<u>Original Article</u>

# The Effect of Active Compounds and Trace Elements Extracted from Artemisia Fruit on Some Liver Enzymes in Humans

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#### Abstract

Artemisia is a perennial wild shrub with large branches and compound leaves. Artemisia contains about 400 types, and its medical importance is due to the presence of many active substances and compounds such as volatile oils, alkaloids and flavonoids, glycosides, saponins, tannins, and coumarins. This study was designed to study the effect of the aqueous extract of the fruit of the Artemisia plant on the organs of the body, as well as to know its ability to activate the hepatic enzyme alanine transaminase (ALT/GPT). The fruit of this shrub was extracted using the measurement technique gas chromatography-mass spectrometry (GC/MASS) and organic solvent hexane and ethyl acetate in one to one ratio. It contained 21 compounds, a high percentage of their terpenes, essential aromatic oils, alkaloids, and phenolic compounds. The results showed a significant improvement in the enzyme (ALT/GPT) level after adding different concentrations of hot aqueous extract to the fruit of the Artemisia plant. The fruit of the Artemisia plant can be used to treat many diseases and improve the activity of liver enzymes.

Keywords: Artemisia, Alanine transaminase (ALT/GPT), Trace elements

# **1. Introduction**

I Artemisia is the most important genera of the wellknown compound family (Asteraceae), an evergreen shrub growing slate, reaching a height of 30 to 50 cm. Inflorescences are racemose, small, seated, ovalshaped, yellow, many-angled, and glossy (1). The leaves are small, alternately pinnate, and often compound. It is gray in color, small in size, and elongated with a narrow longitudinal slit (2). It is classified into: subclass Asteridae, order Asterales, family Asteraceae, and subfamily Asteroideae Family Anthemideae, subfamily Artemisiinae, genus Artemisia- L (3). This plant is used in traditional folk medicine because it has several therapeutic properties; several types grow in the desert plains in many countries. Artemisia fruit taken from the western desert region of Iraq has been used, prompting researchers to test various extracts from this plant to treat physiological disorders and detect the active substances to which this activity is attributed (4). It has been used as a treatment for many diseases since the oldest years, where its fruit is used in treating febrile illnesses and soaked to relieve diabetes and expel worms (5).

It is also burned to purify homes of unpleasant odors and expel vermin. It is used as a wash for eyes infected with ash and antiseptics against various germs and fungi. Its powder is also used to treat skin diseases, wounds, and burns, improve the human body's immunity, and treat respiratory diseases (6). The current study aims to find a treatment using the aqueous extract of the fruit of the Artemisia plant, which does not leave side effects on the sick person, and an alternative to treating chemical compounds and knowing the enzymatic activity of the aqueous extract of the fruit of Artemisia plant (7).

Therefore, this study was designed to study the effect of the aqueous extract of the fruit of the Artemisia plant on the organs of the body, as well as to know its ability to activate the hepatic enzyme Alanine Transaminase (ALT/GPT).

#### 2. Materials and Methods

# 2.1. Prepare Artemisia Plant

Artemisia plant is cut, collected, and dried in the shade at room temperature (30-25 C) for a week, taking into account the continuous daily monitoring to prevent the occurrence of rotting, and then the fruit is taken and ground by an electric grinder and kept in glass containers away from light, heat, and moisture until use.

#### **2.2. Prepare the Extract**

The aqueous extract was prepared by dissolving 50 g of Artemisia fruit powder in two volumetric flasks, each of one liter, and adding 500 ml of sterile distilled water for dissolution. The first flask, the cold aqueous solution, left it. The second flask is the hot solution, which was taken and placed in the shaking incubator at a temperature of 50 °C for 48 hours. After that, both extracts were filtered using filter paper (Wattman1). The filter was centrifuged for 6 minutes at 4000 rpm.

# 2.3. Estimation of pH

The aqueous extract prepared in the aforementioned step 2 was taken to measure the pH. A pH meter was used to determine the aqueous extract's pH value.

# **2.4.** Estimation of the Quality and Percentage of the Elements

3 gm of dried Artemisia leaf powder was taken and placed in a glass beaker, 8 ml of nitric acid and 2 ml of hydrochloric acid at a concentration of 60% were added and left until the next day after covering it with an hour bottle, then the mixture was placed in a sand bath at a temperature of 80  $^{\circ}$ C for about 6 hours Until the

ingested substance turns white. Then the volume is completed to 50 ml with distilled water free of ions, where the elements (Zn, Ni, Cu, Cd, Cl, Pb) were estimated by a flameless atomic absorption device (8).

