

The Potential Anti *Helicobacter pylori* and antioxidant effects of *Artemisia Judaica*

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ABSTRACT

Artemisia judaica (*AJ*) is one of the common species of the genus *Artemisia* that grows in Saudi Arabia desert and Sinai, Egypt where animals graze on it. It is widely used in traditional medicine and by Bedouins there. (*AJ*) has anthelmintic, antibacterial, antiinflammatory, analgesic and antipyretic effects. The present study aimed to (1) elucidates the antibacterial action of *AJ* against *H. pylori* and different other bacterial species (2) delineate the potential antibacterial mechanism of action of *AJ* in comparison with tetracycline and cefotaxime (3) measure the trolox equivalent antioxidant capacity (TEAC) of the *AJ* water extract. Preparation of the (*AJ*) extracts was done by three different methods two of them are usually performed by population in Middle East by boiling of the shade-dried herb in water as tea (decoction), or soaked in tap water for over night (infusion), other method was done by concentrating the aqueous extract of *Artemisia judaica* under vacuum. The results of this study revealed that (*AJ*) has neither antibacterial effects either against *H. pylori* nor any other bacterial species. On the other hand the extract of *AJ* prepared by any of the above mentioned methods shows significant ($p < 0.005$) antioxidant action as compared with blank and related to trolox antioxidant capacity.

INTRODUCTION

Artemisia judaica L (*AJ*) is a desert medicinal plant commonly used as tea by population of Saudi Arabia, and in Egypt Sinai. In the traditional medicine of the Arabic area; *Artemisia judaica* is used in the treatment of gastrointestinal disorders, enhanced eyesight, cardiovascular health, capillary strength, and structure of connective tissue, appearance of skin, and immune systems as well as decreased risk of atherosclerosis, cancer, and arthritis (Abdalla and Abu-Zagra, 1987; Khafagy et al., 1988)

Artemisia judaica, belongs to the family Compositae, and is a fragrant shrub that grows widely in the Arabian area. Phytochemical analysis of *A.J* shows that it is a rich

source of flavonoids including apigenin, cirsimaritin, and various novel compounds (Khafagy and Tosson, 1968; Saleh, 1985; Saleh et al. 1987)

Artemisia family extract showed significant higher antioxidant effect (Liu et al.2004). There are also evidences that *Artemisia* family can improve diabetes mellitus in addition to their antimicrobial and anti fungal effects (Salwa et al. 2009).

Free radicals generation is involved in many disorders include non-steroidal anti-inflammatory drugs (NSAIDs)-induced peptic ulcers (El-Sayed et al. 1998). *Helicobacter pylori* (*H. pylori*) is the bacteria responsible for most ulcers and many cases of stomach inflammation (chronic gastritis). The bacteria can weaken the protective coating of the stomach, allowing digestive juices to irritate the sensitive stomach lining. (Chey et al. 2007). The present study is designed to use *AJ* extract to protect against the free radicals production and to evaluate its role against *H. Pylori* and many other bacteria species including *E. coli*. It is also intended to delineate the antibacterial mechanism of action of *AJ* and comparing its action with that of cefotaxime and tetracycline. The antioxidant capacity of the *AJ* was also investigated by using the trolox equivalent antioxidant capacity.

Aim of the work

- To elucidate the antibacterial action of *AJ* against *H. pylori* and different other bacterial cultures in vitro.
- To delineate the potential antibacterial mechanism of action of *AJ* in comparison with tetracycline (as a bacteriostatic) & cefotaxime (as a bactericidal).
- To measure the trolox equivalent antioxidant capacity (TEAC) of the *AJ* water extract in-vitro.

Work Design

- The effect of the watery extract of *AJ* has been tested on different bacterial colonies in cultures including the *H. Pylori* species.
- The effect of the watery extract of *AJ* versus clarithromycin and amoxicillin has been investigated against various types of bacteria in culture
- The total antioxidant capacity of the extract is measured by using the trolox equivalent antioxidant capacity technique.

