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An Investigation into the Antioxidant Effects of Frankincense Aqueous Extract and its Influence on Acetyl Cholinesterase in Alzheimer's patients in Diwaniyah

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Abstract*. The present study aims to prepare the aqueous extract of the frankincense plant and then identify the most important biologically active substances in it, and study the effect of these substances against free radicals (2,2-diphenylpicrylhydrazyl (DPPH)) and Total Antioxidant Capacity. Then the effect of extract was tested on the blood serum of Alzheimer's patients in the nursing home in Diwaniyah Governorate, where 30 people were taken. 10 of them were healthy and were considered a positive control groupG1, and 20 of them were Alzheimer's patients, where 10 of them were considered a negative control groupG2, and the other 10 Alzheimer's patients had their serum treated with the extract of frankincenseG3. The effect of the extract on malondialdehyde and acetylcholinesterase enzyme was tested. The current study showed that extract contains biologically active substances such as (Flavonoids, Phenols, Tannins Triterpenoids, Steroids and Saponins) and these substances had an effective effect in inhibiting the radical (DPPH) where the inhibition rate reached 88% at (250mg/ml) and Total Antioxidant Capacity was ($8.8*10^3$ µmol/L) at the same concentration. That results also showed the significant rise in the concentration malonaldehyde in Alzheimer's patients (G2, G3) when compared with G1. When the G3 was treated with the aqueous extract, a decrease in the concentration of malonaldehyde (3.23µml/l) was observed. The significant reduction in the Activity of the Acetylcholinesterase enzyme (3.97U/ml) was also observed in the (G3) as compared with(G2) (12.297U/ml). This is due to the biologically active substances in the extract, which indicates the possibility of using this extract to treat Alzheimer's patients in the future.*

Keywords: *Antioxidant, Alzheimer's Disease, Acetyl Cholinesterase, Frankincense.*

1. INTRODUCTION

These plants Boswellia carterii (BC) products oleoresins, often identified as Frankincense, the have long being to use in traditional and Ayurveda medicine. Plants have documented times past of use as anti-inflammatory agent in various countries. Boswellia species are trees that resemble shrubs and are found in China, India, the Middle East, and the Horn of Africa. But according to recent studies, Boswellia oleoresins and compounds generated from them may be helpful in treating a variety of illnesses. In vitro studies have established antibacterial and anti-cancer possessions of Boswellia oleoresin extracts against a variety of cancer cell types. via processes like cell cycle arrest and apoptosis. By inhibiting free radicals and reactive oxygen generated by oxidative stress, Boswellia practiced antioxidant systems .

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Figure 1. Boswell a carterii (Frankincense)

The Oxidative effects of Free Radicals and other Oxidants in biological systems can be alleviated by Antioxidants. Protein, fats, carbs, organic acids, vitamins, polyphenols, flavonoids, beta-carotene, and terpenoids are all found in Boswellia carterii. Amino acids, addition to the recognized flavonol glycosides and the terpene lactones. Both amino and acid groups are present in chemical substances referred to as amino acids (AAs) . Plant have a broad collection of secondary metabolites named flavonoids which are known as antioxidant compound Alzheimer's disease is the greatest communal type of the dementia the aging, secretarial intended for (60% to 70%) of suitcases. Lipid metabolism and the Oxidative Stress are key mechanisms in Alzheimer's disease. The exact cause of this ailment is unknown at this time; however, Acetylcholine (ACh) hydrolysis is thought to have a starring role. It can damage ACh diffusion from the presynaptic membrane and prevent neurotransmitter rousing special effects on the postsynaptic membrane that important for nerve conduction.

