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Determination Of Biological Activity of The of Azadiracta Indica A. Juss. Grown In Somalia

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ABSTRACT

This study was undertaken for screening biological activity of *Azadirachta indica* A. Juss. growing in Somalia. The study was aimed to investigate the biological activity namely: antioxidant activity, total phenol content, total flavonoid content and as well as its Phytochemical composition, 43mg of essential oils (EOs) were extracted from 65g of air-dried plant by hydrodistillation for 3hrs and analyzed with gas chromatography-mass spectrometry (GC-MS), gas chromatography-flame ionization detector (GC-FID), and the total of 31components represented 90.6 % of the total oil were identified. The main components were □-Elemene (32.6 %), germacrene-B (30,1%), whereas the MeOH and Water extracts were employed with High-Pressure Liquid Chromatography (HPLC) to determine phenolic compounds of the plants. The phenolic substances were identified and quantified after comparison with reference standards. Th radical scavenging effect of the extract was analyzed spectrophotometrically with DPPH and TEAC assay respectively. The scavenging effect was fully active in TEAC assay and in all DPPH assays except for the essential oil extracts. Total phenolic content in the extracts was determined by spectrometric method applying the Folin- Ciocalteu assay. The result was ranged from 0.108 to 0.139mg gallic acid/mL and was slightly greater than that in total flavonoid, the Flavonoid contents of the plant was also determined spectrophotometrically using rutin as a standard reference compound with aluminum chloride colorimetric assay. It ranged from 0.003 to 0.052 mg/ml. This study is of significance in providing information on the

Azadirachta Indica A. Juss native to Somalia and it appears to be a rich source of antioxidants and phytochemical studies may supplement the remedial needs of the human.

Keywords : *Azadiracta indica* A. Juss, Somalia, Biological activity, GC-MS/FID, HPLC, Essential oil, HPLC,

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TEAC, Trolox equivalent antioxidant capacity ; EO, essential oil; GA, gallic acid; GAE, gallic acid equivalent; GC–FID, gas chromatography—flame ionization detector; HPLC, High performance liquid chromatography; RE, rutin equivalent; TE, Trolox equivalent; EC50, Half maximal effective concentration.

I. INTRODUCTION

Medicinal plants have attracted more interest in a variety of disciplines recently (Ibrahim, Mohammed, Isah, & Aliyu, 2014; Patel, Venkata, Bhattacharyya, Sethi, & Bishayee, 2016). Especially, they play an important role as primary health care in most African countries (Edeoga, Okwu, & Mbaebie, 2005; Ibrahim et al., 2014). There are a wide variety of medicinal plants found in African countries that belong to a several families. Meliaceae family is one of the most native plants that have been used in folk medicine in these countries. Due to its important medicinal properties, people have used them traditionally in the treatment of various ailments. Neem (*Azadirachta indica* A.Juss., locally called 'Geed Hindi' or 'Geed Talal' as the name indicates the tree is originally from India) belongs to Meliaceae family. The different parts of the tree of Neem are extensively used for malaria, intestinal complaints, high fever, skin diseases, head lice, the treatment of eczema and furuncles, intestinal worm infections, and respiratory system problem etc. in folk medicine in tropical and subtropical area of Asian and African countries. People use traditionally it to clean their teeth, ease constipation, improve the appetite etc. (Adebayo & Krettli, 2011; Bhowmik, Chiranjib, Tripathi, & Kumar, 2010; Chinsembu, 2015; Dastan, Pezhmanmehr, Askari, Ebrahimi, & Hadian, 2010; Hossain, Al-Toubi, Weli, Al-Riyami, & Al-Sabahi, 2013; Muthaura, Keriko, Derese, Yenesew, & Rukunga, 2011; Tewari, 1992). Neem is used in modern medicine because of biological and pharmacological activities such as antiseptic, antipyretic, anti-inflammatory, antidiabetic, anti-ulcer, antibacterial, analgesic, anti-arrhythmic, anti-tubercular, diuretic, spermicide, anti-arthritic, anti-protozoal, antiviral, and antifungal effects, insecticide, antiparalitic, repellent agent, anti-feedant, anti-hormonal, anti-cancer and antioxidant properties; and blood sugar lowering effect (Bhowmik et al., 2010; Biswas, Chattopadhyay, Banerjee, & Bandyopadhyay, 2002; Devatha, Jagadeesh, & Patil, 2018; Forster & Moser, 2000; Patel et al., 2016; Tiwari, Verma, Chakraborty, Dhama, & Singh, 2014; Vietmeyer, 1992). The National Research Council (USA) refers to it as 'a tree for solving global problems' (Arora, Singh, & Sharma, 2008; Su & Mulla, 1999).

