Effect of Silver Nan particles on Microbial Activity of Teucrium Polium Extracts

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ABSTRACT

In this research we study the effect of different extracts of Teucrium polium (cold water, hot water and alcohol extraction), effect of silver nanoparticles and the combination effect of Teucrium polium extracts with silver nanoparticles against different pathogenic microorganisms Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus volgaris, Staphylococcus aureus, Streptococcus and Candida albicans. The results showed that the effect of cold water extract on microbial activity only inhibited the growth of P. aeruginosa. In case of hot water extract there is no effect on any type of microorganisms used in this study unlike the ethanolic extract it is affect on the growth of all microorganisms used except *P.* volgaris and E. coli. In the using of silver nanoparticles it inhibits the growth of all the microorganisms used in this research but in different ratios. In case of combination the effect will increase with assistant of silver nanoparticles except in *P.* aeruginosa and K. pneumonia the effect of silver nanoparticles alone is higher

Keywords: Teucrium Polium, Silver Nanoparticles, Microbial Activity.

دراسة تأثير جزيئات الفضة النانوية على الفعالية المايكروبية لمستخلصات نبات الجعدة

الخلاصة

في هذا البحث تم دراسة تاثير المستخلصات المختلفه لنبات الجعدة (الاستخلاص باستخدام الماء البارد, باستخدام الماء الحار والاستخلاص باستخدام الكحول), ودراسة تأثير جزيئات الفضه النانوية, وايضا دراسة تأثير من مزيج مستخلص نبات الجعده مع جزيئات الفضة النانوية على فعالية بعض الاحياء المجهرية (Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus volgaris, Staphylococcus aureus, Streptococcus and Candida albicans). وأظهرت النتائج ان تأثير المستخلص الذي تم استخلاصه بالماء البارد يمنع فقط نمو بكتريا P. Aeruginosa. وفي حالة الاستخلاص الذي تم بالماء الحار لايوجد اي تأثير على نمو الاحياء المجهرية المستخدمة في هذا البحث على عكس استخدام المستخلص الذي تم عن طريق الكحول فقد اعطى تأثير على جميع الاحياء المجهرية المستخدمة في هذا البحث ماعدا P. volgaris وE. Coli. عند استخدام جزيئات الفضة النانوية قد تم منع جميع انواع الاحياء المجهرية المستخدمه في هذا الدراسة وبمعدلات مختلفه. اما

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في حالة استخدام مزيج من مستخلص نبات الجعدة وجزيئات الفضه النانويه فأن تاثير المزيج على منع نمو الاحياء المجهرية قد ازداد ماعدا بالنسبة لبكتريا P. aeruginosa وK. pneumoniae والذي كان تاثير جزيئات الفضة النانونية لوحده اقوى من مزيج المستخلص مع جزيئات الفضة النانوية في منع نمو البكتريا.

INTRODUCTION

Teucrium polium is a durable, wild-growing, flowering grass plant that can grow to 10-30 cm, has a callous white exterior, and abounds in southwestern Asia, Europe, and North Africa [1]. Teucrium polium has been long recognized in folk medicine in the treatment of many pathophysiological conditions, such as gastrointestinal disorders, inflammations, diabetes and rheumatism. Its extract has been shown to induce hypotensive [2], anti-inflammatory, hypoglycemic, antispasmodic, antibacterial and antipyretic effects [3].

The development of new resistant strains of bacteria to current antibiotics [4] has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides [5]. Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging [6], sensing, targeted drug delivery [7] and gene delivery systems and artificial implants [8]. The new age drugs are nanoparticles of polymers, metals or ceramics, which can combat conditions like cancer [9] and fight human pathogens like bacteria [10].

Silver has long been known to exhibit a strong toxicity to a wide range of micro-organisms [11]; for this reason silver-based compounds have been used extensively in many bactericidal applications [12]. Silver compounds have also been used in the medical field to treat burns and a variety of infections [13]. Several salts of silver and their derivatives are commercially employed as antimicrobial agents [14]. However, the bactericidal property of these nanoparticles depends on their stability in the growth medium, since this imparts greater retention time for bacterium– nanoparticle interaction. There lies a strong challenge in preparing nanoparticles of silver stable enough to significantly restrict bacterial growth [15].

MATERIALS AND METHODS

Preparation of plant extract

Aerial parts of Teucrium polium (TP) was dried for 7–10 days at room temperature. The dried plant material was extracted in three ways:

1-Extract by water

The dried plant ground to powder. 20 g of powder was extracted with 100 ml distilled water by maceration at 32 ˚C for 48 h and shaked intermittently and then the solution was filtered twice and evaporated at 35 ˚C. The concentrated plant extract used by dissolved in distilled water just before experiments [16].