# **2.5. Chemical Detection**

The following chemical detections were made for active chemical components of Artemisia fruit powder Molish test, Benedict, and Iodine test to detect the presence of multiple glycosides. Detection of ninhydrin for amino acids. Biuret test to detect proteins and aqueous ferric chloride (FeCl<sub>3</sub>) to detect phenols (9). A turbidity test was used to detect resins using ethyl alcohol at a concentration of 95% and ethanol alcohol with potassium hydroxide at 50% to detect flavonoids. Marquise detection (prepared by adding 40% formaldehyde to 10 ml of concentrated sulfuric acid) and picric acid to detect alkaloids. The vigorous shaking method was used to detect soaps. Finally, the extract is boiled, left to cool, and lead acetate is added to it at 1% to detect tannins (10).

# **2.6. Determination of Serum (ALT/GPT) after** Addition of the Extract of the Fruit of Artemisia

Prepare the stock solution for extract at a concentration (100 mg/ml) and from this solution, a series of solutions for aqueous extract of cold and hot Artemisia fruit concentrations( 0.1, 0.01, 1, 10, and 100 mg/ml). All these solutions are kept in the refrigerator until use search experiences.

Blood samples were collected from 30 patients (who suffered from a disorder in liver enzymes), and the mean age of patients (was 45-68) years. Blood samples (5ml) were collected from all subjects by venipuncture and left for 45 minutes for clotting, centrifuged to get the serum refrigerated until you start working. One ml of the aqueous extract of cold and hot Artemisia fruit powder in different concentrations was added to the serum of patients. UV-assay measured alanine transaminase (ALT/ GPT) enzyme according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) without pyridoxal phosphate activation (11).

# 3. Results and Discussion

In tables 1 and 2, laboratory studies of the chemical detections of the active compounds contained in the aqueous (cold and hot) extracts of Artemisia plant powder proved that it contains the active compounds glycosides. Where Molish's detection showed the appearance of a

purple ring in the solution indicating the presence of sugars, as well as the appearance of the light blue color in Benedict's detection showed a positive indication of the presence of reducing sugars in a large amount for the hot aqueous extract than in the cold aqueous extract through the formed orange precipitate (12).

Table 1. Chemical detection of active compounds for cold aqueous extract of Artemisia fruit

Active chemicals	Test Name	Detection product	Result
Clusseides	Iodine test	A dark blue color	+ve
Glycosides	Molish test	Violet ring	+ve
	Benedict test	A brown precipitate	+ve
Proteins	Biuret, Reagent	No purple color. The color did not change	-ve
Phenolic Compound	Aqueous, ferric chloride, FeCl <sub>3</sub> (1%)	A green precipitate	+ve
Tannins	Lead Acetate (1%)	A light yellow precipitate	+ve
Resins	Ethanol+Boiling (DW)	Turbidity in a small amount	+ve
Flavonoids	EtOH+KOH 50% 50%	A yellow precipitate immediately, a small amount	+ve
Alkaloids	Picric acid, reagent	Yellow precipitate (a little)	+ve
P <sup>H</sup>	5.93	Acidic solution	
Saponins	Fast shaking	The appearance of a light foam (1.2cm)	
2 ml chloroformTerpenes2 ml glacial acetic acid 2ml H2SO4 Leave it for 10 minutes		A dark brown solution	+ve

(+) indicates positive detection (presence of the active compound)

(-) indicates negative detection (lack of active compound)

Table 2. Chemical detection of active compounds for hot aqueous extract of Artemisia fruit

Active chemicals	Test Name	Detection product	Result
	Iodine test	A dark blue color	+ve
Glycosides	Molish test	Violet ring	+ve
	Benedict test	A brown precipitate	+ve
Proteins	Biuret, Reagent	No purple color. The color did not change	-ve
Phenolic Compound	Aqueous, ferric chloride, FeCl <sub>3</sub> (1%)	A green precipitate	+ve
Tannins	Lead Acetate (1%)	Light yellow precipitate in large quantity (double the amount of cold)	+ve
Resins	Ethanol+Boiling (DW)	Turbidity in large quantity (double the amount of cold)	+ve
Flavonoids	EtOH+KOH 50% 50%	A yellow precipitate a large amount	+ve
Alkaloids	Picric acid, reagent	Yellow precipitate (a little) alkaloids in greater quantity	+ve
P <sup>H</sup>	5.65	Acidic solution	
Saponins	Fast shaking	The appearance of a light foam (1.5cm)	+ve
Terpenes	2 ml chloroform, 2 ml glacial acetic acid, 2ml H <sub>2</sub> SO <sub>4</sub> , Leave it for 10 minutes	A dark brown solution	+ve

