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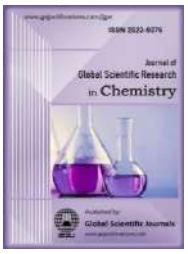

Spectroscopic Methods for Determination of Acetylcholine in Sagebrush Plant

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ABSTRACT

The research includes separating the active compound from the leaves and stems of sagebrush by analytical methods, laying and extraction with solvent, then diagnosing the resulting solution by spectroscopic methods. The aim of the study is to ascertain the presence of the active substance acetylcholine as a chemical compound in the sage plant and to prove it chemically.

1. Introduction

Acetylcholine compound (ACh) figure (1): The name acetylcholine is derived from its structure. Acetylcholine was the first neurotransmitter to be identified (1). It was discovered by Henry Hallett Dale in 1914, and its existence was later confirmed by Otto Loewi. Both were awarded the Nobel Prize in Physiology or Medicine in 1936 for their discovery .It is a chemical compound made up of acetic acid and choline The chemical name according to IUPAC Name is 2-acetyloxyethyl(trimethyl)azanium;chloride , the Molecular Formula: C7H16NO2+, Molecular Weight: 146.21g/mol (2), soluble in organic solvents like ethanol,ether.acetone ..etc. ,but its salts dissolved in distilled water .Spectral determination Information : GC-MS(3) , FTIR Spectra, ATR-IR Spectra , Raman Spectra(4,5), Gas chromatography (6), matrix-assisted laser desorption ionization time-of-flight mass spectrometry(7), g Liquid Chromatography–Tandem Mass Spectrometry (8), Electrochemical(Analysis (9), Ion-Pair Liquid Chromatography with Amperometric Detection (10), Ultraviolet spectroscopic (11), NMR (12), Vibrational spectroscopy analysis (13),Flow injection analysis (14).

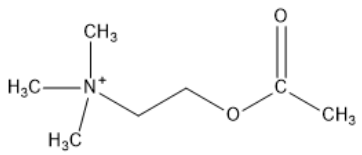

Figure 1: Chemical structure of Acetylcholine compound

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Sagebrush plant : Figure (2) the common sage is Salvia officinalis , Scientific name is Artemisia tridentate ,Artemisia tridentata Sagebrush species had several different names, sage, wormwood .Five species classified as herbaceous, these species are Fringed sage, Fuzzy sage, Birdfoot sage, Porter's sage, and Bud sagebrush (Artemisia frigida, A. papposa, A. pedatifida, A. porteri, and A. Spinescens)(15),Application Analyses on this plant are : near-infrared reflectance spectroscopy (16),paper chromatography (17) , UV-light fluorescence (18), chemical treatments :physical :chemical parameters and staticalanalyses (19), chemical perspiration analysis (20) , Eadie-Hofstee method (21) , Pattern analysis(22), hydraulic redistribution for soil (23),Biological activites (24) , MSD ChemStation (25)…etc.

Figure.2: The real photo for sagebrush plant sample.

it is a shrub or small tree from the family Asteraceae. It is a coarse, hardy silvery-grey bush with inconspicuous yellow-green flowers and grows in arid sections of the western United States and Western Canada. It is the primary vegetation across vast areas of the Great Basin desert and is an indicator species for high desert (above 1,500 ft.). Like others in this genus, it has highly aromatic foliage (26, 27,28).Sagebrush plant consist of 16% proteins, 15% fats, and 47% carbohydrates. Sage suppresses the enzyme responsible for breaking down acetylcholine in the brain, which causes Alzheimer's. Sage also contains antioxidants, and one of the most important important compounds in sage is the volatile oil that contains the compound Thujone, this plant composed largely of camphor (20%-45%), camphene (3%-21%), 1,8-cineole (12%-30%), thujone (6%), and borneol (5%). Sesquiterpene lactones are major components of the nonvolatile phytochemicals found in it (29,30).In this study of this scientific research on the natural sage plant available in the Iraqi markets, using chemical treatment and some spectroscopic methods FT-IR and using RP-HPLC technology.

2. Reagent and Materials

Experimental

The sage plant was purchased from the perfume shop in the Iraqi markets in Baghdad, Iraq .weighted 1 g from plant sample and dissolves it in

100 ml acetyl acetate, Leave the front solution for the whole night., 48 hours, a week, then make an extraction using the Soxhlet device and extract the sample six times at room temperature and each time analyze the extracts used FT-IR to obtained six spectrum and using RP-HPLC technique for the good extract.

RP-HPLC Analysis

HPLC model SYKAMN (Germany) It was used to analyses add detection of acetyl choline .The mobile phase was 50mM ammonium formate in 2% methanol water containing 5mM Sodium dodecyl sulfate (PH = 8.2) (A) and methanol with 0.01% ammonium hydroxide (B) flow rate at 1.2ml/min ,Column was C18 –ODS and the detector UV-Vis at 247nm (31).

