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Biological activity of some bioactive compounds extracted from the leaf of neem trees (Azadirachta indica) cultivated in Makkah, Saudi Arabia

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Biological activity of some bioactive compounds extracted from the leaf of neem trees (*Azadirachta indica*) cultivated in Makkah, Saudi Arabia

Abstract

Neem (*Azadirachta indica*) is a very beneficial traditional medicinal plant which is broadly distributed all over the world. This work aimed to evalute the antioxidant capacity of two extracts of neem leaf soluble phenolics neem leaf (SNL) and insoluble-bound leaf (IBL). The total soluble and insoluble-bound content of phenolics, flavonoids, tannins, and anthocyanins were determined. The antioxidant capacity of the two extracts was assessed by applying various methods viz radical scavenging assays such as radical anion 2,2'-azinobis-(3-ethylbenzothiazoline-6 sulphonate) ($ABTS^{*+}$ scavenging), 1,1-diphenyl-2-picryl hydrazyl (DPPH scavenging) assay, reducing power activity and iron chelation methods. Moreover, the extracts were assessed for the inhibition of low-density lipoprotein (LDL) oxidation, DNA protection activity by using pBR322 plasmid DNA, and the antimicrobial activity. The result revealed that both SNL and IBL extracts have antioxidant activity but SNL antioxidant potintial was higher than that of IBL and it was associated with higher phenolics, flavonoids, tannins, and anthocyanins contents in SNL than IBL. MALDI-TOF-MS analysis to the neem leaf extract demonstrated the presence of various bioactive compounds. Both extracts have the ability to inhibit LDL oxidation and DNA scission as well as both extracts has antimicrobial activity against gram-positive bacteria whereas no antimicrobial potential was recorded against gram-negative bacteria and dermatophyte fungus. Ultimately; this study indicates that the phenolic leaves extract of *A. indica* had significant antioxidant activity. Therefore, the extract of *A. indica* leaves can be used in pharmaceutical applications and as a natural antioxidant instead of a synthetic antioxidant.

Keywords: *Azadirachta indica*, neem, antioxidant capacity, LDL oxidation, DNA protection, antimicrobial activity

Statements and Declarations

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Competing Interests

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Tasahil S. Albishi and Tahani A. Alqahtani. The first draft of the manuscript was written by Tasahil S. Albishi and Tahani A. Alqahtani commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

All the data created or analyzed during the time of this investigation are presented in this publication.

1 Introduction

Azadirachta indica is a fast-growing evergreen tree belongs to family Meliaceae, it grows in tropical and subtropical regions and it is native to the entire Indian subcontinent and Southeast Asia, including Pakistan, Thailand, Sri Lanka, Indonesia, and Malaysia [1]. Now *A. indica* is widely cultivated in many countries all over the world such as Philippines and Australia [2]. In 1971, approximately 50,000 of *A. indica* trees were cultivated in the plains of Arafat in Makkah Al-Mukarramah, Saudi Arabia, to provide shade for the millions of Haj pilgrims [3, 4, 5]. *A. indica* trees were chosen because they are adapted to grow under harsh climate such as in Saudi Arabia where high temperature and low availability of water [6, 7] *A. indica* tree has a deep tap root that can reach up to 30 m deep that helps it to reach to groundwater, as well as lateral roots extending up to 15 m from the trunk, helping for anchoring the tree in the soil [8, 9**].**

According to World Health Organization more than 80% of the individuals in developing nations based on ethnomedicines for health care. But approximately 50% of the World's population still depends on ethnomedicines from plant source [10]. *Azadirachta indica* is known as (Neem) the name was derived from (Nimba) the expression that was used by Ancient Sanskrit to refer "good health" as it was though that *A. indica* brings good health to those who take them [11, 12, 13]. In India, *A. indica* is considered as the panacea of all diseases, and it is called the 'Divine Tree' and "Village Dispensary" [14]. In 1992, the US National Academy of Sciences published a report entitled "Neem: A Tree for Solving Global Problems" [15]. In addition, the *A. indica* tree has been dubbed the "Tree of the 21st Century" by the United Nations [16], this indicates the importance of *A. indica* tree.

Numerous studies reported that neem contains various bioactive compouns that was identified by the modern high-throughput techniques [17]. These compounds fall into two main groupes isoprenoids and non-isoprenoids [18]. Isoprenoids sub-divided into diterpenoids and triterpenoids [19]. Diterpenoids include protomeliacin, azadirone, gedunin, amoorstatin with vepinin, vilasinin, and C-Seco meliacins [20, 21, 22]. All neem tree parts including root, stem, leaves, seeds, flowers and fruits are rich with bioactive ingredients and have medicinal properties [23]. The leaves of *A. indica* have a bitter taste and a strong odor due to the presence of various bioactive compounds, including azadirachtin, azadirone, nimbandiol, nimbolide, nimbin, and azadirachtanin, these compounds exhibit various biological activities, such as antimicrobial, antiviral, anti-inflammatory, and antioxidant properties [24]. The leaves of *A. indica* have been traditionally used in Ayurvedic medicine for treating various ailments, such as leprosy, intestinal worms, epistaxis, and skin ulcers [14].

Secondary metabolites of plant such as phenolic and flavonoids compounds play essential defense roles in plant, the synthesis of these metabolites is strongly affected by the environmental factors like temperature, salinity, drought, light in addition to genetical, nutritional and hormonal factors [25]. Plant phenolic compounds are responsible for its antioxidant potential [26]. As phenolic compounds can inhibit oxidation process by scavenging free radicals or chelate metal ions [27, 28, 29].

Phenolic compounds present in plant could be classified into two majore groups flavonoids and non -flavonoids. Flavonoids compounds consist of two phenolic rings and an oxygenated heterocycle. It includes anthocyanins, flavonols, flavanols, flavones, and chalcones. Non-flavonoids compounds are

monocyclic acids and could be classified into two sub-groups: phenolic acids and stilbenes, phenolic acids are further subdivided into benzoic acids and hydroxycinnamic acids [30]. The cinnamic acids derivatives have higher antioxidant activity than benzoic acids ones. This is attributed to the binding of −CH=CH–COOH of the cinnamic acid to the ring by double bond that increases free radicals stabilization ability than –COOH group in benzoic acid [31].

Neem phenolic contents are strongly affected by geographical location and other abiotic factors [32] so the current work intended to provide light on possibility of using leaf extracts of A. indica grows in Saudi Arabia as an effective source of antioxidants in biological model systems.

2 Materials and methods

2.1 Plant material

Leaves of *A. indica* were obtained from a local flowering plantation in the Al-Awali, Makkah Al-Mukarramah, Saudi Arabia in May 2024

2.2 Samples collection

The leaves of *A. indica* were soaked in tap water for five minutes to get ride of soil and then repeatedly rinsed with distilled water. The leaves were left to be air dried in the dark at room temperature. Finally, the leaves were gronded with electric vacuum packed and stored in -20ºC [33].

2.3 Methods

2.3.1 Extraction of phenolics

Extractions and fractionations of phenolic compounds, both soluble and insoluble-bound, were carried out in accordance with 34 and 35. 150 mL of a methanol: acetone: water (7:7:6, v/v/v) mixture was ultrasonicated for 20 minutes at 30°C with 5g of freeze-dried leaves. The resultant slurries were collected after being centrifuged for five minutes at 4000 x g. Under the same circumstances, the residue was repeatedly extracted. The combined extracts were centrifuged, and the residue was set aside to identify the insoluble bound phenolics while extracts were examined for soluble phenolics. Organic solvents were removed from the supernatants by vacuum evaporation at 40°C. The interfering lipids were removed by adjusting the aqueous phase to pH 2 prior to hexane extraction. Following four extractions using a 1:1 v/v ratio of diethyl ether to ethyl acetate, the soluble phenolic acids were vacuum-dried by a rotary evaporator, then the extract was dissolved in 5ml methanol (80%). Initially, the residue was mixed for 4hr under nitrogen while being dissolved in 50ml of 4M NaOH. After that, the mixture was acidified to pH 2, centrifuged, and diethyl ether: ethyl acetate (1:1, v/v) was used to extract the bound phenolics.