The US Academy of Sciences is now placing great importance on the *Azadirachta indica* tree, because the United Nations has declared that the *Azadirachta*

indica tree as 'the tree of the 21st century' because of its multipurpose use In India, people refer the neem tree in several names: 'Arishtha' which means 'reliever of sickness', it is also called as a 'Sarvorogya nivarini', 'a pharmacotherapy for all disease' (Arora et al., 2008).

Phenolic compounds are thought as natural versatile molecules with promising therapeutic applications due to their antioxidant activity and other health benefits which especially reduce the risk of many oxidative stress related diseases such as cancers, diabetes and cardiovascular and other health protective effects like antimicrobial, anticancer, anti-inflammatory, anti-mutagenic etc. Phenolic acids have also potential for pharmaceutical, biomedical analysis and in drug discovery (Chukwuma et al., 2019; Ge, Li, & Lisak, 2019; Kumar & Goel, 2019; Masullo, Montoro, Mari, Pizza, & Piacente, 2015; Milevskaya, Prasad, & Temerdashev, 2018). For these reasons; rapid, reliable and efficient methods are important for the determination of these compounds. Certain high performance liquid chromatography (HPLC) have been proposed for the determination of phenolic compounds (Chukwuma et al., 2019; Kumar & Goel, 2019; Milevskaya et al., 2018; Öztürk, Tuncel, & Tuncel, 2007). Although a few HPLC methods have been proposed for profiling of *Azadirachta indica* which was collected from different countries, there has been no study on the qualitative and quantitative determination of phenolic compounds of *Azadirachta indica*. Moreover, none of them use internal standard and none of them was collected in Somalia (Malar et al., 2019; Wandscheer et al., 2004).

So far the essential oils are the products of plant secondary metabolism and consist mainly of volatile components such as monoterpenes and sesquiterpenes and, to a slight extent of aromatic and aliphatic compounds and plays a crucial role in defining the properties of the medicinal plants and remains of great scientific interest. Due to their extreme volatility and pungency, neem oil is used in traditional medicine for treating various health problem such as transmitted diseases that are in all tropical and subtropical countries and started gaining popularity as well (Kalita, Bora, & Sharma, 2013; Sharma, Ansari, & Razdan, 1993). However, the neem oils had hexadecanoic acid as the main compounds and other six structure as minor components such as n-alkanes, aromatics, esters, nitrogen and sulfur compounds (Kurose & Yatagai, 2005), the organosulfur compounds such as cis and trans isomers of n-propyl 1-propenyl disulfide, respectively were observed as major compounds by GC/MS whereas di-npropyl-, n-propyl 1-propenyl-, and di-1-propenyl trisulfides and tetrasulfides, and n-propyl butyl disulfide were also identified as manor derivetivies of organosulfur compounds (Babatunde et al., 2019; Balandrin, Lee, & Klocke, 1988; Barros et al., 2014). Nevertheless, the essential oils of *Azadirachta indica* was not studied in details qualitatively and quantitatively in elsewhere using GC/MS and GC–FID techniques, Somalia as well.

The literature review reveals that even though *Azadirachta indica* has several components showing beneficial effects, its proficiency of each phytochemical should be further investigated and specific mechanisms need to be studied to determine the best neem phytochemical constituent required to prevent and treat cancer and other illnesses. Human clinical trials need precludes clinical drug development from this medicinal and dietary plant for the future. It was reported that very limited information is available on neem components. It was also emphasized that *Azadirachta indica* showed significant promise for preventing and treating certain diseases and development of *Azadirachta indica*-based products as drugs (Patel et al., 2016). That is why there is an urgent need to generate data about *Azadirachta indica*. To the best of our knowledge, *Azadirachta indica* plant which was collected in Somalia has not been previously done any research that is why there is an urgent need to generate data about *Azadirachta indica*. The aim of this work is,

therefore; to investigate the biological and pharmacological activity such as antioxidants, total phenol content and total flavonoid contents of the extracts of the *Azadirachta indica* native to Somalia as well as it's the chemical composition as a future primary data.

II. METHODS AND MATERIAL

2.1- Plant material

The leaves of *Azadirachta indica* A. Juss were collected from the regions around Mogadishu area of Somalia in 2019, the plant was collected by biologist Mr. Ahmed Abdulkadir Khalif, (Modern University, Mogadishu, Somalia) and identified by Botanist Dr. Ömer Koray Yaylacı (Anadolu University). The Voucher specimens was deposited at Herbarium of the Faculty of Pharmacy with (ESSE No. 15551), Anadolu University in Eskişehir, Turkey. The collected plant sample was stabilized for one week in the laboratory at room temperature to dry it before being used for experiments. The information on the collection sites are given in Table 1. The leaves were then subjected to screening for biological activities and phytochemical profile. The analysis of the sample was done at Medicinal Plant, Drug and Scientific Research Center (AÜBIBAM), Anadolu University in Eskişehir, Turkey.

