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INHIBITORY EFFECT OF OIL PULLING (COCONUT OIL AND SESAME OIL) ON *Helicobacter pylori*

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Abstract

Helicobacter pylori associated chronic gastritis and peptic ulcers are treated by antibiotic based therapeutic regimens to which resistant strains of the bacteria became increasingly frequent all over the world. The aim of the current study was to test the *in vitro* effect of plant derived substances on *H. pylori* growth. Coconut and sesame oils in concentrations of 25 %, 50 %, 75 % and 100 % were prepared and tested separately and in mixture on clinical isolates of *H. pylori* from patients having chronic gastritis and a positive rapid Urease test. Hexane 40 % (w/v) was used as a control. The results showed that without dilution both coconut and sesame oils have inhibitory activity on the growth of *H. pylori* with greater activity of coconut oil compared to sesame oil (26 and 9 mm inhibition zones respectively). After dilution, coconut oil continued to exert inhibitory activity with inhibition zones of 16, 20 and 22 mm for the concentrations of 25, 50 and 75 % respectively in contrast to sesame oil which showed no any activity at concentrations of 25 and 50 % dilutions. Mixing the two oils together increased the inhibitory effect on *H. pylori* to result in inhibition zones of 28 and 24 mm for the concentrations of 75 and 100 % respectively. The control solution Hexane showed no activity. In conclusion, higher concentrations of coconut oil alone or in a mixture with sesame oil are superior to sesame oil in inhibiting the *in vitro H. pylori* growth reflecting the potential for its use in the therapy of peptic ulcer disease.

Key words: *Helicobacter pylori*, Coconut oil, Sesame oil and Inhibitory activity.

1. Introduction

Helicobacter pylori are well known bacteria that provoke chronic gastric inflammation which could result in the development of a spectrum of serious gastric diseases like peptic ulcer, gastric carcinoma and lymphoma. These conditions are avoidable when the infection can be prevented or eradicated through the use of proper antibiotic based therapies currently available (Ayala *et al*., 2014). Acquired resistance of *Helicobacter pylori* to a variety of conventional therapy becomes a worldwide problem leading to increased risk of infection which necessitate the continuous updating of medical guidelines required for the management of the infection (Malfertheiner *et al.*, 2012).

Traditional medicine systems have been utilized by human since ancient times as therapeutic agents particularly in developing countries and are still in use because in these countries medical services and availability of advanced chemically based medicinal treatment are suboptimal. Plant derived natural products are promising sources for the development of the new therapeutic agents that are effective against infection (Al Mofleh, 2010). Hundreds of scientific publications are available on the medical journals that investigate the anti - *Helicobacter pylori* activity of several types of herbal products. Examples of these are the anti -

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Helicobacter pylori effect of 13 Malagasy medicinal plants (Cassel Beraud *et al*., 1991). Anti - *Helicobacter pylori* activity of Chinese (Higuchi *et al.*,1999), Mexican study (Ankli *et al*., 2009; Castillo Juarez *et al.*, 2009; Robles - Zepeda *et al*., 2011), Iranian (Hajimahmoodi Mand *et al*.2011) Taiwanese (Wang YC,2015) African(Fabry W and *etal*.1996),(Ndip RN and *et al*., 2007) and Greek (Stamatis *et al.*, 2003). It was claimed that oil pulling can cure many systemic diseases such as migrane, diabetes and asthma as well as improving the strength of teeth (Ashokan *et al*., 2009; Singh *et al.*, 2011). In this present study, the effect of oil pulling (namely coconut oil and sesame oil) was investigated to discover it's *in vitro* anti - *Helicobacter pylori* activity with the aim to identify the role of these plant derived products in the therapy of peptic ulcer disease.

2. Materials and Methods

Clinical isolates of *Helicobacter pylori* were obtained from patients with chronic gastritis who had been referred to the Endoscopy Unit at Al-sadder Hospital, Basrah, Iraq. Antral biopsies from those patients with positive rapid Urease tests were cultured in the Microbiology laboratory using the selective modified Columbia Urea agar (MCUA) (Al-Sulami *et al*., 2008). Coconut oil and Sesame oil of known concentration were obtained from local market in India. Hexane 40 % (w/v) was used as a diluents solution to obtain concentrations of 25 %, 50 %, 75 % and 100 % of both oils as described by Al-Shamma *et al*., 2010).

Bacterial suspension preparation

Few single pure colonies were taken to a sterile normal saline tube with turbidity adjusted to approximately 1.5×10^8 cfu/ml in comparison to McFarland turbidity standard.