2.3.2 Estimation of total phenolic content

The procedure outlined by 35 was followed to measure the total phenolic content. One milliliter of the Folio-Ciocalteu phenol reagent has been added to 1ml of methanolic extracts. After adding 1 mL of sodium carbonate (75 g/L) and distilled water (7ml) the mixture was incubated for 45 minutes at room temperature. The optical density was recorded at 725 nanometers.

2.3.3 Estimation of total flavonoid content

The aluminum chloride method of [37] was followed. Distilled water (4ml) was added to 1ml of extracts or a standard quercetin solution (0.75, 1.5, or 3 mg/mL). Subsequently, 5% sodium nitrate (0.3 ml) was added followed by of 10% aluminum chloride (0.3 mL). After adding two ml of NaOH (1M) solution the optical density was measured at 510.

2.3.4 Estimation of condensed tannins

The vanillin assay method described by 38 was followed to measure the amount of condensed tannins. The aqueous extract (1ml) was added to 2ml of freshly prepared 70%vanillin–sulfuric acid then incubated at 20°C for 15 minutes. The mixture's optical density was recorded at 500 nm. The condensed tannin content was calculated by following equation:

Where [CT] is the condensed tannin content (mg Cya/g DW); A is the absorbance of the sample; V

$$[\mathrm{CT}] = \frac{A \times V \times D_f \times V' \times M}{\varepsilon \times v \times m}$$

is the volume of the reaction medium; V^1 is the volume of the aqueous extract recovered after extraction with diethyl ether; v is 0.5 mL; D_f is the dilution factor; M is the molecular weight of cyanidin; m is the weight of the dry matter; and ε is the molar extinction coefficient (34,700 M^{-1} cm^{-1}).

2.3.5 Estimation of hydrolyzable tannin content

The method of 39 was slightly modified to measure the amount of hydrolyzable tannins [40]. One ml of sample was added to 5ml heated potassium iodate 2.5% the mix was incubated for 7min at 30Cº, the optical density was measured at 550 nm.

2.3.6 Estimation of total anthocyanin content

The anthocyanin content was determined using the 41 technique. Each extract (0.5 mL) was diluted separately using 2.5 mL of 0.025 M potassium chloride (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5). After 15 minutes at room temperature, the optical density of the two solutions were recorded at 520 and 700 nm. The anthocyanin content was measured as following:

$$Anthocyanins\ content\ (\mathrm{mg}\ /\ (100g\ of\ dry\ matter) = A * MW * DFI(\varepsilon * \mathrm{W})$$

Where A= absorbance (A_{520nm}-A_{700nm}) pH 1.0 - (A_{520nm}-A_{700nm}) pH 4.5, MW= molecular weight of cyanidin-3-glucoside (C_{15} H_{11} 0_6, 449.2), DF= dilution factor, ε = molar absorptivity (26900), and W= sample weight (g).

2.3.7 Estimation of antioxidant activities of soluble and insoluble- bound phenolics extracted from leaves of *A. indica*

$ABTS^{*+}$ radical scavenging capacity

It is measured according to 42 as modified by 43. The leaf extracts dissolved in phosphate buffer saline (PH 7.4, 0.15 M sodium chloride) was added to radical solution of 2,2'-azinobis-(3-ethylbenzothiazoline-6 sulphonate) and 2.5 mM 2,2'-azobis-(2-methylpropionamidine) dihydrochloride in 2.5 mM phosphate

buffer saline (PH 7.4, 0.15 M sodium chloride) (PBS).After six min the absorbance was recorded at 734 nm.

DPPH radical scavenging capacity (DRSC)

It was carried out according to 44 using electron paramagentic resonance (EPR). By mixing 500µL of suitably diluted soluble and bound phenolic extracts in methanol with 2ml of a 0.18 mM DPPH solution in methanol.

Reducing power activity

The reducing power activity of leaf extracts is measured following 45 method. The extract (1ml) was added to 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide solution. Then incubation at 50 °C in a water bath for 12 min and centrifuge. After that, 2.5 mL of a 10% (w/v) trichloroacetic acid solution was added followed by 0.5 ml of a 0.1% (w/v) ferric chloride solution and 2.5 mL of distilled water were added to the supernetant. The absorbance was recorded at 700 nm.

Iron (II) chelation capacity

The method of 46 was employed to determine the extract's chelation of ferrous ions. Leaf extract (0.5ml) was mixed with 1.85 mL of methanol, 0.05 mL of 1 mmol/L ferrozine. Then incubation at room temperature for 10 min. The absorbance was recorded at 562 nm. The following equation was used to determine the extracts' iron chelation capacities:

$$\boldsymbol{FeII\ chelation\ capacity}\% = (\mathbf{1} - \boldsymbol{sample\ absorbance}) * \mathbf{100}/\boldsymbol{blank\ absorbance}$$

2.3.8 Measuring the antioxidant properties of *A. indica* leaf extracts in biological model systems

Inhibition of human low-density lipoprotein (LDL) oxidation character generated by cupric ions

It was determined based on the methodology outlined by 47. The leaf extracts was dissolved in phosphate buffer (0.1 mg/mL) and mixed with diluted low-density lipoprotein (LDL) cholesterol (0.04 mg LDL/mL) then a solution of cupric sulfate ($CuSO_4$) (0.1 mL, 100µM) was added to start the reaction. Using a diode array spectrophotometer, the production of conjugated dienes (CD) was determined at 234 nm.

Inhibition of DNA strand scission

The capacity of *A. indica* leaf extracts to inhibite scission of supercoiled plasmid DNA (pBR322) strand caused by peroxyl radical was measured following 45 procedure. The extract was mixed with H_2O_2, $FeSO_4$, supercoiled pBR322 DNA For one hour. DNA retention (%) was used to calculate the inhibitory impact of extracts:

$$\boldsymbol{DNA\ retention}\ \% = \frac{\boldsymbol{Supercoiled\ DNA\ contnt\ in\ sample}}{\boldsymbol{Supercoiled\ DNA\ contnt\ in\ control}} * \mathbf{100}$$

Antimicrobial activity of *A. indica* leaves extract

It was evaluated following 48 technique. Using the well-diffusion bioassay technique against five pathogenic microbial strains with the minimum inhibitory concentrations (MICs) of the extracts. The pathogenic microbial strains of gram-negative bacteria, *Escherichia coli* ATCC 25,922, gram-positive bacteria (*Bacillus cereus* ATCC 11,778 and *Staphylococcus aureus* ATCC 25923), and two dermatophyte fungus (*Candida albicans* and *Rhodotorula glutinis*), were acquired from the culture collection of the Research Laboratories Unit, Faculty of Science, Umm Al-Qura University, Makkah, Saudi Arabia.

2.3.10 MALDI-TOF-MS analysis of structural phenolics of *A. indica* leaves extracts

Phenolic compounds identification was carried out according to 49. Using the matrix assisted laser desorption ionization-time of flight-mass spectrometry/mass spectrometry (MALDI-TOF-MS/MS). The spectra were recorded on a Bruker Reflex III instrument (Bremen, Germany). The irradiation source was a pulsed nitrogen laser with a wavelength 337 nm and a laser pulse duration 3 ns. An accelerating voltage of 20.0 kV and a reflection voltage of 23.0 kV were employed in the negative reflection mode.

