

Anti-Alzheimer's and Antioxidant Effects of *Anacardium Occidentale* Oil: Profile Analysis Using GC/FID and HPLC

İşıl Nihan KORKMAZ^{1*} 

¹ Muş Alparslan University, Faculty of Applied Sciences, Department of Plant Production and Technologies, Mus, Türkiye

*Corresponding author: nihan.korkmaz@alparslan.edu.tr

Abstract

Determining the biological profiles of oils is important for a healthy life and the treatment of common global diseases. In this study, the anti-Alzheimer's and antioxidant properties of *A. occidentale* oil were investigated using various methods. The inhibitory effects of *A. occidentale* oil on acetylcholinesterase and butyrylcholinesterase, which are linked to neurodegenerative diseases, were also determined. Phenolic compounds in *A. occidentale* oil were determined by HPLC chromatography, and sixteen different phenolic compounds were identified. The chemical components of oil were evaluated by GC/FID analysis, and the predominant essential oils were oleic acid (41.25%), linoleic acid (37.95%), and palmitic acid (15.03%). The radical scavenging ability of *A. occidentale* oil was investigated using ABTS and DPPH bioanalytical radical scavenging methods. The results revealed strong radical scavenging properties with IC₅₀ of 4.33 µg/mL for ABTS radicals and 14.74 µg/mL for DPPH radicals. The research was further extended by examining the reducing abilities of Cuprac and FRAP. *A. occidentale* oil showed inhibitory effects on acetylcholinesterase (IC₅₀: 13.86 µg/mL) and butyrylcholinesterase (IC₅₀: 16.11 µg/mL). The study will form the basis for future research on the antioxidant properties and enzyme inhibition of oils obtained from different sources of medical and industrial importance.

Keywords

Anacardium occidentale, Chromatography, Antioxidant Activity, Enzyme Inhibition

Anacardium Occidentale Yağının Alzheimer Karşıtı ve Antioksidan Etkileri: GC/FID ve HPLC Kullanılarak Profil Analizi

Işıl Nihan KORKMAZ^{1*} 

¹ Muş Alparslan Üniversitesi, Uygulamalı Bilimler Fakültesi, Bitkisel Üretim ve Teknolojileri Bölümü, Muş, Türkiye

*Sorumlu yazar: nihan.korkmaz@alparslan.edu.tr

Özet

Yağların biyolojik profillerinin belirlenmesi, sağlıklı bir yaşam ve yaygın küresel hastalıkların tedavisi için önemlidir. Bu çalışmada, *A. occidentale* yağının Alzheimer karşıtı ve antioksidan özellikleri çeşitli yöntemler kullanılarak araştırılmıştır. *A. occidentale* yağının nörodejeneratif hastalıklarla bağlantılı olan asetilkolinesteraz ve bütirilkolinesteraz üzerindeki inhibitör etkileri de belirlenmiştir. *A. occidentale* yağındaki fenolik bileşikler HPLC kromatografisi ile belirlenmiş ve on altı farklı fenolik bileşik tanımlanmıştır. *A. occidentale* yağının kimyasal bileşenleri GC/FID analizi ile değerlendirilmiş ve baskın uçucu yağlar oleik asit (%41.25), linoleik asit (%37.95) ve palmitik asit (%15.03) olmuştur. *A. occidentale* yağının radikal süpürücü özelliği, ABTS ve DPPH biyoanalitik radikal süpürücü yöntemleri kullanılarak araştırılmıştır. Sonuçlar, ABTS radikalleri için 4.33 µg/mL ve DPPH radikalleri için 14.74 µg/mL IC₅₀ ile güçlü radikal temizleme özellikleri ortaya koydu. Araştırma, Cuprac ve FRAP'ın indirgeyici yeteneklerinin incelenmesiyle daha da genişletildi. *A. occidentale* yağı, asetilkolinesteraz (IC₅₀: 13.86 µg/mL) ve bütirilkolinesteraz (IC₅₀: 16.11 µg/mL) üzerinde inhibitör etkiler gösterdi. Çalışma, tıbbi ve endüstriyel öneme sahip farklı kaynaklardan elde edilen yağların antioksidan özellikleri ve enzim inhibisyonu üzerine gelecekteki araştırmalar için temel oluşturacaktır.

Anahtar kelimeler

Anacardium occidentale,
Kromatografi,
Antioksidan
Aktivite,
Enzim İnhibisyonu

1. INTRODUCTION

Medicinal plants have bioactive compounds that play an important role in reducing the progression of diseases and often in complete recovery due to the presence of different chemical substances in different compositions found as secondary metabolites in one or more parts of the plants. The primary reason for the pharmacological use of medicinal plants is their abundance of secondary metabolites. For this reason, they are used in many neurodegenerative diseases, such as Alzheimer's disease (AD). Some of the metabolites found in different parts of plants may protect against neurodegeneration. Many natural compounds exhibit anti-Alzheimer's activity through specific pharmacological mechanisms, such as targeting acetylcholinesterase (AChE) [1].

Radicals containing at least one unpaired electron, or non-radical reactive compounds with the potential to oxidize biomolecules, are referred to as reactive oxygen species (ROS). Oxidative stress has played a significant role in the early stages of many diseases, including neurodegenerative diseases, the aging process, and cancer [2]. To prevent oxidative stress, the body relies on an antioxidant system. These systems work together to defend against the harmful effects of oxidative stress [3]. Antioxidant properties are particularly important components of the body's defense systems, protecting it from damage caused by ROS. While ROS occur naturally during cellular biotransformation, excessive amounts cause oxidative stress [4,5]. This can damage biomolecules, contributing to cellular dysfunction and diseases such as diabetes, cancer, immunodeficiency, and obesity. Even at low concentrations, antioxidants provide an effective defense by neutralizing ROS and reducing oxidative stress [6]. Numerous studies have reported that certain compounds possess antioxidant properties, thus reducing the likelihood of many diseases, such as neurodegenerative diseases [7]. In this context, there has been an increased interest in researching new plant-based antioxidants that can effectively combat free radical damage [8].

