Faculty of Agriculture & Veterinary Medicine, Thamar University

Yemeni Journal of Agriculture & Veterinary Sciences

Available online at; WWW.YJAVS.COM

DOI: xxxx.xxxx

In vitro study on antimicrobial efficacy of Rumex nervosus L. extracts on *Staphylococcus aureus* , *Escherichia coli*, and *Candida albicans*

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Article history:

Received

٢٠ May 2014

Accepted

25 June 2014

ABSTRACT

Rumex nervosus L. (Polygonaceae) crude extracts were evaluated for antibacterial and antifungal activities . Methanolic and aqueous crude extracts of seeds and leaves were prepared. The tested microorganisms were clinical isolates of *Escherichia coli* (G-), *Staphylococcus aureus* (G+), and *Candida albicans*, The antimicrobial effects were detected by total colonies count and by using well diffusion method. Methanol and water leaves extracts showed a notable effects on the three microbes , while seeds extractions showed low affectivities. Methanol extraction also had more effective than aqueous one,The results showed that *E.coli* isolates are more sensitive to the crude extracts than *S.aureus.*

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Keywords: *R.nervosus* ,leaves extraction, methanol extraction , antimicrobial effecacy

INTRODUCTION

In spite of different synthetic drugs, peoples still have trust to use herbals and plant materials to treat their infections and diseases. Medical plants were investigated around the world , beside the best ways for extraction and estimation the effective compounds(Islam,et al;2006 ; Thakare,2004 ; Adejumo and Bamidele ;2009 ; Essam, et al; 2008 ; Chhabra,et al; 1984 ; Anesini and Perez ;1993)

One of these plants is the genus *Rumex* (Polygnoceae). It has more than 200 species distributed in temperate regions(www.@efloras.org). *Rumex* spp. is a perennial or less commonly annual shrubs. The antimicrobial activity of different species related to the genus were detected (Al-Dubai and Al-khulaidi; 1996; Farrukh ,2010 ; Panduraju, et al;2009 ; Senha,et al;2011; . Mostafa and Eman;2011). The plant was used in other cases in folk medicine such as antiflammotary, antitumor, astringent ad anti dermatitis (Ramzi, et al;2010 ; Fatima, et al;2009). Demirezer ,1993 reported the usefulness of Rumex as diuretic, cholalogue, tonic, and laxative agents. Leaves, stems, and roots of some species are used to treat pneumonia, cough, abscesses, stomach- ache, and small pox (Abdelnaser;2005).

Al-Dubai and Al-Kulaidi (1996) recorded *R.nervosus* as one of the medical plants in Yemen, and the recent study was design to investigate and compare between the effects of water and methanol extractions of leaves and seeds of *R.nervosus* on local pathogenic isolates of *E.coli*, *S.aureus*, and *C.albicans*.

MATERIALS AND METHODS

The plant extractions

Seeds and leaves were collected from Ebb province at march 2010, they were brought immediately to the lab. The Plant samples were washed thoroughly by tap water, dried at room temperature for two days far from sun light. Fine powder of dried plant materials was obtained by electric grinding machine. Extracts concentrations were prepared by soaking a determinate weight (gm) of seeds and leaves powder in sterile distilled water and methanol to obtain 25%, 50%, and 75% concentrations. The glass containers were shake by orbital shaker for 24 hours, after that the mixtures were filtrated by sterile stainless mesh, then by whatman no. 1 filter paper by using handle vacuum pump.

The filtrates were dried in water bath (8 h./70 C^o), and the thick extracts were kept in dark vials with screw caps at 4 C^o until using (Farrukh,et al 2010).

The tested microorganisms

From private clinics in Thamar province, the clinical microbes samples were collected by sterile swaps from euro genital tract. The swabs were cultured after sampling directly on culture medium by streaking method.