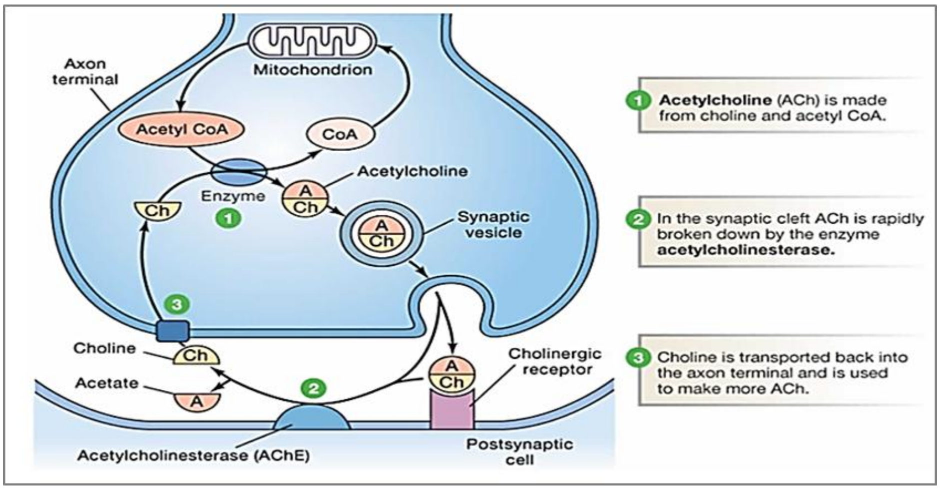

Figure 2. Acetylcholine(Ach) metabolism in cholinergic nerve terminals

Acetyl cholinesterase inhibitors (AChEIs) are a crucial treatment option for Alzheimer's disease because they can postpone the breakdown of acetylcholine (ACh). Because of its multifaceted etiology, the alzheimer's disease has proven difficult in remedy. Different antagonists and inhibitors of important proteins and enzymes involved in the diseases etiology have been investigated as impending treatment medicines. It has the potential to enhance therapeutic efficacy in a synergistic manner. It has the potential to improve the therapeutic effectiveness of single-target medicines by working synergistically. Alternatively, it can alleviate side effects.

2. MATERIALS AND METHODS

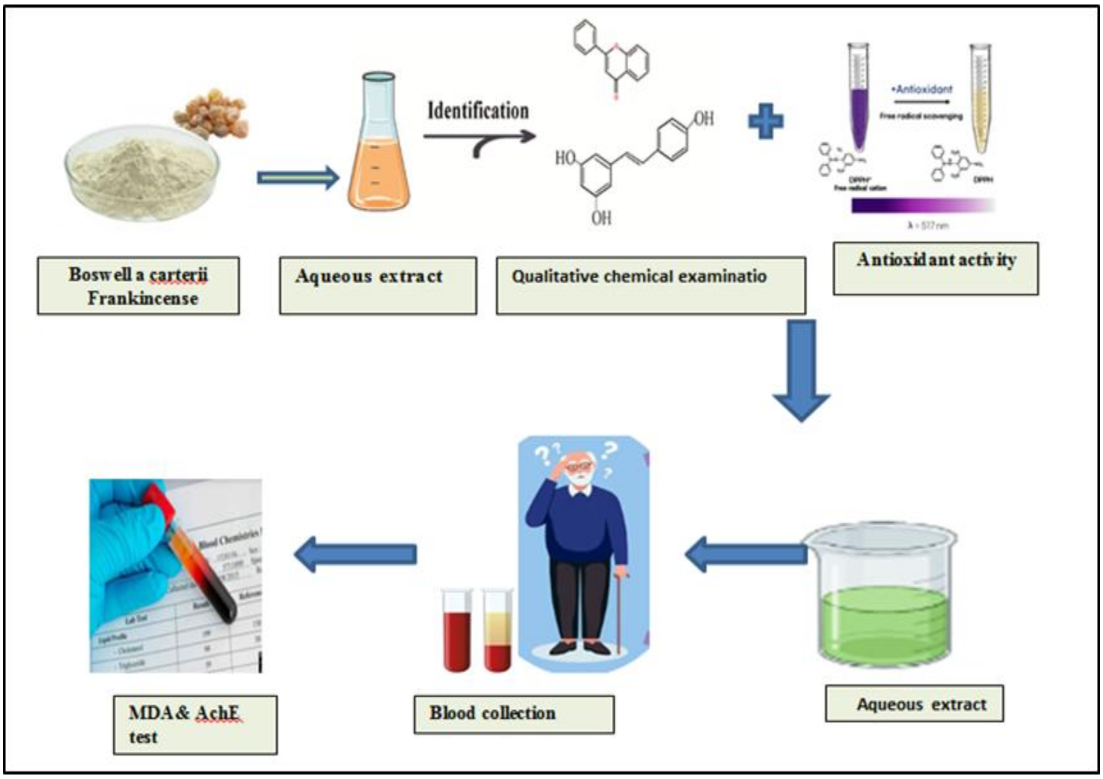

Figure 3. Experimental Design

Frankincense was obtained from herbalist's shops in Diwaniyah market in an amount of 1 kg, then it was purified from impurities, then ground and the necessary extraction and tests were carried out on it.

- Cold aqueous extraction:

The cold aqueous extract of frankincense was prepared using the (Harborne, .1973) method.

