

## In vitro the Antiprotozoal Activity of *Zingibar officinale* Alcohol Extract and Metronidazole in *Entamoeba gingivalis* which Isolated from Patients with Periodontal Disease

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### Abstract

*Entamoeba gingivalis* is parasite found in mouth, causes dental disease. This parasite discovered in dental plaque, the last studies found relation between *E. gingivalis* and periodontal disease. In present study collected 30 samples collected from patient with age (20-30) years, result show infection with this parasite 46.6%. These samples are cultured in TYSGM-9 media, and then treated with different concentration of alcoholic extract ginger and metronidazole which show inhibited growth of parasite at (25, 50, 75, 100 µg/ml. )

**Keywords:** *Entamoeba gingivalis*, ginger, metronidazole.

### الخلاصة

يوجد طفيلي *Entamoeba gingivalis* في الفم مسببا المرض للأسنان. اكتشفت هذا الطفيلي في الكلس الموجود بين الأسنان، إذ وجدت الدراسات الأخيرة علاقة بين الاصابه بهذا الطفيلي وأمراض اللثة. تم في الدراسة الحالية جمع 30 عينة من اسنان المرضى الذين تتراوح اعمارهم بين (20-30) سنة، إذ كانت نسبة الإصابة بهذا الطفيلي 46.6%. تم زرع هذه العينات في وسط زرع TYSGM-9 ، وبعدها عوملت العينات مع المستخلص الكحولي للزنجبيل و عقار الفلاجيل (25 و 50 و 75 و 100 ميكروغرام / مل) إذ وجد ان اكثر تركيز لتثبيط نمو الطفيلي هو تركيز 100 ميكروغرام / مل.

الكلمات المفتاحية: المتحولة اللثوية، الزنجبيل ، عقار الفلاجيل

### Introduction

Periodontitis have different forms of the generality oral disease. About 5% to 20% of people are affected by intense periodontitis. (Burt, 2005) the Parasite has not an intermediate host to infect the host and transmitted through contamination of food and drinks (Idowu *et al*, 2006).

Study was conducted show that *Entamoeba gingivalis* was only protozoan found in saliva and plaque. (Lucht *et al*, 1998) Metronidazole effects on different protozoans like *Entamoeba histolytica* and most Gram-negative and Gram-positive anaerobic bacteria. (Collin *et al*, 1997) *E. gingivalis* is oversensitive to Metronidazole. (Azzouni *et al*, 1994) Ginger is the rhizomes of the *Zingibar officinale* Roscoe; it's used as spice and medicine for some cases like diarrhea, headaches, cold, stomach disorder, nausea and others cases. (Baliga *et al*, 2011; Baliga *et al*, 2012; Haniadka *et al*, 2013) it also has strong anxiolytic and activities against emetic. (Vishwakarma *et al*. 2002). this herbal is safety for many people especially pregnant women. Clinical tests prove ginger have effect against chemotherapy like vomiting and nausea. (Westfall *et al*, 2004)

## Material And Method

### A/ sample collection:

30 samples collected from Private clinic by using sterile cotton swap from patients with periodontitis to detect *Entamoeba gingivalis*.

### B/ Isolation procedures

#### a / examine under microscope:

Used wet mount and normal saline to detect parasite and examined directly under microscope. (Onyido *et al*, 2011).

#### b/ used culture:

Used TYSGM-9 (Trypticase-Yeast Extract-Serum-Gastric Mucin) medium which prepare from Potassium phosphate dibasic 2.8 gm, Potassium phosphate monobasic 0.4 gm, Sodium chloride 7.5 gm, Casein digest peptone 2.0gm, Yeast extract (BBL) 1.0 gm, distilled water 970 ml. then incubated parasite at 37°C for 48-72 hours (Diamond *et al.*, 1982), in this study does not use animals.

#### C/ Prepared extract of ginger and metronidazole:

Ginger powder gets from market (50gm). Mix powder with 100ml ethanol (99.9%) in an electric blender for 30 min. This suspension was filtered. Then methanol was removed in a rotary evaporator to produce a dry powder. To obtain concentrations 12.5µg/ml, 25µg/ml, 50µg/ml and 100 µg/ml, powder was dissolved in ethanol (Naz *et. al.*, 2007).

#### D/Prepare dilution of Metronidazole

Bought dissolved Metronidazole 500mg/ml from pharmacy, which prepare from it concentrations 12.5µg/ml, 25µg/ml, 50µg/ml and 100 µg/ml.

#### E/ Used spectrophotometer at 540nm.

## Result

30 samples were collected from patients aged 20 to 30 years, found 46.6% was positive with *Entamoeba gingivalis*. All samples were positive when cultured in TYSGM-9.

In present study was investigated *in vitro* effect of Ethanol Extract of ginger (*Zingiber officinale*) on the growth and motility of *Entamoeba gingivalis* compared to the standard drug metronidazole with significant at the 0.05 level

Growth of parasite show in figures 1 and 2. Take drop of broth and calculate dead *E. gingivalis* / 100, which show 53% of *E. gingivalis* was killed with ethanol extract of ginger and 89% of this parasite killed with metronidazole.