3. Results

Analytical testing sample screens, profiles and data interpretation are available on FTIR to identify chemical compounds in extract products. FTIR give quantitative and qualitative analysis for organic and inorganic samples. Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The spectra produce peaks of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components. FTIR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information. The work plan is to conduct the placement process for periods, A sample of the sage plant sample solution dissolved in acetate after leaving it for 48 hours Figure (3).

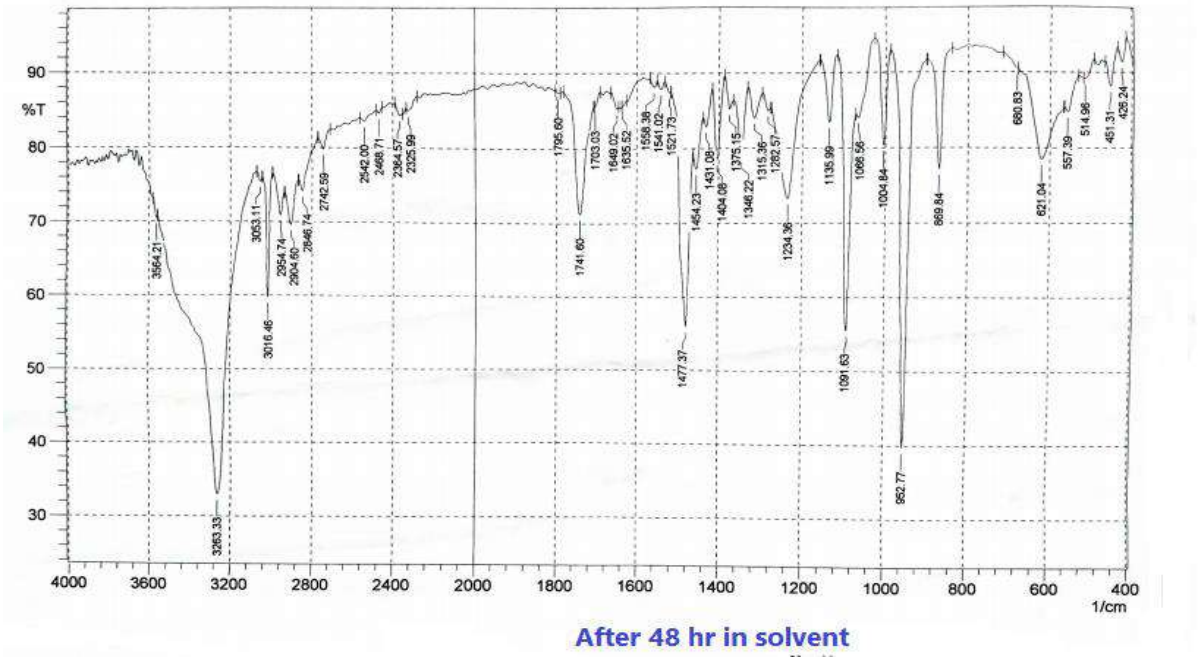

Figure .3 : FTIR spectrum of Saga plant solution after 48 hr.

The plant solution, after leaving it for 72 hours, is dissolved in acetyl acetate, Figure (4) .