The plates were incubated at 37 C° . The growing yeasts were re-cultured on nutrient agar medium + chloramphenicol solution and pure culture of Candida albicans was identified following germ tube and chlamydospore forming testes. Bacterial isolates were purified, and identified by the standard methods .The antimicrobial effects of all extracts were detected firstly by colony count(CCT). While the comparison among antimicrobial activity of plant extracts were determined by well diffusion methods. Standard aseptic microbiological methods were followed throughout this antibacterial study.

RESULTS AND DISCUSSION

Although there are several studies about numerous species of *Rumex*, (Panduraju,et al; 2009 ; Senha,et al;2011; Mostafa,et al;2011 ;Fatima,et al;2009 ; Abdelnaser,et al;2005 ;Suzan and Avni;2007) but there are a few concern *R.nervosus* especially in our region.

The results of recent study showed that the crude methanol leaves extractions gave higher antimicrobial inhibitions than water leaves extractions by well test method (8,13,18) mm and (10,17,23) mm respectively (table-1) and by TCC (fig.1) .The antimicrobial effects of crude leaves extracts was reported also by Getie et al(2003) in Ethiopia who explain high antimicrobial activity of *R.nervosus* crude leaves extract. The effect of different solvents on the antimicrobial efficacy levels was explain previously .

Table .1 The inhibition zones(mm) of methanol and water extracts of *R.nerosus* leaves.

Testing Microorganism	Inhibition zone (mm) / mean of two wells					
	Aqueous%			Methanol%		
	25	50	75	25	50	75
S.aureus	6	13	18	10	17	23
E.coli	6	11	16	8	10	19
C.albicans	5	10	16	5	12	20

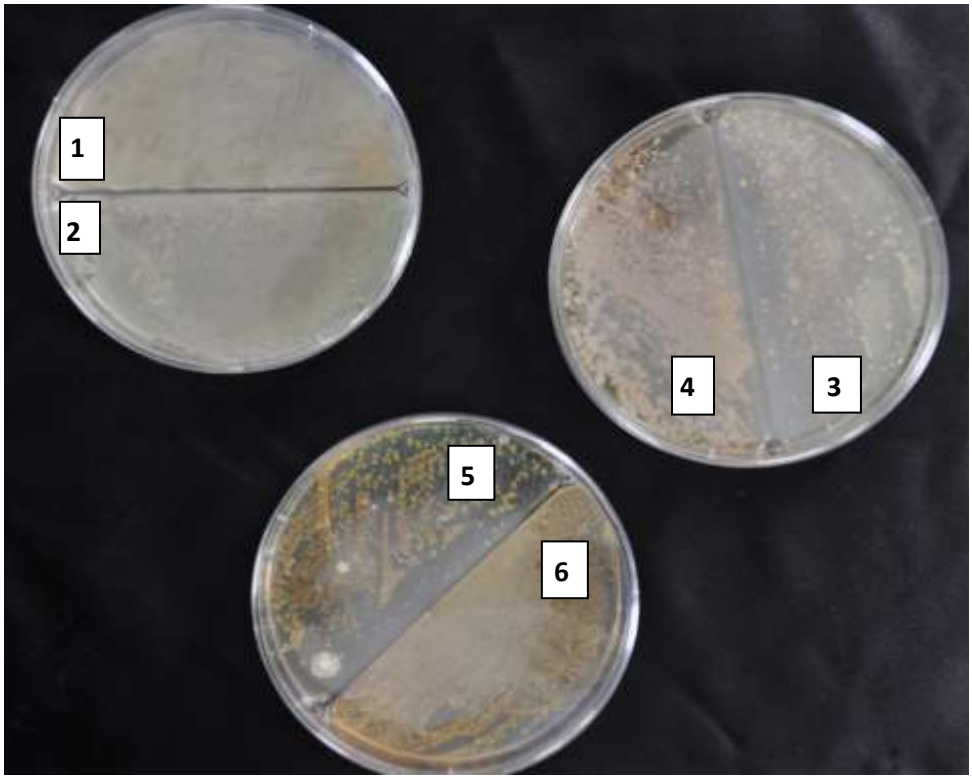

Fig.1- The antimicrobial effects of methanol(M) and water extracts of *R.nervosus* leaves on total colonies count witch: **1&2** (M)and (W) extracts against *C.albicans* ; **3&4**(M)and (W) extracts against *E.coli* **;** **5&6**(M)and (W) extracts against *S .aureus*

The effect of different solvents on the antimicrobial efficacy levels was explained by Ozturk and Ozturk (2007) who reported that water extracts of *R.caucasicus* and *R.alpinus* had no effects on 121 strains tested bacteria, while the methanol extracts effected on all isolates .Mostafa et al(2011) recorded that the pattern of inhibition showed highly significant variation according to different solvents used for *R.visicarius* extractions.