Dementia, which primarily affects the elderly, is a very serious neurodegenerative disorder characterized by the deterioration of memory and cognitive functions. It is considered one of the public health challenges of the 21st century [9–11]. This makes it imperative to delve deeper into the complexities of this insidious disease and pursue innovative research and intervention pathways [12]. AD deprives individuals of their individuality, abilities, impact on society, and valuable memories, and imposes an emotional and economic burden on their environment and societies [13]. According to World Health Organization (WHO) data, millions of people are affected by this condition, and the current number is expected to triple by 2050. The pathogenesis of AD is not fully understood, but among the specific pathological findings is a decrease in acetylcholine (ACh) levels in the brain [14]. It has been suggested that ACh levels increase in patients due to an effective immune response designed to alleviate the reported symptoms [15]. The first factor identified in Alzheimer's disease is the inhibition of

acetylcholinesterase (AChE). AChE exists in two classes: acetylcholinesterase and butyrylcholinesterase (BChE). AChE is found in the brain and neuronal synapses. It is a neurotransmitter that transmits nerve signals and is vital for memory. When these two enzymes are inhibited by various inhibitors, the acetylcholine compound in nerve endings will be restored to functional levels, allowing nerve impulses to be transmitted. This, in turn, may partially address the treatment of diseases such as AD, amnesia, and other conditions. Therefore, inhibiting AChE is a promising approach for Alzheimer's disease treatment [16].

Given this information, AChE has become a target of current pharmaceutical research, resulting in the development of many drugs currently in clinical use for the treatment of AD. AChE inhibitors account for the majority of drugs approved by the FDA [17,18]. Numerous studies are underway to investigate the problems arising from overuse of currently available drugs and the numerous side effects associated with their long-term use. Therefore, the development of new pharmaceuticals for the treatment of AD has become a pressing issue [19].

The use of natural products for neurodegenerative diseases is based on traditional knowledge. Different countries have used their local flora and fauna to treat brain-related diseases according to their cultural traditions. In this context, cashew, grown primarily in India, is the fruit of a tree in the Anacardiaceae family and is known in medical and pharmaceutical literature as *A. occidentale*. The fruits of this plant are known for their memory-enhancing properties. Historical sources indicate that many scientists used *A. occidentale* to enhance memory [20].

A. occidentale L. is a tropical tree belonging to the Anacardiaceae family and is particularly known for its cashew nuts and fruit. Among the main bioactive components of this plant are anacardic acids, tannins, flavonoids, and other phenolic compounds. These compounds are known to have anti-inflammatory and antioxidant properties. Additionally, some preliminary studies have suggested that cashew extracts may have positive effects on anxiety. However, these findings need to be confirmed in larger, controlled studies [21,22]. Research has shown that cashew tree extracts, like those of both peanuts and apples, offer potential health benefits. These benefits include antidiabetic, anticancer, and gastroprotective effects. It has also been reported as a potential herb for treating diabetes, myocardial infarction, asthma, rheumatoid arthritis, hemorrhoids, liver, esophageal, breast, oral cavity, and heart diseases, CNS disorders, and cancers such as hepatocellular carcinoma and breast carcinoma. It is a rich source of antioxidants with high free radical scavenging properties. Research on its antidepressant effects is ongoing. *A. Occidentale* contain biologically active phytochemicals. These compounds may have positive effects on the nervous system by exhibiting anti-inflammatory and neuroprotective properties. Anacardic acid and polyphenols, in particular, may offer potential benefits in

alleviating psychological disorders such as anxiety [21]. However, the exact mechanism underlying its various effects has not been fully determined [21].



Figure 1. Sample image of *A. occidentale* used in the study.

The high polyphenol content, one of the most important quality parameters of oil, can be transferred into the oil without being damaged by the cold-pressing method. The main advantage of oils obtained with this method is that the bioactive components are transferred into the oil without being damaged [23,24]. There are inhibitory and antioxidant studies in the literature on the effects of *A. occidentale* leaves, bark extracts, and extracts on AD. However, the direct inhibitory effects of *A. occidentale* cold-pressed oil on AChE and BChE, as well as some antioxidant methods, remain lacking [25,26]. In light of this information, this study investigated the in vitro inhibitory effects of cold-pressed *A. occidentale* oil on AChE and BChE, and evaluated its antioxidant and anti-AD effects holistically to elucidate the underlying inhibitory mechanisms as a potential drug candidate for AD treatment. We also determined the essential oil and polyphenol content of the oil using GC/FID and HPLC analytical methods.

2. MATERIAL AND METHOD

2.1. Chemicals and Instruments

All chemicals used within the scope of the study were supplied from Sigma-Aldrich and Merck. *A. occidentale* oil, Tito brand, originating in Turkey, is commercially available as a cold-pressed oil.

2.2. Analysis of Polyphenol Profiling with HPLC

One of the most commonly used techniques in the literature for the determination of phenolic compounds in natural sources is the HPLC technique. HPLC analysis was carried out at Muş Alparslan University Central Research Laboratory using an Agilent 1260 Infinity II with a C18 5 μ m, 4.6 x 250 mm column at 28 $\pm$ 4°C column temperature. An Agilent 1260 Infinity II Diode Array Detector WR (DAD) was used as the detector. The flow rate was set at 0.7 mL/min. The content was measured at 272, 280, 310 nm with a 20 μ L injection volume. The mobile phases used were: mobile phase A (1% Acetic Acid) and mobile phase B: 100% Acetonitrile). Gradient rates were 0.0 min (90% acetic acid, 10% acetonitrile), 28.0 min (60% acetic acid, 40% acetonitrile), 39.0 min (40% acetic acid, 60% acetonitrile), 50.0 min (10% acetic acid, 90% acetonitrile), and 55.0 min (90% acetic acid,

10% acetonitrile). The flow rate of the mobile phases was maintained for 60 min (10 min column wash).