2.4 Statistical analysis

Each experiment was carried out three times, and the mean ± deviation of the results was given. Analysis of variance (ANOVA) and Tukey's multiple range tests were used to calculate the significance of differences among the results at $p < 0.05$ [50].

3 Results and discussion

Neem (*A. indica*) extracts have many applications as anti-inflammatory, antioxidant, anticancer, diabetic regulation, immune system improvement and insect repellent [51]. The beneficial effects of *A. indica* extracts mainly contributed to the presence of bioactive ingredients [52]. So our study aimed to investigat the physicochemical parameters of neem cultivated in Makkah Al-Mukarramah, Saudi Arabia. Our results, revealed that leaf extract of *A. indica* leaf contain several active phenolic compounds (Table 1) such as flavonoids, condensed tannins, hydrolyzable tannins, anthocyanin. The concentration of soluble fraction (SNL) was higher than Insoluble-Bound phenolics fraction with the highest values was recorded for soluble anthocyanin and hydrolyzable tannins, 76.42 and 70.16 mg/g DW respectively.

Table1 Soluble and insoluble-boubed contents of total phenolics (mg/g DW), flavonoid (mg/g DW), condensed tannins (mg/g DW), hydrolyzable tannins (mg/g DW) and anthocyanin (mg/g DW) of *A. indica* leaf extracts. Data are means of three replicates ± SE.

Phytochemicals	Soluble phenolics	Insoluble-Bound phenolics
Total Phenolics	84.33±11.2[a]	46.93±3.91[a]
Total Flavonoid	32.54±5.73[d]	12.66 ±1.75[d]

Condensed Tannins	50.19 ±9.53[c]	18.94 ±0.87[c]
Hydrolyzable Tannins	70.16 ±5.16[b]	20.11 ±3.13[b]
Total Anthocyanin	76.42 ±19.12[b]	21.11 ±3.17[b]

Columns with different letters are significantly different at $p \leq 0.05$.

Methanol (70%) was found to be the most effective solvent in a prior study to extract antioxidant components from *A. indica* plants [53]. While, 54 proved that the most effective extraction solvent for phenolic compounds from *A. indica* leaves was ethanol (70%), followed by methanol> Ethyl acetate> 80% Ethanol> Pure ethanol> N-Butanol> 60% Ethanol> 50% Ethanol> and Water (in concentrations within the range of 13.75, 13.54, 12.66, 12.27, 11.95, 10.99, 9.57, 6.64, and 4.70 mg/g, respectively). Our results showed that the mixture of methanol/ acetone/ water (7:7:6, v/v/v) resulted in higher concentration of phenolic compounds as compared with methanolic extract used by 54. The concentration of phenolic compounds in plant is highly affected by the environmental conditions under which plant grow, in this context the high temperature and low water availability condition in Saudi Arabia may put neem plant under stress condition and resulting in phenolic compounds biosynthesis acceleration [55]. The higher phenolic compounds content in the methanolic (soluble phenols) extract result in higher antioxidant capacity value measured by different methods (Table 2).

Table 2 $ABTS^{*+}$ and DPPH radicals scavenging capacity, reducing power and iron chelating activity of *A. indica* leaves extracts. Data are means of three replicates ± SE.

:

Sample	$ABTS^{\bullet+}$ scavenging (mmoles trolox eq/g of leaves extract)	DPPH· scavenging (mmoles trolox eq/g leave extract)	Reducing Power (moles trolox eq/g leave extract)	Iron chelating activity (µmoles EDTA eq/100g leave extract)
SNL	6.46±0.18[a]	25.59±3.27[a]	1341.71±44.86[a]	2374.55±139.82[a]
IBL	0.55±0.14[b]	6.50 ±0.23[b]	713.25±28.89[b]	683.91 ±79.41[b]

Columns with different letters are significantly different at $p \leq 0.05$.

The study of [56] reported that the antioxidant capacity should be measured by more than one technique and we cannot depend on a single test to assess the antioxidant potential of any plant extract. In the current study, the total antioxidant capacity of *A. indica* leaf extracts was assessed using radical scavenging assays, included DPPH· and $ABTS^{\cdot+}$ scavenging methods. The DPPH free radical could be reduced by the antioxidant compounds existing in the plant extract forming di-phenyl hydrazine which is a yellow colored compound so can give a measure to the antioxidant capacity of the plant extract. Indeed, phenolic compounds can donate hydrogen present in phenolic hydroxyl groups and scavenge free radicals to stop the chain of peroxidation reaction [57, 58]. The DPPH values of soluble phenolic and insoluble-bound phenolics extracts were 25.59 and 6.50 mmoles trolox eq/g leaf extract respectively (Table 2). Another method used in measuring antioxidant capacity for *A. indica* leaf extracts is the reducing power activity, this method evaluate the ability of the extract to form complexes with metals like iron and copper. In our study, Ferric reducing antioxidant power (FRAP assay) was used, in which

ferric 2, 4, 6-tripyridyl-s-triazine complex [Fe^{3+}-$(TPTZ)_2$] $^{3+}$ could be reduced to ferrous complex [Fe^{2+}-(TPTZ)2] $^{2+}$ by the antioxidants present in the extract [59]. The soluble phenolic extract recorded higher FRAP value than insoluble-bound phenolics extract (Table 2). Our result is in agree with 60 who revealed that the increase in total phenolic and flavonoids compounds increased the antioxidant potential of *Azadirachta indica* A. Juss measured by DPPH and FRAP methods.

Metal chelation capacity is another method was applied on *A. indica* leaf extracts, it determined the potential of the extracts antioxidants to chelate metal ions like ferrous iron (Fe^{2+}). Ferrous iron helps in the formation of ROS, as Fe^{2+} is highly reactive and it is considered one of the most essential lipid oxidation prooxidant [59]. Moreover, transition metal ions such as Fe^{2+} can induce lipid peroxidation by Fenton reaction, in addition to the formation of more reactive peroxyl and alkoxyl radicals from breakdown of lipid hydroperoxides. Therefore, the substances that can chelate iron such as phenolica and flavonoids are known to have antioxidant impacts and stimulating the defense against oxidative stress [61, 62].

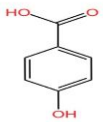
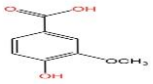
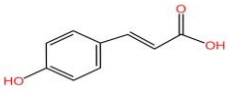
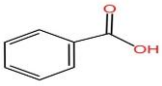
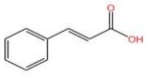
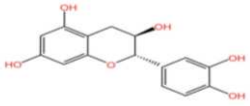
The antioxidant activity of neem extracts are a consequence of the phenolic contents [63, 64]. Phenolic compounds of plants prestent in two forms free and bound forms [65]. The bound forms found as glycosides, esters and insolublebound complexes [66]. In the current data the soluble phenolic extract has higer concentration of phenolic, flavonoids, condensed tannins, hydrolyzable tannins and anthocyanin content than insoluble-bound phenolics extract (Table 1) and this was associated with higher antioxidant capacity for soluble phenolic extract measured by different techniqes (Table 2).