Table 1. Botanical name, collection sites and date of the plant material.

Botanical name and herbarium record	Collection site	Collection date
Azadirachta indica A. Juss ESSE No. 15551	Halane Village, Wadajir District, Mogadishu Latitude: 2° 1'2.80"N Longitude: 45°17'16.02"E Range: 753 m	January, 2009

2.2. Chemicals

Gallic acid (GA), protocathechic acid (protoCA), p-hydroxy benzoic acid (p-OH-BA), vanillic acid (VA), caffeic acid (CA), chlorogenic acid (ChA), syringic acid (SA), p-coumaric acid (p-CouA), ferulic acid (FA), o-coumaric acid (o-CouA), trans-cinnamic acid (tr-CinA), gallic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), sodium persulfate ($Na_2S_2O_8$), rutin hydrate, aluminum chloride, acetic acid (HA), Folin-Ciocalteu's phenol reagent (FC reagent), sodium carbonate, 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox), propylparaben as internal standard (IS), (MeOH), ethanol, n-hexane and formic acid were supplied from Sigma Aldrich (St. Louis, MO, USA) and Merck (GmbH, Darmstadt, Germany) and they were analytical grade and were used without further purification.

2.3. Apparatus

UV-spectrophotometer (UV-1601, UV-Visible spectrophotometer, Shimadzu), 96-flat bottom well plate cells, Microtiter plate assay was performed with Biotek Powerwave XS microplate reader, Accelerated Solvent Extractor (ASE), Rotary Evaporator, Clevenger Apparatus, Agilent 5975 GC-MS system (Agilent, USA; SEM Ltd., Istanbul, Turkey) was equipped with the HP-Innowax FSC column (60m×0.25mm id with 0.25μm film thickness, Agilent, USA). The GC–FID analysis was performed with capillary GC using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey). Ultrapure water (0.05 μS/cm) was obtained from a Direct-Q® Water Purification System (Germany). For the HPLC analysis, an HPLC system (a model 600 E HPLC pump, 717 plus autosampler, 996 photodiode array detector, and data processor of a Millenium 32 from Waters Corp. Massachusetts, USA) was used. For the determination of phenolic acids, C18 column (Ultrasphere, Teknokroma, Barcelona, SP) (100 x 4.6 mm, inner diameter 3 μm) was employed.

III.EXPERIMENTAL ANALYSIS

3.1. Extraction Procedure

3.1.1. Essential oils (EOs)

The air-dried plant material (leaf) was subjected to hydro distillation by using Clevenger type apparatus for (3h) according to European pharmacopeia. The oil obtained were then diluted with n-hexane (10%, v/v) and subjected to GC/MS and GC–FID techniques to determine the chemical profiles of the oil extracts.

3.1.2. Accelerated Solvent extractor (ASE)

The leaf was extracted with accelerated solvent extraction method (pressurized liquid extraction) sequentially using n-hexane, MeOH and water as solvents. The working conditions of the ASE techniques for the extraction with n-hexane and MeOH were as follows: Heat at 5 min, static at 5 min, Flush% 50%, purge 100%, temperature of 70 °C, pressure applying 1500 psi, while with water was temperature of 100 °C, pressure applying 1500psi. All n-hexane and MeOH extracts were concentrated to dryness by rotary vacuum evaporation while water extracts were also concentrated to dryness by lyophilization process.

3.1.3. Maceration extraction at room temperature

Maceration with water was applied to get the folk medicine preparation, the extract was directly concentrated to dryness by lyophilization process then the extracts was subjected to screening for biological activities and determine the phenolic compound of the extracts using HPLC techniques. The extraction procedure was done in different ways as it is mention on above and the yield obtained from each was then summarized in Table 2.

Table 2. Percentage of yield from extraction procedure

Weight of row plant (g)	Extraction Method	Solvent	%yield (w/w)
10	ASE	n-hexane	0.62
10	ASE	MeOH	8.16
10	ASE	Water	13.25
65	Hydrodistillation	Water	0.043
10	Maceration At room temperature	Water	15.87

3.2. GC-MS/FID Analysis

The chemical composition of oil extracted from the leaves of *Azadirachta indica* growing in Somalia were reported earlier in the section of 'EXPERIMENTAL' with GC-FID/MS techniques. The oil yeilds from 65g air-dried plant was 43 % (w/w).