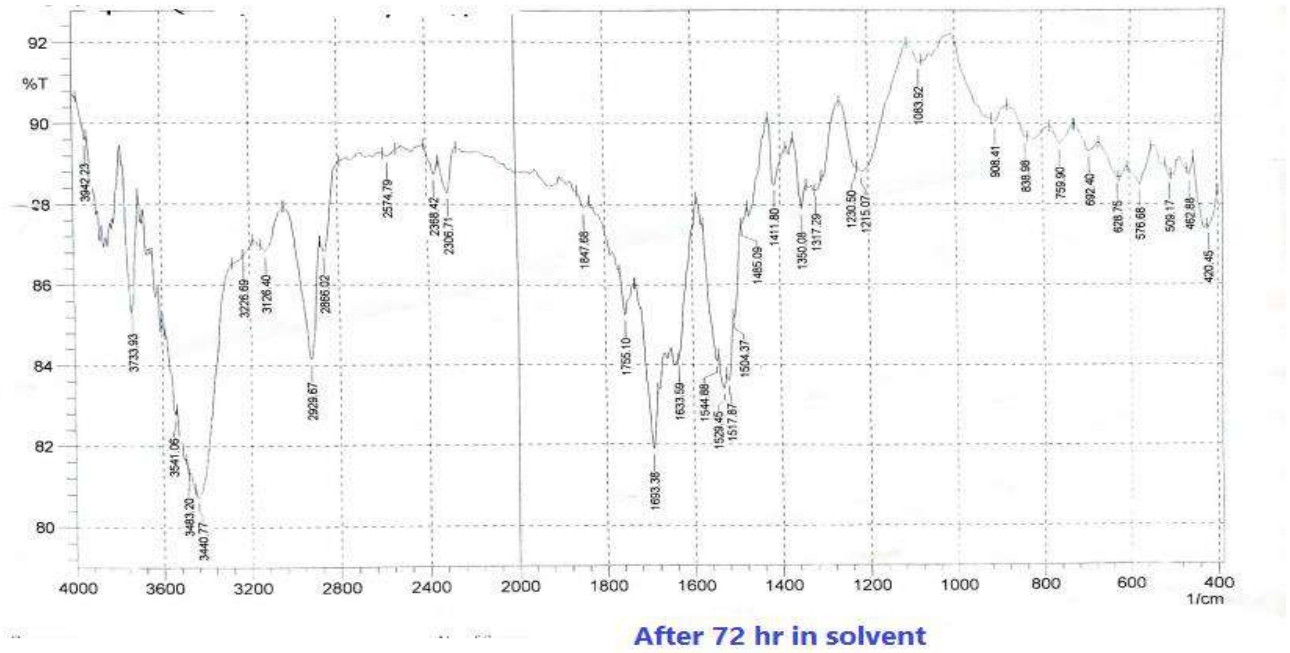

Figure .4 : FTIR spectrum of Saga plant solution after 72 hr .

The solution of the plant after leaving it for a week is dissolved in acetyl acetate solvent, Figure (5) .

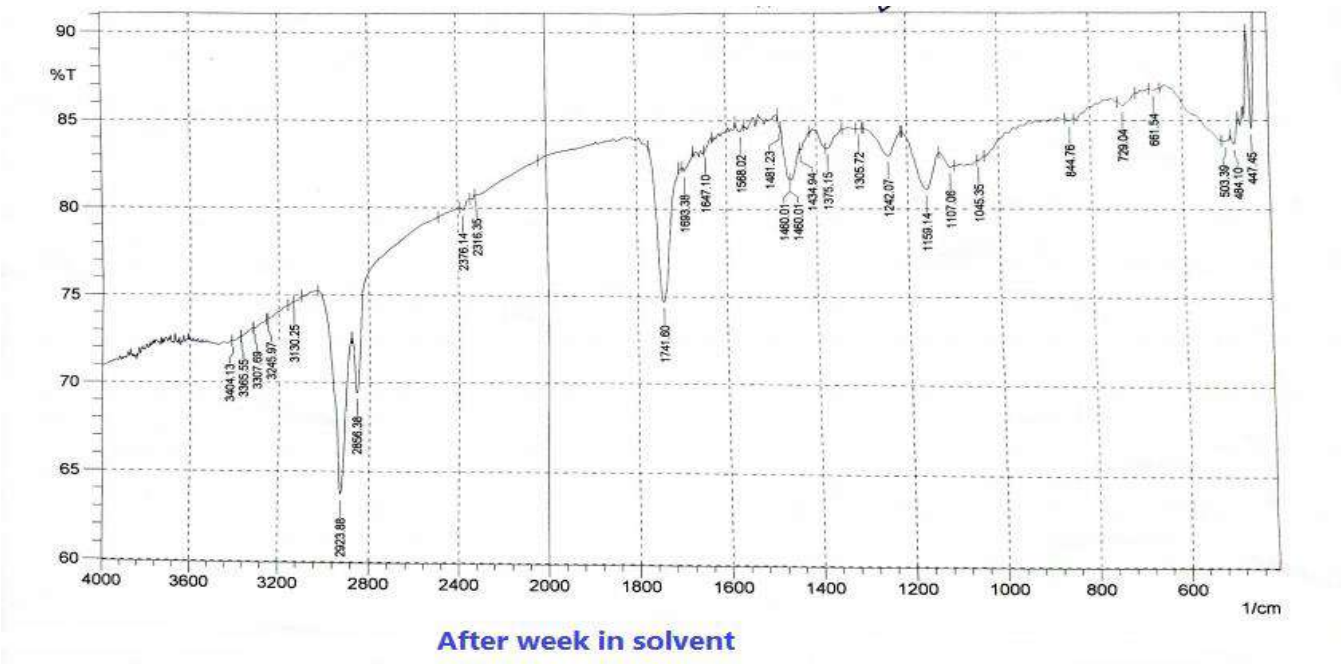

Figure .5: FTIR spectrum of Saga plant solution after week .