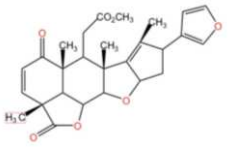
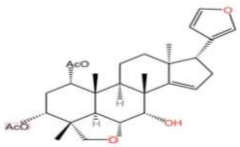
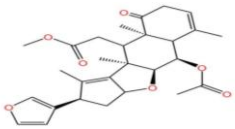
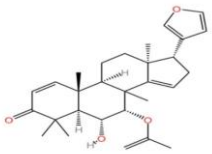
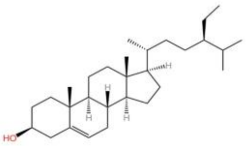
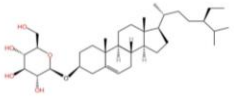
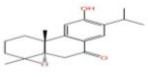
Various studies proved that *A. indica* leaves methanolic extract contain phenolic content, flavonoids and proanthocyanidin such compounds have antioxidant as well as antimicrobial activities [63, 67, 68]. The structure of phenolic compounds as well as number and arrangement of their hydroxyl groups determine their antioxidant activity [69, 70] so the total antioxidant capacity of an extracts dependes not only on the amount of phenolic compouds in the extract but also on their types. It was reported by [23] that the antioxidant capacity of *A. indica* leaf methanolic extract is directly contributed to the phenolic content and the number of phenolic hydroxyl groups on their ring structures which gives it the high ability to scaveng ROS [71].

In the present work, MALDI-TOF-MS analysis showed that the methanolic extract of *A. indica* leaf contains various bioactive components with different chemical structures (Table 3), and this will give the extract various biological activities. It was reported that neem contains about 60 types of biologically active ingredient including terpenoids and steroids [72]. Nimbolide is a bioactive compounds found in *A. indica* leaf extract (Table 3), it is a main component of neem leaves also known as limonoids, it is a tetranortriterpenoid and exhibited various biological activities such as anti-feedent, anti-malarial, antimicrobial and anti-cancer activity [73]. Vilasinin is a type of limonoids which is a hexacyclic tetranortriterpenoid and has various medicinal applications [6]. Also, *A. indica* leaf extract contain sugiol which is a diterpenoid and it has many biological activities like antimicrobial, antioxidant, anti-inflammatory, anti-carcinoma, antiviral, and cardiovascular agent [74]. Sugiol recorded high antioxidant activity via various systems including radical scavenging as DPPH, nitric oxide, superoxide anion, and

hydroxyl radical scavenging additionaly the reducing power activity [75]. Catechins is another bioactive constituent of *A. indica* leaf extract (Table 3) which is a flavonol compound [76] and recorded the highest ABTS-scavenging capacity, the highest stoichiometry of Fe^{3+} reduction in the FRAP assay when compared with flavonoids and standard antioxidants, GSH and ascorbic acid [77]. Vanillic acid is a derivative of benzoic acid, it has high antioxidant capacity so it used in different pharmacological activities such as cardioprotective, anti-inflammatory, immuno-stimulating, antiapoptotic, hepatoprotective and neuroprotective [78].

Table 3 Mass/Spectral characteristics of phenolics and other natural compounds identified from *A. indica* leaves extracts

	MW	Chemical structure	IDENTIFICATION
1	138.122		p-Hydroxybenzoic acid
2	168.15		Vanillic acid
3	164.160		p-Coumaric acid
4	122.123		Benzoic acid
5	148.16		trans-Cinamic acid
6	290		Catechin

7	466.5		Nimbolide
8	428.6		Vilasinin
9	482.6		Nimbinene
10	452.6		Nimocinol
11	414.7		B-Sitosterol
12	576.8		beta-Sitosterol glucoside
13	300.4		Sugiol

Measuring the antioxidant properties of *A. indica* leaf extracts in biological model systems

Atherosclerosis is a main factor that causes cardiovascular disease and leads to increase mortality. Oxidized low-density lipoprotein (oxLDL) is the principal cause for atherosclerosis [79]. LDL-cholesterol oxidation inhibition assay can be used to evaluate the anti-atherogenic potential of antioxidants to prevent cardiovascular disease [80]. In the present investigation, copper sulfate was used to intiate LDL oxidation, then conjugated dienes (CD) the oxidation products was measured the inhibitory activity, as the decrease in CD concentration indicates the decrease in oxidation of phospholipids. Both phenolic extracts of *A. indica* leaves has an inhibitory effect on low-density lipoprotein (LDL) oxidation (fig. 1) but soluble phenolic extract recorded higher activity than the insoluble-bounded phenolic extract this could be attributed to the higher phenolic compounds contents (Table 1) that was associated with higher antioxidant capacity of SNL extract than IBL extract (Table 2).

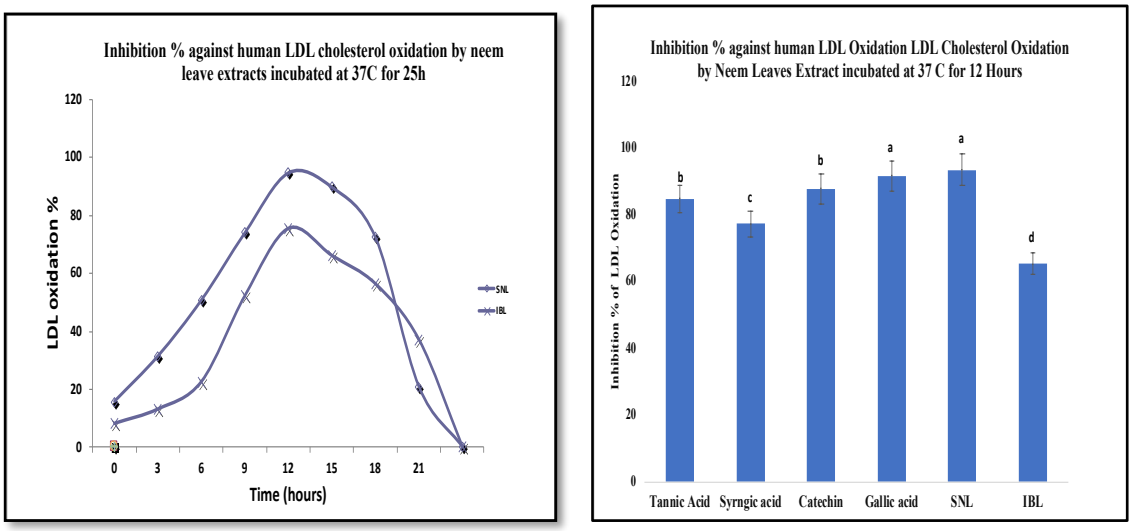


Fig.1 Effect of soluble (SNL) and insoluble-bound (IBL) *A. indica* leaves extracts on inhibition of the human low-density lipoprotein (LDL) cholesterol oxidation initiated by cupric ions.

The oxidation of low density lipoprotein (LDL) is mainly stimulated by free-radicals [81-84]. So several polyphenols have the ability to reduce LDL oxidation via free radicals scavenging or chelating prooxidant metals [85, 86, 30]. From the out come of the obtained results in (Table 3) the leaf extract of neem contain many active compounds like p-Coumaric acid which is one of hydroxycinnamic acid derivatives that have high antioxidant activities and it was reported by [87] that hydroxycinnamic acid derivatives including p-Coumaric acid succeeded to inhibit peroxidation of human low density lipoprotein. Also, Catechin present in neem leaf extract (Table 3) inhibited peroxidation of LDL by 51.1% and when a mixtures of phenolic compounds are used give synergistic more effective response [88]. In this context, 51 reported that the *A. indica* leaves extract that contain various phenolic compounds significantly inhibits LDL oxidation due to its phenolic contents. In addition, the *A. indica* leaves phenolic acids can be considered safe for potential food applications, such as functional meals meant to improve health [89]. Interestingly, 90 was reported that the oral administration of neem leaf ethanol extract to diabetic rats recorded a decrease in low-density lipoproteins LDL by 38.98% and the very-low-density lipoproteins by 27.5% as compared with diabetic untreated control.