3.2.1. Identification of compounds

Identification of individual compounds of oil were made by (i) Computation of the relative retention indices (RRI) of the compounds on polar column under predetermined temperture conditions for n-alkanes (C8–C40), and compare with the ones with the same chromatographic conditions, with those of authentic compounds or literature data, (ii) Computer matching with validated commercial mass spectral libraries Wiley GC/MS Library 7.0 (Wiley, New York), MassFinder software 4.0, Adams Library, and NIST Library. (Schepetkin et al., 2015), and comparison of the recorded spectra with data from literature reviewied and their retention indices with accurate compounds or with those reported in the literature was confirmed (Adams, 2007). For quantification aims, relative area percentages obtained by FID were used without the use of correction factors.

3.3. HPLC Analysis

3.3.1. Procedure

The analysis was performed using a gradient program including two solvent systems (A: MeOH: water: formic acid (10: 88: 2 v/v); B: MeOH: water: formic (90: 8: 2 v/v)). Initial condition was 100% A; 0–15 min, changed to 100% A; 15–20 min, to 85% A; 20–30 min, to 50%; 30–35 min to 0% A; 36–42 min, then back to 100% A. The flow rate was 1 mL and the injection volume was 10 µL. The signals were detected at 280 nm. Standard phenolic acids were prepared in a solvent consisting of water: MeOH (50: 50; v/v). Propylparaben was used as internal standard (IS). The validated method (Öztürk et al., 2007) was applied to the standart solution including phenolic compounds and samples. Extracts of the plant based material were prepared as detailed in the 'EXPERIMENTAL' section.

3.4. Antioxidant screening

3.4.1. Determination of antiradical activities of *Azadirachta indica* antioxidants by DPPH

The antioxidant activity of the extracts was determined against DPPH, the test extracts were dissolved in MeOH to obtain the concentration of 10mg/ml, Then the 10mg/ml concentration were diluted with MeOH as (10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, 0.3125 mg/ml, 0.15625 mg/ml, and 0.078125 mg/ml). 0.1 mg/mL of standard gallic acid were also prepared with MeOH and used as a positive control, 100 µL of (extract/ standard) solution was mixed with 100 µL DPPH solution (0.08 mg/mL in MeOH) in 96-flat bottom well plate cells. After incubation at room temperature for 30 min in dark place, the mixture was analyzed through spectrophotometer with wave length 517 nm. The experimental analysis was performed three times in each as replicate) (Brand-Williams, Cuvelier, & Berset, 1995; Chen, Bertin, & Froldi, 2013; Yur et al., 2017). The data obtained from spectrophotometer was then acquired using SigmaPlot Software 12.0 (San Jose, CA), BioDataFit® version 1.02 (Castro Valley, CA). Then, the free radical scavenging activity of the samples was expressed as % inhibition and calculated using the following formula:

$$^{0}/_{0}\, Inh = (\frac{AB-AS}{AB}) \times \%100$$

Where, A_B: means the absorbance of blank reagent (all reagents except the target sample) where A_S: means the absorbance of sample and the result obtained from it was then used to calculate EC50.

3.4.2. Determination of ABTS radical cation scavenging activity (TEAC assay)

Trolox equivalent antioxidant capacity of the sample was evaluated with $ABTS^{+1}$ according to the procedure described by (Re et al., 1999; Yur et al., 2017). 7 mM of ABTS and 2.5 mM of sodium persulfate were prepared with 10ml of ultrapure water, then the mixture was allowed to stand in the dark place at room temperature for 16hr until the ABTS radical cation ($ABTS^{+1}$) was formed and being ready to use for experiments. 10 ml of aliquot $ABTS^{+1}$solution were diluted with absolute ethanol and introduced to UV-spectrophotometer to adjust the absorbance reading in between of 0.70-0.80 at 734 nm at room temperature. Trolox standards with concentration (3, 2, 1, 0.5, 0.25, 0.1 mM) were also prepared with MeOH, 0.1 mg/mL of standard gallic acid were also prepared in MeOH and used as a positive control, 10 µL of the sample (extract/standard) solution was taken and mixed with 990 µL of ($ABTS^{+1}$) solution in tube, and blank solution was prepared by mixing 10 µL of MeOH with 990 µL of ($ABTS^{+1}$) solution which was used as control. Then the mixtures were incubated for 30 min at room temperature, and then the mixture was used to run spectrophotometer with 734 nm the result obtained from it was plotted as a function of concentration of antioxidants of Trolox for the standard reference data then expressed as percentage inhibition of absorbance. All determinations were carried out at least three times, and in triplicate. Then the $ABTS^{+1}$scavenging activity of the samples was expressed as a Trolox equivalent antioxidant capacity and calculated using this equation: ($y= 33.272x- 0.9153$, $r^2 = 0.9981$)

