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ANTI-*HELICOBACTER PYLORI* ACTIVITY OF THREE MEDICINAL PLANTS (*CINNAMOMUM ZEYLANICUM, SYZYGIUM AROMATICUM* AND *ZINGIBER OFFICINALE)*

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ABSTRACT

In this study, three medicinal plants; cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) and ginger (*Zingiber officinale)* extracts were examined and screened for anti-*Helicobacter pylori* activity.

Ninety-six percent of methanol was used for the extraction of these plants. It has been shown that *Zingiber officinale* contains high total phenolic compounds content (110 mg GAE / 100 g extract) in comparison to *Cinnamomum zeylanicum* (98.2 mg GAE / 100 g extract) and *Syzygium aromaticum* (98 mg GAE / 100 g extract).

The antioxidant activity of our extracts measured by FRAP method indicates that *S. aromaticum* had a high antioxidant activity (107.2 mg AAE / 100 g extract) relative to *Z. officinale* (105.1 mg AAE / 100 g extract) and *C. zeylanicum* (58 mg AAE / 100 g extract).

All studied plants demonstrated strong anti-*H. pylori* activity with inhibition zone diameter ranged from 10 to 36 mm.

KEYWORDS: *Cinnamomum zeylanicum, Syzygium aromaticum, Zingiber officinale, Helicobacter pylori,* antioxidant activity, antibacterial activity

INTRODUCTION

Helicobacter pylori is a Gram-negative spiral-shaped bacterium that was first isolated by Barry Marshall and J. Robin Warren. Since its discovery in 1983, the microorganism has been associated with the etiopathogenesis of several diseases of the digestive system, such as gastritis, peptic ulcer disease and gastric cancer (Marshall et Warren, 1983).

The current commonly prescribed regimen for the eradication of *H. pylori* infection includes a triple therapy, which combines the antibiotic clarithromycin (CLR) and amoxicillin (AMX) with a proton pump inhibitor such as omeprazole. This regime is associated with side-effects and fails to eliminate infection in 30% of patients. Medicinal plants are the source of natural compounds called phytochemicals that possess antimicrobial and anti adhesive properties (Lúcia Cogo et *al*., 2010). They have been used as traditional remedies in treating gastrointestinal diseases and their anti-*H. pylori* activity has been widely demonstrated in *vitro* **(**Sudan et *al*, 2013; Cortès-Rojas et *al.*, 2014; Alkushi et *al*., 2013).

Clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum zeylanicum*) has been used for centuries as food preservatives and treatment of gastrointestinal disorders (Nassan et *al*., 2015). Ginger (*Zingiber officinale)* is one of the widely used anti-inflammatory and antioxidant spices (Alkushi et *al*., 2013). The aim of this study is to evaluate the *in vitro*

anti-*Helicobacter pylori* activity of these three medicinal plants which are used in Algeria traditional medicine for gastro intestinal illness.

MATERIAL AND METHODS

Plant Material

Cinnamon pipes (*Cinnamomum zeylanicum*), clove floral button (*Syzygium aromaticum*) and rhizome of ginger (*Zingiber officinale)* were purchased from local markets of our city.

Bacterial Strain

Helicobacter pylori (SAN 158 responsible of gastric cancer and 26695 responsible of gastric ulcer), were obtained from the Laboratory of Bacteriology of the National Research Center of *Campylobacter* and *Helicobacter* (CNRCH), Pellegrin Hospital, University of Bordeaux, (France). These strains were stored at 4°C in transport media (port-pylorus), BioMerieux.

Preparation of Extracts

10 g of the plant material was powdered in a laboratory mill, then transferred into extraction tubes and mixed with 100 ml of 96% methanol (v/v) by maceration at room temperature for 24h. The extracts were obtained after centrifugation at 3000 rpm for 10 minutes and were filtered through What man filter paper and the residue was re-extracted after 48 h, the. Combined supernatants were evaporated to dryness under vacuum at 40^{o}C using Rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4^{o}C (Owen and Johns, 1999).

Determination of Total phenolic Compounds Content

The concentration of phenolic compounds in plant extracts was determined using spectrophotometric method (Singleton et *al*., 1999). Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, with 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCo_3$. The samples were thereafter incubated in a thermostat at 45^{o}C for 45 min.

The absorbance was determined using spectrophotometer at λ max = 765 nm. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolic compounds was read (mg/ml) from the calibration line; then the content of phenolic compounds in extracts was expressed in terms of gallic acid equivalent (mg GAE/ 100 g extract).

Phytochemical Tests

Screening of the above three selected medicinal plants for various phytochemical constituents (tannins, saponosids, flavonoids, alkaloids, terpenoids, cardiac glycosides, sterols and triterpenes) were carried out using standard methods (Bhargava et *al*., 2012; Bruneton.,1999; Malla et *al*., 2013 ; N' Guessan et *al*., 2009).