Moreover, the antioxidant property of neem leaf extracts was evaluated by its capacity to inhibit plasmid supercoiled DNA (pBR322) strand scission. The oxidation of supercoiled DNA is stimulated by hydroxyl and peroxyl radicals [91, 92]. The potential of phenolic extracts as antioxidants has been examined by its ability to scavenge free radicals that induce DNA strand scission [49, 93, 94]. Both neem extracts possesses an inhibitory effect on DNA strand scission (fig. 2 and 3a,b), but SNL extract was the most effective and recorded (95.76%) inhibition of a DNA strand scission while IBL extract recorded approximately 51.43% and Galic acid which used as a positive standard recorded 91.69% inhibition of DNA scission (fig 2)

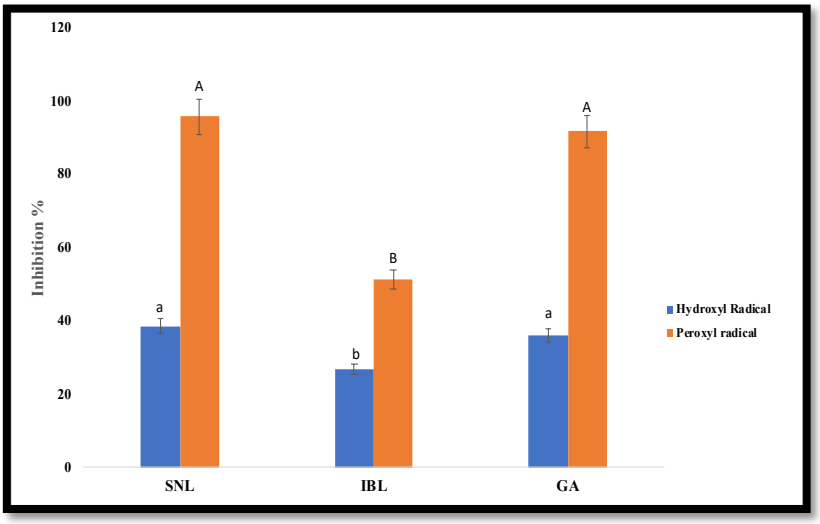
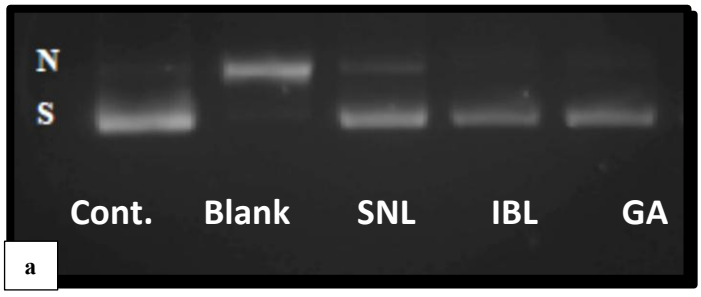


Fig. 2 The effect of soluble (SNL), insoluble-bound (IBL) phenolic extracts of *A. indica* leaves and Galic acid (GA) on the inhibition percentage of DNA strand scission caused by peroxyl or hydroxyl radicals. Data are means of three replicates ± SE. Columns with different letters are significantly different at $p < 0.05$.



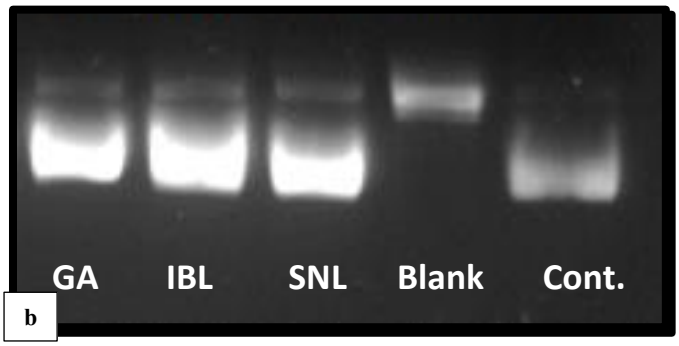


Fig. 3 Inhibitory effects of soluble (SNL) and insoluble-bound (IBL) phenoic extracts of *A. indica* leaves and Galic acid (GA) on DNA nicking caused by hydroxyl radicals (a) and peroxyl radical (b). DNA with nicked (N) and supercoiled (S) ends.

The free radicals scavenging activity of phenolic extracts of *A. indica* leaf on plasmid DNA damage (fig. 3a, b) recorded a significant decrease in nicked DNA (N) while native supercoiled (S) DNA increased. Both extractes are DNA protector but SNL recorded higher protection for DNA than IBL extract due to the higher content of phenolic compounds that have the ability to scavenge free radicals. Moreover, the existence of phenolic compounds which contain ortho-dihydroxy structure in the B-ring such as catechin (Table 3) gave neem extract high protective effect against DNA damage as these compounds exerts high scavenging activities [95].

Various human diseases such as mutagenesis, cancer, atherosclerosis, diabetes and neurodegenerative are caused by free radicals and oxidative damage of the DNA molecule that result in base modification and strand scission [96, 97]. Thus, neem extracts may have various beneficial applications in the biological system due to their ability to inhibit DNA scission.

Antimicrobial activity of *A. indica* leaves extract

From the out come of the obtained data, gram-positive bacteria were more sensitive to the *A. indica* leaf extracte with zone of inhibition (1.5 cm with *Bacillus cereus* ATCC 11,778, and 1.2 cm with *Staphylococcus aureus* ATCC 25923) (Table 4 and fig. 4), whereas no antimicrobial potency recorded against gram-negative bacteria (*Escherichia coli*) and dermatophyte fungus (*Candida albicans*, and *Rhodotorula glutinis*).

Table 4 Antimicrobial activity of *A. indica* leaf extract against *Bacillus cereus* (*B. cereus*), *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli*), *Rhodotorula glutinis* (*R. glutinis) and Candida albicans* (*C. albicans)*

	B. cereus	***S. aureus***	***E. coli***	***R. glutinis***	***C. albicans***
Zone of inhibition (Cm)	1.5	1.2	0.0	0.0	0.0

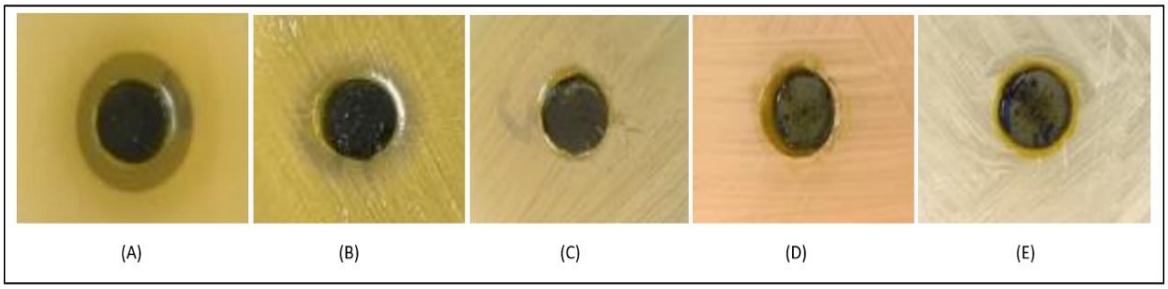


Fig. 4 *The effect of* A. indica *leaf extract on zone of growth inhibition on the cultures of (A)* Bacillus cereus*, (B)* Staphylococcus aureus*, (C)* Escherichia coli*, (D)* Rhodotorula glutinis*, and (E*) Candida albicans *after adding.*