MATERIALS AND METHODS

Materials:

Artemisia judaica dried leave were purchased from a local herbal market in Riyadh city, KSA

Bacteria: *Staphilococcus aureus*, *Ehirichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Streptococcus group B*. and *Helicobacter pylori*

Various strains of bacteria were isolated & identified in its specific cultures in vitro. This was done in the KFMC microbiology laboratories.

Antibiotics: cefotaxime and tetracycline were generously obtained KFMC hospital Pharmacy.

Culture media like blood Agar medium, Mueller Hinton Agar & Saborod dextrose agar were obtained from KFMC hospital laboratories

Chemicals:

- Trolox, a water-soluble vitamin E analog.
- 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)

Instruments:

- Spectrophotometer, (Pharmacia LKB, Ultrospec III – England)
- Rotary evaporator, Heidolph Instruments GmbH & Co.KG Walpersdorfer Str. 12, D-91126 Schwabach, Germany
- Glass tools and polyethylene tubes

METHODS:

Preparation of *Artemisia judaica* extract:

Hot water extraction (decoction):

It is a usual popular preparation. It is done according to the methods described by (Abdel-Salam et al. 2009): Approximately 50 g of *Artemisia judaica* material is cut into small pieces, milled and placed in a flask (2 L) with 1000 mL of distilled water and boiled for 15 min, then the mixture is filtered twice, first through cheese-cloth (50% cotton/50% polyester) and then through filter paper (Whatman No. 2). The final concentration of the preparation of *Artemisia judaica* is 5% as a total solids. The amount of obtained *Artemisia judaica* extract is preserved in sterile dark bottles (500 mL) in a cool environment (4°C) until further use. All these processes were done in the KFMC and FOM laboratories.

Soaked preparation (infusion):

Approximately 50 g of *Artemisia judaica* material is cut into small pieces, milled and placed in a flask (2 L) with 1000 mL of distilled water for over night then the mixture is filtered twice.

Preparation of Aqueous Extract of *Artemisia judaica* under vacuum:

One hundred and fifty milliliters of distilled water were added to 30 grams of the shade-dried herb, and then the mixture boiled for about 20 minutes. The whole mass was filtered and concentrated under vacuum to about 30 ml. The concentration of the dried *A. judaica* was 1 g/ml in the final extract. The extract was stored in refrigerator in well-closed dark glass container.

Bacteriological Study:

Culture medium used for susceptibility test:

For *Helicobacter pylori* and *Streptococcus* group B we used Blood Agar medium.

For *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* we used Mueller Hinton Agar.

For *Pseudomonas aeruginosa* we used Saborod dextrose agar.

Antibiotics used for the comparison:

Tetracycline and Cefotaxime versus *Artemisia judaica* extract in three concentrations 5%, 10% and 20%.

METHOD

Different bacterial species were collected from the microbiology laboratory in the hospital of King Fahad Medical City in Riyadh-Kingdom of Saudi Arabia. The strains were isolated from different medical specimens by the hospital lab. and identified by morphology of the colonies and the biochemical reactions using analytical profile index (API). The identified isolates were kept frozen in -80°C refrigerator in order to maintain the strain for research.

The bacterial strains were cultured on the suitable medium for the susceptibility test for each strain, and then a pharmaceutical disk saturated with the selected antibiotic under investigation is placed on the surface of the medium inoculated on the whole surface with the test organism.

5, 10 & 20 % of *Artemisia judaica* extract were prepared by the above mentioned two different methods. Then a disk of sterile filter paper is saturated with the different concentrations of the filtrate of the plant extract and placed on the surface of the agar medium which has been inoculated earlier with the organism under investigation.

The diameters of the circular "halo" (technically known as a "plaque," or zone of inhibition) surrounding the disks of antibiotics or the plant extracts are measured for comparison of the sensitivity of different bacterial species to the agents used.