The solution was taken for a week, filtered, and the resulting solution was taken to the extraction step by means of the Soxhlet device for the purpose of extracting it several times, Figures (6,7,8,9,10,11) explain It

shows that the solution was extracted and the number of extraction times was 6, and each time extraction we measured the extracted substance by FT-IR .

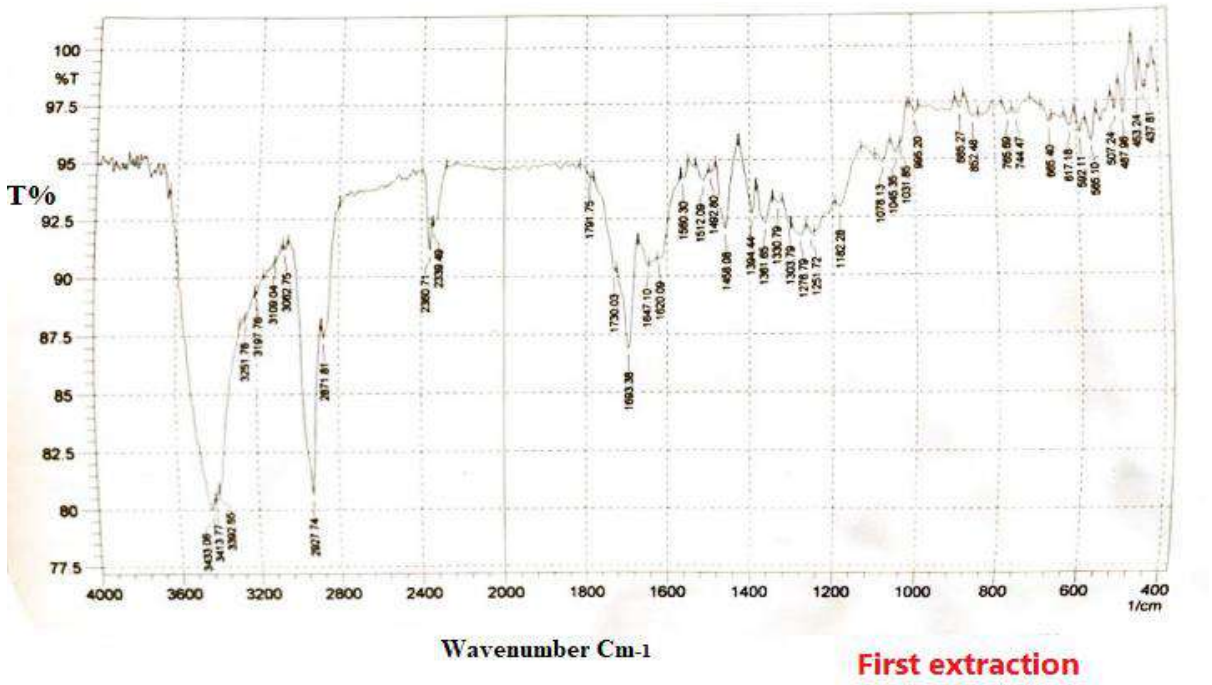

Figure .6: FTIR spectrum of Saga plant solution after week , The first extraction process by Soxhlet device.

