
ANTIBACTERIAL ACTIVITY OF ROOT EXTRACTS OF *RUMEX NERVOSUS* GROWING IN YEMEN

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Abstract

Roots of *Rumex nervosus* growing in the mountains around Ibb university, Yemen Republic, were collected in February 2008, washed under tap water, cut into small pieces and dried using hot air oven at 60°C until a constant weight was obtained. The dried plant material was then ground into a fine powder and successively extracted till exhaustion with petroleum ether 60/80°C, diethyl ether, chloroform, acetone and methanol. Extraction was carried out by cold soaking to avoid breakdown of active principles. The obtained extracts yielded gummy materials after gentle drying. Filter paper discs were impregnated with 10 mg of each of the extracts obtained and the antibacterial activity was checked by the disc-plate diffusion method. The results obtained have revealed that except for the methanol extract, all the other extracts exhibited varying antibacterial activities against the tested pathogenic bacteria. The obtained results indicate that *Rumex nervosus* growing in Yemen can be used in future as a source of active antimicrobial agents for industry and medicine.

Key words: *Rumex nervosus*, Antibacterial, Successive extraction

Introduction

Al-Dubaie and Al-Khulaidi (1996) reported that *Rumex nervosus* is rich in its content of secondary metabolites like oxalic acid, potassium oxalate, tannins, resins, rumicin, vitamin C, anthraquinones, flavonoids and anthracine glycosides, so it could be used for many purposes in folk medicine of Yemen to clean blood, as a diuretic, against bleeding, as an antiseptic for eyes, to treat tuberculosis and abdominal infections.

Einhellig and Ramussen (1973) reported on the presence of three phenolic substances in *Rumex crispus* that could inhibit germination of *Amaranthus retroflexus*, grain sorghum, and field corn. The same authors added also that this may be the reason that in the quadrates where *Rumex crispus* grow, the other plants biomass get reduced growth. Munavu *et. al.* (1984) could isolate and characterize major anthraquinone pigments of *Rumex abyssinica* and added that in Ethano-medicine of Kenya, the roots of this plant are used for the treatment of abscesses and abdominal pains. The active substances that could be identified in this plant included

flavonoids, anthraquinone derivatives, naphthalene derivatives and triterpenoids. *Rumex acetosa*, which is used in some parts of the world as a green salad exhibits also antimicrobial, antiviral and anticancer activities. Litvinenko and Muzychkina (2003) revealed that the roots of many species of *Rumex* that grow in Kazakhstan are rich in biologically active substances and that these substances belong to various phytochemical classes. Kupeli *et al.* (2004) reported that *Rumex* generally have antioxidant and antibacterial activities and that it is medicinally used as a gastroprotective and anti-inflammatory crude drug. Jang *et al.* (2005) claimed that *Rumex japonicus* root extracts are used as a Chinese drug for the treatment of many diseases including jaundice, constipation, scabies and uterine hemorrhage. The biologically active substances from this plant were proved to have antioxidant, antimicrobial and cytotoxic activities. Elzaeawely *et al.* (2005) investigated both the antibacterial and anti oxidant activity of hexane, chloroform, ethyl acetate as well as ethanol and water extracts of the aerial parts of *Rumex japonicus* and could achieve the results that ethyl acetate extract exhibited the maximum antibacterial effect against the tested bacteria (*Bacillus subtilis*, *B. cereus*, and *E. coli.*). This potent antibacterial activity, as suggested by the same authors, may be due to the high content of phenolic compounds like pyrogallol and pyrocatechin which could be detected in this type of extracts.

The purpose of this study is to screen the successive root extracts of *Rumex nervosus* growing in Ibb Mountains, republic of Yemen for antibacterial activity against some common pathogenic bacteria. It is to be mentioned that although *Rumex* is being commonly and widely used in folk medicine of Yemen for treatment of gastric problems, it has never been subjected to such study in Yemen. However, some fruitful experiments in this field but with other plants belonging to the flora of Yemen were conducted by other authors such as Awadh *et al.* (2001), Mothana and Lindequest (2005), Hussein E. A. (2006), Mothana R. A. *et al.* (2006), Al-Fatimi *et al.* (2007) and Mothana *et al.* (2007).

Materials and Methods

Successive extraction

Fifty grams of root dry powder were subjected to successive extraction using the following organic solvents: petroleum ether, diethyl ether, chloroform, acetone and methanol (96%). The extraction was carried out by soaking the powder in excessive amount of the solvent with continuous and gentle stirring at room temperature for a suitable period of time. This was repeated until the powder was completely

exhausted. After filtration, the organic solvents were evaporated to dryness using gentle heating. The extracts were then dissolved in hot methanol-water (8:2) to give a final concentration of 200 mg/ml. and were stored in dark bottles at 4°C until needed.

