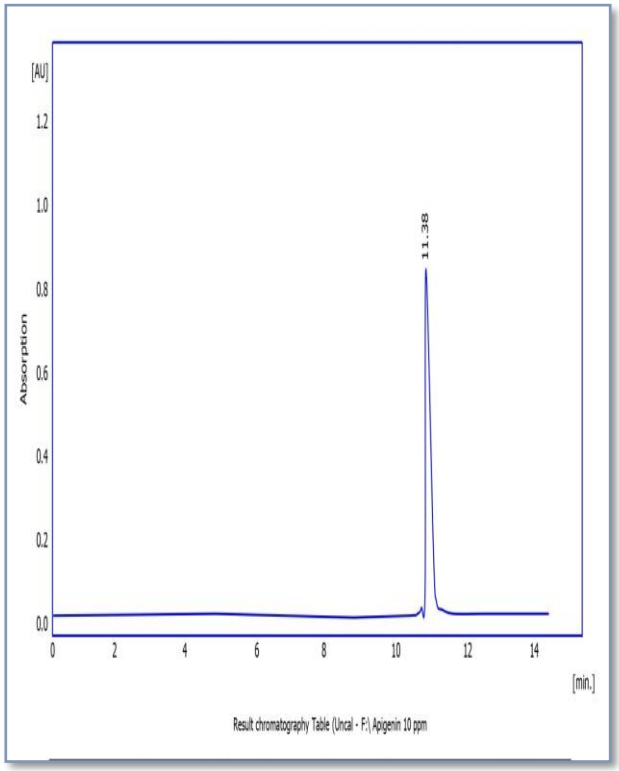
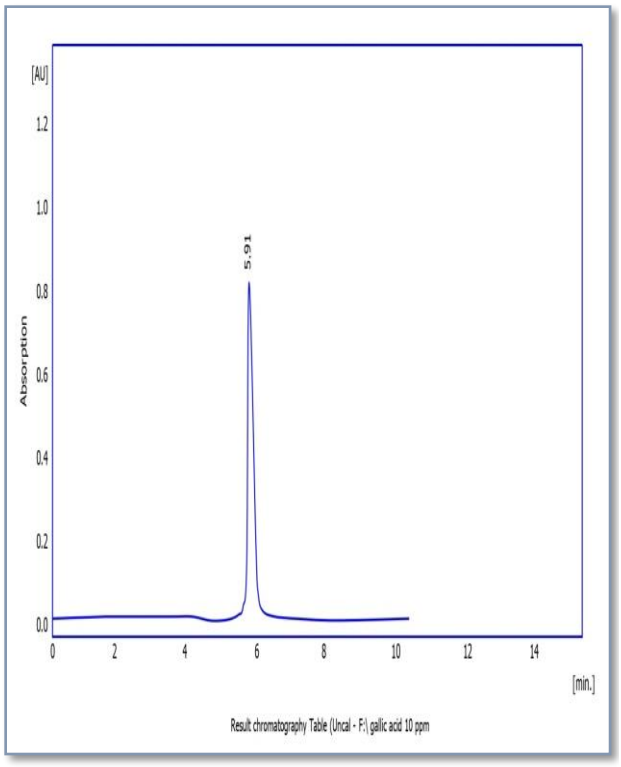