3.5. Determination of total flavonoid content

The total flavonoid contents was determined by using rutin as standard reference compound with aluminium chloride, for the preparation of calibration curve of rutin, standards with concentration (0.8, 0.6, 0.4, 0.2, 0.1 mM) were prepared with MeOH, 80 µL of (plant extract (2 mg/mL)/ standards/) were taken and mixed with 80 µL aluminium chloride (2% v/v) then diluted with 1.85mL of absolute ethanol, the mixture is then incubated for 40 minute at room temperature, the blank solution was also prepared from 1 drop of 15% (v/v) acetic acid , 80 µl plant extract and 1.85 mL absolute ethanol, after 40 minute, the mixture was analyzed with spectrophotometer with 415 nm, the absorbance obtained from it was then compared with calibration curve of rutin for the standard reference data (Miliauskas, Venskutonis, & Van Beek, 2004; Yur et al., 2017). All experiment was carried out in

triplicate. Then the total flavonoid content in the plant extract in rutin equivalent (RE) was calculated by using this formula: (y= 0.7935x+ 0.0126, r^2 = 0.9998).

3.6. Determination of total phenol content

The total phenol content was evaluated as gallic acid equivalent using FC reagent, for calibration curve of gallic acid 20µL aliquots 0.8, 0.6, 0.4, 0.2, and 0.1 mM Methanolic gallic acid solution were mixed with 300 µL of FC reagent, 20µl sample (2 mg/ml) in MeOH and 300 µl of FC reagent were mixed and 1560 µl of ultrapure water was added, then the mixture was allowed to stand in the dark at room temperature for 1-8 minute, 300 µl of 20% (m/v) sodium carbonate was then added and the mixture was again incubated in the dark place for 2 hours, and then the color generated in the mixture was used to run spectrophotometer with 760 nm the absorbance result obtained from it was then compared with calibration curve of gallic acid for the standard reference data (Singleton, Orthofer, & Lamuela-Raventós, 1999; Yur et al., 2017). All determinations were carried out at least three times, and in triplicate. Then, the total phenol content of the samples was expressed as gallic acid equivalent and calculated using this equation: (y= 0.4015x+ 0.0074, r^2 = 0.9941).

IV. RESULTS AND DISCUSSION

We present the result obtained from the screening of so-called leaf extracts of *Azadiracta indica* A.Juss., the screening platform combines biological activity and its phytochemical composition, GC-MS/FID and HPLC analysis were performed for associating active compounds of *Azadiracta indica* A.Juss., collected in Somalia.

4.1. GC-MS/FID Analysis

The results obtained from GC-MS/FID Analysis were summarized in Figure 1 and Table 3. GC-MS/FID technique allowed us to determine qualitative and quantitative analysis of *Azadirachta indica* EOs. The detected volatile compounds with their, retention indices, relative percentages and method of identification is given in Table 3 based on their elution with the HP-Innowax FSC column. Different components were found with GC-MS/FID analysis and resulted with 31 as the key components of 90.6% of the total oils, the main components and their respective abundances were identified as γ-Elemene (32.8%), Germacrene-B (30.1 %), β-Caryophyllene (5.2%), Phytol (2.9%), Hexadecanoic acid (2.2%),γ-Muurolene (2.1%),α-Humulene(1.6%), Germacrene D (1.5%),β-Elemene(1.5%), Pentadecanal (1.4%), Nonacosane (1.3%), Eudesma-4(15),7-diene-1-b-ol (0.4%), Juniper camphor (0.4%), 1,6-Germacradien-5α-ol (0.1%), 3,4-Dimethyl-5-pentylidene-2(5H)-furanone (0.1%), Hexahydro-farnesylacetone (0.3%), (E)-Nerolidol(1.2%), Neophytadiene isomer I43,38 (0.5%), Selina-3,7(11)-diene (1%), δ-Cadinene (0.5%), β-Bisabolene (0.3%), γ-Amorphene (0.1%), epi-Zonarene (0.2%), (Z)-β-Farnesene (0.3%), β-Copaene (0,2%), cis-α-Bergamotene (0.4%), β-Bourbonene (0.6%), α-Bourbonene (T%), α-Copaene (0.7%), δ-Elemene (0.6%) and α-Cubebene (0.1%), although the the essential oil compositions of the leaves neem tree has been studied previously (Davies, 1990; Kurose & Yatagai, 2005), but the essential oil compositions of the Azadirachta indica leaves collected in Somalia were reported here for the first time.

