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Research Article

EVALUATION OF *ROSMARINUS* SPECIES EXTRACTED BY DIFFERENT SOLVENTS AGAINST MOSQUITO LARVAE, *BIOMOPHALARIA* SPECIES AND DIFFERENT PATHOGENIC BACTERIA

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ABSTRACT

The botanical extracts were used as a scours of insecticidal, molluscicidal and antimicrobial agents. Many authors try to improve the potency of these extracts by using different solvents. The pet-ether and ethanolic extracts of *Rosmarinus* species were tested as insecticidal and molluscicidal agents against *Culex pipiens* and *Biomophalaria alexandrina*. The LC_{50} of pet-ether and ethanolic extracts against mosquitoes were 511.84 & 721.81 ppm respectively. While, LC_{50} of these extracts against snails were 236.81 & 276.16 ppm respectively. From those results the snails appear more susceptible to both extracts than mosquitoes. Both mosquitoes and snails more susceptible to pet-ether extract than ethanolic extract. petroleum ether and ethanol extracts of *Rosamarinus* sp in different concentrations (5, 10, 15 and 20%) were evaluated for their possible antibacterial activity against six pathogenic bacteria, *Staphylococcus aureus* and *Entrococcus faecalis* (gram positive bacteria), *Escherichia coli*, *Proteus sp*, *Acetobacter sp* and *Pseudomonas aeruginosa* (gram-negative bacteria). The petroleum ether and ethanol extracts of *Rosamarinus* sp. in different concentrations exhibited antibacterial activity against all tested organisms (except *Acetobacter sp* was resistant at all concentrations of petroleum ether extract). Findings were compared to those produced by Gentamycin (10 μg) and Tetracyclin (30 μg), reference antibiotic

Keywords: Botanical Extracts, *Rosmarinus, Mosquitto larvae, Biomophalaria*

1. INTRODUCTION

Snails' species are associated with transmission parasitic disease as intermediate host. Schistosomiasis is a parasitic disease that affects 200 million people in different countries [1]. Snail control with molluscicides has been one of the effective methods used for rapid and effective control of disease. Bait formulation of different molluscicides, would be an effective tool for selective killing of the snail with minimal adverse effect on the environment. The high cost of synthetic molluscicides, used in the control of the intermediate snail hosts, has resulted in renewed interest in plant molluscicides [2, 3].

Mosquitoes transmit serious human diseases, causing millions of deaths every year. Among these diseases, malaria, yellow fever, dengue and dengue hemorrhagic fever, filariasis and Rift Valley fever at endemic and epidemic areas in many countries [4-6]. Plants may be alternative sources of mosquito control agents [7-14].

The environmental problems caused by overuse of pesticides have been the matter of concern for both scientists and public in recent years. The reasons for these problems refer to the high toxicity, non-biodegradable properties of pesticides and the residue in soil, water resources and crops that affect public health. Thus, on the one hand, one needs to search the new highly selective, biodegradable pesticides and environmental friendly pesticides [8, 14-17].

Biological control stands to be a better alternative to the chemical controls aimed against snails. The search of herbal preparations that do not produce any adverse effects in the non-target organisms and are easily biodegradable remains a top research issue for scientists associated with alternative molluscicides control [18, 19].

Plant extracts show antibacterial effects [20] and antifungal activity against wide range of fungi [21-23].

Natural antimicrobials can be derived from different parts of the plant (barks, stems, leaves, flowers and fruits), various animal tissues or from microorganisms [24]. Although some therapeutic benefits can be traced to specific plant compounds, herbs contain mainly active constituents that, together, combine to give the plant its therapeutic value. Consequently, it is believed that the whole plant has more effective healing properties than its isolated constituents [25]. Bioactive compounds such as glycosides, alkaloids and terpenes are examples contained in some plants and could be used as drugs and antimicrobial agents [26]. Many extracts and essential oils have been derived from plants and found to have antibacterial, fungicidal and insecticidal properties [27].

Recently, natural products have been evaluated as sources of antimicrobial agents with efficacies against a variety of microorganisms, so alternative strategies are sought that do not use antibiotics to reduce pathogenic bacteria fungi from foods and patients. Plants have been in use for thousands of years to conserve food and treat health diseases [28-33].

The aim of the present study was to evaluate insecticidal, molluscicidal and antimicrobial activities of *Rosmarinua* species extracted by different solvents to explore full potential use of that plant as insecticide, molluscicide and antibiotic in future.