Test microorganisms:

The pathogenic microbes used in this study were: *Staphylococcus aureus* NCTC 7447, *Escherichia coli* BPP 01, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* CS25. Tested bacteria were cultured using nutrient broth and assayed on nutrient agar medium containing Petri-dishes.

Assay of antimicrobial activity:

This was carried out by the disk-diffusion method described by Salie F. *et al.* (1996). Five cm (diameter) filter paper disks were impregnated with 50µl of the extract (equivalent to 10 mg of dry extract). After the organic solvent was completely evaporated, the discs were put on the surface of solid agar medium seeded with test bacteria in 9 cm diameter Petri-dishes. All the plates were incubated at 37°C for 24 h. The experiment was performed 3 times under strict aseptic conditions. Bacterial growth was determined by measuring the diameter of the zone of inhibition and the mean values were calculated.

Results and Discussion

Successive extraction resulted in a yield of 230.18 mg extract per gram dry root powder. The liest amount of extract was obtained with diethyl ether (6.32 mg) while the highest amount of extract was obtained with methanol (147.04 mg). Results of the present study (table 1 and plate 1) revealed that methanol extract failed to exhibit any effect against the tested bacterial strains while, on the other hand, the best antibacterial activity was detected in the chloroform extract. The gradual and successive extraction process could extract the active antibacterial agents before extraction with methanol. Diameter of the inhibition halo using chloroform extract was 16 mm with *E. coli*, 25 mm with *S. aureus*, 23 mm with *K. pneumonia* and 21 with *P. aeruginosa*. Diethyl ether extract exhibited an antibacterial effect against all the tested bacterial strains and the diameter of the inhibition halo ranged from 11 to 14 mm, the effect is thus considered less pronounced than with chloroform extract. Following diethyl ether extract comes acetone. The effect of petroleum ether extract was slight and with *P. aeruginosa* no inhibition zone was even possible to be

detected. Results of the present study may document the cumulated information observed by trial and error of people who use this plant in folk medicine. The results obtained may also synergize and agree with the results obtained by other authors like Munavu R. M. *et al.* (1984), Al-Dubaie and Al-Khulaidi (1996), Kupeli E. *et al.* (2004) and Jang D. S. *et al.* (2005) in the extracts of aerial parts and root systems of *Rumex* exhibit antibacterial activity. Elzaeawely A. A. *et al.* (2005) suggested that the antibacterial activity of *Rumex* may due to its contents of phenolic substances.

The results obtained in this study may indicate the wealth of the flora of Yemen with plants having some potent antibacterial agents as it was observed in other studies that were carried out by Awadh A. N. *et al.* (2001), Mothana and Lindequest (2005), Hussein E. A. (2006), Mothana R. A. *et al.* (2006), Mothana R. A. *et al.* (2007) and Al-Fatimi M. *et al.* (2007).

Studies should be continued with potent and promising extracts to isolate, purify and identify its active ingredients.