Biochemical study:

Measurement of antioxidant capacity:

TEAC (Trolox equivalent antioxidant capacity) assay. The method is based on the ability of antioxidant molecules to quench the long-lived 3-ethylbenzothiazoline-6-sulfonic acid (ABTS $\cdot+$), a blue-green chromophore with characteristic absorption at 734 nm, compared with that of Trolox, a water-soluble vitamin E analog. The addition of antioxidants to the preformed radical cation reduces it to ABTS, determining a decolorization. A stable stock solution of ABTS $\cdot+$ is produced by reacting a 7 mmol/L aqueous solution of ABTS with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use (Pellegrini et al 1999). At the beginning of the analysis day, an ABTS $\cdot+$ working solution is obtained by the dilution in ethanol of the stock solution to an absorbance of 0.70 ± 0.02 AU at 734 nm, verified by a Hewlett-Packard 8453 Diode Array spectrophotometer (HP, Waldbronn, Germany), and used as mobile phase in a flow-injection system, according to Pellegrini et al 2003. Results are expressed as TEAC in mmol of Trolox per L (water extract) of sample. All these processes were done in the FOM, KFMC laboratories.

Statistical analysis:

Data were analyzed by using the Student t- test described by Sendecor and Cochran (1969). p value < 0.05 was considered significant.

Results

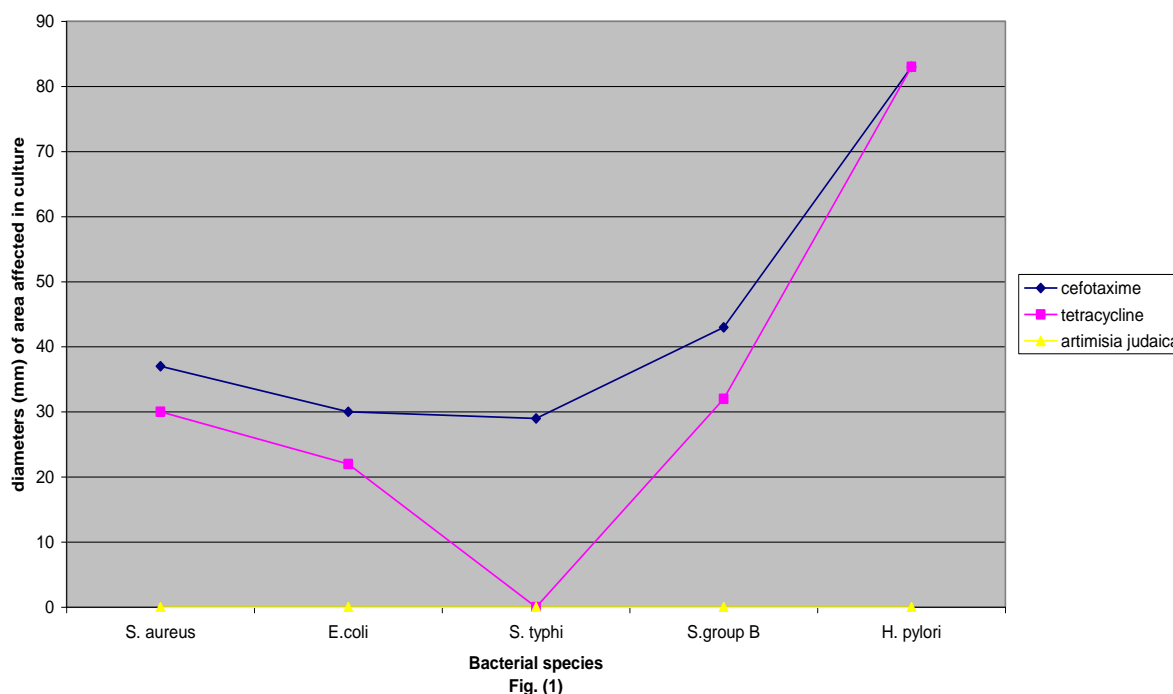
Bacteriological Results:

The sensitivity of the *Helicobacter pylori* and different other bacterial species were tested against 5%, 10% & 20% of *Artemisia judaica* extracts and compared with two antibiotics of known mechanism of actions cefotaxime, inhibits cell wall synthesis, and tetracycline which inhibits bacterial protein synthesis. Unfortunately we did not find any effect for *Artemisia judaica* extract prepared by the above mentioned two methods of preparations and even in high enough 20% concentrations on the growth of any of the used strains of bacteria in different culture media compared with cefotaxime and tetracycline Tab. (1) and Fig. (1)