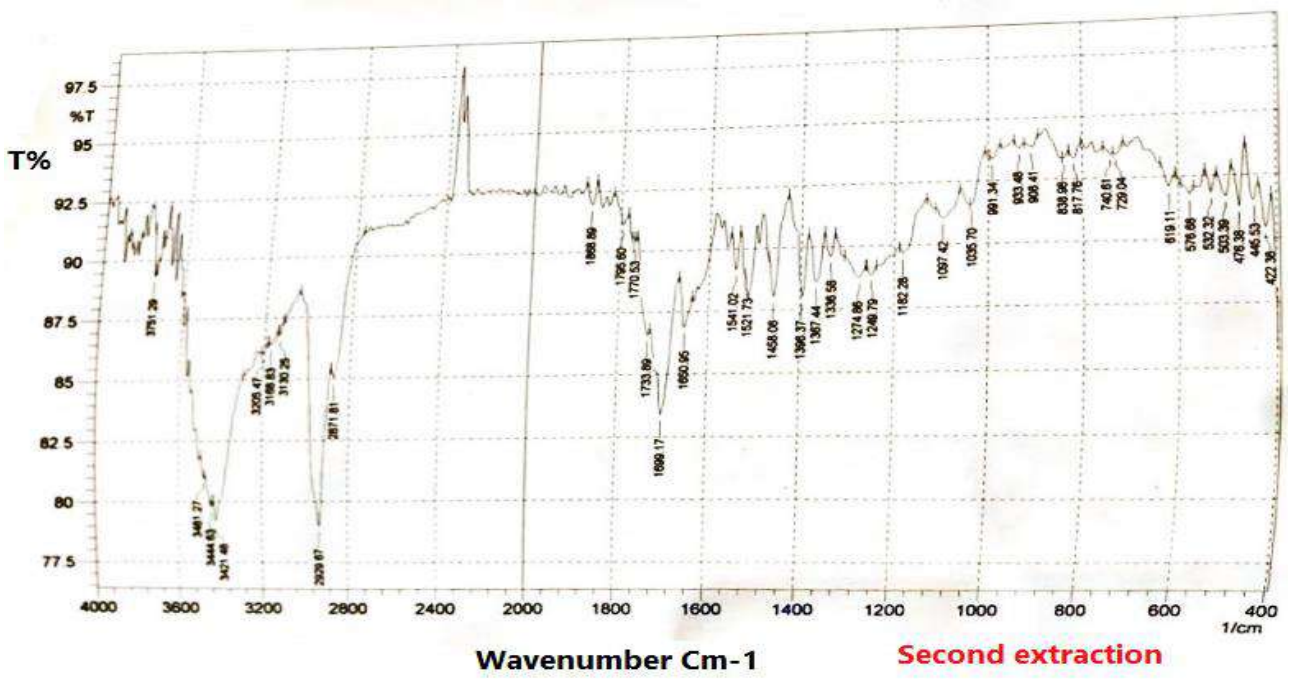

Figure 7 : The second extraction process by Soxhlet device.

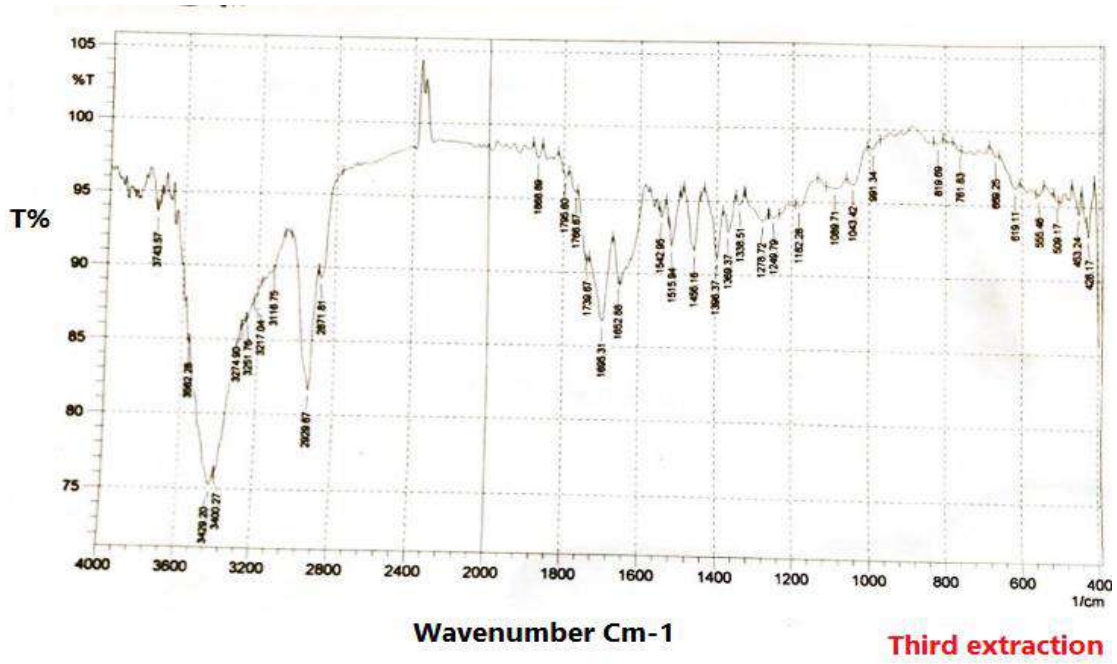

Figure 8 : The third extraction process by Soxhlet device.