2. MATERIALS & METHODS

2.1. Tested compounds

The tested medicinal plant (*Rosmarinus* sp.) was washed to avoid dusts and dirt then lift to dry under shade in the laboratory. Dried plant (whole plant) were cut into small pieces and ground in an electric grinder. Hundred grams of the resulting powdered materials of each plant were extracted with ethanol absolute and petroleum ether. The extractions were accomplished by means of a Soxhlet apparatus. The solvent extracts of each plant were evaporated and dried under vacuum using a rotary evaporator of water bath adjusted at 60-70ºC. The resulted dry crude extracts were storage at 4ºC in screw capped vials, until use.

2.2. Tested mosquitoes

***Culex pipiens* (*Culicidae: Diptera*).**

Provided by collecting from Tabuk area and transferred to the research laboratory of Biology Department – Science Collage – Tabuk University where self-perpetuating colonies were established and maintained during the present study, according to the method described by Kamel [8]. Late third larval instars were used for toxicological studies.

2.3. Efficiency of plant extracts on mosquitoes

Preliminary, toxicological bioassay tests were carried out to the selected plant extracts on tested insects as a modification for the method described by Kamel [9] and Wright [34] their LC_{50} and LC_{95} values were determined as well as their slope function, according to Finney [35] and WHO [36].

2.4. Tested parasite

Adult *Biomphalaria alexandrina* (Shell diameter: 12-14 mm) was subjected to current study. Uninfected snails, that is, those that did not show patent trematode infections, were maintained in the laboratory conditions for seven days before being used in our molluscicidal tests.

2.5. Efficiency of plant extracts on snails

Ten snails were then allocated to each of the groups and immersed in either untreated dechlorinated tap water or aqueous extract treated dechlorinated water (positive & negative controls). Preparations of the plant extracts and toxicity test protocols were adapted from those described by Brackenbury [37].

2.6. Test microorganisms and culture preparation

Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus sp* & *Acetobacter sp* and Gram positive bacteria *Staphylococcus aureus* and *Entrococcus faecalis* were obtained from "Culture Collection of Antibiotic Resistant Microbes (CCARM)" Military Hospital Tabuk.

2.7. Antimicrobial assay

2.7.1. Determination of antibacterial activity

Antimicrobial activity of the crude extracts was determined by Agar well assay methods as described by different authors [38-41].

The inoculum size of each group of bacteria was prepared by using nutrient broth to give a concentration of 1×10^8 bacteria per milliter. The suspension (100µl) was spread onto the surface of Mueller Hinton Agar (MHA) medium. Wells (5 mm in diameter) were cut from the agar with a sterile borer and 50µl extract solutions were delivered into them. Negative controls were prepared using sterile DMSO and gentamycin & tetracycline were used as positive reference standards to determine the sensitivity of each microbial species tested. The treated plates were stored in a refrigerator at 4˚C for at least six hours to allow diffusion of the extracts into the agar while arresting the growth of the test microbes [42]. The plates were then incubated for 24-48 hours at 37˚C. Antimicrobial activity was determined by measuring the diameters of inhibition zones (DIZ) in mm. All tests were performed in triplicates and the developing inhibition zones were compared with those of the reference discs. The means and standard deviations (±SE) of (DIZ) was done.

3. RESULTS AND DISCUSSION

3.1. Insecticidal studies

3.1.1. Evaluation of the larvicidal activity of Rosmarinus species extracts on mosquito larvae

These experiments were carried out to evaluate the potency of *Rosmarinus* sp extracted by two different solvents (pet-ether & Ethanol) against *Culex pipiens* larvae. The results in table 1 and Fig. 1 showed that pet-ether extract was more potent than ethanolic extract according to their LC50. The confidential limits of each of the tested extract were statistically calculated for LC50 and LC95 at P=0.05.

Table 1: Larvicidal activity of *Rosmarinus* sp.extracts on *Culex pipiens* larvae

Plant	Solvent	LC 50 (Co. Limits)	LC 95 (Co. Limits)	Slope Function
Rosmarinus sp.	Pet-ether	511.84 (458.45-571.42)	1576.69 (1222.76-2034.32)	3.4
	Ethanol absolute	721.56 (675.66-770.57)	1313.38 (1159.0-1488.46)	6.3

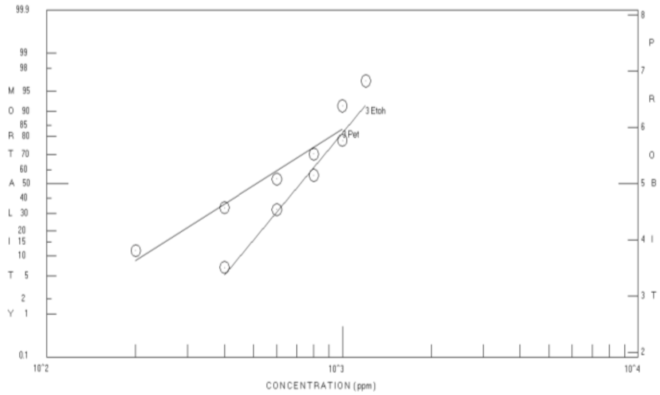

Fig. 1: Susceptibility of *Culex pipiens* larvae to *Rosmarinus* sp. extracts

Rosmary essential oil is used as antioxidant, antimicrobial and insecticidal agents [43, 44]. The potency of the crude plant extracts is often attributed to the complex mixture of active compounds [13]. Major components of rosemary crude extract were found to be eucalyptol and camphor [45]. The pet-ether extract more potent because of the main components of plant more dissolve in pet-ether than ethanol [46].

