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## In vitro the antiprotozoal activity of *Allium sativum* extract and metronidazole in *Entamoeba gingivalis* which isolated from patients with periodontal disease

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### Abstract

*Entamoeba gingivalis* is a parasitic protozoa of the oral cavity, The *E. gingivalis* is transferred between humans directly by oral contact or through the common usage of crockery. The infective form is trophozoites. The last studies found relation between *E. gingivalis* and periodontal disease. In present study collected 30 samples collected from patient with age (21\_30) years, result show infection with this parasite 53.3 %. These samples are cultured in TYSGM-9 media, and then treated with garlic (*Allium sativum*) extract alcoholic and metronidazole which show inhibited growth of parasite at 100 µg/ml.

**Keywords:** *Entamoeba gingivalis*, *Allium sativum*, metronidazole.

### Introduction

*Entamoeba gingivalis*, is a cosmopolitan amoeba that is present in the human oral cavity. It is a protozoon with a simple development cycle which has diagnosis only one stage –the trophozoite, which is characterised by a variable shape. It does not create cysts [1]. *E. gingivalis* was originally isolated and described by Gros[2], but subsequent studies on this parasite are scarce, outdated, and controversial, mainly due to the difficulty in maintaining *E. gingivalis* in vitro [3,4]. To date, the pathogenicity of *E. gingivalis* has not been demonstrated. The impact of the infection on the course of inflammatory processes in the oral cavity may be supported by the fact that *E. gingivalis* occurs more frequently amongst people with alterations of the mucous membrane of the oral cavity, inflammation of the palatal tonsils and paranasal sinuses, as well as amongst those with bad oral cavity hygiene and the sick with a lowered body immunity [5]. Up to 95%

of the population with poor oral hygiene may be infected with *E. gingivalis* [6]. However, indirect contamination may occur through sharing food, cups, cutlery and other fomites [7]. In the oral cavity, this protozoon occurs mainly on gingival edges, in interdental spaces, carious lesions, paranasal sinuses, the alveolar pyorrhoea, tonsillar crypts, and in bronchial mucus. It has also been found in the contents of lung abscesses. If the protozoon is found in bronchial secretion, it is necessary to differentiate between *E. gingivalis* and *E. histolytica*, which is based on the ability of *E. gingivalis* to phagocytose leucocytes [8]. Metronidazole, a molecule previously demonstrated to be active against anaerobic bacteria and parasites [9]. Several studies indicated the effectiveness of this antibiotic against *E. gingivalis* [10,11]. Garlic (*Allium sativum*) is one of the edible plants which has generated a lot of interest during human history as a medicinal panacea, this useful plant has several therapeutic uses, including lowering blood pressure and reducing the high level of cholesterol in the blood and acts as an anticoagulant, garlic as well as working to expel intestinal worms and inhibit amoeba dysentery and Giardia growth in addition to its effectiveness against a wide range of microorganisms including many Gram-negative and Gram-positive bacteria, fungi, protozoa and viruses [12,13,14]. Chemical analyses of garlic cloves have revealed an unusual concentration of sulfur-containing compound (1–3%) [15].

## **MATERIAL AND METHOD:**

### **A/ sample collection:**

30 samples collected from dental patients in Private clinic to detect *Entamoeba gingivalis*.

### **B / examine parasite under microscope:**

By using sterile cotton swap and examined directly under microscope to find the parasite. [16]

### **C/ used culture:**

In current study used TYSGM-9 (Trypticase-Yeast Extract-Serum-Gastric Mucin) medium to incubate *E. gingivalis* at 37°C for 48-72 hours. This media preparation from Potassium phosphate dibasic 2.8 gm, Potassium phosphate monobasic 0.4 gm, Sodium chloride 7.5 gm, Casein digest peptone 2.0 gm, Yeast extract (BBL) 1.0 gm, distilled water 970 ml. then incubated parasite at 37°C for 48-72 hours [17].

### **C/ used extract of Garlic ( *Allium sativum* ) :**

Garlic was dried at room temperature for several days then crushed to get powder, take (50 gm) from it to mix with 100 ml 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 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml, powder was dissolved in ethanol (Naz et. al., 2007).

### **D/Metronidazole**

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

### **E/ spectrophotometer have been used at 540 nm.**

## **Results**

After examination of collected samples, found 53.3 % 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 *Allium sativum* on the growth and motility of *Entamoeba gingivalis* compared to the standard drug metronidazole with significant differences at the 0.05 level. Growth of parasite shown in diagram 1 and 2. Take drop of broth and calculate dead *E. gingivalis* / 100, which show 55% of *E. gingivalis* was killed with ethanol extract of Garlic and 86% of this parasite killed with metronidazole.