Table 1. effects of cefotaxime, tetracycline versus *Artemisia judaica* against different bacterial species

Agent	Cefotaxime	Tetracycline	<i>Artemisia judaica</i> 5-20 %
Organism			
<i>Staphilococcus aureus</i>	37mm	30mm	0mm
<i>Ehirichia coli</i>	30mm	22mm	0mm
<i>Salmonella typhi</i>	29mm	0mm	0mm
<i>Pseudomonas aeruginosa</i>	14mm	12mm	0mm
<i>Streptococcus group B</i>	43mm	32mm	0mm
<i>Helicobacter pylori</i>	83mm	83mm	0mm

Sensitivity of bacteria strains to artemisia judaica vs antibiotics



Biochemistry

RESULTS:

By using trolox standard antioxidant curve (coefficient correlation value = -0.993) Fig (2) as a reference to the antioxidant capacity of the *AJ* extract preparations, it was found that all types of preparations produced a significant TEAC ($p < 0.005$) compared with blank. in this regard also the concentrated vacuum extract produced a significant ($p < 0.05$) antioxidant effect compared with decoction (tea) and soaked (infusion) preparations Tab. (2) Fig. (3)

Table 2. the trolox equivalent antioxidant capacity of *AJ* preparations

	1 st set	2 nd set	3 rd set	Mean ± SD
Vacuum ext.	0.447	0.392	0.399	0.413±0.030*
Tea	0.287	0.308	0.313	0.303 ± 0.0137
Soaked	0.232	0.290	0.251	0.258 ± 0.029