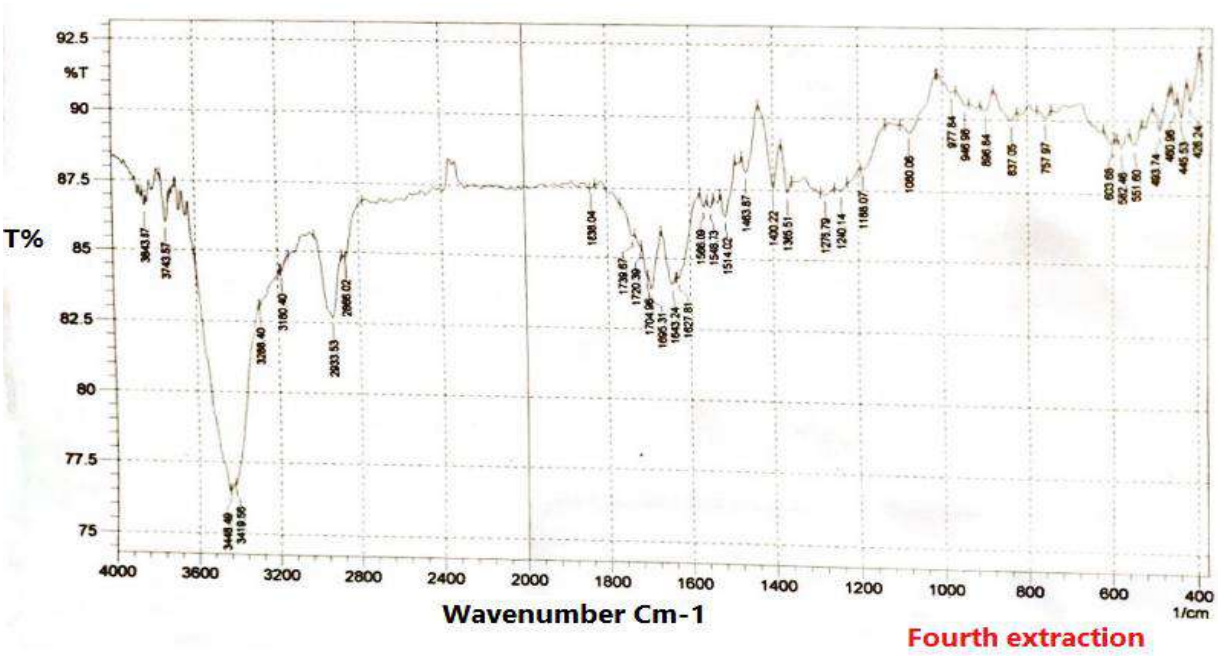

Figure 9 : The fourth extraction process by Soxhlet device.

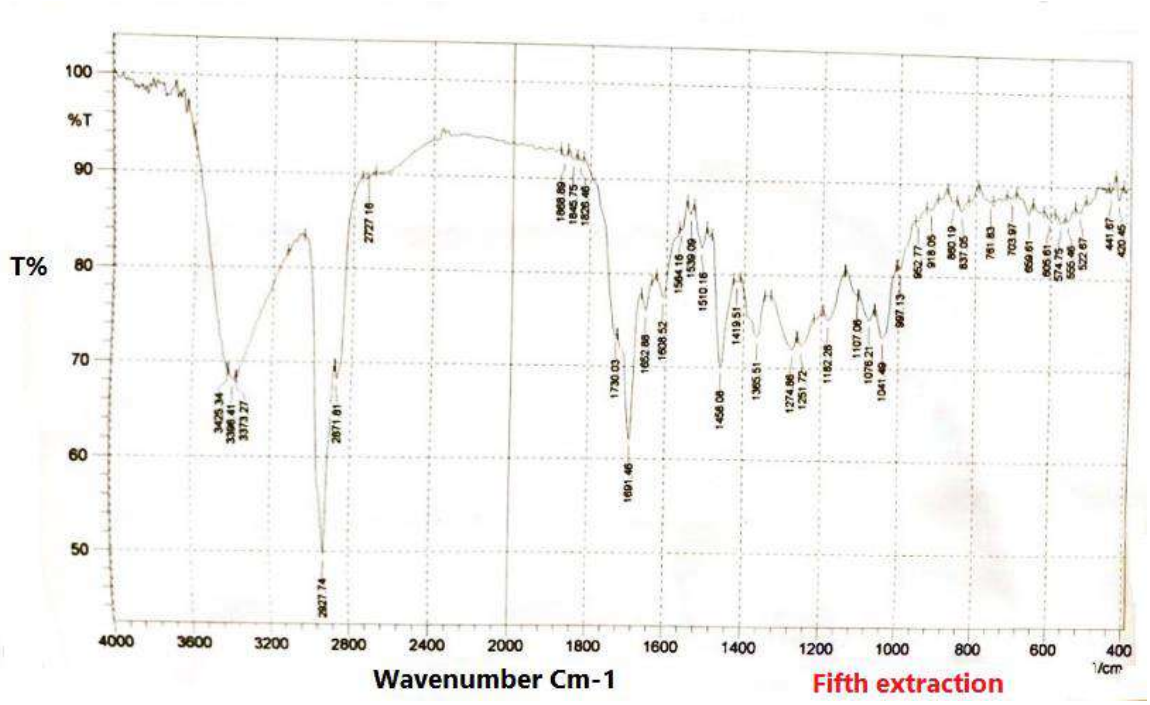

Figure 10 : The Fifth extraction process by Soxhlet device.

