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Cover Page Footnote

The author would like to sincerely thank the personnel of laboratory of Department of Nature and Life Sciences, University of Ibn Khadoun, Tiaret, Algeria.

IN VITRO ANTI-*HELICOBACTER PYLORI* AND ANTIOXIDANT ACTIVITIES OF PLANTS USED IN ALGERIAN TRADITIONAL MEDICINE FOR GASTROINTESTINAL DISORDERS

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ABSTRACT

It has been recognized that gastric colonization with *Helicobacter pylori* (*H. pylori*) induces a risk of developing ulcer disease and gastric cancer. The interest of this research relates for the first time to the study of the Anti-*Helicobacter pylori* activities of three medicinal plants methanolic extracts, namely *Allium sativum*, *Allium cepa* and *Foeniculum vulgare*. The activity of the methanolic extracts was assessed against two strains of *H. pylori* (SAN158 and 26659) by the disc diffusion method on Muller Hinton agar. The content of phenolic compounds (TPC) was determined by the Folin-Ciocalteu test and the antioxidant activity was evaluated through the FRAP method (Ferric reducing antioxidant power). All plants extract were inhibitory towards both strains, with *F. vulgare*'s showing the strongest activity. The highest TPC, along with the best antioxidant capacity, was exhibited by the *F. vulgare* methanolic extract (1.44 mg GAE (gallic acid equivalents) / g ± 0.2 and 0.009 mg EAA (ascorbic acid equivalent) / g ± 0.001, respectively). This study showed that *Allium sativum*, *Allium cepa* and *Foeniculum vulgare* bioactive substances may be effective against *Helicobacter pylori*.

Keywords: *Allium, Foeniculum vulgare*, antibacterial activity, *Helicobacter pylori*.

INTRODUCTION

Helicobacter pylori is a pathogenic bacterium, recognized for their implications in gastroduodenal diseases; duodenal and gastric ulcer, gastric cancer and lymphoma of MALT (mucosa-associated lymphoid tissue). In general, antibiotics cause disruption of the human gastrointestinal microflora. In addition, there are cases of resistance of the bacteria to the treatment of antibiotics or an allergy of patients to antibiotics leading to severe complications. These prompt us to look for alternative agents instead of antibiotics and to develop a more effective and safer therapy with new natural molecules. Throughout the world, and for a long time, medicinal plants have occupied an important place in the medicine and in culinary preparations of peoples (Bouzouita et al., 2008). The last decades have been marked by the particular interest in medicinal plants as sources of natural bioactive substances (El-Haci et al., 2012). Algeria, by its biogeography, offers great ecosystem diversity (Ennadir et al., 2014). Therefore, Algerian populations have long been dedicated to traditional medical practice and have acquired a strong know-how in this area. The plants: Onion (*Allium cepa*), garlic (*Allium sativum*) and fennel (*Foeniculum vulgare*) are among the ancient foods cultivated in Algeria and known for their medicinal properties and applications. Moreover, they are a considered as a rich source of phytonutrients which have important elements of the Mediterranean diet (Khan et al., 2011). They are used to treat a variety of diseases related to the digestive, the neural, the cardiovascular, and the

respiratory systems (Dorant et al., 1996). Our work is concerned with the study of the Anti-Helicobacter activity of extracts from *Allium sativum* L., *Allium cepa* L. and *Foeniculum vulgare* L. cultivated in Algeria (Atherton, 2006). Importance of the study is related to the fact that *H. pylori* is the main etiological agent of peptic ulcer diseases, and its presence is a risk factor for the development of the chronic atrophic gastritis, an inflammatory condition that paves the way to gastric adenocarcinoma (Parsonnet et al., 1991). Our work has also measured the antioxidant activity and total phenolic content of the plants extracts.

MATERIALS AND METHODS

The Extraction

The fresh plants material from *A. cepa, A. sativum* and *F. vulgare* was bought from the market in the town of Tiaret (Algeria), the plants used in the experiment were identified by Dr. Miara MD. a botanist from the faculty of biological sciences at the university of Tiaret. The vegetables (onions, garlic clove and fennel) was separated from impurities, cut into small pieces, and dried in open air protected from light. The dry matter was ground and sieved. The extraction was by maceration of 10 g dry matter of material with 100 mL of 96 % methanol (v/v) at room temperature for 24 h. The extracts were obtained after centrifugation at 3,000 rpm for 10 minutes and filtering of supernatants through Whatman paper. The extracts were evaporated using rotary evaporator and kept at 4°C (O'Gara et al., 2000).