- Qualitative chemical examination of the extract:

The solution of sample extract was made by dissolving (2 g) of extract in 20 mL of 96% ethanol. Then the active compounds were identified such as Flavonoids, Phenols and Tannins, Triterpenoids, Saponins and Steroids

DPPH Method

This experiment uses a spectrophotometer to determine the effectiveness of Antioxidants to decrease(DPPH) (2,2-diphenylpicrylhydrazyl), another radical aren't typically seen in the living systems at 517 nm. Five concentrations of the aqueous extract of the Frankincense (250,200,150,100,50mg/ml) were prepared, and the inhibitory activity of these concentrations was measured and compared with the activity of vitamin C, which is the strongest reference for antioxidants.

Total Antioxidant Capacity

Electron transfer and hydrogen atom transfer are two tests commonly employed to assess antioxidant activity. The CUPRAC (Cupric Reducing Antioxidant Capacity) approach is the chromogenic oxidant test for vitamins C and E that employs the Cu(II)-neocuproine (Cu(II)-Nc) reagent. Then 30 minutes, the measured absorbance at 450 nm in mixture of the antioxidant solution (whether undiluted or after acid hydrolysis) and solutions of $CuCl_2$, neocuproine (Nc), and ammonium acetate at pH 7.

Collected of Blood Samples and Experimental Design.

Blood samples were collected from patients (20) with Alzheimer's disease who were in Diwaniyah a nursing home, according to the evaluation of two neurologists. they were divided them into two groups 10 of them serving as negative control groups G2. As for the other 10 they were considered a third group, their serum samples were treated with frankincense extract G3. Samples were also taken from healthy 10 persons of the same age group and in the same place and were considered a control group G1.

Determination of Malondialdehyde (MDA) concentration in serum

The spectrophotometric measurement of the color that took place during the reaction of MDA with Thio Barbituric Acid (TBA) in 540 nm served as basis for the principle of this technique, which is explained by Guidet and Shah (1989).

Determination the Activity of Acetylcholine Esterase (AchE) in the serum

By applying the (MHO) Modifier Method. At a wavelength (412 nm) Absorbance was read before and after the base material was applied for all 3 minutes of enzyme reaction with the base substantial.

3. RESULTS AND DISCUSSION

The Yield of Extraction and Phytochemical Screening Test.

The yield of extraction was determined by calculating the proportion of pure extract Frankincense to crude Frankincense, the percentage was for the aqueous extract (23.5 %). Numerous active compounds with hydroxyl groups, carbonyl groups, and double bonds were found in the plant extract, according to analysis. These compounds are acknowledged to have a multiplicity of biological activities, comprising antimicrobial, antioxidant and anti-inflammatory, properties, in addition to their chemo preventive significance. The chemicals' biological activity provides evidence the therapeutic abilities of the extract under investigation show (Table 1).

Table 1. Phytochemical screening test results about Frankincense aqueous extract

No.	Phytochemical Tested	Results	Description
1	Flavonoids	+	Formed red color
2	Phenols and Tannins	+	Formed blue-black color
3	Triterpenoids and Steroids	+	Formed Purple-brown color
4	Saponins	+	There is a little forth

Evaluation of the antioxidant activity of Frankincense extract

Based on the ability to inhibit the DPPH radical The antioxidant activity was evaluated, as the characteristic violet color of this radical changes to yellow, the results showed that highest inhibition rate (88%) was at the maximum in concentration of 250 mg/ml. The changes in the inhibition percentage of the DPPH radical for the concentrations of extract are shown in the figure (4).