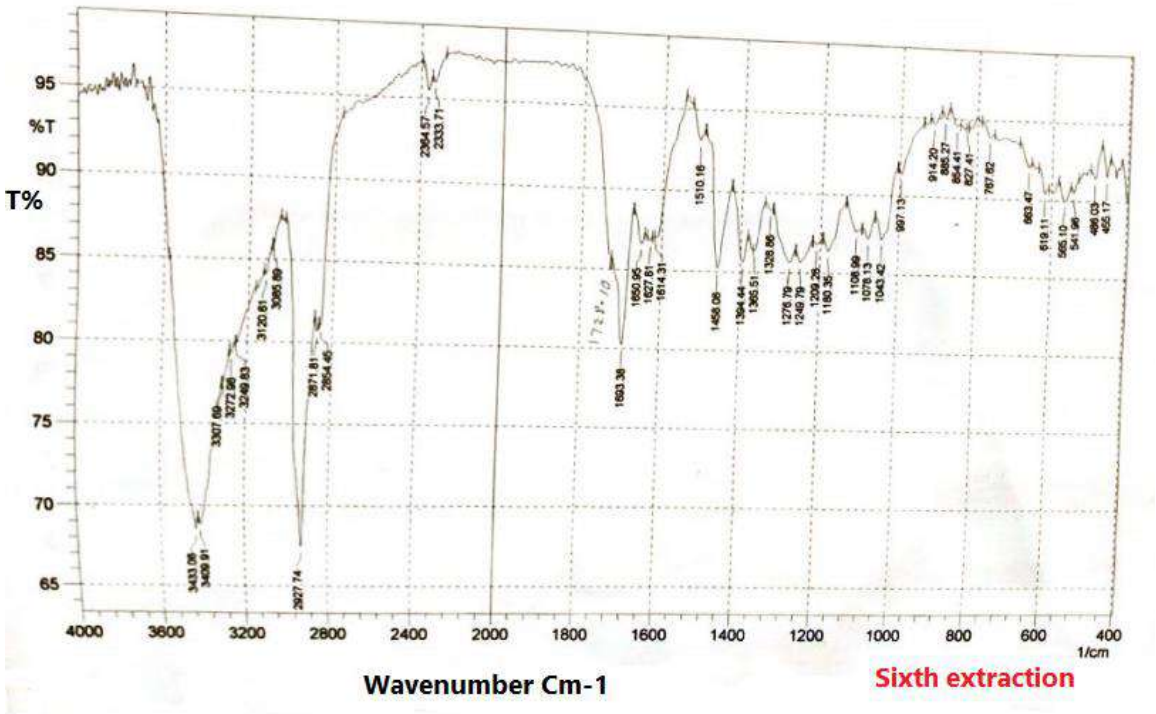

Figure 11 : The six extraction process by Soxhlet device.

After the step of layering and taking the product of the solution and extraction six times for this resulting solution, it was found through the spectrum taken for each time of the extraction that the acetylcholine compound does not appear and only functional groups appear for each of what is present in the extracted plant solution.

And to cut off doubts with certainty and make sure whether there is acetylcholine in the plant solution or not? ,The extracted material was taken in the Soxhlet device to be examined by the high-performance liquid chromatography technique .the conditions of method : The UV visible absorption spectrum of standard Acetylcholine solution in mobile phases of ammonium formate (2% v/v/v) methanol and 5mM Sodium dodecyl sulfate (PH = 8.2) (A) and methanol with 0.01% ammonium hydroxide (B) flow rate at 1.2ml/min ,Column was C18 –ODS and the detector UV-Vis recorded wavelength at 247nm . A sample of the pure standard of the compound acetylcholine was taken and examined in a technique RP- high-performance liquid chromatography , obtained chromatograms 12 and the results are shown in Table 1.