In vitro Anti-Helicobacter Activity

The two strains (SAN 158 and 26695) of *H. pylori* considered in this study were donated by the laboratory of Bacteriology at the National Research Centre of Campylobacter and *Helicobacter* (CNRCH), University of Bordeaux 2 (France) isolates from gastric biopsies of patients with gastroduodenal disease and which are resistant to antibiotics such as Gentamicin, Erythromycin, Amoxyline, Lerofloxacum. The susceptibility test was based on the agar diffusion method. An inoculum of each strain *H. pylori* (SAN 158 and 26695) was prepared by the transfer fresh colonies in tubes containing sterile saline with the adjusting the turbidity to 2.0 McFarland units (approximately $6x10^8$ CFU/mL). Half a millilitre of the bacterial suspension was spread on the surface of a Muller Hinton agar medium supplemented with 10% of blood defibrinated sheep. Filter paper disks of six mm of diameter impregnated with 10 µl of each extract of different concentrations (50 and 100 mg/ml) were placed onto the surface of the inoculated agar plates, then, were incubated at 37ºC under microaerophilic conditions for 3-5 days. The antibacterial activity was expressed as the mean diameter of the growth inhibition zone around the disks (Makni et al., 2001; Satrani et al., 2007; Balaji et al., 2012). Antibiotics amoxicillin 25 µg and vancomycin 30 mg were the positive controls while methanol alone was the negative control.

Total Phenolic Content Determination and Reducing Power

To determine the TPC, a spectrophotometric method was used (Singleton and Rossi. 1965) with a calibration curve obtained using gallic acid as the standard, expressing it as mg of gallic acid equivalent (GAE)/100 g of extract.

The reducing power was measured according to Oyaizu (1986). Ascorbic acid was used as the positive control and results were expressed as ascorbic acid equivalent per unit weight (mg AAE/100 g).

Statistical Analysis

Excel (Microsoft Corporation, USA) was used for the statistical analysis. Data are presented as mean ± SD.

RESULTS AND DISCUSSION

Total Phenolic Content and Reducing Power

The average TPC by the three plant species ranged from 0.85 to 1.44 mg GAE/100 g Extract. In *A. sativum*, it was 0.85 mg GAE/g of extract (Table 1). The highest TPC for *A. sativum* was observed with values up to 22.83 mg GAE/100g Extract by Kallael et al., (2014). Conversely, the average TPC for A. cepa was 1.21 mg GAE/100 g Extract, alike the value seen by Sulaiman et al., (2011). Many other authors found higher values.
At last, the content in *F. vulgare* extract was 1.44 mg GAE/100 g Extract, lower than the 37.2 mg found by Chatterje et al., (2012).

Table 1: Total Phenolic Compounds Content in methanolic plants extracts

Plant	Total phenolic Compounds Content (Mg GAE/100g Extract)
A. sativum	0,85 ± 0,4030
A. cepa	1, 21 ± 0,5303
F. vulgare	1,44 ± 0,5303

Different factors such as geographical, climatic, genetic, the degrees of maturation of the plant and the duration of storage have a strong influence and effect on the polyphenols content within same species (Pedneault et al., 2001; Fiorucci 2006). Ferric Reducing Ability of Plasma (FRAP) is a reliable *in vitro* test to investigate the antioxidant activity of many compounds, based on the ability of polyphenols to reduce ferric iron to ferrous iron. One of the antioxidant mechanisms is the reducing power (Karagozlerl et al., 2008). As shown in table **2** a significant difference between extracts were recorded with the highest FRAP activity displayed by *F. vulgare* (0.96 ± 0.13 mg AAE/100 g Extract). The general low reducing power of our extracts can be explained by the fact that their TPC is not rich in strong antioxidant compounds. Indeed, values may also vary depending on the standard used. In the case of *A. sativum*, antioxidant activity was lower than those obtainable using either gallic acid. Our results were close to Brahma (2009), which used ascorbic acid as a standard and to Sulaiman et al., (2011) with used gallic acid. The activity of the *F. vulgare* extract was inferior to those obtained by Rawson et al., (2011) using trolox as a standard (79.61 ± 1.969 mg TE/100 g).