2.3. GC/FID Analysis of Essential Oil of *A. Occidentale* Oil

The composition of *A. occidentale* oil was determined using the analytical method described by the AOCS Ce 1–62 method. Analysis was performed using GC-FID only. No MS detector was used. Fatty acids were identified according to retention times reported in the literature for standard FAME (fatty acid methyl esters) mixtures and quantified by the peak area normalization method (% area) [27].

2.4. Analyses to Determine Antioxidant Parameters of *A. occidentale* oil

To determine the antioxidant potential of *A. occidentale* oil, (ABTS) radical scavenging activity, Cu²⁺-Cu⁺ (Cuprac reduction capacity, (DPPH) free radical scavenging activity and FRAP reduction capacity tests were performed [28].

2.4.1. 2,2-Azino-bis (3 – ethylbenzo – thiazoline - 6-sulfonic acid) (ABTS) radical scavenging activity

The antioxidant activity of *A. occidentale* oil was determined using the trolox equivalent antioxidant capacity test with ABTS^{•+} radical cation by making minor modifications to the Re method. Absorbance was determined at 734 nm. Ascorbic acid, α -tocopherol, and trolox were used as control compounds [29].

2.4.2. Cu²⁺-Cu⁺ reduction capacity (Cuprac method)

The Cu²⁺ reducing capacity of *A. occidentale* oil was determined by making minor modifications to the method developed by Apak et al. (2006) [30]. Absorbance values were measured at 450 nm and the obtained data were recorded [31].

2.4.3. 1,1-Diphenyl 2-picryl hydrazyl (DPPH) free radical scavenging activity

DPPH analysis of *A. occidentale* oil was performed using the 1,1-Diphenyl 2-picryl hydrazyl (DPPH) free radical scavenging method based on the Blois (1958) method. Absorbance was measured at 517 nm. DPPH scavenging activity was expressed as % inhibition [32].

2.4.4. FRAP reduction capacity

The FRAP reducing capacity of prepared stock solutions of *A. occidentale* oil was determined by a modified version of the Oyaizu method [33]. Absorbance at 593 nm was recorded against the blank [34].

2.5. Activity Assays of Cholinesterases

The inhibitory effects of *A. occidentale* oil on AChE and BChE activities were measured with commercially available enzymes from Electrophorus electricus and

Equine serum, respectively, using simple modifications of Ellman's spectrophotometric method. Absorbance was measured spectrophotometrically at 412 nm [35,36].

2.6. In Vitro Inhibition Studies

To examine the inhibitory effect of *A. occidentale* oil, activity analysis was performed at five different inhibitor concentrations. Control enzyme activities measured without inhibitor were taken as 100%, and activity measured in the presence of inhibitor was calculated as % activity [37,38]. Graphs of activity % vs. [*A. occidentale* oil] were then plotted, and the IC₅₀ value was calculated from the equation in this graph. Tacrine (TAC) was used as the reference inhibitor against this enzyme [39,40].

3. RESULTS

With the increasing number of people affected by diseases resulting from neurodegenerative disorders, it is necessary to develop new drug candidates that are more effective, reliable, and have fewer side effects against the biological targets of the central nervous system (CNS). While the pathophysiological cause of AD, a lethal CNS disorder, remains unclear, studies in the forebrain suggest that the cholinergic pathway is involved. The cholinergic hypothesis is that inhibition of the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which hydrolyze the depleted cholinergic neurotransmitters, leads to the development of neurotransmitters. Targeting cholinesterase inhibition

with small molecules improves cognitive function and slows the progression of AD [41]. Only a few small molecules targeting AD have been approved by the FDA. Although memantine, rivastigmine, galantamine, and donepezil are currently in clinical use, they have been reported to have various long-term side effects. Therefore, increased research has focused on the design and pharmacological activities of safer potential anti-Alzheimer's agents [19].

GC/FID was used to conduct sensitive qualitative analysis of the components of *A. occidentale* oil. In this study, a total of 8 compounds were identified in *A. occidentale* oil. Among them, oleic acid (41.25%), linoleic acid (37.95%), palmitic acid (15.03%), stearic acid (4.37%), behenic acid (0.46%), arachidic acid (0.44%), myristic acid (0.30%), and gondoic acid (0.20%) were the most prevalent components in *A. occidentale* oil. (Table 1 ve Figure 2).

Table 1. GC/FID analysis of *A. occidentale* oil revealed various compounds in the sample and their respective percentages

RT	Formula	Contents (%)
19.227	Myristic acid (C14:O)	0.30
24.306	Palmitic acid (C16:O)	15.03
32.057	Stearic acid (C18:O)	4.37
32.907-33.807	Oleic acid (C18:1, cis-9)	41.25
36.324-36.552	Linoleic acid (C18:2, cis-9,12)	37.95
38.594	Arachidic acid (C20:O)	0.44
40.979	Gondoic acid (C20:1, cis-13)	0.20
48.096	Behenic acid (C22:O)	0.46