3.2. Molluscicidal studies

3.2.1. Evaluation of the molluscicidal activity of Rosmarinus species extracts on adult snails

Biomphalaria alexandrina is intermediate host of Schistosomiasis. Although snail control might be an effective method of controlling Schistosomiasis, there has been a general lack of control initiatives, largely due to the cost of available molluscicides. In present study the results showed rosemary extracts, by pet-ether and ethanol appear different degree of potency as shown in table 2 & fig. 2. The results obtained from the present study indicated that rosemary plant extracts can be used as ecofriendly molluscicidal agent. Similarly, Bakry [47] reported that *Euphorbia splendens, Atriplex stylosa* and *Guayacum officinalis* have molluscicidal activity against *Biomphalaria alexandrina*. Also, Vijay P [19] studied the molluscicidal property of *Eclipta alba, Balanites aegyptiaca* and *Cissus quandragularis* against the snail *Lymnaea acuminate* and the author concluded that the ethanolic extract of *E. alba* may be used for the pest management. The pet-ether extract was more potent than ethanol extract; this result was attributed to the chemical components of each extract because of the polarity of each solvent. The major components of rosemary plant were terpenoids, tannins, cardiac glycosides, flavonoids reducing sugar and saponins as stated by [46].

Table 2: Molluscicidal activity of *Rosmarinus* sp. extracts on *Biomphalaria alexandrina*.

Plant	Solvent	LC 50 (Co. Limits)	LC 95 (Co. Limits)	Slope Function
Rosmarinus sp.	Pet-ether	236.81 (213.89-262.16)	627.30 (515.23-764.14)	3.9
	Ethanol absolute	276.16 (251.31-303.45)	699.57 (569.93-859.09)	4.1

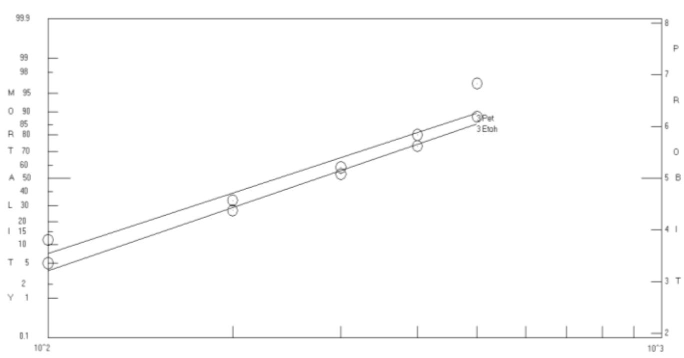

Fig. 2: Susceptibility of *Biomphalaria alexandrina* to *Rosmarinus* sp. extracts

3.3. Antibacterial studies

3.3.1. Antibacterial activity of ethanolic extract of Rosmarinus species

The effect of *Rosmarinus* sp extract was examined against six strains of pathogenic bacteria as shown in table 3 and fig. 3. The results showed that the ethanolic extract of *Rosmarinus* sp has activity against either gram-positive or gram-negative bacterial species. The extract showed the strongest inhibition against *E. coli* followed by *Pseudo. aeruginosa* with an inhibition diameter of (28 mm and 24.6 mm) respectively.

The moderate activity of *Rosmarinus* sp extract was demonstrated against *Staph. aureus* and *Acetobacter sp* at the concentration of 20% and the minimum zone of inhibition against *Ent. faecalis* (15 mm)and *Proteus sp* (14 mm) respectively.

In a study by Shama [27] on the methanolic extract of *Rosmarinus* officinalis leaves, it was found that the extract was active against *Staph. aureus*, *E. coli* and *Proteus vulgaris* at concentrations 12.5% and 25% and *Pseudo. aeruginosa* at a concentration of only 25% .

3.3.2. Antibacterial activity of petroleum ether extract of Rosmarinus species

Pet-ether extract of *Rosmarinus* sp was assayed and found to show the maximum antibacterial activity against *E. coli* and *Ent. faecalis* (DIZ, 25.3 mm and 23 mm respectively), followed by *Proteus sp, Pseudo. aeruginosa* and *Staph. aureus* with inhibition diameter (20 mm, 18 mm and 17 mm respectively) and on activity against *Acetobacter sp*. (table 3 and fig. 4).