2- Extracted by boiling

25 g was heated in 300 ml of distilled water for 15 min at 95 ºC, followed by rapid filtration through a cellulose filter and then What man No.1 filter paper and dried on 40 ºC [17]. The dried extract was dissolved in distilled water just before experiments.

3- Alcoholic extraction

30 gm the plant powder extracted with 200 ml of 95% ethanol by using Sox let for 48 hour, followed by rapid filtration through What man No.1 filter paper and dried on 40 ºC [18]. The dried extract was dissolved in distilled water just before experiments.

SYNTHESIS OF SILVER NANOPARTICLES

Aqueous solution of silver nitrate ($AgNO_3$) at concentration of 0.02 mmol/mL was prepared and used for the synthesis of silver nanoparticles. 10 mL of *Teucrium polium* (TP) extract was added into 90 mL of aqueous solution of 0.02mmol/mL silver nitrate for reduction into Ag+ ions and exposed to bright sunlight at 50 °C; the change of color takes place within few minutes from yellowish to reddish brown color [19].

Biological Assay to Evaluate Combined Effect

The silver nanoparticles synthesized using Teucrium polium (TP) extracts was tested for antimicrobial activity by agar well diffusion method against different pathogenic microorganisms Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella pneumoniae (K. pneumoniae), Proteus volgaris (P. volgaris) (Gram negative), Staphylococcus aureus (S. aureus), Streptococcus (Gram positive) and Candida albicans (C. albicans) (Yeast). Mueller – Hinton agar (MHA Oxoid) was prepared, sterilized and cooled to 45°C, then distributed into Petri dishes. Inoculum of 10^6 cfu/ml for each strain according to McFarland turbidity standards was inoculated by swabbing on the Mueller-Hinton agar plates, and then wells of 5mm diameter were prepared in each agar plate.

The wells were loaded with 100µl of Plates were incubated at 37°C for 24hrs, and at the end of the period, the inhibition zones were measured in mm.

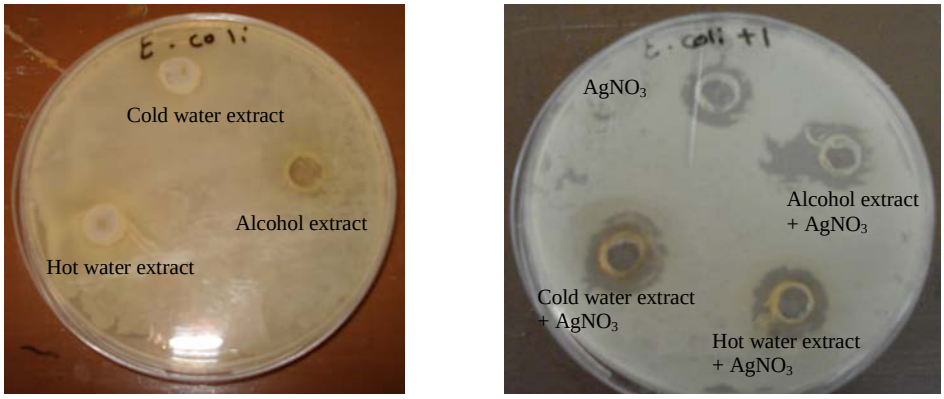
RESULTS AND DISCUSSION

Based on the inhibitory zone diameters the results in Table (1) showed that the effect of Teucrium polium cold water extract on microbial activity only inhibited the growth of P. aeruginosa Figure (2-A). In case of hot water extract there is no effect on any type of microorganisms used in this study unlike the ethanolic extract it is affect on the growth of all microorganisms used except P. volgaris and E. coli Figure (1-A, 2-A, 3-A, 4-A, 5-A, 6-A & 7-A) and this is agree with Zerroug et al. according to E. coli, but did not agree according to P. aeruginosa [20]. Also according to S. aureus and P. aeruginosa agree with Khaled et al. [21], but disagree with them according to E. coli. And disagree with Mansour and Soudabe [22] who reported T. polium inhibit the growth of E. coli O157 in the early stages of its growth. Cells were more vulnerable to the inhibitory effects of plant extracts during exponential growth versus other growth phases showed that T. polium effected greater inhibition of *E. coli O157*, a major gastrointestinal pathogenic microbe.

Table (1) Effect of Teucrium polium extracts without silver nanoparticals ($AgNO_3$) on microbial activity.