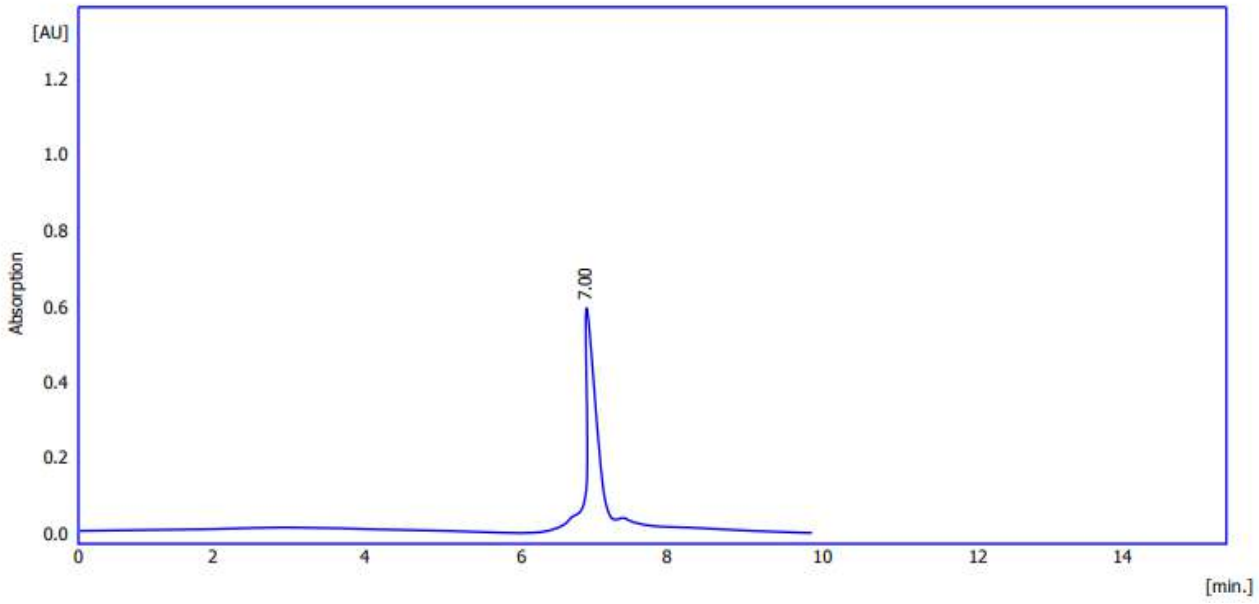

Figure 12. Chromatogram of standard pure Acetylcholine

Table 1 Separation result of standard Acetylcholine

Compound	Reten.Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	WOS [min]
Acetylcholine	7.00	250.49	714.55	100.00	100.00	0.30
	Total	250.49	714.55	100.00	100.00	

In order to resolve the issue, a reversed phase high-performance chromatography test was conducted for the extracts solution, and table 2 shows the obtained results, which shows the absence of acetylcholine in the sagebrush plant.

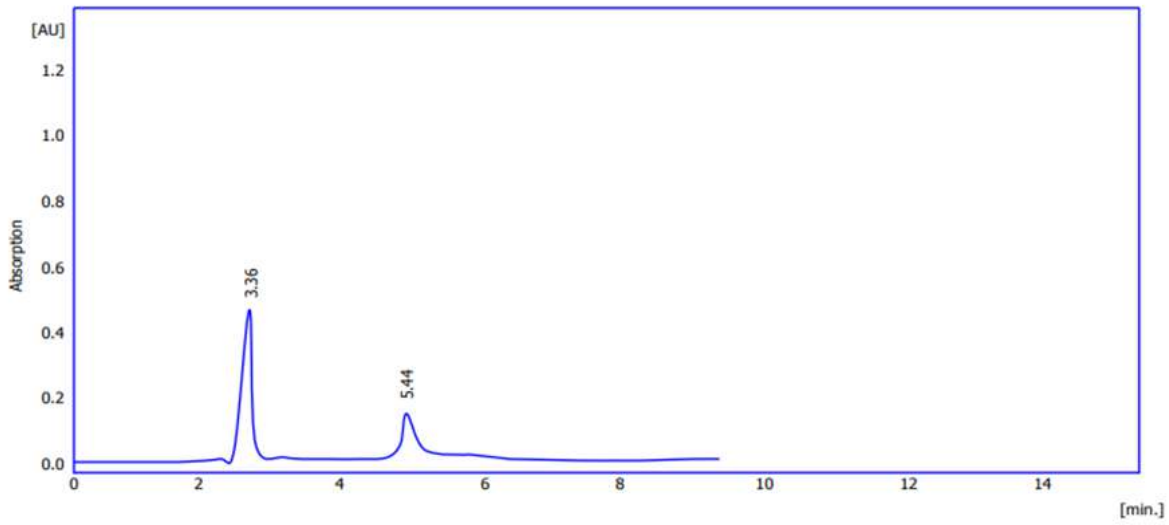

Figure 13. Chromatogram of sample solution.

Table 2: Separation result of Extract saga plant solution

Compound	Reten.Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	WOS [min]
Extract Saga solution	3.36	2458.25	688.58	80.00	80.00	0.35
	5.44	125.89	66.58	20.00	20.00	0.19
	total	2584.15	755.16	100.00	100.00	

4. Conclusion

Through this chemical study using spectroscopic methods are Fourier transform infrared spectroscopy (FTIR) and Reverse phase high performance liquid chromatography. It was found chemically that there is no acetylcholine in the sage plant.

5. Acknowledgments

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