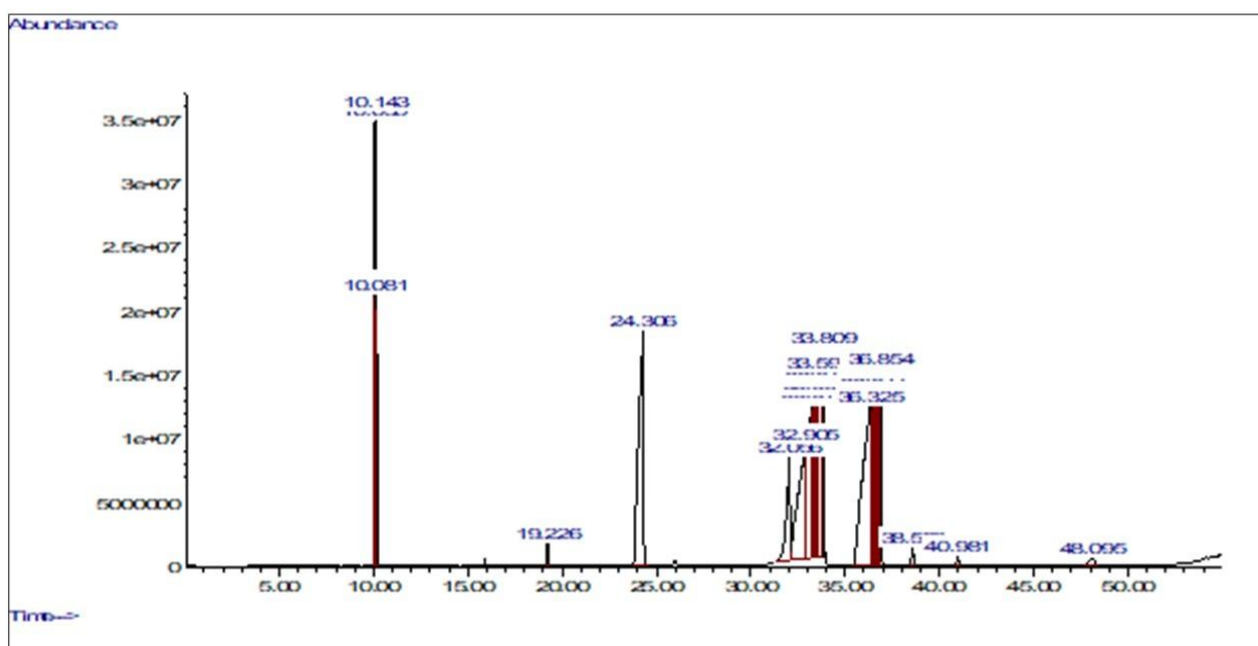


Figure 2. GC/FID analysis of *A. occidentale* oil identified various compounds in the sample and their respective percentages.

To collect data on the chemical components of *A. occidentale* oil, it was subjected to phytochemical

component analysis using HPLC and very significant results were obtained.