Table 3. Main chemical constituents of the *Azadirachta indica* A. Juss leaf essential oils

No	RRI	Compound	Relative percentages , %
1	1466	α-Cubebene	0.1
2	1479	δ-Elemene	0.6

3	1497	α-Copaene		0.7
4	1528	α-Bourbonene		t
5	1535	β-Bourbonene		0.6
6	1545	*cis*-α-Bergamotene		0.4
7	1600	β-Elemene		1.5
8		β–Copaene		0.2
9	1612	β-Caryophyllene		5.2
10	1650	γ-Elemene		32.8
11	1668	(*Z*)-β-Farnesene		0.3
12	1677	*epi*-Zonarene		0.2
13	1687	α-Humulene		1.6
14	1704	γ-Muurolene		2.1
15	1726	Germacrene D		1.5
16	1733	γ-Amorphene		0.1
17	1741	β-Bisabolene		0.3
18	1773	δ-Cadinene		0.5
19	1796	Selina-3,7(11)-diene		1
20	1846	Neophytadiene isomer I 43,38		0.5
21	1854	Germacrene-B		30.1
22	2041	Pentadecanal		1.4
23	2050	(*E*)-Nerolidol		1.2
24	2161	Hexahydro-farnesylacetone		0.3
25	2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone		0.1
26	2202	1,6-Germacradien-5α-ol		0.1
27	2320	Juniper camphor		0.4
28	2368	Eudesma-4(15),7-diene-1-b-ol		0.4
29	2622	Phytol		2.9
30	2900	Nonacosane		1.3
31	2931	Hexadecanoic acid		2.2
			Total	90.6

* RRI calculated against n-alkanes (C9–C40) on polar HP-Innowax column

MS = mass-spectrometry

t= trace

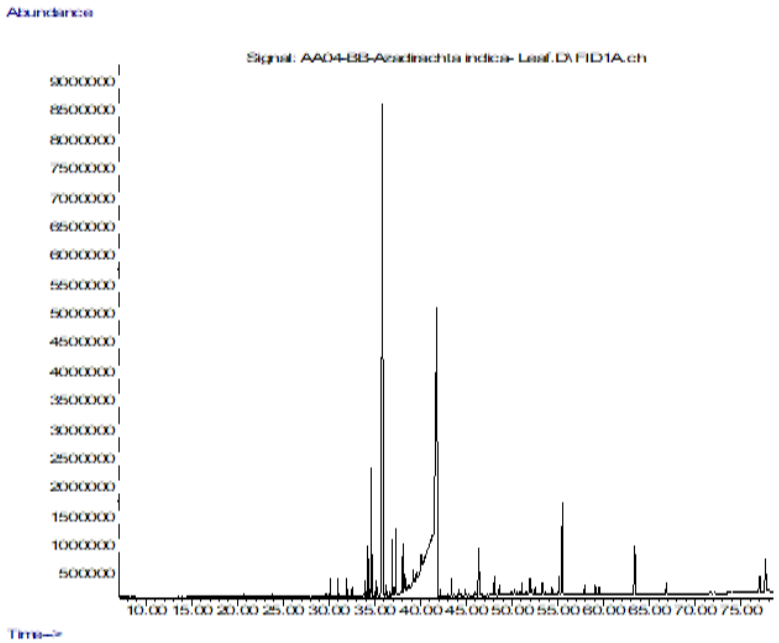

Figure 1. The GC-MS/FID spectrum of the Azadirachta indica A. Juss leaf essential oils.

4.2. HPLC Analysis

The chromatogram of the retained compounds of standard phenolic mixture and the extracts were presented in Figure 2. As highlighted in the chromatograms and the validated method (Öztürk et al., 2007), 1.70 x 10-5 M p-OH-BA was quantified in methanolic extract of *Azadirachta indica* A. Juss and 1.60 x 10-5 M p-OH-BA and 1.05 x 10-5 M protoCA in the ones extracted with water by maceration technique.