Such result was in accordance with the finding of 98 who revealed that leaves and bark extracts of *A. indica* have an inhibitory effect on *Staphylococcus aureus* growth while none of the two extracts had any effect on *E. coli*. On the other hand, 63 proved that *A. indica* crude extract has high antimicrobial activity against *S. aureus* and *E. coli.* Indeed, many recent studies have demonstrated the effectiveness of the *A. indica* tree extracts against bacteria and fungi [99-102]. Such effect of *A. indica* leaf extract could be contributed to the existence of many bioactive compounds that are structurally and chemically diverse, which include phenols, flavonoids, tannins, triterpinoids, and steroids (Table 3) [103]. Also, 103 proved that *Azadiracta indica* leaf extract was very effective against *P. aeroginosa, S. aureus, E. coli and S. typhi*. It is worth mentioning that *A. indica* leaf extract contain ß-sitosterol (Table 3) which is a polyphenolic favonoid and has a lot of applications as anti-fungal and antibacterial comound [104, 105]. Moreover, p-hydroxybenzoic acid and trans-cinnamic acid recorded in *A. indica* leaf extract (Table 3), have antibacterial activity against most of Gram + ve and some of Gram - ve bacteria [106].

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References

1. Mafara SM and Bakura TL (2021) Multiple potentials of neem tree (*Azadirachta indica*); a review. Bakolori Journal of General Studies 11(1): 1-2.
2. Singh KK (Ed.). (2009) Neem: a treatise. IK International Pvt Ltd.
3. Ahmed S, Bamofleh S, Munshi MT (1989) Cultivation of neem (*Azadirachta indica*, meliaceae) in saudi arabia. Economic Botany 43(1): 35-38.
4. Mardiha MAU, Al-Shyhaibani NA (2014) Prospect of Neem Plantation at Arafat, Saudi Arabia. Current World Environment 9(1): 81-86.
5. Bader A, Abdelhady MI, Shaheen U, El-Malah Y, Abourehab MAS, Barghash MF (2015) *Azadirachta indica* as a source for antioxidant and cytotoxic polyphenolic compounds. Biosciences Biotechnology Research Asia 12(2): 1209-1222.02.

6. Saxena N, Kumar Y (2009) Chemistry of azadirachtin and other bioactive isopreniods from Neem (*Azadirachta indica* A. Juss). In: Neem, a Treatise Singh KK, Phogat S, Dhillon RS, Tomar A (ed). IK International Publishing House Pvt. Ltd., New Delhi, India. 2009, pp 208–230.
7. Mondal E, Chakraborty K (2016) Azadirachta indica-A tree with multifaceted applications: An overview. Journal of Pharmaceutical Sciences and Research 8(5): 299.
8. Vietmeyer ND (1992) Neem: a tree for solving global problems. Report of an ad hoc panel of the Board on Science and Technology for International Development, National Research Council. National Academy Press.
9. Dhillon RS, Ahlawat KS, Pundeer JS (2008) Botany of neem. Neem-A Treatise 30-43.
10. Oyebode O, Kandala NB, Chilton PJ, Lilford RJ (2016) Use of traditional medicine in middle-income countries: a WHO-SAGE study. Health Policy Plan 31:984–991.
11. Arumugam A, Agullo P, Boopalan T, Nandy S, Lopez R, Gutierrez C, Narayan M, Rajkumar L (2014) Neem leaf extract inhibits mammary carcinogenesis by altering cell proliferation, apoptosis, and angiogenesis. Cancer biology and therapy 15(1):26-34.
12. Omóbòwálé TO, Oyagbemi AA, Adejumobi OA, Orherhe EV, Amid AS, Adedapo AA, Nottidge HO, Yakubu MA (2016) Preconditioning with *Azadirachta indica* ameliorates cardiorenal dysfunction through reduction in oxidative stress and extracellular signal regulated protein kinase signalling. Journal of Ayurveda and Integrative Medicine 7(4):209-17.
13. Patel SM, Venkata KC, Bhattacharyya P, Sethi G, Bishayee A (2016) Potential of neem (*Azadirachta indica* L.) for prevention and treatment of oncologic diseases. In Seminars in cancer biology (Vol. 40, pp. 100-115). Academic Press.
14. Singh P, Tiwari M (2021) Review on *Azadirachta Indica* Int J Pharma Life Sci 2(1 Part A): 28-33.
15. Agrawal DP (2001) Medicinal properties of neem: new findings. History of Indian science and.
16. Ray A (2012) Potential properties used and scope of *Azadirachta indica* in human health care. Research Journal of Science and Technology 4(2): 55-58.
17. Atawodi SE, Atawodi JC (2009) *Azadirachta indica* (neem): a plant of multiple biological and pharmacological activities. Phytochem Rev 8:601–620.
18. Tiwari R, Verma AK, Chakraborty S, Dhama K, Singh SV (2014) Neem (*Azadirachta indica*) and its potential for safeguarding health of animals and humans: A review. J Biol Sci 14:110–123.
19. Seriana I, Akmal M, Darusman Wahyuni S (2019) Neem leaves extract (*Azadirachta indica* A. Juss) on male reproductive system: a mini-review. IOP Conf Series 399:012106
20. Tan QG, Luo XD (2011) *Meliaceous limonoids*: chemistry and biological activities. Chemical reviews 111(11):7437-522.
21. Haldar S, Kolet SP, Thulasiram HV (2013) Biocatalysis: fungi mediated novel and selective 12-β or 17-β hydroxylation on basic limonoid skeleton. Green Chem 15:1311–1317
22. Ponnusamy S, Haldar S, Mulani F, Zinjarde S, Thulasiram H, Kumar AR (2015) Gedunin and azadiradione: Human pancreatic alpha amylase inhibiting limonoids from neem (*Azadirachta indica*) as anti-diabetic agents. PLoS ONE 10 (10):0140113.

23. Kumar R, Sharma S, Devi L (2018) Investigation of total phenolic, flavonoid contents and antioxidant activity from extracts of *Azadirachta indica* of Bundelkh and Region. Int J Life Sci Scienti Res eISSN 2455(1716):1716.

24. Sharma A (2023) Review on the medicinal importance of nimb (*Azadiracta indica*) in prospective of ayurveda.

25. Strack D (1997) Phenolic metabolism. In Plant Biochemistry, Dey PM, Harborne JB Eds, Academic Press San Diego pp. 387-416.

26. Cai Y, Luo Q, Sun M, Corke H (2004) Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 74: 2157-2184.

27. Pokorney J. Introduction. In Pokorny J, Yanishlieva N, Gordon MH (2001) (Eds.), Antioxidants in food: practical applications, Cambridge: Wood head Publishing Limited pp. 1-3

28. Pitchaon M, Suttajit M, Pongsawatmanit R (2007) Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chem 100: 1409-1418.

29. Balasundram N, Sundram K, Samman S (2006) Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem 99: 191-203.

30. Anwar S, Khan S, Almatroudi A, Khan AA, Alsahli MA, Almatroodi SA, Rahmani AH (2021) A review on mechanism of inhibition of advanced glycation end products formation by plant derived polyphenolic compounds. Molecular Biology Reports 48:787-805.

31. Cuppett S, Schnepf M, Hall C (1997) Natural antioxidant are they a reality? Natural antioxidants: chemistry, health effects, and applications. AOCS Press, Champaign

32. Ghimeray AK, Jin C, Ghimire BK, Cho DH (2009) Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta Indica* A. Juss grown in foothills of Nepal. Afr J Biotechnol 8:3084–3091.

33. Albishi T (2018) Bioactivities of wood polyphenols: antioxidants and biological effects (Doctoral dissertation, Memorial University of Newfoundland).