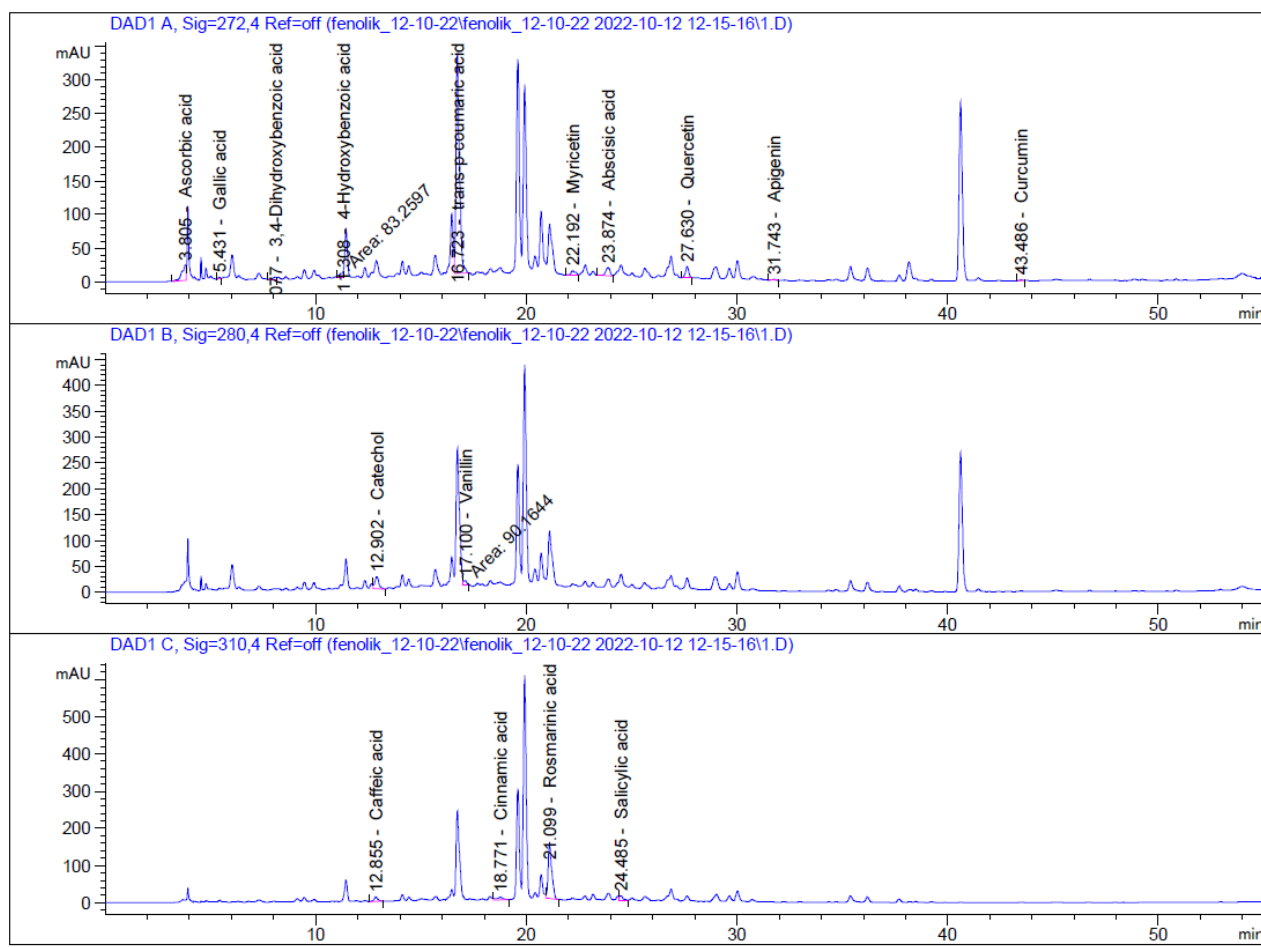


Figure 3. Phytochemical composition using HPLC to collect data on chemical components of *A. occidentale* oil.

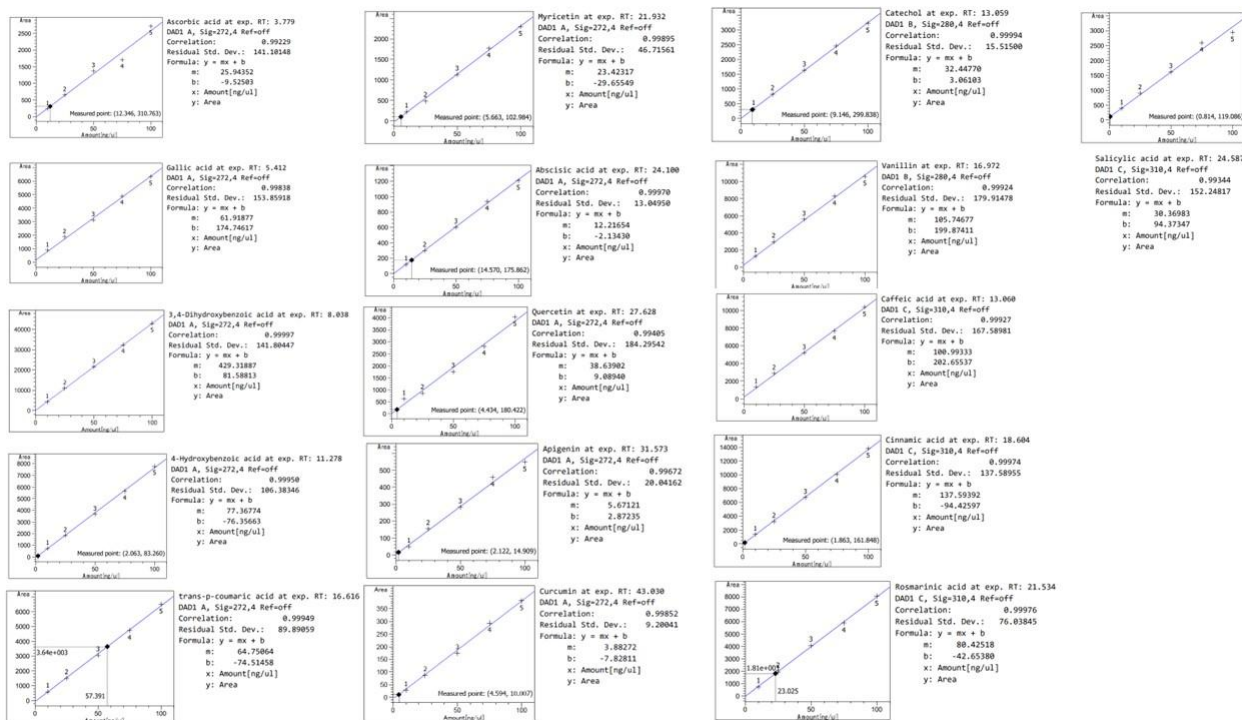


Figure 4. Calibration curves of secondary metabolites screened in the study

In this study, 16 phenolic compounds were tentatively identified from *A. occidentale* oil. HPLC analysis

identified ascorbic acid, gallic acid, 3,4-Dihydroxybenzoic acid, 4-Hydroxybenzoic acid, trans-p-

coumaric acid, Myricetin, Absciscic acid, Quercetin, Apigenin, Curcumin, Catechol, Vanillin, Caffeic acid, Cinnamic acid, Rosmarinic acid, and Salicylic acid (Figure 3, Figure 4).

Recent studies have shown that natural organic compounds also exhibit potent neuroprotective effects. These findings suggest that incorporating these compounds into dietary practices may play a critical role in improving overall health and preventing various diseases. The multifaceted effects of compounds such as quercetin and apigenin in regulating oxidative stress and enhancing cholinergic signaling provide an integrated approach to neuroprotection. By effectively inhibiting AChE, these compounds help maintain ACh levels, which are essential for cognitive function impaired in AD and related disorders [42].

The effects of curcumin on patients with AD have been investigated. It has also been extensively studied in modern medicine for the treatment of various medical conditions, including cystic fibrosis, hemorrhoids, gastric ulcers, colon cancer, breast cancer, atherosclerosis, liver disease, and arthritis. It has been used in various types of treatments for dementia and traumatic brain injury. Curcumin also has a potential role in the prevention and treatment of AD. Curcumin, with its antioxidant, anti-inflammatory, and lipophilic properties, improves cognitive function in AD patients. Increasing evidence suggests that cerebral dysregulation and abnormal inflammatory reactions caused by oxidative stress, free radicals, beta-amyloid, and biometal toxicity contribute to the fundamental processes in AD pathology. Curcumin's diverse effects, including the reduction of beta-amyloid plaques, delaying neuronal degradation, metal chelation, anti-inflammatory, antioxidant, and reduction of microglia formation, have led to improved overall memory in AD patients [43].