When the crude extractions of seeds were tested ,the inhibition zones of water and methanol were either very low or showed no effects (table-2) and (fig.2).

Table..2: The inhibition zones(mm) of methanol and water extracts of *R.nervosus* seeds .

Testing microorganism	Inhibition zone (mm) / mean of two wells					
	Aqueous%			Methanol%		
	٢٥	50	75	25	50	75
S.aureus	0	0	2>	0	2>	2>
E.coli	0	2>	2>	2>	2>	2>
C.albicans	0	2>	0	0	2>	2>

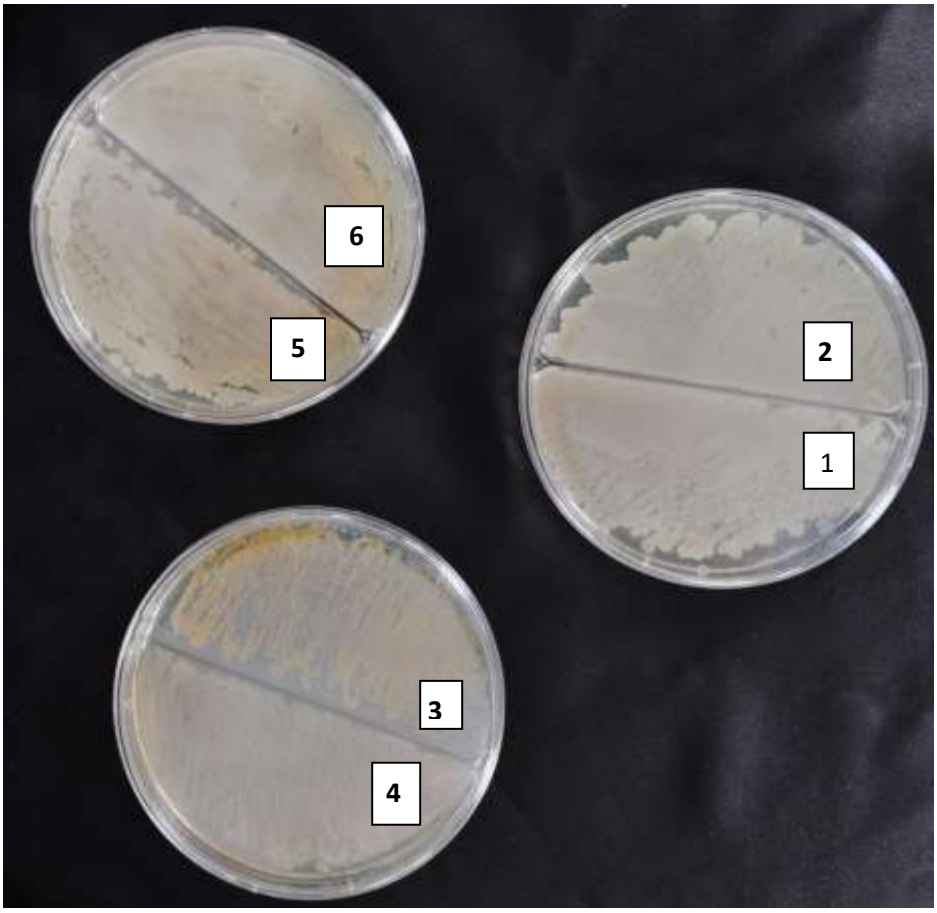

Fig.2- The antimicrobial effects of methanol(M) and water(W) extracts of *R.nervosus* seeds on total colonies count witch: 1&2 (M)and (W) extracts against *C.albicans* ; 3&4(M)and (W) extracts against 5&6(M)and (W) extracts against *S .aureus E.coli* ;