34. Krygier K, Sosulski F, Hogge L (1982) Free, esterified, and insoluble-bound phenolic acids. Composition of phenolic acids in cereal and potato flours. Journal of Agricultural and Food Chemistry 30(2): 337-340.

35. Naczk M, Shahidi F (2006) Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. Journal of Pharmaceutical and Biomedical Analysis 41(5): 1523-1542.

36. Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. American journal of Enology and Viticulture 16(3): 144-158.

37. Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64(4): 555- 559.

38. Scalbert A, Mila I, Expert D, Marmolle F, Albrecht AM, Hurrell R, Tomé D (1999) Polyphenols, Metal Ion Complexation and Biological Consequences. In Plant Polyphenols Springer, Boston, MA, 2, 545-554.

39. Bossu CM, Ferreira EC, Chaves FS, Menezes EA, Nogueira ARA (2006) Flow injection system for hydrolysable tannin determination. Microchemical Journal 84(1-2): 88-92.

40. Ben Mahmoud S, Saad H, Charrier B, Pizzi A, Rode K, Ayed N, Charrier-El Bouhtoury F (2015) Characterization of sumac (*Rhus tripartitum*) root barks tannin for a potential use in wood adhesives formulation. Wood Science and Technology 49: 205-221.

41. Giusti MM, Wrolstad RE (2001) Unit F 1.2: anthocyanins. Characterization and measurement with UV -visible spectroscopy. In: Wrolstad, RE, editor. Current protocols in food analytical chemistry. New York: John Wiley and Sons. P. F1.2 .1 - 1.2. 13.
42. Van den Berg R, Haenen GR, van den Berg H, Bast AALT (1999) Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. Food Chemistry 66(4): 511-517.
43. Siriwardhana SS, Shahidi F (2002) Antiradical activity of extracts of almond and its by-products. Journal of the American Oil Chemists Society 79(9): 903-908.
44. Madhujith T, Shahidi F (2006) Optimization of the extraction of antioxidative constituents of six barley cultivars and their antioxidant properties. Journal of Agricultural and Food Chemistry 54: 8048-8057.
45. Albishi T, John JA, Al-Khalifa AS, Shahidi F (2013) Antioxidant, anti-inflammatory and DNA scission inhibitory activities of phenolic compounds in selected onion and potato varieties. Journal of Functional Foods 5(2): 930-939.
46. Liyana-Pathirana CM, Shahidi F (2006) Importance of insoluble-bound phenolics to antioxidant properties of wheat. Journal of Agricultural and Food Chemistry 54(4): 1256-1264.
47. Ambigaipalan P, Shahidi F (2015) Antioxidant potential of date (*Phoenix dactylifera* L.) seed protein hydrolysates and carnosine in food and biological systems. Journal of Agricultural and Food Chemistry 63(3): 864-871.
48. Owayss AA, Elbanna K, Iqbal J, Abulreesh HH, Organji SR, Raweh HS, Alqarni AS (2020) In vitro antimicrobial activities of Saudi honeys originating from *Ziziphus spina-christi* L. and *Acacia gerrardii* Benth. trees. Food science and nutrition 8(1): 390-401.
49. Albishi T, Banoub JH, de Camargo AC, Shahidi F (2019) Wood extracts as unique sources of soluble and insoluble-bound phenolics: Reducing power, metal chelation and inhibition of oxidation of human LDL-cholesterol and DNA strand scission. Journal of Food Bioactives 8.
50. Snedecor GW (1980) Analysis of variance. Snedecor GW, Cochran WG. Statistical Methods 215-237.
51. Islas JF, Acosta E, Zuca G, Delgado-Gallegos JL, Moreno-Treviño MG, Escalante B, Moreno-Cuevas JE (2020) An overview of Neem (*Azadirachta indica*) and its potential impact on health. Journal of Functional Foods 74: 104171.
52. Devi J, Sharma RB (2023) Medicinal Importance of Azadirachta indica: An Overview. Journal of Drug Delivery and Therapeutics 13 (6):159-65.
53. El-Sayed MM, Maher ME, Eman AE, Mosad AG (2009) Total phenolic contents and antioxidant activities of Ficus sycomorus and Azadirachta indica. Pharmacologyonline 3: 590-602.
54. Shewale S, Rathod VK (2018) Extraction of total phenolic content from *Azadirachta indica* or (neem) leaves: Kinetics study. Preparative Biochemistry and Biotechnology 48(4): 312-320.
55. Chalker-Scott L, Fuchigami LH (2018) The role of phenolic compounds in plant stress responses. In Low temperature stress physiology in crops (pp. 67-80). CRC press.
56. Alam N, Bristi NC, Rafiquzzaman M (2013) Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharm J 21:143–152.
57. Soares JR, Dins TCP, Cunha AP, Ameida LM (1997) Antioxidant activity of some extracts of Thymus zygis. Free Radic Res 26:469–478.

58. Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA (2004) Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem 84:551–562.

59. Gülcin I (2012) Antioxidant activity of food constituents: an overview. Archives of toxicology 86:345-91.

60. Vats S. (2016) Effect of initial temperature treatment on phytochemicals and antioxidant activity of *Azadirachta indica* A. Juss. Applied biochemistry and biotechnology 178:504-12

61. Pisoschi AM, Pop A (2015) The role of antioxidants in the chemistry of oxidative stress: A review. European journal of medicinal chemistry 97:55-74.

62. Gulcin İ (2020). Antioxidants and antioxidant methods: An updated overview. Archives of toxicology 94(3): 651-715.

63. Pokhrel B, Rijal S, Raut S, Pandeya A (2015) Investigations of antioxidant and antibacterial activity of leaf extracts of *Azadirachta indica*. Afr J Biotechnol 14:3159–3163.

64. Al-Hashemi ZSS, Hossain MA (2016) Biological activities of different neem leaf crude extracts used locally in Ayurvedic medicine. Pac Sci Rev A: Nat Sci Eng 18:128e131.

65. Robbins RJ (2003) Phenolic acids in foods: an overview of analytical methodology. J Agric Food Chem 51:2866–2887

66. Ross KA, Beta TS, Arntfeld D (2009) A comparative study on the phenolic acids identifed and quantifed in dry beans using HPLC as afected by diferent extraction and hydrolysis methods. Food Chem 113:336–344

67. Vergallo C, Panzarini E, Din L (2019) High performance liquid chromatographic profling of antioxidant and anti-diabetic favonoids purifed from *Azadirachta indica* (neem) leaf ethanolic extract. Pure Appl Chem 91:1631–1640.

68. Deka H, Das S, Lahan JP, Yadav RNS (2013) In-vitro free radical scavenging, antioxidant and antibacterial activity of *Azadirachta Indica* A. Juss. of Assam. Advances in Life Sciences 3:1–4

69. Barclay LR, Vinqvist MR, Mukai K, Itoh S, Morimoto H (1993) Chain-breaking phenolic antioxidants: steric and electronic effects in polyalkylchromanols, tocopherol analogs, hydroquinones, and superior antioxidants of the polyalkylbenzochromanol and naphthofuran class. The Journal of Organic Chemistry 58(26):7416-20.

70. Chen JH, Ho CT (1997) Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. Journal of agricultural and food chemistry 45(7):2374-8.

71. Cao G, Sofic E, Prior RL (1997) Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. Free Radical Biol Med 22: 749-760.

72. Trivedi A, Fatima N, Husain I, Misra A (2019) An update on the therapeutic potential of neem and its active constituents: A panacea for all diseases. Era's Journal of Medical Research 6 (1):110-7.