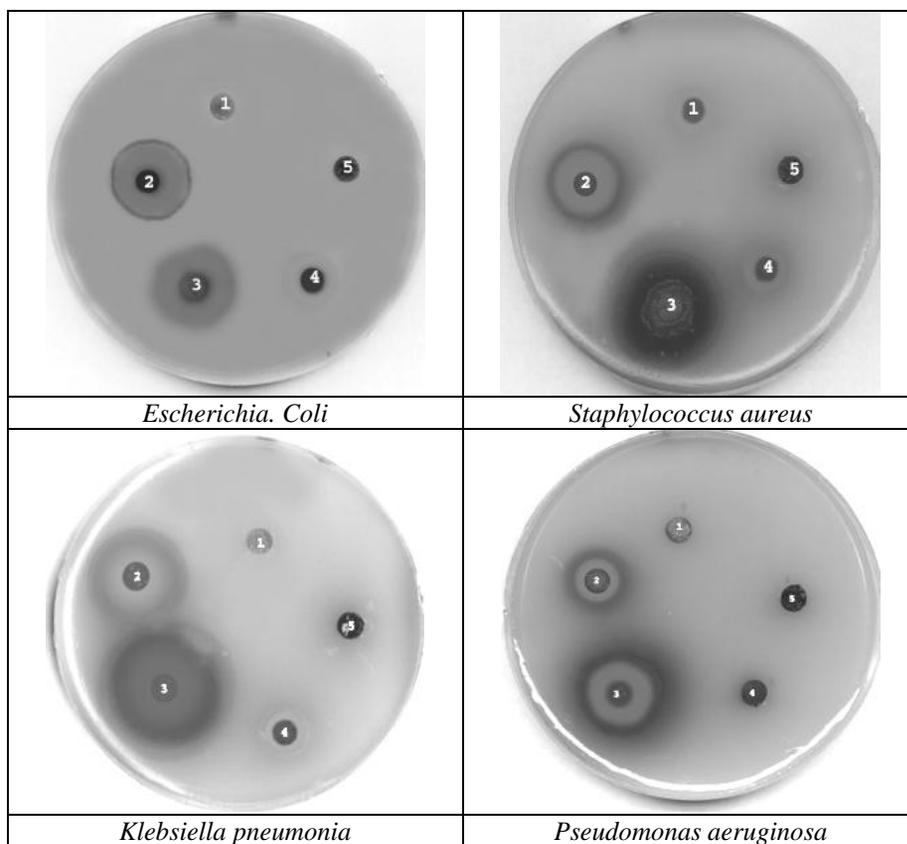
Conclusion

Roots of *Rumex nervosus* were collected from plants growing in Ibb mountains, Ibb, Yemen republic. After the roots were washed and dried, they were ground into a fine powder and successively extracted with petroleum ether, diethyl ether, chloroform, acetone and methanol. The successive extracts were dried and checked for their antibacterial activity against pathogenic bacterial strains, *Staphylococcus aureus* NCTC 7447, *Escherichia coli* BPP 01, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* CS25. The most potent extract was chloroform followed by diethyl ether extract, acetone, and then the petroleum ether while methanolic extract exhibited no activity. Roots of *Rumex nervosus* thus may be promising as a source of antibacterial agents for food preservation and medicinal purposes.

Table (1): Weights (mg/gm dry powder) and antibacterial activities of successive extracts of *Rumex nervosus* (diameter of inhibition zones (mm) of growth of some pathogenic bacteria). Each value is a mean of 3 determinations. Diameter of filter paper disc 5 mm.

| Type of extract | Wt. of extract mg/g. dry powder | <i>E.</i> <i>coli</i> | <i>S.</i> <i>aureus</i> | <i>K.</i> <i>pneumonia</i> | <i>P.</i> <i>aeruginosa</i> |
|-----------------|------------------------------------|--------------------------|----------------------------|-------------------------------|--------------------------------|
| Petroleum ether | 9.57 | 6 | 8 | 8 | 00 |
| Diethyl ether | 6.32 | 14 | 13 | 14 | 11 |
| Chloroform | 7.68 | 16 | 25 | 23 | 21 |
| Acetone | 89.54 | 09 | 09 | 10 | 6 |
| Methanol | 147.04 | 00 | 00 | 00 | 00 |

Plate (1): Antibacterial activity of *Rumex nervosus* root successive extracts by the disc-diffusion method.



(1) Petroleum ether (2), Diethyl ether, (3) Chloroform,
(4) Aceton, (5) methanol

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الملخص العربي

النشاط الضد بكتيري لمستخلصات جذور نبات العثرب النامي في اليمن

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لقد تم في تلك الدراسة تجميع جذور نبات العثرب النامية في جبال إب - الجمهورية اليمنية. بعد غسل و تجفيف الجذور تم طحنها طحناً جيداً ثم أجرى لها إستخلاصاً متعاقباً بواسطة المذيبات العضوية : بترول إيثيرى 40-60 و إيثير ثنائى الإيثيل و كلوروفورم و أسيتون و ميثانول. أجرى بعد ذلك تجفيف المستخلصات و دراسة نشاطها الضد بكتيري باستخدام بكتيريا ستافيلوكوكس أوريوس و إشيريشيا كولاي و سيدوموناس إيروجينوزا و كليبيلا نيومونيا. أثبتت الدراسة أن أكبر المستخلصات فعالية ضد تلك السلالات البكتيرية المرضية هو كلوروفورم ثم مستخلص الإيثير ثنائى الإيثيل فأسييتون ثم الإيثير البترولى $40-60^{\circ}\text{C}$ بينما لم يكن للمستخلص الميثانولى تأثير يذكر. إن النتائج المتحصل عليها تبشر بإمكانية استخلاص مواد ذات طبيعة ضد بكتيرية من جذور نبات العثرب و استخدامها في الصناعات الغذائية و الدوائية.