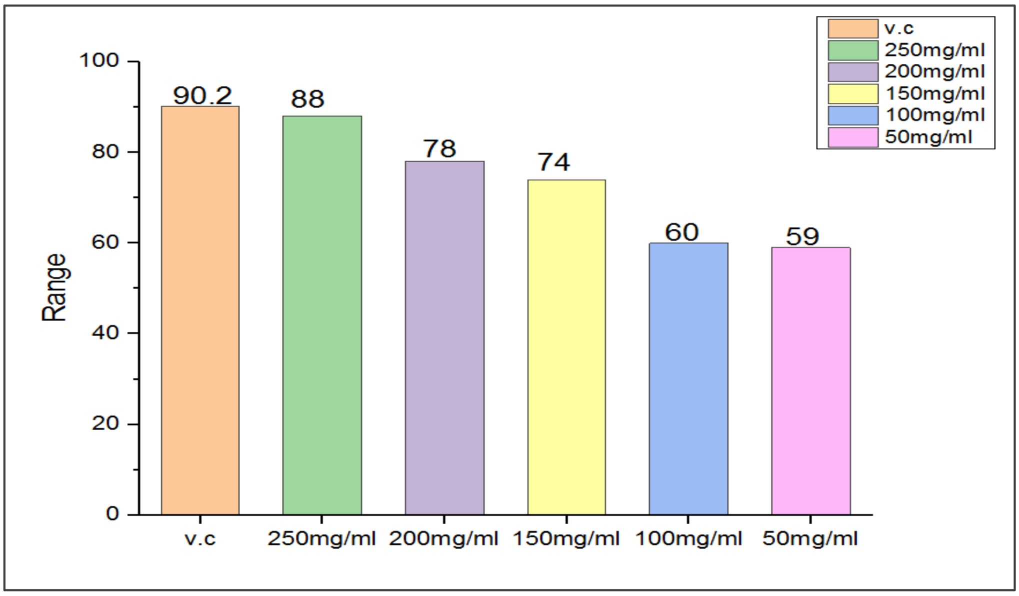

Figure 4. The percentage of inhibition the DPPH radical by Frankincense extract

The reason for the appearance of these results might be due to the attendance of a high percentage of effective phytochemical compounds which act as free radical scavengers in the aqueous extract. The most prominent of these compounds are phenols and flavonoids which can action as Antioxidants and have the strong Antioxidant Activity, these compounds work to quench the DPPH radical. Free radicals oxidize flavonoids to produce less effective and more stable flavonoid radicals. This is because it interacts with free radicals since it contains hydroxyl groups, which give the free radical an electron to make it more stable.

Evaluation of Total Antioxidant Capacity of Frankincense extract

Free radical scavengers' is another name for antioxidants. Frankincense extract exhibits antioxidant activities as it has the capacity to scavenge free radicals (Table 2).

Table 2. Total Antioxidant Capacity of Frankincense

No.	Concentration	Total antioxidant capacity µmol/L
1	250mg/ml	$8.8*10^3$
2	200mg/ml	$8.62*10^3$
3	150mg/ml	$7.92*10^3$
4	100mg/ml	$7.00*10^3$
5	50mg/ml	$6.90*10^3$

Because of these characteristics, frankincense is useful in the treatment of many oxidative stress-related illnesses. There is a relationship between the presence of the phytochemical compounds and the Antioxidant Activity of Frankincense. Phenolic substances, such as flavonoids and tannins, are always assumed to have important antioxidant capacity due of their abundance of hydroxyl groups(OH)., which are effective hydrogen givers and can quickly oxidize ROS. Flavonoids and tannins are important free radical scavengers that stop the production of additional ROS in a termination reaction Additionally, it has been suggested that phenolic compounds, which scavenge free radicals and inhibit ROS, contribute to antioxidant action. The composition of the extracting solvent, the different biochemical properties, and the polarity of dissimilar antioxidant substances that may or may not dissolve in specific solvent all have a significant impact on the total amount of antioxidant substances that can be successfully extracted from their source. The results of the current study showed this, using water as a polar solvent to dissolve as many polar antioxidant molecules as possible, including flavonoids, phenols, and other chemicals.

Evaluation of the Malonyldialehyde in blood of Alzheimer's patients

Elevated the MDA heights are observed in the serum of Alzheimer's patients G2. The plasma MDA concentrations were higher in the AD group than in the control groupG1 Also, a clear improvement was observed in the G3, which was treated with the aqueous extract. show (figure 5).