Curcumin possesses a wide range of biological activities with considerable safety. The antioxidant and inhibitory potential of curcumin against key enzymes involved in human pathology, namely AD (AChE) and (BChE), was investigated. DPPH and H₂O₂ assays were used to assess the antioxidant capacity of curcumin, and its enzyme inhibitory activity was assessed in vitro using standard procedures. Curcumin exhibited excellent antioxidant activity with an IC₅₀ value significantly lower than the reference vitamin C. In terms of enzyme inhibitory activity, curcumin exhibited excellent inhibitory activity against AChE and BChE. The findings showed that curcumin was significantly less potent than the reference galantamine against AChE but more potent than the reference galantamine against BChE and BChE. These results indicate that curcumin exhibits promising antioxidant and anti-Alzheimer's properties and may be used as a potential natural remedy against these diseases[44].

Quercetin belongs to the flavonoid family, found in fruits, vegetables, green tea, and even most plants with antioxidant activities. Quercetin has anti-inflammatory, anticancer, and anti-prostatic activities, as well as beneficial effects on high cholesterol, kidney transplantation, asthma, diabetes, viral infections, lung, schizophrenia, and cardiovascular diseases. Quercetin has the potential to scavenge hydroxyl radicals (OH[•]), hydrogen peroxide (H₂O₂), and superoxide anion (O₂^{•-}). These ROS inhibit the processing of lipids, proteins, amino acids, and deoxyribonucleic acid (DNA), leading to epigenetic changes. ROS play a vital role in the progression of AD, and quercetin has been proposed as an optimal choice for overcoming cellular and molecular signals in regulating normal physiological functions [45]. The present study reports the anticholinesterase and antioxidant activities of compounds (bergapten, pimpinellin, umbelliferone, quercetin, rutin, and kaempferol) isolated from the aerial parts, fruits, flowers, roots, and stems of *Leiotulus dasyanthus*. Antioxidant activities of the samples were measured by the DPPH method. Anticholinesterase activity was evaluated by the Ellman method. The dichloromethane fraction of the root showed significant inhibition of AChE (49.66%) and BuChE (92.21%) at 20 µg/mL. The dichloromethane fractions of the root possess significant antioxidant and anticholinesterase activity. Further studies on the roots will be important for improving the use of this plant for pharmaceutical and food research. Rosmarinic acid has been given with its antioxidant activity and mechanism of action in previous studies [46]. In light of this information, the inhibitory effects of *A. occidentale* oil, used specifically to enhance memory, on the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were determined. The results are presented in Table 2.

The IC₅₀ value of *A. occidentale* oil for AChE was calculated as 13.86 µg/mL (r²: 0.9673) and for BChE as 16.11 µg/mL (r²: 0.9355). On the other hand, tacrine, the standard inhibitor for both cholinergic enzymes, showed values of 8.82 µg/mL and 2.54 µg/mL (r²: 0.9836) against AChE and BChE. During the measurements, Tacrine, a synthetic compound, was used as a positive control and the IC₅₀ values of this compound and *A. occidentale* oil were compared. The findings showed that *A. occidentale* oil inhibited both enzymes and at a level closer to the standard substance (Table 2, Figure 5).

Table 2. *A. occidentale* oil inhibition results against AChE and BChE enzymes

Compound	Enzyme	IC ₅₀ (µg/mL)	r ²
<i>A. occidentale</i> oil (µg/mL)	AChE	13.86	0.9673
<i>A. occidentale</i> oil (µg/mL)	BChE	16.11	0.9355
TAC	AChE*	8.82	0.9836
TAC	BChE*	2.54	0.9878

*Tacrine was used as a positive control for a AChE and BChE enzyme and taken from the reference of [47,48].

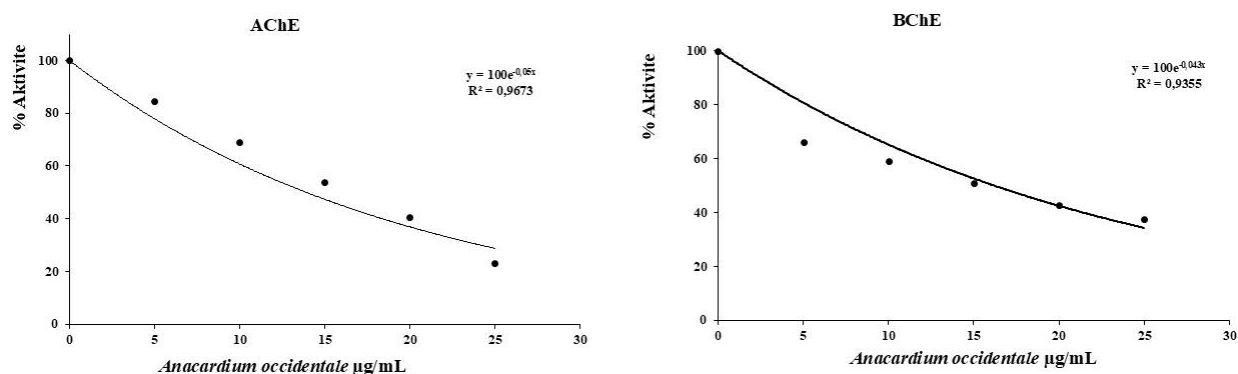


Figure 5. Half maximal concentration (IC_{50} ; $\mu\text{g/mL}$) values of *A. occidentale* oil against AChE and BChE enzymes.