Figure 4: HPLC analysis for standards phenolic compound







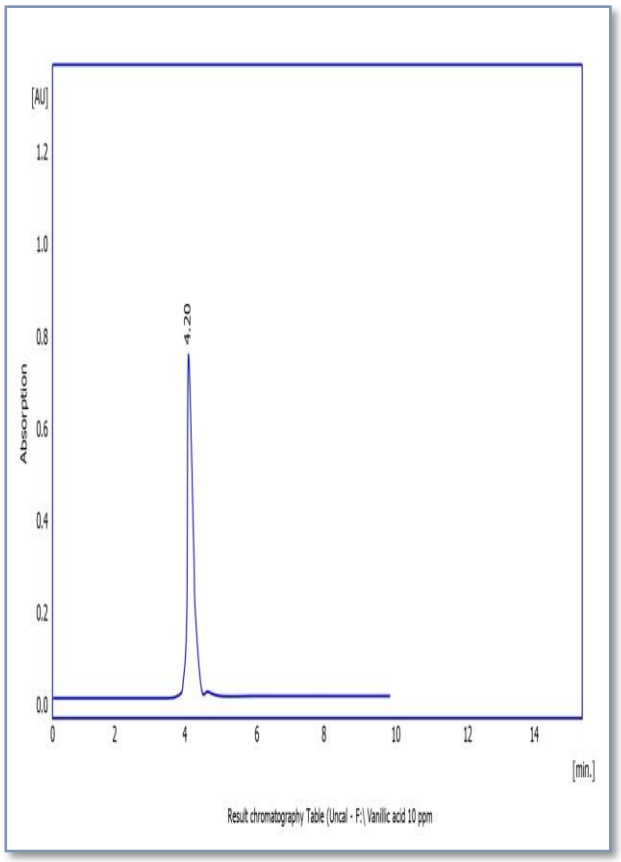
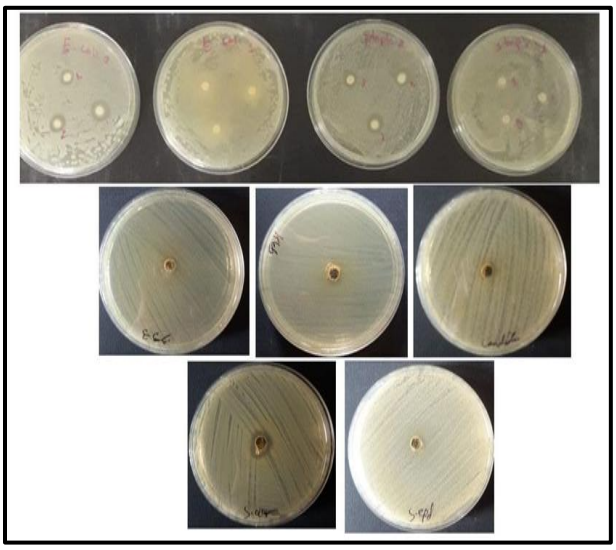


Table 3: Values the standard deviation of the aqueous, alcohol extracts and ethyl acetate of the Boswellia carterii

Sample No.				S1	S2	S3	S4
Aqueous Extract	*Gram-positive*	*S.aureus*	*Mean*	-	-	-	-
			Sd	-	-	-	-
		S.epidermidis	*Mean*	-	-	-	-
			Sd	-	-	-	-
	Gram-Negative	*E .coli*	*Mean*	-	-	-	-
			Sd	-	-	-	-
		Klebsiella sp.	*Mean*	-	-	-	-
			Sd	-	-	-	-
	Fungi	*C.albicans*	*Mean*	-	-	-	-
			Sd	-	-	-	-
Alcohol Extract	*Gram-positive*	*S.aureus*	*Mean*	*20*	*9*	*22*	-
			Sd	*16*	-	*17*	-
		S.epidermidis	*Mean*	*10*	-	*9*	-
			Sd	*12*	*10*	*12*	-
	Gram-Negative	*E .coli*	*Mean*	*17*	*15*	*14*	-
			Sd	*1.44*	*1.56*	*1.34*	-
		Klebsiella sp.	*Mean*	-	-	-	-
			Sd	-	-	-	-
	Fungi	*C.albicans*	*Mean*	*21*	*8*	*22*	-
			Sd	*1.12*	*1.52*	*1.94*	-
Ethyl acetate Extract		*S.aureus*	*Mean*	-	-	-	-

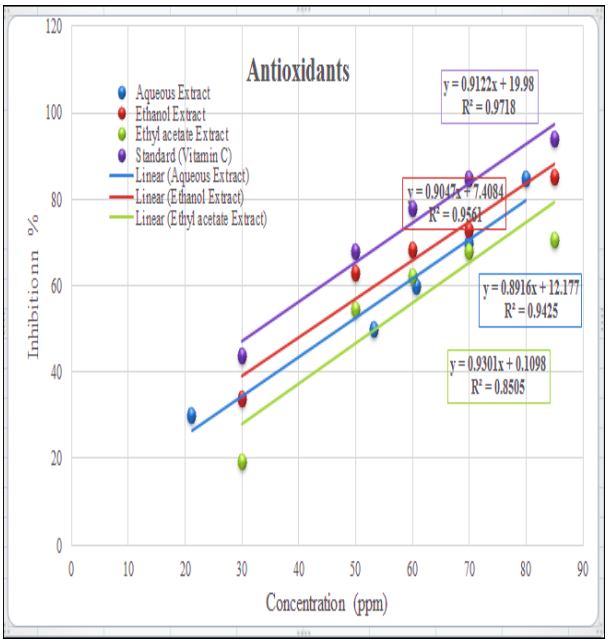
	Gram-positive		***Sd***	-	-	-	-
		S.epidermidis	***Mean***	-	-	-	-
			Sd	-	-	-	-
	Gram-Negative	***E .coli***	***Mean***	-	-	-	-
			Sd	-	-	-	-
		Klebsiella sp.	***Mean***	-	-	-	-
			Sd	-	-	-	-
	Fungi	***C.albicans***	***Mean***	-	-	-	-
			Sd	-	-	-	-