Well diffusion agar

Well diffusion method was utilized to detect the inhibitory activity of different concentrations of coconut and sesame oils separately through spreading bacterial suspension of 1.5×10^8 cfu/ml over Mueller Hinton agar plates using sterile cotton swabs. Three wells with a diameter of 6 mm were made on the surface of each previously cultured Mueller Hinton agar plate. Wells were then filled separately with 50 microliter from each concentration of both oils preparations. Control wells containing only Hexane of comparable concentrations were also prepared. Mixtures of both oils in similar concentrations (i.e., 25 %, 50 %, 75 % and 100 % of each) were also tested in a similar fashion. Plates were incubated aerobically at 37 ºC for 24 hrs, after which the diameter of inhibition zones around wells were recorded in millimeters. Tests were performed in triplicate (Valgas *et al*., 2007).

3. Results

With pure solutions (without dilution), both coconut and sesame oils showed inhibitory effect on the growth of *Helicobacter pylori*. While, the control solution Hexane showed no inhibitory activity (Table - 1). The inhibition zones for pure preparation of coconut and sesame oil were 26 mm and 9 mm respectively (Table - 2 & 3).The minimum inhibition zone for coconut oil was 16 mm at a concentration of 25 % while for sesame oil it was 8 mm at a concentration of 75 % (Table - 2 and 3).Unlike coconut oil, sesame oil showed no inhibitory activity against the growth of *Helicobacter pylori* at concentrations of 25 % and 50 % (Table - 4).

Table - 1: The inhibitory effect of coconut oil, sesame oil extracts and Hexane (as control) on *Helicobacter pylori*

Type of extract	Microorganisms
Coconut oil	Sensitive
Cesame oil	Sensitive
Hexane	Resistant

Table - 2: The inhibitory effect of coconut oil extract on *Helicobacter pylori*

Concentration	Inhibition zone of *Helicobacter pylori* (mm)
25 %	16 mm
50 %	20 mm
75 %	22 mm
100 %	26 mm

Table - 3: The inhibitory effect of sesame oil extract on *Helicobacter pylori*

Concentration	Inhibition zone of *Helicobacter pylori* (mm)
25 %	0
50 %	0
75 %	8 mm
100 %	9 mm

When the two oil preparation were mixed together, the zone of inhibition increased to 28 mm at 100 % concentration to 24 mm at 75 % concentration (Table - 4).

Table - 4: The inhibitory effect of coconut - sesame mixture on *Helicobacter pylori*

Concentration	Inhibition zone of *Helicobacter pylori* (mm)
100 %	28 mm
75 %	24 mm

4. Discussion

Medicinal plants and various essential oils are good sources of natural antimicrobial agents. Chinese tea and Iranian grown plants for examples were proved to have *in vitro* inhibitory effects against *Helicobacter pylori* bacteria (Ingolfsdottir *et al*., 1997; Yee *et al.*, 2000; Li *et al.*, 2005). The same is also true for essential oils which were used since ancient times in several communities all over the world for their traditional healing effects mediated through their biological activities. Many preclinical studies have documented antimicrobial, antioxidant, anti-inflammatory and anti-neoplastic activities of essential oils in a number of cells and animal models. However, there is paucity in the use of these essential oils in human studies which limits their effective and safe use as therapeutic agents (Sharifi Rad *et al.*, 2017).

Coconut, *Cocos nucifera* L., is a tree which is cultivated for its multiple uses in nutritional, industrial and medicinal values (Deb Mandal and Mandal, 2011). The composition of crude coconut oil includes a variety of constituents like triacylglycerols (the major component representing 90 %), free fatty acids, partial glyserides, phospholipids, sterols, tocopherols, pigments, volatiles, trace metals and oxidized products. During purification, objectionable impurities such as free fatty acids, phospholipids, pigments, trace metals, the products of oxidation and proteinaceous impurities are removed without considerable loss of neutral oils and tocopherols which are the natural antioxidants. The majority of saturated fat in coconut oil was lauric acid (Mensick *et al.*, 2003). Exposure to lauric acid might be the most inhibitory component to growth of bacteria (Shilling, 2013).

The highest proportions of fatty acid in sesame oil are Linoleic acid, omega 6 and Oleic acid (Vadlapudi and Naidu, 2010). In the current study, higher concentrations of both coconut and sesame oils showed greater inhibitory effects on *Helicobacter pylori* than with diluted solutions. This might be explained by the fact that active components required to inhibit *Helicobacter pylori* are present in small quantities and with serial dilution their amounts became insufficient to prevent bacterial growth. This concept was also highlighted by Vadlapudi and Naidu (2010) who stated that active compounds for bacterial inhibition might be present in insufficient quantities in the extract to show activity and so large doses are needed to prove the activity. On the other hand, the active component might present in high quantities but other constituents exert antagonistic effects of the bioactive compounds that purification and isolation of these active compounds are required to show their real activity.

An interesting finding in the current study was the augmentation of the antibacterial activity after mixing together the two oil substances. This could indicate the increase in the amount of active antibacterial components available in the mixture which powerfully inhibit the bacterial growth. Further purification and utilization of specific constituents of the tested oils are required in the future to evaluate their actual antibacterial activities against *Helicobacter pylori* and their therapeutic potentials.

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