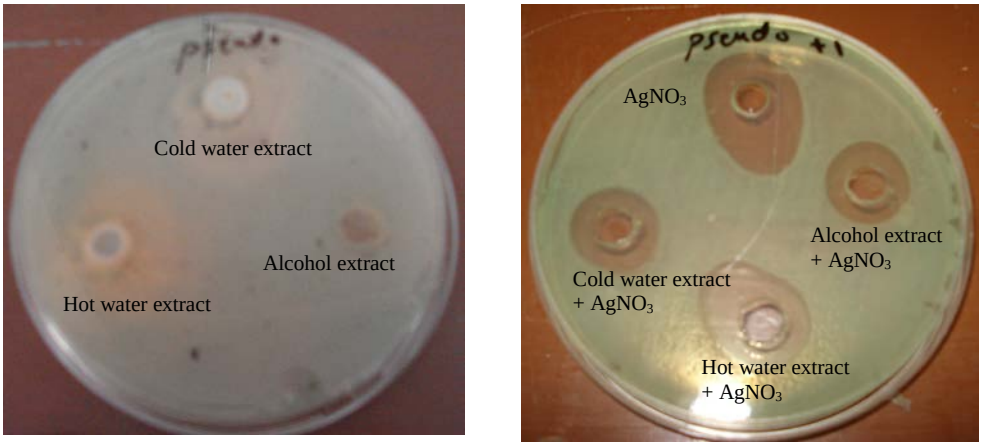
	cold water	**hot water**	**alcohol**
E. Coli	0	0	0
Proteus	0	0	0
Pseudomonas	11	0	11
Klebsella	0	0	13
Staphylococcus aureus	0	0	12.5
Streptococcus	0	0	12
Candida albicans	0	0	15

Inhibition zone in mm



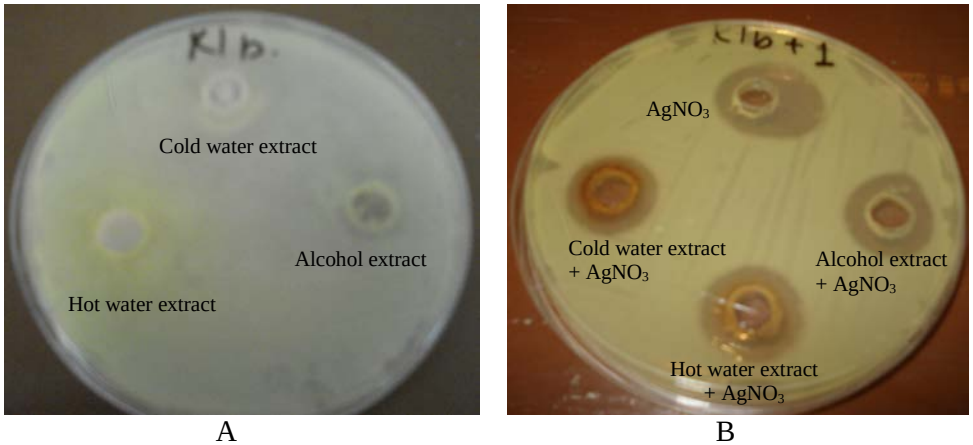
A B

Figure (1) Effect of -A- different T. polium extracts on Escherichia coli, B- different T. polium extracts with nanoparticles.



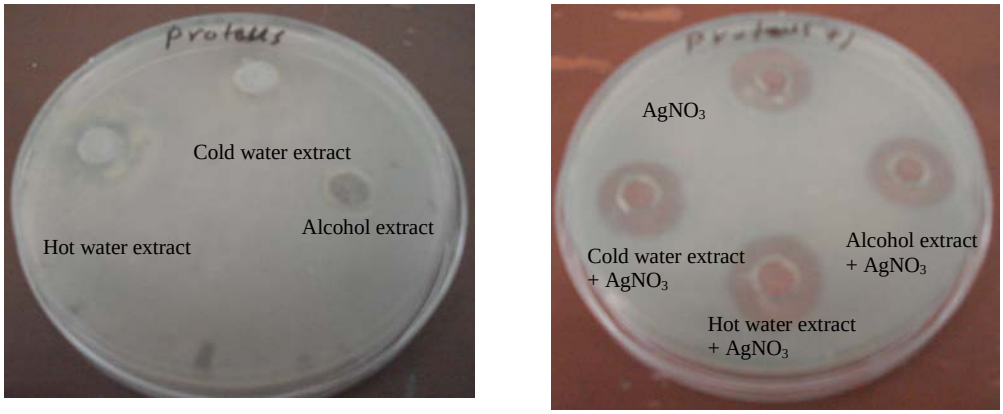
A B

Figure (2) Effect of -A- different T. polium extracts on Pseudomonas aeruginosa, B- different T. polium extracts with nanoparticles.