Figure 5: Inhibition zones caused by Boswellia carterii extracts Against Staphylococcus aureus, Staphylococcus



Microorganisms can be inhibited through a variety of mechanisms, including cell membrane damage, protein synthesis inhibition, and disruption of biological processes and cell membranes by particular enzymes. The presence of compounds like -Pinene and -Pinene (pinene-type monoterpene hydrocarbons), which are involved in the disruption of the membrane cell wall by the lipophilic compounds, has an impact on the nature of the bacterial cell wall [45] Additionally, bacteria like Escherichia coli are resistant to antibiotics because gram-negative bacteria's cell walls have a high lipid content (up to 20%) compared to gram-positive bacteria's lipid content of 0-2%. Positive gram-positives, on the other hand, The antibacterial substances present in extracts are more sensitive to microorganisms [40]. According to research, Boswllia alcohol extract has more antibacterial action against gram-positive bacteria than gram-negative bacteria that are resistant to each of the three extracts water and ethanol [46]. Free radical scavenging activity The DPPH radical scavenging assay is a sensitive antioxidant assay substrate that is unaffected by the polarity of the substrate A hydrogen radical or an electron can be taken up by the stable free radical DPPH to create a stable diamagnetic molecule The extracts' high phenolic content may explain for their potent antioxidant properties. Based on these results, antioxidant activity against DPPH radicals was shown by both the extracts and the mixed sample three extractions showed composite action antioxidant efficacy against DPPH radicals. The fascinating anti-oxidant stress activity of gingko leaf extracts is therefore revealed by this exploratory study, suggesting its potential use as a medicinal source for the treatment and prevention of disease. It has therapeutic and pharmaceutical properties that can treat diseases brought on by free radicals. Fig (6) Data indicated that the three extracts contain a good percentage of antioxidants when compared to vitamin C, a potent and efficient antioxidant [47].

Figure 6: DPPH Free radical scavenging activities of Boswellia carterii extracts compared with Vitamin C.



A: Vitamin C (IC50=32.909 ppm), B: Ethanol Extract (IC50= 42.421ppm), C:

Aqueous Extract(IC50=53.64ppm)

D:Ethyl Acetate Extract (IC50= IC50=77.078ppm) the outcomes demonstrated that water extracts, ethanol and ethyl acetate from carterii boswellio leaves had DPPH radical scavenging action. According to their diverse concentrations of 10 g/ml to 60 g/ml, the ethanol extract showed the highest percentage of scavenging activity. Boswellia carterii leaf extracts' strong DPPH scavenging activity is consistent with reports [48] The increasing intensity of the color is directly proportional to the inhibition of DppH[49] and phenols. This high content of flavonoids and phenols is the primary driver of their antioxidant activities. Ethanol also showed more extraction of phenolic content than aqueous extraction and ethyl acetate extraction, so ethanol extract showed the best antioxidant properties. The increasing concentration of the extract is increasingly preventing DPPH activity. Ethanol extracts also had a clear and apparent effect on bacterial inhibition compared to aqueous and ethyl acetate extracts.

CONCLUSIONS

This study established that Boswellia carterii leaf extracts contain significant levels of phenols, flavonoids, and amino acids. The abundance of flavonoids and phenols is the primary driver of their antioxidant activities. Ethanol also showed 99% more extraction of phenolic content than aqueous extraction, so ethanol extract showed the best antioxidant properties. The extract's concentration is limiting DPPH activity more and more as it grows. In comparison to aqueous extracts, ethanol extracts also appeared to have a clear influence on bacterial inhibition.

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