P < 0.005

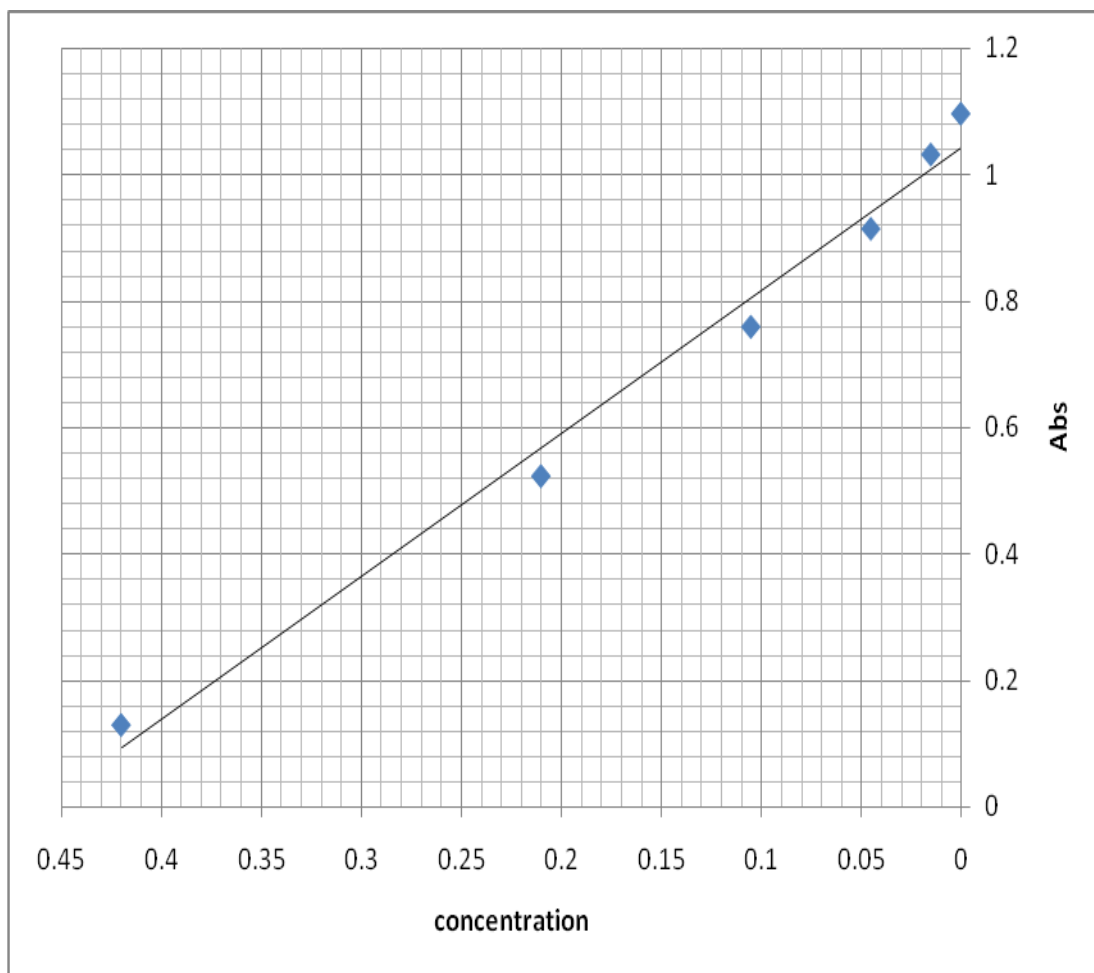


Fig 2. Trolox standard antioxidant capacity curve; it is noticed that as the trolox increased more of the ABTS is scavenged and subsequently the absorbance decreased

DISCUSSION

Artemisia judaica is one of the common species of the genus *Artemisia* that grows in middle east deserts where animals graze on it. It is widely used in folk medicine and is recommended as a healer plant in traditional medicine by Bedouins in Saudi Arabia desert and Sinai, Egypt. The volatile oil prepared from the flowering branches of *Artemisia judaica* has anthelmintic, antiinflammatory, analgesic and antipyretic effects; in addition to antimicrobial activity against *Staphylococcus aureus*, *Candida albicans* and *Rodotorula ruba* (Batanouny et al. 1999)

Aqueous extract of the ground aerial parts of *Artemisia arborescens* induced a concentration dependent reduction in the amplitude of the phasic contractions and tone of the rat ileum (Abu Zarga, 1995). The flavone isolated from *A. judaica* (circsimaitin), has the same effect (Abdalla and Abu Zarga, 1987). The aqueous extract of *A. herba-alba* produced initial hyperglycaemia which was followed by hypoglycaemia in normal and alloxan –treated rabbits and mice (Marrif et al. 1995).

Methanol extract of *Artemisia pallens* induced significant blood glucose lowering effect (Subramoniam et al. 1996). Artemisinin (a compound isolated from *Artemisia annua*), is a potent cancer chemoprevention agent (Lai et al. 2006).