73. Rajendran P, Renu K, Abdallah BM, Ali EM, Veeraraghavan VP, Sivalingam K, Rustagi Y, Abdelsalam SA, Ibrahim RI, Al-Ramadan SY (2024) Nimbolide: promising agent for prevention and treatment of chronic diseases (recent update). Food and Nutrition Research. 15.

74. Bajpai VK, Sonwal S, Hwang SK, Shukla S, Khan I, Dey DK, Chen L, Simal-Gandara J, Xiao J, Huh YS, Han YK (2021) Sugiol, a diterpenoid: Therapeutic actions and molecular pathways involved. Pharmacological Research 163:105313.

75. Bajpai VK, Sharma A, Kang SC, Baek KH (2014) Antioxidant, lipid peroxidation inhibition and free radical scavenging efficacy of a diterpenoid compound sugiol isolated from *Metasequoia glyptostroboides*. Asian Pacific Journal of Tropical Medicine 7(1):9-15

76. Yang CS, Landau JM, Huang MT, Newmark, HL (2001) Inhibition of carcinogenesis by dietary polyphenolic compounds. Ann Rev Nutr 21: 381–406.

77. Grzesik M, Naparło K, Bartosz G, Sadowska-Bartosz I (2018) Antioxidant properties of catechins: Comparison with other antioxidants. Food chemistry 241:480-92.

78. Sharma N, Tiwari N, Vyas M, Khurana N, Muthuraman A, Utreja P (2020) An overview of therapeutic effects of vanillic acid. Plant Arch 20(2):3053-9.

79. Wengrofsky P, Lee J, Makaryus AN (2019) Dyslipidemia and its role in the pathogenesis of atherosclerotic cardiovascular disease: implications for evaluation and targets for treatment of dyslipidemia based on recent guidelines. In Dyslipidemia Intech Open.

80. Zhang S, Li L, Chen W, Xu S, Feng X, Zhang L (2021) Natural products: The role and mechanism in low-density lipoprotein oxidation and atherosclerosis. Phytotherapy Research 35(6): 2945-2967.

81. Klatt P, Esterbauer H (1996) Oxidative hypothesis of atherogenesis. Journal of Cardiovascular Risk 3: 346–351.

82. Mertens A, Holvoet P (2001) Oxidized LDL and HDL: antagonists in athrerothrombosis. The FASEB Journal 15: 2073–2084.

83. Pryor WA (2000) Oxidation and artherosclerosis. Free Radical Biology and Medicine 28: 1681–1682.

84. Upston JM, Kritharides L, Stocker R (2003) The role of vitamin E in atheroscerosis. Progress in Lipid Research 42: 405–422.

85. Bhuyan U, Handique JG (2022) Plant polyphenols as potent antioxidants: Highlighting the mechanism of antioxidant activity and synthesis/development of some polyphenol conjugates. Studies in Natural Products Chemistry 75: 243-266.

86. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, ... and Sharifi-Rad J (2020) Life style, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Frontiers in physiology 11: 694.

87. Cheng JC, Dai F, Zhou B, Yang L, Liu ZL (2007) Antioxidant activity of hydroxycinnamic acid derivatives in human low density lipoprotein: mechanism and structure–activity relationship. Food chemistry 104(1):132-9.

88. Cirico TL, Omaye ST (2006) Additive or synergetic effects of phenolic compounds on human low density lipoprotein oxidation. Food and Chemical Toxicology 44(4):510-6.

89. Kumaresan S, Karthi V, Senthilkumar V, Balakumar BS, Stephen A (2015) Biochemical constituents and antioxidant potential of endophytic fungi isolated from the leaves of *Azadirachta indica* A. Juss (Neem) from Chennai, India. J Acad Ind Res 3(8): 355-361.

90. Zuraini A, Vadiveloo T, Hidayat MT et al (2006) Effects of neem (*Azadirachta indica*) leaf extracts on lipid and C-reactive protein concentrations in cholesterol-fed rats. J Nat Remedies 6: 109–14. http://doi.org/10.18311/jnr/2006/451.

91. Peng H, Shahidi F (2022) Enzymatic synthesis and antioxidant activity of mono-and diacylated epigallocatechin gallate and related by-products. Journal of Agricultural and Food Chemistry 70(29): 9227-9242.

92. Hossain A, Senadheera TR, Dave D, Shahidi F (2023) Phenolic profiles of Atlantic sea cucumber (*Cucumaria frondosa*) tentacles and their biological properties. Food Research International 163: 112262.

93. Akbari B, Baghaei-Yazdi N, Bahmaie M, Mahdavi Abhari F (2022) The role of plant-derived natural antioxidants in reduction of oxidative stress. Bio Factors 48(3): 611-633.

94. Čižmárová B, Hubková B, Tomečková V, Birková A (2023) Flavonoids as promising natural compounds in the prevention and treatment of selected skin diseases. International Journal of Molecular Sciences 24(7): 6324.

95. Rice-Evans C, Miller NJ, Paganga G (1997) Antioxidant properties of phenolic compounds. Trends Plant Sci 2: 152–9.

96. Marnett LJ (2000). Oxyradicals and DNA damage. Carcinogen 21: 361- 370.

97. Olinski R, Gackowski D, Foksinski M, Rozalski R, Roszkowski K,Jaruga P (2002). Oxidative DNA damage: Assessment of the role in carcinogenesis, atherosclerosis, and acquired immunodeficiency syndrome. Free Radic Biol Med 33: 192-200.

98. Francine U, Jeannette U, Pierre RJ (2015) Assessment of antibacterial activity of neem plant (*Azadirachta indica*) on Staphylococcus aureus and Escherichia coli. J Med Plants Stud 3(4): 85-91.

99. Faujdar SS, Bisht D, Sharma A (2020) Antibacterial potential of neem (*Azadirachta indica*) against uropathogens producing beta-lactamase enzymes: A clue to future antibacterial agent? Biomedical and Biotechnology Research Journal (BBRJ) 4(3): 232-238.

100. Al-Sarraj FM (2021) A Review on the impacts of *Azadirachta indica* on Multi-drug Resistant Extended Spectrum Beta Lactamase-positive of Escherichia coli and Klebsiella pneumonia. Advancements in Life Sciences 8(3):228-32.

101. Altayb HN, Yassin NF, Hosawi S, Kazmi I (2022) In-vitro and in-silico antibacterial activity of *Azadirachta indica* (Neem), methanolic extract, and identification of Beta. d-Mannofuranoside as a promising antibacterial agent. BMC plant biology 22(1):262.

102. Hussain U, Sami AJ, Rafique S, Khan MI, Shahid AA (2023) Methanolic Extract of Neem Plant Inhibits NS3 and NS5A Nonstructural Proteins of HCV 3A Genotype. Pakistan Journal of Zoology 1:55(3).

103. Vinoth B, Manivasagaperumal R, Rajaravindran M (2012) Phytochemical analysis and antibacterial activity of *Azadirachta indica* A. Juss. International Journal of Research in Plant Science 2(3): 50-55.

104. Mahmoud DA, Hassanein NM, Youssef KA, Abou Zeid MA (2011) Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. Brazilian Journal of Microbiology 42: 1007-1016.

105. Jaisinghani RN (2017) Antibacterial properties of quercetin. Microbiol Res 8:6877

106. Cho JY, Moon JH, Seong KY, Park KH (1998) Antimicrobial activity of 4-hydroxybenzoic acid and trans 4-hydroxycinnamic acid isolated and identified from rice hull, Biosci Biotechnol Biochem 62: 2273-2276.

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Author Contributions

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Data availability

All the data created or analyzed during the time of this investigation are presented in this publication.