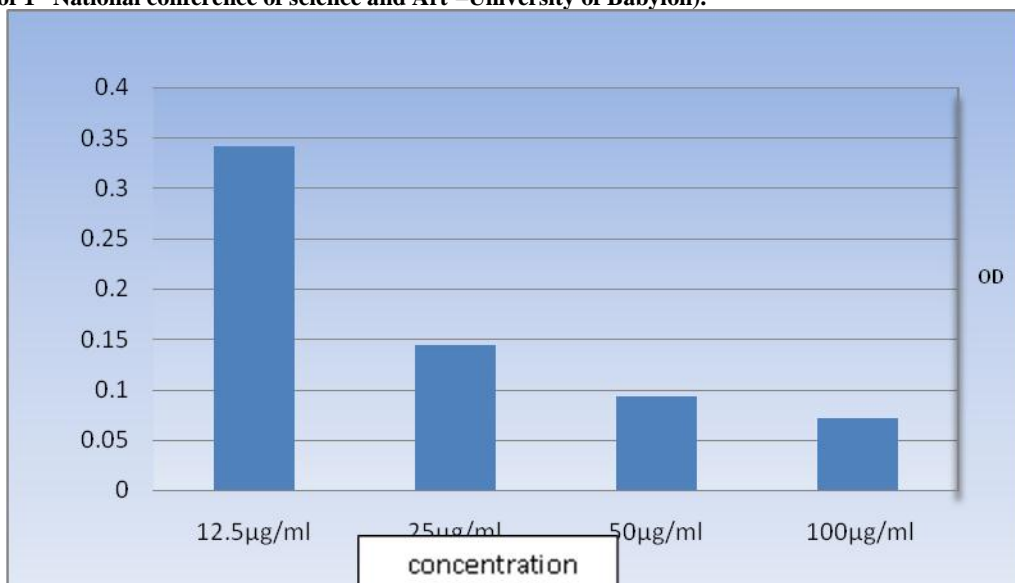


Figure 1: the effect of alcoholic extract of *Allium sativum* on *Entamoeba gingivalis*

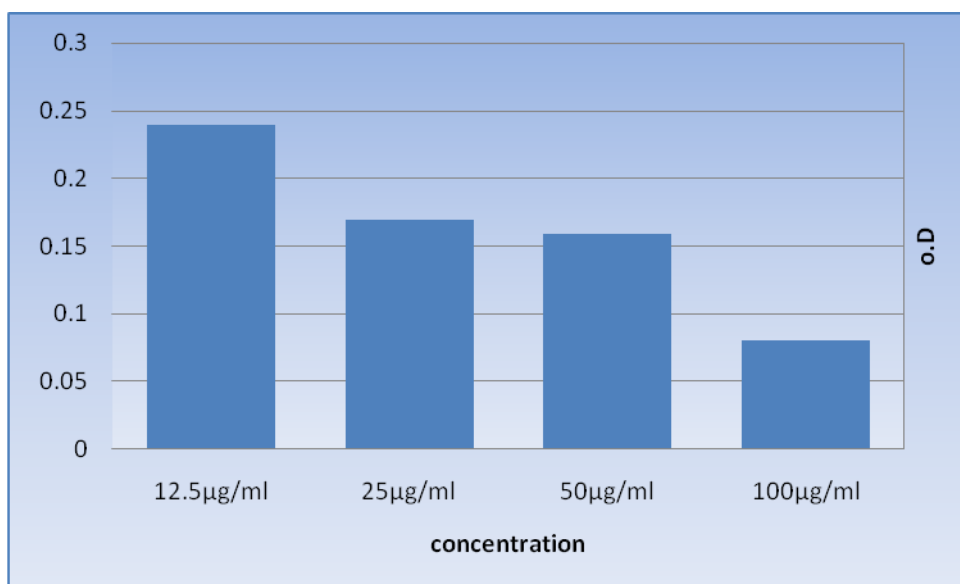


Figure 2: the effect of metronidazole on *Entamoeba gingivalis*

### Discussion

The present study was aimed at determining the prevalence of *Entamoeba gingivalis* between patients with periodontal disease and to enhance the role of plant extracts in the treatment of parasitic infections as an alternative to medical drugs and avoid its side effect. Our research has shown that 53.3 % of surveyed people are infected with *E. gingivalis*. Our result was closely to another study in Babylon city which show that the rate of infection with *E. gingivalis* was 53% [18]. On the other hand, this result was higher than another study which refers to infection with *E. gingivalis* was 31.67% [19]. While the incidence of this parasite was higher in another study reached to 72% [1]. The incidence of this parasite, as well as the intensity of the infection, is correlated with oral hygiene status and the use of various preparations intended for maintaining oral cavity hygiene or probable these parasites are opportunists particularly lesions gingivalis and periodontal pockets [19,16].

After cultured parasite and deal with extract of garlic, noted growth of parasite was inhibition in 100 µg/ml (0.071) in compare with metronidazole 100 µg/ml (0.080) (figure 1). This result show the effect of garlic on growth of parasite like another research which proves the effect of garlic as inhibited for parasite and microorganisms [20,21]. In other studies, used garlic against *Entamoeba histolytica* which is very sensitive to garlic extract [22,23]. Other studies have found that garlic extract also very efficiently inhibited the growth of other protozoan parasites such as *Giardia lamblia*, *Leishmania major*, *Leptomonas colosoma*, and *Crithidia fasciculata* [24].

The antimicrobial activity of garlic is believed to be due to a sulphur-containing compound known as allicin [22]. Metronidazole was killed *E. gingivalis* (figure 2) and this result was similar to another result in other research which insures Metronidazole killed the parasite [25]. Another study also proved that the number of *E. gingivalis* trophozoites was also reduced after metronidazole treatment [26]. While other study prove metronidazole treat amoebiasis [27].

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