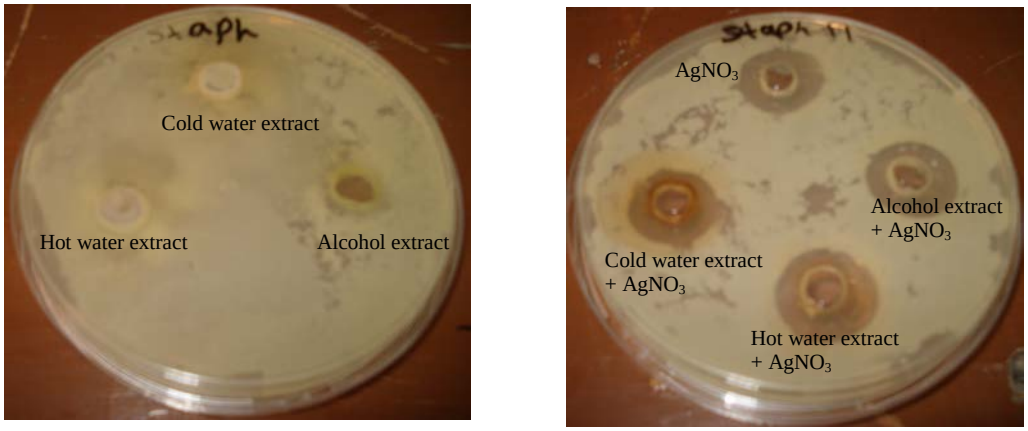
A B

Figure (3) Effect of -A- different T. polium extracts on Klebsiella pneumoniae, B- different T. polium extracts with nanoparticles.



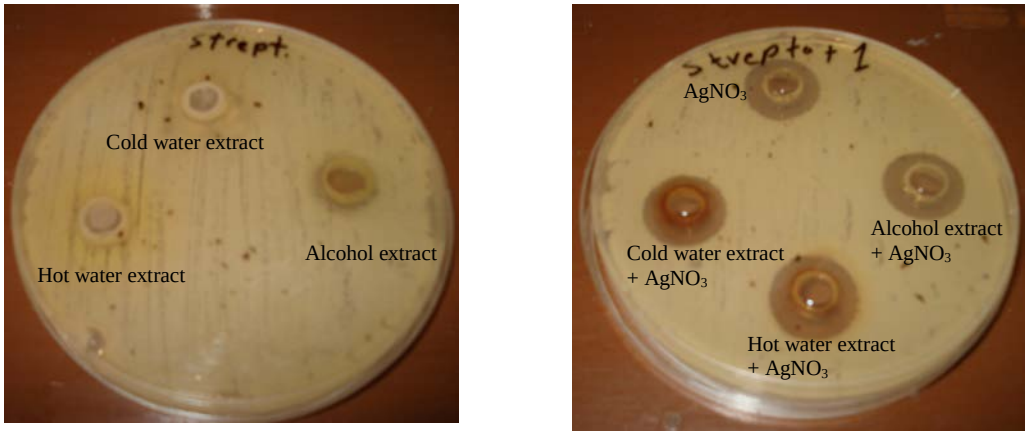
A B

Figure (4) Effect of -A- different T. polium extracts on Proteus volgaris, B- different T. polium extracts with nanoparticles.



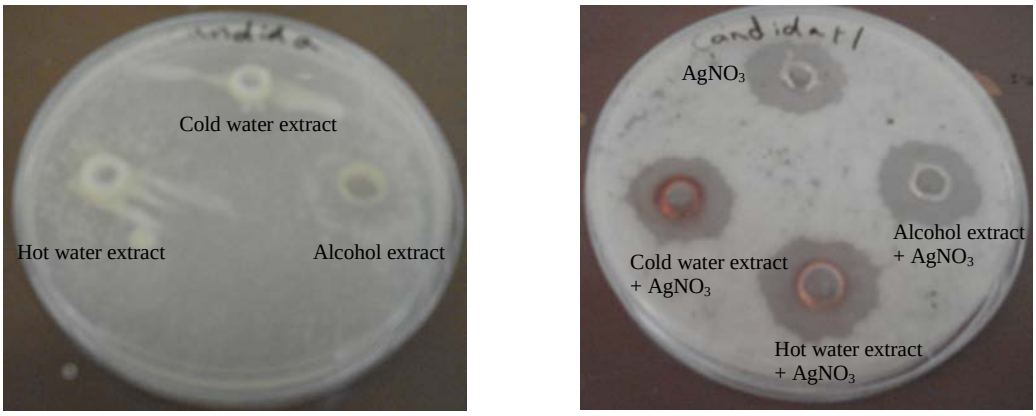
A B

Figure (5) Effect of -A- different T. polium extracts on Staphylococcus aureus, B- different T. polium extracts with nanoparticles.