Evaluation of Antioxidant Activity

The antioxidant capacity of each sample was determined by the method given by Oyaizu (1986) with some modifications. Methanolic solution of each extract was diluted in distilled water and mixed with 2.5 ml of the phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of $K_3Fe\ (CN)_6$ at 1%. The mixture was incubated at 50 °C for 30 min. 2.5 ml of

$C_2HCl_3O_2$ at 10% is added and centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant of each sample is mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%). Vitamin C (L-ascorbic acid) was used as antioxidant standard; the absorbance of the resulting solution was measured by spectrophotometer at 595 nm and was expressed in terms of ascorbic acid equivalent (mg AAE/100 g extract).

Evaluation of Antibacterial Activity

An inoculum of each strain *H. pylori* (SAN 158 and 26695) was prepared by transferring their fresh colonies in tubes containing sterile physiological saline solution and adjusting the turbidity to the 2.0 McFarland standards. This turbidity produces a suspension that corresponds to approximately 6.0×10^8 CFU/ml of *H. pylori*. The disk diffusion test was used to analyze the susceptibility of our strains *H. pylori* (26695 and SAN 158*)* against different plant extracts.

The bacterial suspensions were spread-plated onto Columbia Agar plates supplemented with 10% defibrinated sheep blood. Filter paper disks of 6mm diameter impregnated with 50µl of each extract solution were placed onto the surface of the inoculated agar. The plates were incubated at 37ºC under microaerophilic conditions and observed after 3 to 5 days. The antibacterial activity was expressed in terms of the mean diameter of the inhibition zone around the disks (Lúcia Cogo et *al*., 2010).

RESULTS AND DISCUSSIONS

Total Phenolic Compounds Content

The obtained results presented in table 1, indicated that *Z. officinale* had high total phenolic compounds content compared to *S. aromaticum* and *C. zeylanicum*.

Table 1: Total phenolic compounds content in methanolic plants extracts

Plant	Total phenolic compounds content (mg GAE/100g extract)
Z. officinale (Ginger)	110 ± 0.37
S. aromaticum (Clove)	98 ± 0.45
C. zeylanicum (Cinnamon)	98.2 ± 0.45

NB: Results are expressed as means ± standard deviation

The average recorded value of the total phenolic compounds content in the methanolic extract of *Z. officinale* is (110 ± 0.37 mg GAE / 100 g extract) (tab1). This result is significantly higher than those found by Phatak et *al*. (2015), which recorded a value of (18 ± 0.09 mg GAE/ 100 g extract). In the same plant, Fahmi (2014) recorded higher polyphenols contents in the order of (264 ± 1 mg GAE / 100 extract). Our result is comparable to this found by Maizura et *al*. (2011), it was (101.56 mg GAE / 100 g extract).

The mean value of total phenolic compounds content in the methanolic extract of *S. aromaticum* is (98 ± 0.45mg GAE / 100 g extract). It is superior to that shown by Phatak et *al.* (2015) which found values of (32 ± 0.23 mg GAE / 100g extract). On the other hand, Abdou (2011) recorded higher total phenolic compounds content which is of the order of (171.8 mg GAE / 100 g extract). Our results are close to those found by Sultana et *al*. (2011), which was (81.04 ± 0.78 mg GAE / 100 g extract).

Concerning *C. zeylanicum,* it has been shown that its total phenolic compounds content was **(**98.2 ± 0.45 mg GAE / 100 g extract). This value is significantly higher than given by Phatak et *al*. (2015), which give values of (21 ± 0.12 mg GAE / 100 g extract). Shan et *al*. (2005) recorded higher total phenolic compounds content which is in the order of (11.90 mg GAE /100 g extract).

Phenolic compounds are plants secondary metabolites considered as very important plant constituents due to the presence of one or more hydroxyl groups on their aromatic ring. They possess multiple biological properties such as antitumor, anti-inflammatory anti mutagenic and antimicrobial properties, and these activities might be related to their antioxidant activity (Keerti et *al*., 2014; Mishra and Sharma, 2014).

Phytochemical Tests

The obtained results indicate that the methanolic extract of our plants contains tannins, flavonoids, terpenoids, cardiac glycosides, saponosides, alkaloids, sterols and triterpenes.These results are confirmed with the studies of Womeni et *al*. (2013); Bhargava et *al*. (2012); Sudan et *al*. (2013); Mishra and Sharma (2014) and Jayaprakasha and Rao (2011).

These compounds were proven to exhibit anti-bacterial, anti-fungal, anti-viral and anti-carcinogenic (Bhargava et *al*., 2012).

Antioxidant Activity

The results of the antioxidant activity are illustrated in the following table:

Table 2: Antioxidant activity of methanolic extracts of our plants

Plant	Antioxidant activity (mg AAE/100 g extract)
Z. officinale (Ginger)	105.1
S. aromaticum (Clove)	107.2
C. zeylanicum (Cinnamon)	58

The results shown is table 2 indicated that the *S. aromaticum* had high antioxidant activity (107.2 mg AAE / 100 g extract) compared to ginger (105.1 mg AAE / 100 g extract) or cinnamon (58 mg AAE / 100 g extract).