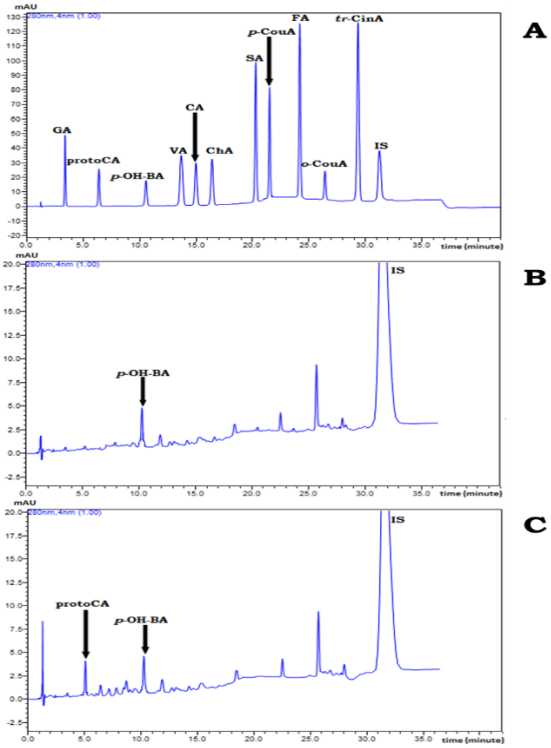

Figure 2. Chromatogram of certain standard phenolic acids (a) and *Azadirachta indica* A. Juss extracts of: Methanolic (b) and maceration with water (c) in the optimum conditions [using a gradient program with the two solvents system (A: MeOH:Water:formic acid (10:88:2, v/v); B: MeOH:water: formic acid (90:8:2, v/v)). The flow rate of 1 mL . min^{-1}, injection volume of 10 mL detecting at 280 nm. Standard phenolic acids were prepared in a solvent consisting of water:MeOH (50:50; v/v). Internal standard: Propylparaben].

4.3. Antioxidants

For DPPH assay, the highest scavenging effect for extracts obtained with ASE method was observed in n-hexane extracts with (EC50= 1.388 mg/ml), whereas water extract (EC50= 0.324 mg/ml) showed the lowest activity level while the essential oil was found to be inactive in this test as evident in Figure 3. In the experiment, all the samples have shown activity towards ABTS radicals with highest TEAC values for essential oil (0.0452mM) obtained with Clevenger type apparatus (Table 4).

Table 4. Radical scavenging activity of the solvent extracts from *Azadirachta indica* native in Somalia

Plant Material	Sample weight	Extracts	%yield (w/w)	DPPH radical EC50: mg/ml	ABTS radical cation (mg TEs/g extract)
Azadirachta indica	10	n-Hexane	0.62	1.388±0.035	0.041±0.001
	10	MeOH	8.16	0.344±0.009	0.033±0.002
	10	Water	13.25	0.324±0.056	0.031±0.001
	65	Essential oils	0.043	NA	0.045±0.0002
	10	Maceration with Water	15.87	0.532±0.032	0.033±0.001

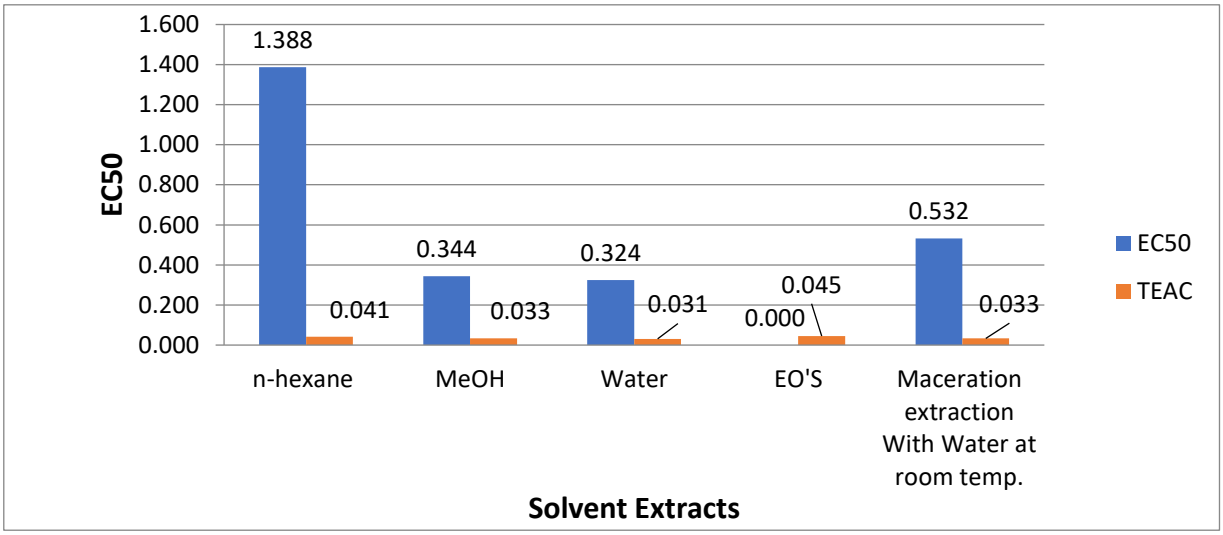

Figure 3: Grgaph of radical scavenging effect DPPH and TEAC from Azadirachta indica

4.4. Total phenol contents and total flavonoid content

Total phenol content values were found to be between 0.108 GAE mg/mL and 0.139 GAE mg/mL. the highest total phenol content was detected in MeOH extracts. Total flavonoid content values were determined as highest in aqueous extracts (0.052 and 0.047 RE mg/mL) and lowest in n-hexane extract (0.003 RE mg/mL) as shown in (Table 5 and Figure 4).