(+) indicates positive detection (presence of the active compound)

(-) indicates negative detection (lack of active compound)

Biuret detection was used to infer the presence of proteins in the extracts, but it gave a negative detection because the violet solution did not appear. It indicates that the aqueous extract of Artemisia fruit does not contain protein compounds (13).

We have detected phenolic compounds (in aqueous  $FeCl_3$  solution), which showed their presence in the aqueous solution due to the appearance of a dark green precipitate (hot is more than cold). An examination of tannins was conducted, which gave a positive detection due to the appearance of a light yellow precipitate (where the quantity of the precipitate in the hot extract is twice the precipitate in the cold extract).

Also, the extracts contain resins (the appearance of turbidity in the solution), alkaloids (the yellow precipitate), and flavonoids, a red solution, and in a tremendous amount for the hot extract than for the cold extract (14). As well as containing terpenes (a brown solution) and Saponins (the appearance of a thick foam). The acidity function of cold and hot aqueous extract of Artemisia fruit was measured, as the pH value of the hot extract was  $P^{H}$ =5.65 and that of the cold extract pH=5.93.

We note that both extracts have acidic properties, but the hot aqueous extract gave a lower value because of the possibility of hot aqueous extract polar active compounds that cause an increase in pH(15).

Table 3 showed a significant increase in the level of the ALT/GPT enzymes, and this increase in the level of this enzyme was directly proportional to the increase in the concentration of the hot extract of Artemisia fruit (16).

**Table 3.** Serum (ALT/GPT) levels before and after addition of the hot extract of the fruit of artemisia in different concentrations (*in vitro*)

Cono (mg/ml)	Mean of( ALT/GPT) (U/L ) ± S.D		
Conc. (mg/ml)	Before	After	
0.01	15.36±4.27	16.01±5.21	
0.1	$15.36 \pm 4.27$	$17.08 \pm 4.11$	
1	$15.36 \pm 4.27$	$18.35 \pm 3.01$	
100	15.36±4.27	20.21±3.61	

This is because the hot aqueous extract can give more compounds, especially those with polar groups, which represent the most significant part of the fruit and are responsible for giving the acidic value and its ability to link with other active groups. In addition, the presence of some mineral elements in the aqueous extract of Artemisia fruit leads to an increase in the activation rate of the enzyme's function, as shown in table 4, similar to the previously published studies (17, 18).

Table 4. Detection	of mineral elements	in aqueous	extract of
	the fruit of artemist	a	

No	Metallic Elements	Concentration (mg/L)
1	Pb	0.3
2	Cl	0.35
3	Cu	0.82
4	Ni	0.1
5	Cd	0.6
6	Zn	0.4

The presence of flavonoid compounds that captures free radicals and tannins activates the enzymes and transporter proteins present in the cell membrane in the body (19, 20). From these studies, we conclude that it is possible to use the fruit of the Artemisia plant or its aqueous extract in the treatment of intestinal infections and cases of diarrhea (21), hypoglycemia, and heart rate regulation, as well as treatment of damage or damage to the stomach wall therefore, we recommend taking advantage of foodstuffs in the treatment of some pathological conditions instead of medicines because they lead to side effects (22).

# 4. Conclusion

We conclude that it is possible to use the fruit of artemisia or its aqueous extract in treating intestinal infections, cases of diarrhea, hypoglycemia, and heart rhythm regulation, as well as the treatment of damage or injury to the stomach wall.

# **Authors' Contribution**

Study concept and design: A. R. M. Acquisition of data: M. R. A. Analysis and interpretation of data: M. R. A. Drafting of the manuscript: A. R. M.

Critical revision of the manuscript for important

intellectual content: A. R. M.

Statistical analysis: M. R. A.

Administrative, technical, and material support: A. R. M.

# **Conflict of Interest**

The authors declare that they have no conflict of interest.

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