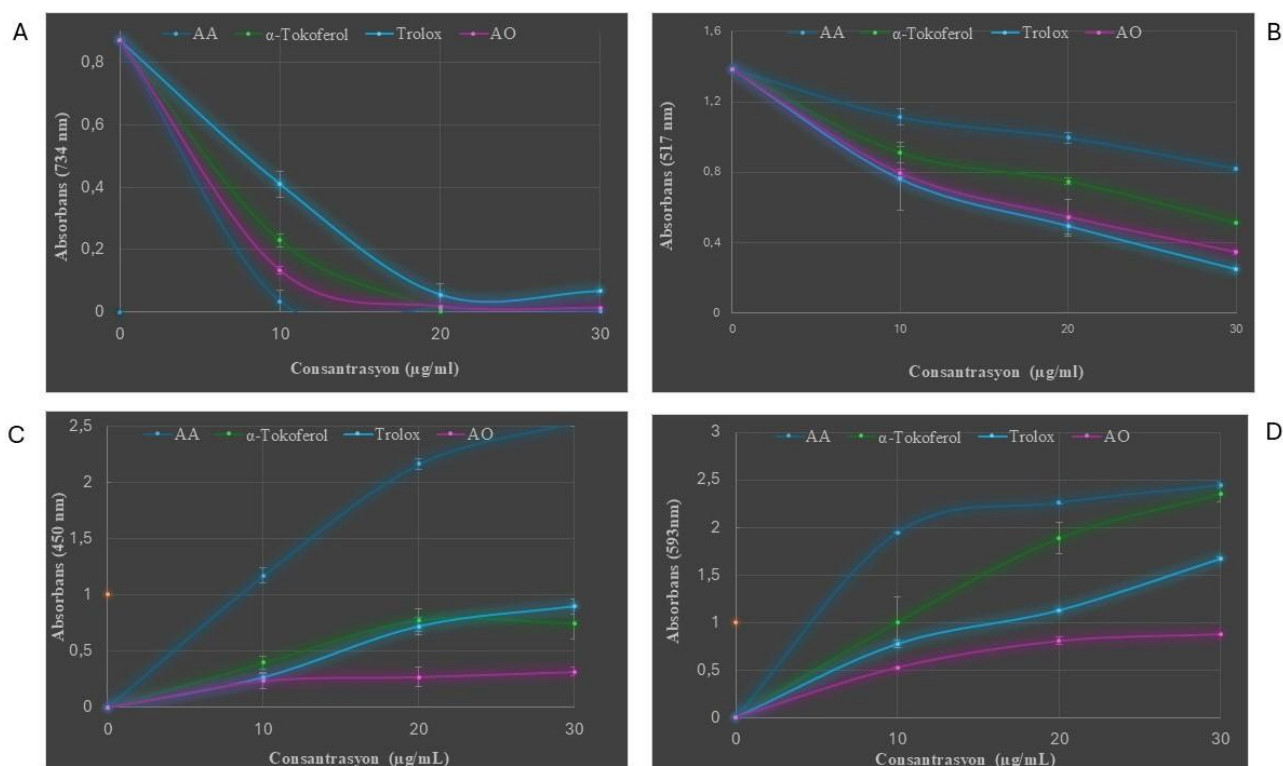


Figure 6. Radical scavenging activity and reducing abilities of *A. occidentale* oil and standard antioxidants. (A) ABTS scavenging activity; (B) DPPH scavenging activity; (C) CUPRAC; (D) FRAP.

Table 3. The reducing power of and scavenging activities (IC_{50}) of *A. occidentale* oil

Antioxidants	ABTS ⁺ scavenging		DPPH [•] scavenging		CUPRAC		FRAP	
	IC_{50}	r^2	IC_{50}	r^2	r^2	$\lambda 450$	r^2	$\lambda 593$
Ascorbic acid	3.39	0.992	38.5	0.9854	0.9829	2.534±0.153	0.9145	2.44±0.186
α-tocopherol	2.86	0.9678	21.0	0.9822	0.9513	0.744±0.105	0.9905	2.358±0.044
Trolox	4.65	0.9315	12.6	0.9962	0.991	0.894±0.035	0.9908	1.673±0.124
<i>A. occidentale</i> oil (AO)	4.33	0.9974	14.74	0.9933	0.9294	0.315±0.067	0.9579	0.878±0.015

In the study, ABTS⁺ was first prepared from ABTS. Then, a reaction mixture was prepared for *A. occidentale* oil and standard antioxidants at different concentrations, and absorbance values were measured at 734 nm. The findings indicated a decrease in absorbance values with increasing

concentrations. The results obtained from ABTS cation radical scavenging experiments conducted at 30 $\mu\text{g/mL}$ concentrations showed consistent trends. The IC_{50} values calculated for the ABTS method were as follows: α Tocopherol (2.86 $\mu\text{g/mL}$) > ascorbic acid (3.39

$\mu\text{g/mL}$) > AO (4.33 $\mu\text{g/mL}$) > trolox (4.65 $\mu\text{g/mL}$) (Table 3).

In antioxidant studies, radical scavenging capacity was determined by determining DPPH scavenging activity. The radical scavenging capacity of solutions of *A. occidentale* oil and standard antioxidants at different concentrations was determined by measuring absorbance values at 517 nm. Absorbance values were observed to decrease with increasing solution concentrations. The IC_{50} value was calculated from the measured absorbance values. A lower IC_{50} value, or one closer to that of standard antioxidants, indicates more effective DPPH scavenging activity [51]. In the DPPH scavenger test, the effective compound was determined as ascorbic acid. IC_{50} values were shown in the following order: trolox (12.6, r^2 : 0.9962) > AO (14.74, r^2 : 0.9933) > α -tocopherol (21.0, r^2 : 0.9822) > Ascorbic acid (38.5, r^2 : 0.9854) (Table 3). To determine the cupric (Cu^{2+}) ion reducing capacity of *A. occidentale* oil solutions at different concentrations, absorbance values at 450 nm were measured. *A. occidentale* oil at a concentration of 30 $\mu\text{g/mL}$ and standard antioxidants were compared. The cupric ion reducing capacity of *A. occidentale* oil was observed to increase proportionally with increasing concentration. This comparison revealed that ascorbic acid (λ_{450} : 2.534) > trolox (λ_{450} : 0.894) > α -Tocopherol (λ_{450} : 0.744) > AO (λ_{450} : 0.315) (Table 3).