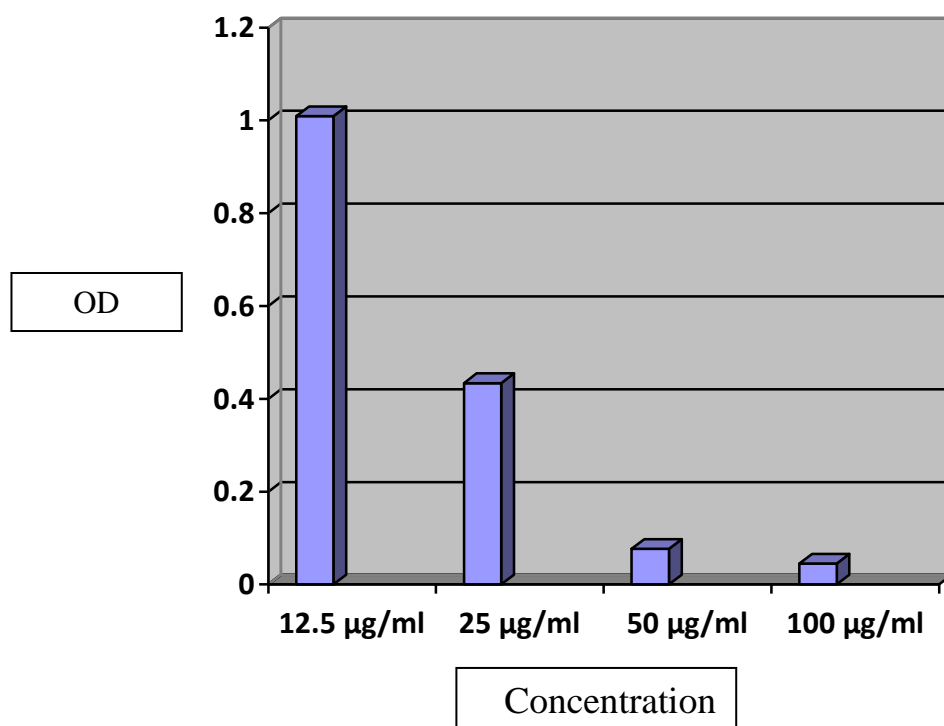


Figure 1: the effect of alcoholic extract of ginger on *Entamoeba gingivalis*

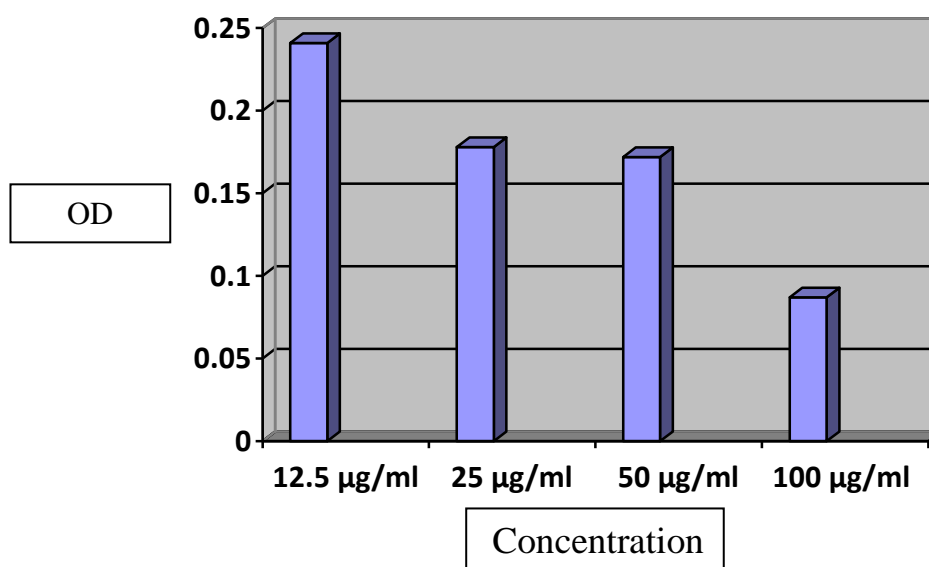


Figure 2: the effect of metronidazole on *Entamoeba gingivalis*

## Discussion

The trophozoite of *Entamoeba gingivalis* ranges in size from 8 to 20  $\mu\text{m}$  and morphologically resembles that of *E. histolytica* found in mouth. In present study samples take from people whom have from continue inflammation in periodontal are more infection with parasite especially *Entamoeba gingivalis*. In this study show rate of infection with parasite among people with age 20-30years, which was 46.6%, this result was closely to another study in Babylon city rate of infection with *E. gingivalis* was 42.9 % (Al-hamiary *et al.*, 2011). while 31.67% rate of infection with parasite in another study (Onyido *et al.*, 2011) in culture noted all of them are grow in 37C° for 48-72 hours (Gannon JT and Linke H A, 1990). Cause of infection with *E. gingivalis* possibly to no oral hygiene (Onyido *et al.*, 2011) or this parasite is opportunists (Talaro and Talaro, 2002).

After cultured parasite and deal with extract of ginger, noted growth of parasite was inhibition in 100  $\mu\text{g/ml}$  (0.045) in compare with metronidazole 100  $\mu\text{g/ml}$  (0.087) (figure1and2) with significant difference. This result show the effect of ginger on growth of parasite like another research which proves the effect of ginger as inhibited for parasite and microorganisms (Aly and Mantawy , 2013). In other study, used ginger against *Haemonchus contortus* which killed all of these worms (Qbal *et al.*, 2001). We don't found any research conformity with this study.

Metronidazole was killed *E. gingivalis* and this result was similar to another result in other research which insures Metronidazole killed the parasite (Eloufir *et al.*, 2014) while other study prove metronidazole treat amoebiasis (Klasco ,2006).

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