Evaluation the Biological Activity of *Ocimum basilicum L.* as Antibacterial Agent in Some Diarrhoeal Bacteria.

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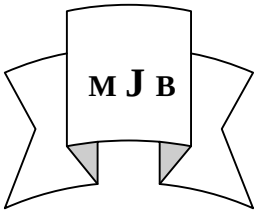

Abstract

The antidiarrhoeal activities of leaf extracts of *Ocimum basilicum L.* is investigated by disc diffusion and tube dilution methods. The extracts were active against, *Escherichia coli , Salmonella typhi, Enterococcus faecalis and Shigella dysenteriae.*

The leaf extract was more active against *S. dysenteriae* and least active against *S. typhi.* The sensitivity of the organisms measured in terms of zone of inhibition ranged from 8 to 19.5 mm. The minimum inhibitory concentrations were from 8 to 50 mg ml^{-1}, while the minimum bactericidal concentration ranged from 16 to 62 mg ml^{-1}.

الخلاصة

أجريت هذه الدراسة لتقييم فعالية مستخلص الماء الحار لنبات*O.basilicum* ضد بعض أنواع البكتريا الممرضة والمسببة للإسهال .وقدتم التحري عن فعالية المستخلص بطريقة انتشار المستخلص في الاكار الحاوي على بكتريا الاختبار،وكذلك تم تحديد التركيز المثبط الأدنى والتركيز القاتل الأدنى وذلك بعمل تخافيف من المستخلص ومعاملتها مع بكتريا الاختبار في وسط زرعي سائل.وكان المستخلص فعالا ضد بكتريا *S. typhi and E.coli , E. faecalis, S. dysenteriae.*

أن مستخلص الأوراق كان أكثر فعاليه ضد بكتريا *S. dysenteriae.* وأقل فعاليه ضد بكتريا *S.typhi*

إن أقطار التثبيط في نمو البكتريا تراوحت بين (19.5 - 8 مليمتر) وتراوح معدلات التركيز المثبط الأدنى بين (50 - 8 ملغم\ )$^{-1}$ بينما كانت معدلات التركيز القاتل الأدنى (62-16 ملغم\ مل$^{-1}$).

Introduction

Medicinal plants constitute effective source in medicine especially when some bacteria shown a relative resistance against some antibiotics.

Herbal medicine shown a very important utility especially in rural population who depend on it as primary health care. The family lamiacea (which the plant of the test belongs to) have an important role in herbal medicine Among many number of medicinal plants [1]**.**

The extracts of the leaves or the whole plant of *Ocimum basilicum* is popular for the treatment of diarrhoea

The problem of increasing rate of resistance of bacteria to appropriate drugs and antibiotics promoted this investigation.

The activity of leaves extract against some of the bacterial species associated with diarrhoea is discussed here.

Materials and method

1-Plant material

Fresh leaves of *O.basilicum* were collected from plants grow in the north and middle of Iraq.

2-Extraction

The dried leaves were milled into a fine powder. The ingredients of the powdered leaves (10 g) were then extracted with (200 mL) of distilled water in a Soxhlet extractor apparatus

at(40-50°C) for(24 hours) according to Mistcher [2]. The resulting extract was sterilized by using a membrane filtration unit.

3-Bacteria of the test

The following bacteria were isolated from stole samples of diarrhoeal patients :*Escherichia coli, Salmonella typhi ,Shigella dysenteriae* and *Enterococcus faecalis* and then identification of these bacteria is down according to Baron [3].

The organisms were maintained on blood agar slopes and brain heart infusion agar at (4°C) and sub cultured on nutrient broth before (24 hours) of the bacterial samples were used [4].

4-Bacterial sensitivity test

By taking (0.1 mL) of each sub cultured bacterial samples(each o.1 ml containing (1.5×10^8 cell per mL) equivalent to McFarland tube number (0.5) according to Barry [5] and each bacterial sample were spread with sterile glass spreader on sterile Muller-Hinton agar medium plates.

Sterile filter paper discs previously soaked in the leaf extract were placed on the plate of each bacterial sample and the plate were incubated at(37ºC)for (24-48 hours). Distilled water was used as a control .Examine for zone of inhibition [6]

5-Determination of MIC and MBC

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts were determined by diluting the extracts to various concentrations (0.0-60.0 mg mL^{-1})by using nutrient broth in test tubes. Each test tube was inoculated with a bacterial suspension containing ($1.5x10^8$)cells per mL and incubated (at 37 ºC) for(24 hours). The MIC was regarded as the lowest concentration of the extract that did not permit any visible growth when compared with plant extract free broths inoculated with each of the bacterial suspensions. The MBC was regarded as the lowest concentration of the extracts that prevents the growth of any bacterial colony on solid medium .The MBC was determined using the method of Rotimi [7]. Tubes that showed no visible growth were streaked on fresh nutrient agar plates, incubated at 37C° for 24h and examined for growth.

Results

The sensitivity of the aqueous extract of *O. basilicum* measured by the zone of inhibition varies from (means + 1 SEM) 8 ±1.8 mm in *S. typhi* to 19.5 ±.6 mm in *S.dysenteriae.* Table 1. The antibacterial activity of the leaf extracts was measured in terms of MIC and MBC. The lowest minimum inhibitory concentration of 8 mg mL^{-1} was against *S. dysenteriae*, while the highest value of 50 mg mL^{-1} was against *S. typhi* Table 2. Also the MBC ranged from 16 mg mL^{-1} in *S.dysenteriae* to 62 mg mL^{-1} in *S. typh* Table 2.

Table 1 Sensitivity of the bacteria to leaf extract of *O. basilicum* determined by the zone of inhibition in mm.

Bacteria of the test	Zone of inhibition* (mm)
S. dysenteriae	19.5 ± 0.6
E.faecalis	15.5 ± 0.3
E. coli	11.5±1.5
S. typhi	8.0±1.8
Sterile distilled water (control)	0
*means ± SEM of 3 values	

Table 2 Antibacterial activities of leaf extracts of *O. basilicum* measured in terms of MIC and MBC

Bacteria of the test	MIC (mg mL $^{-1}$)	MBC (mg mL^{-1})
S. dysenteriae	8	16
E.faecalis	16	31
E. coli	32	45
S. typhi	50	62

*MIC (minimum inhibitory concentration)

* MBC(minimum bactericidal concentration)

Discussion

The aqueous extracts of the leaves of *O. basilicum* contain substances with antibacterial properties. This agrees with the works of Olowokudejo and Sofowora [8, 9]. The extracts were active against the following bacteria of medical importance: *S. dysenteria , E. faecalis, E. coli , and S. typhi.* Some of the infections by these bacteria include diarrhoea, gastrointestinal disorders, and typhoid fever. Resistant strains of these organisms to many pharmaceutical drugs have been widely reported.

The extract was most active against *S. dysenteriae* and least active against *S. typhi*. Previous chemical analyses of the plant showed the presence of thymol and eugenol[10] that might be responsible for the antibacterial properties. The MIC values of the extract were lower than the MBC values, suggesting that the plant extract is bacteriostatic at lower concentrations but bactericidal at higher concentrations. These results offer a scientific rationale for the traditional use of the aqueous extract for the treatment of diarrhoeal diseases.

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