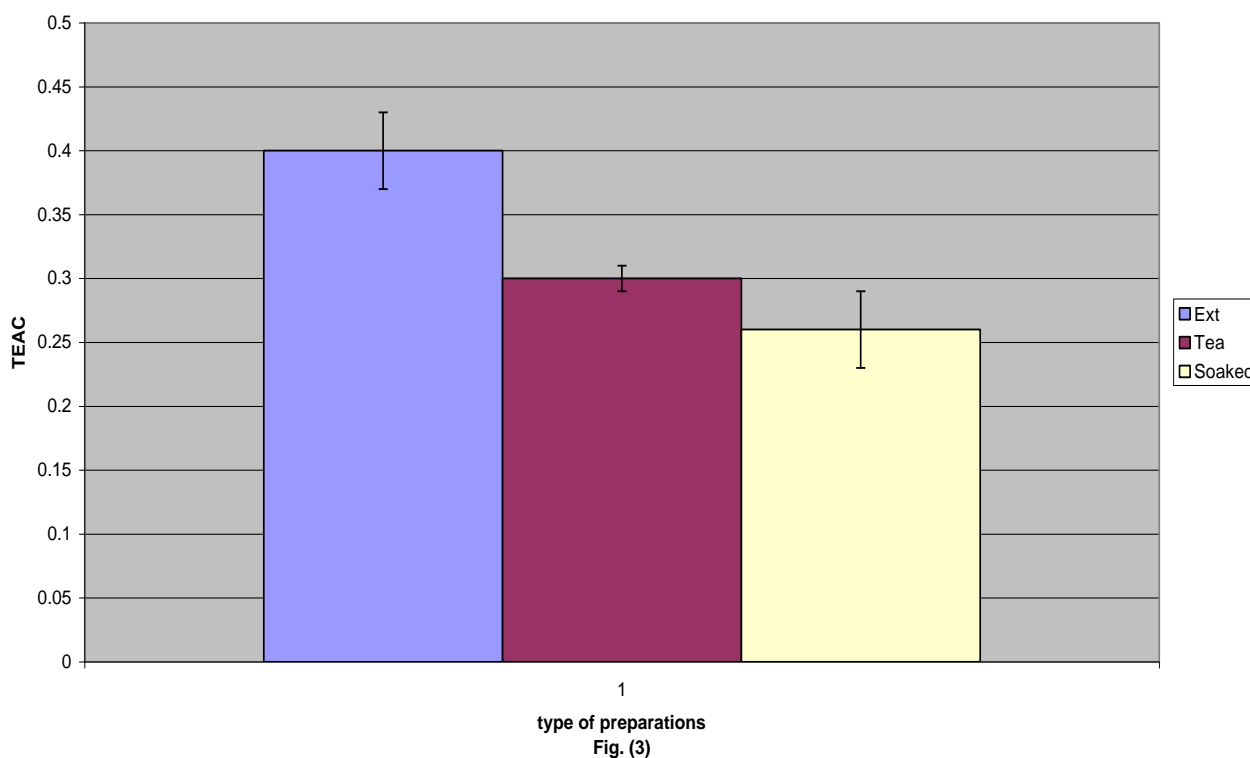
Phytochemical analysis of *Artemisia judaica* shows that it is a rich source of flavonoids including apigenin, cirsimaritin, and various novel compounds (Khafagy and Tosson, 1968; Saleh, 1985; Saleh et al. 1987)

The present study was designed to elucidate the antibacterial action of *AJ* against *H. pylori* and different other bacterial species, to delineate the potential antibacterial mechanism of action of *AJ* in comparison with tetracycline and cefotaxime and to measure the trolox equivalent antioxidant capacity (TEAC) of the *AJ* water extract.

In our present study we used the boiled or soaked water crude extract of the dried leaves of *Artemisia judaica* plant as usually prepared by people of our Arabian area and also we get concentrated preparation under vacuum.

Investigation of the crude extract on bacterial sensitivity in culture and the trolox equivalent antioxidant capacity (TEAC) were run to evaluate the beneficial effects of this

Antioxidant effect of three preparations of *Artemisia Judaica*



plant.

Unfortunately, in our study we have not found any antibacterial effect for the crude extract of *Artemisia judaica* in contrast to the results previously obtained by Charchari et al. 1996 and Batanouny et al. 1999 and this is may be due to different methods of extract preparations or because those authors used a single component of the crude extract after HPLC separation, or it may be also due to different plant species and culture as they used plant grown in Algeria desert while in our study we used the dried leaves of *Artemisia Judaica* plant grown in Saudi Arabia desert.

Because many synthetic antioxidants (butylated hydroxyanisole and butylated hydroxytoluene) have been proved to have undesirable side effects (Jayaprakasha et al., 2007). There has been an increasing interest in the use of medicinal plants rich in

antioxidants to reduce free radicals-induced tissue injury (Zhishen et al., 1999). Phytochemicals content in plants are promising alternatives to synthetic drugs.

Our present study revealed a significant ($p < 0.005$) TEAC for AJ extract preparations, the results which run in parallel with that obtained by Liu et al. (2004). The antioxidant effects of AJ extract open several therapeutic utilities to the plant in senility, Parkinsonism, NSAID-induced peptic ulcers and many other disorders induced by free radicals generation. This study needs more investigations and extensions to evaluate the AJ extracts in the above mentioned disorders.

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