A B

Figure (6) Effect of -A- different T. polium extracts on Streptococcus, B- different T. polium extracts with nanoparticles.



A B

Figure (7) Effect of -A- different T. polium extracts on Candida albicans, B- different T. polium extracts with nanoparticles.

In case of using silver nanoparticles it inhibit the growth of all the microorganisms used in this research but in different ratios the greatest effect was on P. aeruginosa then K. pneumoniae, C. albicans, Streptococcus, P. volgaris, S. aureus, and E. coli respectively Figure (1-B, 2-B, 3-B, 4-B, 5-B, 6-B & 7-B). This is disagreeing with Jun et al. results, Ag nanoparticles were most effective against E. coli. For S. aureus, however, Ag nanoparticles showed a mild growth-inhibitory effect even in high concentration [23]. Also disagree with Ivan and Branka if 10^4 CFU were applied to the plate, a concentration of nanoparticles of 20 μg cm^{-3} completely prevented bacterial growth, and the study shows that silver nanoparticles have excellent antibacterial activity against E. coli [24].

Ag-NPs have been known for it's inhibit and bactericidal activity and more than 85% major pathogens were inhibited by this new material [25].

In this study also we make a combination between *T. polium* three different types of extract (cold water, hot water and ethanol extraction methods) with silver nanoparticles Table (2) and study their effect on microbial growth and the results showed that the effect will increase with assistant of silver nanoparticles except in *P. aeruginosa* and *K. pneumoniae* the effect of silver nanoparticles alone is higher than when it combined with *T. polium* extracts Figure (2-B& 3-B) but in *E. coli* the combination between silver nanoparticles and *T. polium* alcohol extract give the higher growth inhibition Figure (1-B). In case of gram negative bacteria *P. volgaris*, gram positive bacteria *Staphylococcus aureus* and *Streptococcus* and Yeast *Candida albicans* the higher growth inhibition was in the combination between silver nanoparticles with *T. polium* hot water extract Figure (4-B, 5-B, 6-B & 7-B).

Table (2) Effect of Teucrium polium extracts with silver nanoparticals ($AgNO_3$) on microbial activity (inhibition zone in mm).

	AgNo3	cold water	hot water	alcohol
E. Coli	15	15	14	23
Proteus	18	18	20	17
Pseudomonas	30	17	24	16
Klebsella	24	17	19	16
Staphylococcus aureus	17	20	21	19
Streptococcus	19	16	20	18
Candida albicans	21	20	23	20

Inhibition zone in mm

The mechanism of the growth-inhibitory effects of Ag nanoparticles on microorganisms has not been well understood. One possibility is that the growth inhibition may be related to the formation of free radicals from the surface of Ag. Uncontrolled generation of free radicals can attack membrane lipids and then lead to a breakdown of membrane function [26].

The major mechanism through which silver nanoparticles manifested antibacterial properties is by anchoring to and penetrating the bacterial cell wall, and modulating cellular signalling by dephosphorylating putative key peptide substrates on tyrosine residues. Silver nanoparticles act primarily in three ways against Gram-negative bacteria: (1) nanoparticles mainly in the range of 1–10 nm attach to the surface of the cell membrane and drastically disturb its proper function, like permeability and respiration; (2) they are able to penetrate inside the bacteria and cause further damage by possibly interacting with sulfur- and phosphorus-containing compounds such as DNA; (3) nanoparticles release silver ions, which have an additional contribution to the bactericidal effect of the silver nanoparticles. Although bacterial cell lysis could be one of the reasons for the observed antibacterial property, nanoparticles also modulate the phosphotyrosine profile of putative bacterial peptides, which could thus affect bacterial signal transduction and inhibit the growth of the organisms [15].

Integrates nanotechnology and bacteriology, leading to possible advances in the formulation of new types of bactericides. However, future studies on the biocidal influence of this nanomaterial on other Gram positive and Gram-negative bacteria are necessary in order to fully evaluate its possible use as a new bactericidal material.

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