The variations between seeds and leaves extractions related to the variation in the presence and /or amounts of phytochemicals found in plant tissues (Mostafa et al,2011) .Such variation of phytochemicals concentration were known among different species of *Rumex* also. Hussain et al (2010) explain the differences in antimicrobial efficacy between leaves methanol extracts of two species of Rumex (*R.dentatus* and *R.hastatus*) against *S.aureus* and *E.coli* .

Fatima *et al*,(2009) explained no antimicrobial effects of methanol and hexane extracts of *R.dentatus* leaves on *S.aureus* and *E.coli* in compare with stems and roots. This may due to different species were used. The effect of different species of Rumex on bacterial isolates was reported also by (Suzan and Avni ; 2007) who detected that methanol extract of *R.caucasicus* had more effect on (52) pathogenic bacterial isolates than *R.alpinus*. A crud methanol extracts of *R.dentatus* leaves had moderated antibacterial activity against *S.aureus* and high against *E.coli* ,while *R.hastatus* showed low antibacterial effect on both isolates (Farruk,*et al* ;2010).

Leaves extractions showed a less effect on *E.coli* (G-) than *S.aureus* (G+) isolates (table-1). In Saudi Arabia, a twenty aerial plant parts collected from nineteen genera (include *Rumex nervosus*) showed less antibacterial effect on *E.coli* in compare with *S.aureus* ,this agree with our results. The resistance of *E.coli* to the effect of *R.nervosus* extractions in compare with *S.aureus* may related to the different cell wall components for each isolate especially the lipopolysaccharide layer in (G-) cell wall.

In (table-1) , the effects of leaves methanol extracts on the pathogenic isolate of *C.albicans* (yeast) were declared .A same results were reported in Saudi Arabia for *R.nervosus* leaves extract (Essam,et al;2008) ,and in India for *R.nepalensis* roots extract. The positive antifungal activity of different species and different plant parts of *Rumex* were also investigated against filamentous fungi(Farrukh;2010 ; Panduraju;2009 ; Surjeet;2011) .

Finally the present finding enhance farther studies about the active principles present in methanol extract of leaves and other parts of this genus- especially- the species *R.nervosus* is one of a local medical plants which was used in folk medicine in Yemen beside its using as edible plant.

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دراسة الكفاءة ضد الميكروبية لمستخلصات العثرب *Rumex nervosus* L. على *Staphylococcus aureus* و *Escherichia coli* و *Candida albicans* في الزجاج

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جامعة ذمار / كلية العلوم التطبيقية

الملخص:

تم تقييم الأثر ضد البكتيري والضد فطري للمستخلص الخام للـ *Rumex nervosus* L.(Plygonaceae) حيث حُضر المستخلصين الميثانولي والمائي لأوراق وبذور النبات واختبرت فعاليتها ضد عزلات ممرضة من *Staphylococcus aureus* (G+) و *Escherichia coli* (G-) و *Candida albicans* بحساب العدد الكلي للمستعمرات وكذلك بإتباع طريقة قياس قطر التثبيط well diffusion وأظهر المستخلصان الميثانولي والمائي للأوراق تأثيراً مثبطاً لنمو الميكروبات المختبرة بعكس المستخلصين المحضرين من البذور الذين كان فعلهما المضاد للنمو الميكروبي ضعيفا ، وقد كان المستخلص الميثانولي أعلى فعالية من المستخلص المائي في قدرته على تثبيط النمو الميكروبي في جميع الاختبارات الموجبة. أشارت النتائج الى ان عزلات *E.coli* كانت أكثر حساسية من عزلات *S.aureus* لتأثير المستخلص المستعمل.

الكلمات المفتاحية/ *R.nervosus* , leaves extraction ,methanol extraction, antimicrobial efficacy