Contrary to our results, Denre et *al*. (2014) showed in his study on *S. aromaticum* and *Z. officinale* that their reducing power which is (14 ± 0.05 mg AAE / 100 g extract), (48 ± 00.03 mg AAE / 100 g extract) is significantly lower than in our sample.

Many studies have shown good antioxidant activity of *Z. officinale* (Hinneburg et *al*., 2006, Kim et *al*., 2007, Kota et *al*., 2008). Some studies have shown that ginger roots extracts have high antioxidant activity (Chen et *al*., 1986, Shirin and Jamuna, 2010). Jaina et *al*. (2011) found that the antioxidant activity of *S. aromaticum* is in the order of 675.33 ± 5.36 (Mm Fe^{+2}/ g) which is greater than those of *C. zeylanicum* and *Z. officinale* with values of 222 ± 1.98 (Mm Fe^{+2}/ g) and 47 ± 0.65 (Mm Fe^{+2} / g) respectively.

Z. Officinale is a strong antioxidant and can either mitigate or prevent the production of free radicals (Ali et *al*., 2008). The phenolic constituents of *C. zeylanicum* are likely to be responsible for the observed antioxidant activity. Some authors have demonstrated a linear correlation between the content of total phenolic compounds and their antioxidant capacity (Bhargava et *al*., 2012; Alothman et *al*., 2009; Kota et *al*., 2008).

Antibacterial Activity

The obtained results of the antagonism are illustrated in the following table:

Table 3: Inhibition zone diameters of our plants extracts

	IZD (mm)		
Plant / Bacterial strains	***Z. officinale***	***C. zeylanicum***	***S. aromaticum***
***H. pylori* SAN 158**	36	18	34
H. pylori 26695	14	10	29

MDI: Inhibition Zone Diameter

By comparing the results of the three methanolic extracts of our plants, it is clear that the methanolic extract of *Z. officinale* and *S. aromaticum* are more potent than the methanolic extract of *C. zeylanicum*. In addition, *H. Pylori* SAN 158 gives greater value of MDI than *H. pylori* 26695.

The methanolic extract of *S. aromaticum* gives the highest IZD against *H. pylori* 26695 which is 29 mm. On the other hand, the methanolic extract of *Z. officinale* exhibited high activity against *H. pylori* SAN 158 with IDZ of 36 mm.

Anti-*Helicobacter pylori* activity of the extracts may be attributed to the high content of flavonoids, which have been reported to be involved in inhibition of nucleic acid biosynthesis and other metabolic processes (Alothman, 2009).

The antimicrobial potency of *S. aromaticum* is believed to be due to tannins, saponins, phenolic compounds and flavonoids (Keerti et *al*., 2014). Many studies have demonstrated numerous beneficial health effects of *C. zeylanicum*, such as anti-inflammatory properties, anti-microbial activity, reducing cardiovascular disease, boosting cognitive function and reducing risk of colonic cancer (Jayaprakasha et Rao, 2011 Bhargava et *al*., 2012; Alothman et *al*., 2009; Kota et *al*., 2008).

Z. officinale is known as a high resource with phenolic components (Tang and Zhao, 2001). The effective compounds possessing anti-*H. pylori* activity were identified as 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol and phenolic acids and their derivatives (Kim et *al*., 2007).

The aqueous and ethanolic extracts of *Z. officinale* inhibited the growth of antibiotic-resistant *H. pylori in vitro*. (Nostro et *al*., 2005). Both the methanolic extract of the dried powdered turmeric rhizome and curcumin inhibited the growth of all *H. pylori* strains examined *in vitro* (Mahady et *al*., 2002).

Screening of Turkish anti-ulcerogenic folk remedies for anti-*H. pylori* activity, revealed that flowers of *Cistus laurifolius* and *Spartium junceum*, cones of *Cedrus libani*, herbs and flowers of *Centaurea solstitialis* ssp. solstitialis, fruits of *Momordica charantia*, herbaceous parts of *Sambucus ebulus*, and flowering herbs of *Hypericum perforatum*, showed anti-*H. pylori* activity (Yesilada, 1999).

The antimicrobial activity of different Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove have been proved against several tested bacterial and fungal strains (Sofia et *al*., 2007).

The Greek herbal medicine extracts of *Anthemis melanolepis, Cerastium candidissimum, Chamomilla recutita, Conyza albida, Dittrichia viscosa, Origanum vulgare* and *Stachys alopecuros* have been proved to be active against one standards train and 15 clinical isolates of *H. pylori* (Stamatis, 2003).

Successive extracts of *Sapindus mukorossi* and *Rheum emodi* inhibited the growth of 30 resistant clinical isolates of *H.pylori* (*in vitro* and *in vivo*) (Ruggiero et *al*., 2006).

Curcuma longa showed immense therapeutic potential against *H. pylori* infection as it was highly effective in eradication of *H.pylori* from infected mice as well as restoration of *H.pylori* induced gastric damage (Mahady et *al*., 2002).

To the best of our knowledge, there is no extensive global view on exploring medicinal plants for anti-*H.pylori* activity.

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