Effect of Aqueous and Alcoholic Extract of *Hydrastiscanadensis* Plant on Bacteria Isolated from Otitis Media

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Abstract

The results of the current study showed that the effect of the alcoholic extract of the*Hydrastiscanadensis* plant was more effective than the aqueous extract on the bacteria isolated from otitis media at the same concentrations.As the alcoholic extract had the highest effect on *proteusmirabilis*,*Staphylococcusaerues*, *Staphylococcus epidermidis,Escherichia coli*, *Pseudomonas aeruginosa* and *Klepsiella pneumonia*with an inhibition diameter ranging (20, 18, 17, 16 ,16, 15) mm respectively at a concentration of 200 mg/ml, The study also showed that the higher the concentration of the extract, the greater the inhibitory activity against the bacterial species,with significant differences in the probability level 0.05 of the two extracts on the bacterial species, except*Pseudomonas aeruginosa*, *Staphylococcusaerues* there was no significant difference in the effect.

Keywords - *Hydrastiscanadensis, alcoholic extract, aqueous , Bacteria , Otitis media.*

Introduction

Otitis media is one of the main reasons for visiting health centers all over the world, as it causes serious complications, including hearing loss[(1)] , meningitis and brain abscesses[(2)] , if not treated properly. It is an inflammatory disease that affects the lining of the mucous membrane of the middle ear and the associated incision[(3)],which is either acute[(4)] or chronic[(5)]. The causative agent may be bacterial, viral or fungal,[(6)], but more causes of inflammatory middle ear common are infections with bacteria such as *Pseudomonas aeruginosa,Staphylococcus aureus*, *Protusspp,Staphylococcus epidermidis*, *Escherichiacoli*, *Klepsiellapneuomina*[(7)]. The increase in bacterial resistance to antibiotics in recent times has become one of the most serious threats facing the entire world, as some strains have acquired resistance to most antibiotics, which calls for seeking to find new anti-bacterial agents that cause disease to overcome bacterial resistance[(8)].Medicinal plants with antimicrobial properties are alternatives to the increasing problem of bacterial resistance to antibiotics[(9)]. including plant *HydrastisCanadensis*,whichalso known by other names including Goldenseal, Orange root, Yellow puccon, India eye, eye root, Ground raspberry [(10)] .It is a small perennial herb found in the humid forests of Canada and the eastern United States and belongs to the Ranuculaceae family[(11)],the effectiveness of alcoholic extract due to the alkaloids such as berberine, canadine, hydrstine, canadline and others, which have anti-bacterial, antifungal, protozoal and tuberculosis effectiveness as well[(12)]. The efficacy of alkaloids against bacteria has been proven by many studies, where it was found that they have high inhibitory efficacyagainst both Gram positive and Gram negative bacteria[(13)]. They also play an important role in treating a number of diseases such as asthma, analgesic and anti-cancer conditions[(14)].It´s

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mechanism of action is to inhibit bacterial cell division by interfering with DNA replication, targeting enzymes Rna polymerase, gyrase, topoisomerase IV and stopping bacterial replication[15].Berberine has been found to be effective against *Vibriocholera*,*Staph. aurues*, *Klebsiellaspp*,*Shigella*,*Sterptococcuspyogenes*[16].

Materials and Methods

The aqueous extract of the plant in this study were prepared by mixing 40 g of the plant model in 160 ml of sterile distilled water (4: 1 weight / volume), then the plant model was placed with the Blander crushing device inside an ice bath, and the plant samples were stirred by the electro-magnetic stirrer For 60 minutes, in order to disintegrate and tear the cell wall of the samples, leave the mixture in the refrigerator for 24 hours for the purpose of soaking, filter the mixture through several layers of gauze, then filter again using a Buchner bowl funnel and using Whatman No.1 filter papers with vacuum By means of a vacuum, to get rid of (the non-milled parts and the remnants of the fibers), after that the extracts were poured into sterile glass containers and placed in an electric oven at a temperature of 40 ° C until all the water evaporated and to preserve the active substances and after drying the extracts, they were placed in airtight glass containers. Close, labeled, and refrigerated until use[17] . In the same way previously mentioned, the alcohol extract was prepared by adding ethyl alcohol at a concentration of 95% instead of distilled water.