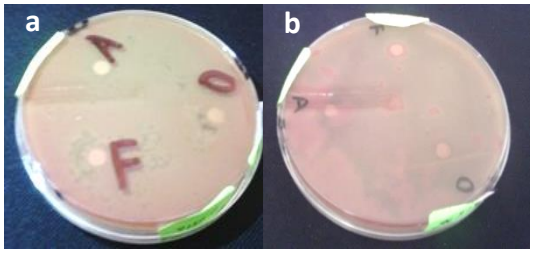
Table 2: Antioxidant activity of methanolic extracts of our plants

Plant	Antioxidant Activity (Mg AAE/100 g Extract)
A. sativum	0.35 ± 0.17
A. cepa	0.55 ± 0.09
F. vulgare	0.96 ± 0.13

In Vitro Anti-Helicobacter Results

All plant extracts demonstrated anti-*H. pylori* activity with inhibition zone diameters (IZD) ranging from 8 to 13 mm. The most active was *F. vulgare* against *H. pylori* SAN 158 followed by *A. cepa* and then by *A. sativum* with IZD of 13 mm, 11 mm, 8 mm, respectively. Activity against the *H. pylori* 26695 was higher by *A. sativum* followed by *F. vulgare* and *A. cepa* with IZDs of 11 mm, 10 mm, and 8 mm, respectively. However, *H. pylori* bacteria was resistant to amoxicilin and sensitive to vancomycin with an IZD of 17 mm (Figure 1).

The activity of the prepared extracts supports, on a scientific basis, the use of these plants by the Algerian folk medicine in the treatment of gastric diseases.



a: *H. pylori* SAN 158 b: *H. pylori* 26695

Figure 1: Zones of inhibition of methanolic extracts from the three plants.

It is well-known that species of the genus *Allium*, especially garlic and onion, have antibiotic activity. Moreover, standard antibiotic against *H. pylori* are often ineffective in high-risk populations (Cellini et al., 1996; Gowsala et al., 1997). Garlic extracts have an effect against *H. pylori* (O'Gara et al., 2000; Cai et al., 2011). Gowsala et al., (1997) demonstrated the effect of thiosulfinates from garlic extracts on the growth of *H. pylori* with a minimum inhibitory concentration of 40 µg/mL. *Allium spp.* mainly contains cysteine sulfoxides and the allinase enzyme that converts cysteine sulfoxides into thiosulfinates. The latter has a strong antibacterial activity on Gram-negative and Gram-positive bacteria (Benkeblia, 2004; Rabinkov et al., 1998). The major compounds of garlic and onion are instead organosulfides (Benzeggouta, 2005). In facts, the most important constituent in garlic is: allicin converted to other sulfur compounds, namely ajoene, mono-, di, and tri-allyl sulfides and vinyldithiins (Sengupta et al., 2004). Garlic also contains a wide range of thiosulfinates apparently responsible for the activity against *H. pylori* (Hiroaki and al. 2014). The antibacterial effect of red onion is associated to the presence of flavonoids, polyphenols, organosulfides, saponins and other secondary metabolites (Al masaudi et al., 2012). In addition, onions contain also quercetin that exhibits anti-*Helicobacter* activity (O'Gara et al., 2000; Corzo-Martinez et al., 2007), which notably extends to reported that many different Gram-positive and Gram-negative bacteria (Ankri and Mirelman, 1999). Dially disulfide, dially tridisulfide, dially tetradisulfide and essential oil rich in these sulfides may have a role in the prevention and treatment of gastric infections (Ehiabhi et al., 2006). For these reasons, regular consumption of onion significantly reduces the risk of stomach cancer (Dorant et al., 1996). Also extracts form fennel may contribute to the prevention of gastritis and stomach cancer (Dorant et al., 1996). In facts, fennel methanolic and ethanolic extracts and essential oils are all inhibitory against *H. pylori* and contain trans-anethole, estragole, fenchone, limonene, α-pinene and γ-terpinene (Mahady et al., 2005; Li et al., 2005; Lim 2013). A significant reduction in cancer mortality is found with 34 % for supplementation with garlic (Hiroaki et al., 2014). In general, the use of medicinal plants would better protect humans against the bacteria responsible for many gastrointestinal disorders (diarrhoea, typhoid, ulcer, etc.) (Tabak and Doukani 2017).

CONCLUSION

This study demonstrated that the methanolic extracts of *A. sativum, A. cepa. and F. vulgare* present in Algeria have a significant TPC with antioxidant activity and were able to inhibit *Helicobacter pylori*, supporting the popular use of these products as anti-*Helicobacter* agents.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest in the publication

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