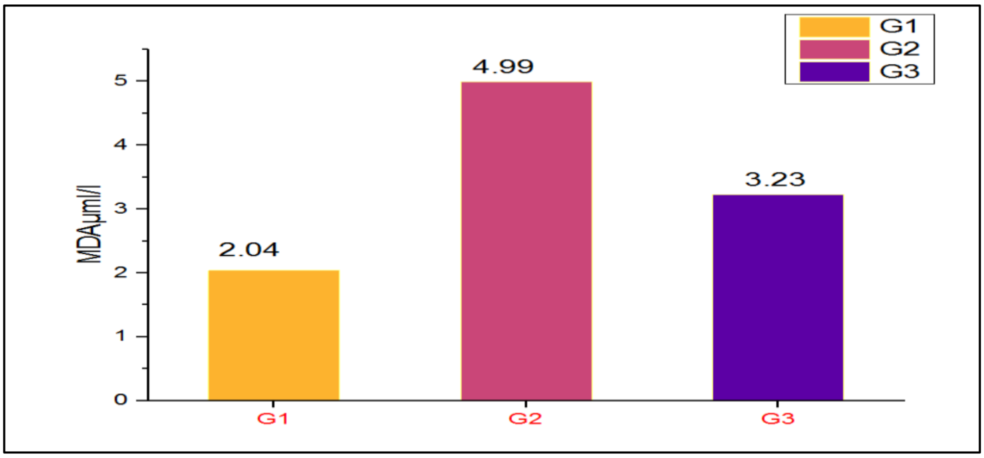

Figure 5. Malonyldialehyde concentration in the blood of Alzheimer's patients

Increased quantities of Oxidants relative to Antioxidant protection mechanisms causes an oxidation of Phospholipids, Proteins, and Nucleic acids such as DNA, which leads to the dysfunction that ends in the death of these cells. Malonyldialehyde concentration is pivotal indicator of increase in Free radicals that the incidence of the lipid peroxidation process, this causes an increase in the changeability of the cell membrane. The hydroxyl radical($OH^{\cdot}$) it's among the radicals that contribute to the breakdown of the Cell membranes. This explains main reason behind the high levels of MDA in the second group in which oxidative stress was stimulated by aging and Alzheimer. Oxidative stress damage is widely present in the AD patients and Malondialdehyde (MDA) is generally present in the brain tissue. That result is consistent with study. on AD patients that showed raised on the Oxidative Stress, and there are complex communications between lipid metabolites and Oxidative Stress.

Evaluation of Acetylcholine Esterase (AchE) Activity in the Blood of Alzheimer's patients

Figure (6) its shows the significant ($P \leq 0.05$) increase in Acetyl Choline Esterase enzyme activity in the blood of Alzheimer's patients group (G2) in comparison with the control group (G1).

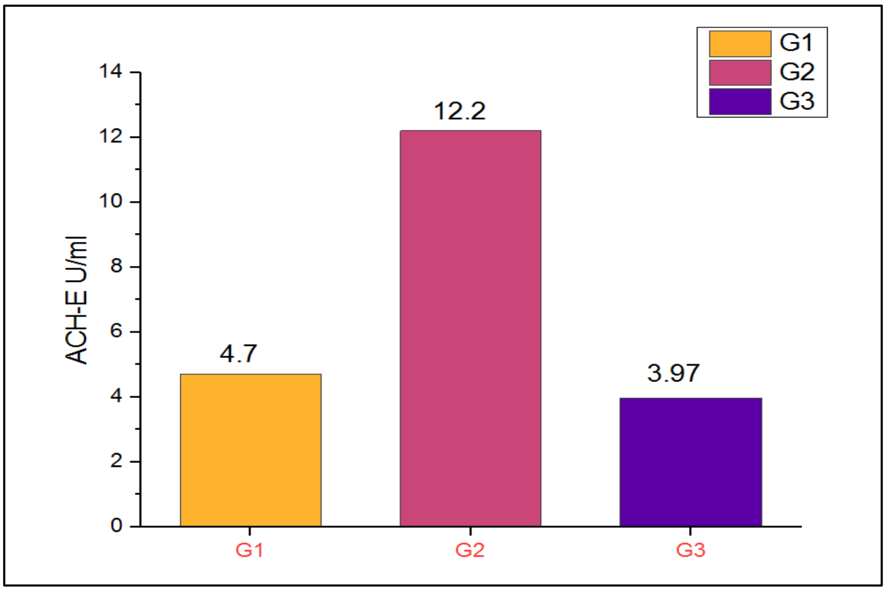

Figure 6. activity of the acetylcholinesterase enzyme

AchE's primary purpose is to reduce Ach dispersion and activation of neighboring receptors; hence, it inhibits the neuronal transmission and synaptic connection. It's furthermore crucial in the brain growth; blocking the AChE reduces ACh fritter, allowing ACh to accumulate. This collecting ACh activates muscarinic and nicotinic receptor, providing some therapeutic relief for memory difficulties in the brain diseases. Amyloid-beta Aβ plaques and oxidative stress have been related to elevated levels of AChE, that can lead to reduced memory deficits and cholinergic transmission in Alzheimer's disease patients due to diminished ACh signaling. current study showed that AD patients' blood levels have been found to be greater in AChE. AChE inhibition has the ability to enhance levels of the ACh, neurotransmitter generated by the cholinergic neurons, that has been shown to improve cognition, learning, memory, and inflammation suppression. Several FDA-approved medications try to inhibit AChE.

The presence of polyphenols, flavonoids, sterols, and alkaloids in group 2 may be connected to the frankincense extract's ability to suppress acetylcholinesterase activity. These substances have been demonstrated to have anti-acetylcholinesterase qualities, according to recent reports.Thus, the mechanism of inhibition can be proposed through the forming of hydrogen bonding between active compounds in the aqueous extract of the Frankincense(such as phenols) and the proton of the hydroxyl group(OH) of the amino acid serine present in active site of enzyme, that leads to inhibition of enzyme because part of the active site is occupied with binding with the active composites in the extract and not with acidic group of tyrosine.

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