The method of researchers Shinkafi and Dauda 2013 was adopted to test the effectiveness of both the aqueous and alcoholic extract of the Golden Seal against the bacterial species isolated from otitis media(*Pseudomonas aeruginosa, Staphylococcus aureus*,*Staphylococcus epidermidis,Escherichia coli, Klepsiellapneuomina , proteus mirabilis)*after being diagnosed with biochemical tests and confirming the diagnosis with the VITEK2 system device. The plates containing Muller Hinton agar medium were prepared for the purpose of implanting a bacterial suspension on them after comparing them with a McFarland tube. The disc diffusion method was used in which disks of Whattman No.1 filter paper with a diameter of 6 mm were prepared and then sterilized with oven at 160 ° C for two full hours. Then the tablets were saturated with concentrations(200, 100, 50, 25, 12.5, 6.25 mg/ml) aiming to test their effectiveness against bacterial isolates, and using sterile forceps, they were placed on the surface of the medium and then incubated at 37 ° C for 24 hours to observe the effectiveness or not, and measure the inhibition diameters in mm compared to chloramphenicol antibiotics as positive control [18].

Results

The results of the current study showed, that the effect of alcoholic extract of goldenseal plant was higher than that of aqueous extract.as showed in table (1), the effect of the alcoholic extract had the largest effect on *P. mirabilis*with aninhibition diameter of 20 mm, The least effect was on bacteria*k. pneumonia*, where the inhibition diameter was 15 mm, while the effect of the aqueous extract was almost non-existent at the same concentration of 200 mg / ml where the maximum effect of the aqueous extract was on isolates with an inhibition diameter of 7 mm for each of*P. aeurginosa*, *Staphylococcus epidermis*, *k. pneumonia* , in which there are statistically significant differences, at a probability level of 0.05.

Table (1) : The inhibition diameters of both the alcoholic and aqueous extract of the *Hydrastis Canadensis* on bacteria isolated from otitis media.

Cont.	Extract concentrations in mg / ml												Bacterial isolates
	6.25		12.5		25		50		100		200		
	Aqu.	Alc.	Aqu.	Alc.	Aqu.	Alc.	Aqu.	Alc.	Aqu.	Alc.	Aqu.	Alc.	
23	0 g	0 g	0 g	0 g	0 g	10 d	0 g	11 c	6 f	13 b	7 e	16 a	P. aeurginosa
24	0 f	0 f	0 f	0 f	6 d	4 e	0 f	10 c	0 f	14 b	6 d	18 a	Staph. aurues
27	0 h	9 e	0 h	11 d	6 g	11.5 cd	0 h	12 c	6 g	14 b	7 f	17 a	Staph. epidermidis
22	0 g	8 e	0 g	10 d	0 g	14 c	0 g	17 b	6 f	13 c	6 f	20 a	P. mirabilis
25	0 e	0 e	0 e	7 d	0 e	9 c	0 e	10 c	6 d	12 b	7 d	15 a	k. pneumonia
26	0 f	0 f	0 f	6 e	7 e	10 d	0 f	12 c	0 f	14 b	6 e	16 a	E.coli

Similar letters mean there is no significant difference at the level of probability 0.05Alc. : Alcohol , Aqu : Aqueous , cont. : control .

Discussion

The study showed that the extract of the golden seal plant had inhibitory activity against the isolated bacterial species of otitis media, especially the alcoholic extract, which was more effective than its aqueous counterpart. This is due to the solubility of the active substances in alcohol more than water, and as the study showed that the lower the concentration of the extract, the less inhibitory activity against bacteria.Udvardy and his colleagues (2015), found that the medicinal effect of golden seal plant is mainly due to the alkaloids such as berberine - hydrastine - which are present mainly in the roots and other alkaloids[19].Inanother study, it was found that the extracts of this plant were effective against germs that cause periodontitis[20] .Junioand his colleagues(2011) also found that extracts dissolved with ethanolic alcohol from the aerial parts of the plant and roots have efficacy against *Staph. aerues* by influencing flow inhibition of ethidium bromide[21]. The aqueous and alcoholic extracts of herbs plants, especially the golden seal, are composed of complex active ingredients with bioactivity that can limit the increase in bacterial species gaining resistance. The mechanism by which bacteria are targeted by using plant extracts in general has not yet been clarified, but there is proposed mechanisms include influencing the genetic synthesis of the bacterial cell, especially DNA and RNA, disrupting the biological membranes of the microorganism, interfering with the nutritional pathways of the microorganism, stimulating clotting of cytoplasmic contents and influencing the phenomenon of quorum sensing (QS) which means cutting off the normal cellular communication of germs [22-23].

Conclusions

Golden seal(*Hydrastis Canadensis*) plant extract has inhibitory efficacy against bacteria isolated from otitis media and can be considered as antibacterial agents.

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