In the FRAP method, which is expected to exhibit an antioxidant effect by donating electrons, Fe^{3+} ions are reduced to Fe^{2+} ions. The resulting Fe^{2+} ions form a blue compound in the presence of tripyridyl triazine (TPTZ), exhibiting an absorbance at 593 nm [52]. It was observed that the reduction capacities of the prepared *A. occidentale* oil solution at different concentrations and also of the standard antioxidant solutions from ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) increased with increasing concentration. Based on these results, the measured values were plotted and compared with each other. In determining the reducing capacity, the results at the 30 $\mu\text{g/mL}$ concentration were compared. The results were determined as follows; Ascorbic acid (λ_{593} : 2.44) > α -Tocopherol (λ_{593} : 2.358) > trolox (λ_{593} : 1.673) > AO (λ_{593} : 0.878) (Table 3).

Antioxidants are substances that have the ability to neutralize reactive oxygen species and free radicals, suppress oxidative stress, and protect cellular components such as DNA, proteins, and lipids from oxidative damage. Oxidative stress is associated with many diseases and the aging process, including cancer, diabetes, and neurodegenerative diseases, making antioxidants a major area of interest in biomedical research. All extracts tested in this study demonstrated significant antioxidant activities in DPPH, ABTS, CUPRAC, and FRAP assays, suggesting that these extracts could be used to prevent oxidative damage. These results are consistent with the initial aim of this study, which was to determine the biological potential and identify the bioactive components present in these extracts. Furthermore, their antioxidant properties may play an important role in improving their protective and/or therapeutic effects, which could provide

a basis for further mechanistic studies and potential health benefits [53–55].

4. DISCUSSION AND CONCLUSION

In a separate study, the antioxidant and antibacterial activities of *A. occidentale* shell waste were investigated. DPPH radical and ABTS cation radical scavenging experiments were conducted to determine antioxidant activity. The findings showed that cashew nut shell waste extract (150 $\mu\text{g/mL}$) inhibited DPPH and ABTS radicals by $75.5 \pm 1.4\%$ and $97.1 \pm 1.4\%$, respectively, corresponding to $57.1 \pm 1.0\%$ and $56.2 \pm 0.6 \mu\text{mol}$ trolox equivalent antioxidant capacity, respectively [56].

A. occidentale shell fluid (CNSL) is a good source of unique phenols with aliphatic side chains. The 15 carbon atom side chain includes saturated, mono-olefinic, di-olefinic and tri-olefinic [57]. Cardanol, cardol, 2-methyl cardol, and anacardic acid [45], [46]. In addition, other compounds were identified in CNSL using gas chromatography-mass spectrometry, revealing the presence of cardanol (40.26%), cardol (29.95%), phytosterol (10.68%), triacontane (4.66%), and anacardic acid (1.79%) [47]. *A. occidentale* shell fluid (CNSL), a viscous and pungent oil, is one of the richest sources of non-isoprenoid phenolic lipids such as anacardic acids (71–82%), cardanols (2–9%), cardols (13–20%), and 2-methylcardols (1–4%) [58].

A study on *A. occidentale* shell fluid (CNSL) indicated that CNSL provides potent cellular antioxidant protection against hydrogen peroxide and inhibits AChE activity. These properties have been linked to chemical compounds found in the shell, such as anacardic acids, cardol, and cardanol. Therefore, CNSL may be a potential candidate for the treatment of neurodegenerative diseases [59,60].

In literature studies, differences in the essential oil contents of *A. occidentale* shell waste and liquid are noteworthy. Additionally, chemical compounds such as anacardic acids and cardanol, found in *A. occidentale* shell waste and liquid, were not observed in the oil. The potential effects of *A. occidentale* fruit on in vitro anticholinergic AChE and BChE inhibitory activity were investigated. IC_{50} values for AChE and BChE were measured as $12.7 \pm 0.5 \text{ mg/mL}$ and $1.8 \pm 0.1 \text{ mg/mL}$, respectively, while in this study, the IC_{50} values for AChE and BChE were calculated as 13.86 $\mu\text{g/mL}$ and 16.11 $\mu\text{g/mL}$, respectively. AChE inhibitory activity in fruit and oil showed similar values [58,59,61–63].

A. occidentale oil was subjected to a series of in vitro assays to evaluate its antioxidant capabilities and potential inhibitory effects on several metabolic enzymes. AChE and BuChE enzymes are linked to conditions such as AD, diabetes, and glaucoma, respectively. Furthermore, the potential active components in *A. occidentale* oil were determined using HPLC and GC/FID techniques. GC/FID analysis revealed the highest amounts of palmitic acid, stearic acid, oleic acid, and linoleic acid, while HPLC analysis revealed a high amount of curcumin.

A. occidentale is one of the most consumed foods worldwide and boasts numerous health benefits. Its proven biological activity is widely believed to protect against various diseases when included in the diet. This food surpasses many commonly consumed foods in phenolic acids. The antioxidant properties of *A. occidentale* have been investigated using *in vitro* bioanalytical analysis, yielding impressive results. The findings strongly suggest that *A. occidentale* oil may be a valuable source of essential biomolecules vital for biological functions. These findings highlight the potential of *A. occidentale* oil as an important and practical source of biologically important biomolecules, contributing significantly to overall well-being.

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