Table 5. Total phenol contents and total flavonoid contents from *Azadirachta indica* native in Somalia

Plant Material	Sample weight	Extraction solvent	%yield (w/w)	TPC mg gallic acid/mL	TFC mg/ml
Azadirachta indica	10	n-Hexane	0.62	0.123±0.008	0.003±0.001
	10	MeOH	8.16	0.139±0.005	0.032±0.007
	10	Water	13.25	0.126±0.003	0.052±0.004
	65	Essential Oils	0.043	0.108±0.005	-0.014±0.002
	10	Maceration with water	15.87	0.129±0.009	0.047±0.002

*Values stated are means ±STD of three times in each as replicate,

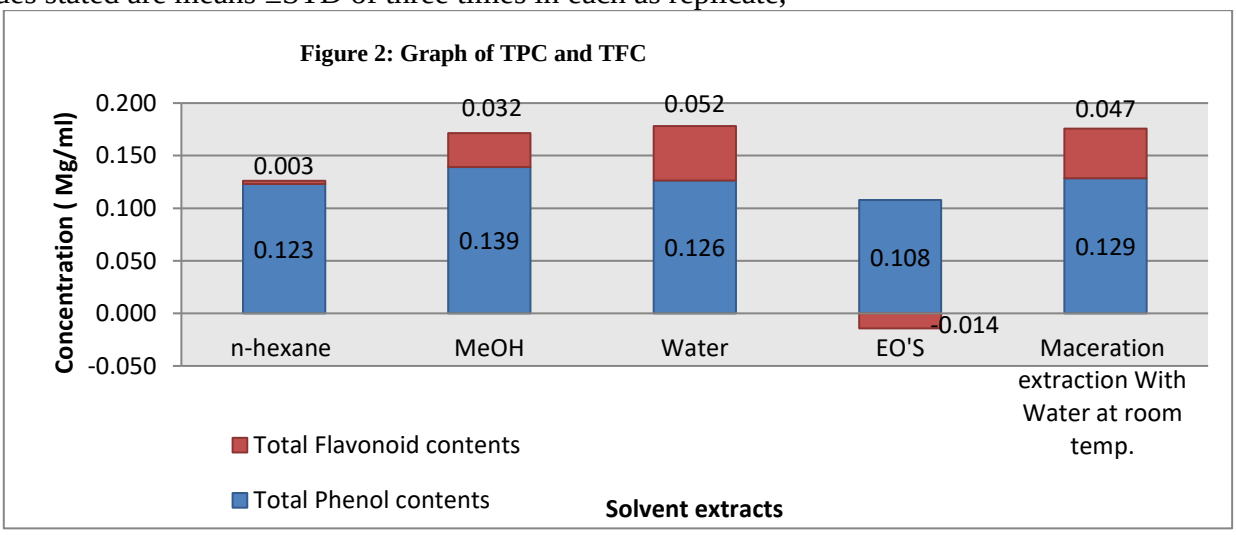

Figure 4: Graph of total phenol contents and total flavonoid contents from *Azadirachta indica*

V. CONCLUSION

The study has shown that the biological activity of the leaf of *Azadirachta indica* native to Somalia was fully active in TEAC assay, phenol and flavonoid and in all DPPH assays except for the essential oil extracts. The powerful natural antioxidant and the effectiveness as a powerful antibiotics features of *Azadirachta indica* could be the reason for its use as a remedy for various ailments as well as its use for domestic purposes. The phytochemical composition of oil extracts was also analyzed with GC-FID/MS techniques and the results have emphasized that the *Azadirachta indica* leaves have a unique source of various types of compounds having diverse chemical structures. The results in this study also demonstrated that the leaf of *Azadirachta indica* contain phenolic acids which indicate their potentials as therapeutic agents. Due to their powerful antioxidative activity against several antioxidant system could also be the reason for its use as treating for human diseases, furthermore, the extracts of this plant can be used as easily reachable source of natural antioxidants in the field of pharmaceutical applications. This study was also conducted to evaluate the biological activity and the chemistry of the leaf part of the *Azadirachta indica* to generate significant primary data and also encourage researchers to explore information about medicinal plants native to Somalia. More extensive research work is needed to develop better understanding and biochemical reference data about plants native to Somalia.

CONFLICT OF INTEREST STATEMENT

All authors of the manuscript declare that they do not have financial/commercial conflicts of interest.

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