Our results are in agreement with those found by other authors such as Nair [25] and Helena [48]. In contrast Shama [27] reported that A narrow range of antimicrobial activity was exhibited by the Pet-ether extract. At all concentrations, it was not active against *staph. aureus*, *Pseudo.aeruginosa* and *C.*

albicans. The highest activity was shown against *E. coli* at 25% with an inhibition zone of 15 mm.

The results obtained in the present study indicate that the ethanol Petroleum ether extract of *Rosmarinus* sp have varied antibacterial activities to the test organisms used. The findings support the idea that many plants are used in the treatment of various diseases whose symptoms might involve microbial infection leading to the discovery of novel bioactive compounds [49-50].

Table 3: Antibacterial activity of Rosmarinus sp. extracts. Each value is the mean of 3 replicates ± S.E.

Bacteria	Conc. (%)	Zone of inhibition (mm)			
		Plant extracts		Positive control	
		Ethanol absolute	Pet-ether	Gentamicin (10 μg)	Tetracyclin (30 μg)
Pseudomonas aeruginosa	5	18.3±0.88	10.6±0.33	22.3±0.33	16.6±0.66
	10	21.3±1.4	14.6±0.33		
	15	23±0.57	15.6±0.33		
	20	24.6±0.33	18±0.00		
Escherichia coli	5	12.6±0.33	12.3±0.33	20.6±0.33	23±0.57
	10	17±0.57	14.6±0.33		
	15	26.3±0.33	22.3±0.33		
	20	28±0.57	25.3±0.33		
Proteus sp	5	8.3±0.33	13±0.00	0±0.00	0±0.00
	10	12±0.57	17±0.57		
	15	13±0.00	18.3±0.33		
	20	14±0.00	20±0.00		
Acetobacter sp	5	12.3±0.33	0±0.00	24.3±0.66	0±0.00
	10	15.6±0.33	0±0.00		
	15	17.3±0.33	0±0.00		
	20	18.3±0.33	0±0.00		
Entrococcus faecalis	5	7±0.00	10±0.57	20±0.00	22±0.00
	10	12±0.57	13.6±0.66		
	15	13.6±0.33	19.3±0.66		
	20	15±0.00	23±0.57		
Staphylococcus aureus	5	12.3±0.33	9±0.57	19.3±0.33	12.3±0.33
	10	16±0.57	12±0.33		
	15	17.6±0.88	14±0.00		
	20	18.6±0.66	17±1.00		

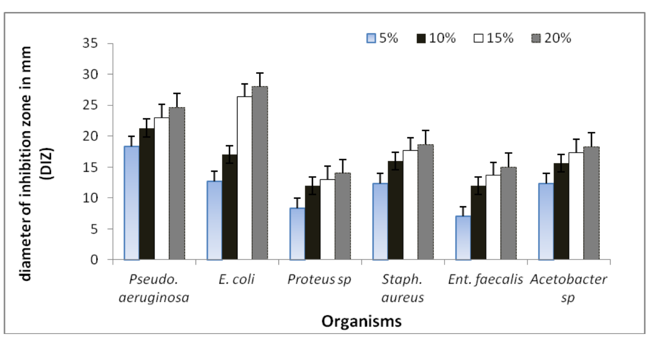

Fig. 3: Antibacterial activity of different concentrations of ethanolic extract of *Rosmarinus* sp against bacterial strains.

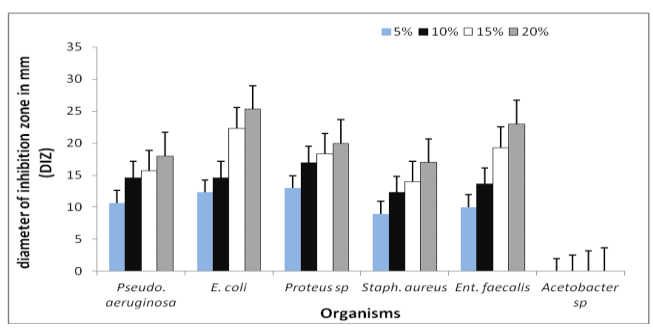

Fig. 4: Antibacterial activity of different concentrations of petroleum ether extract of *Rosmarinus* sp against bacterial strains.

4. CONCLUSION

Finally, from the present work we can conclude that *Rosmarinus* sp extracted by different solvents act as insecticidal agent beside its repellent activity. Also, can use as molluscicides and antimicrobial agents. This study of petroleum ether and ethanolic extracts of *Rosmarinus sp* detected their antimicrobial activity against the pathogens *pseudo. aeruginosa*, *E. coli*, *Proteus sp*, *Acetobacter sp*, *Ent. faecalis* and *Staph. aureus,* they can be recommended for use in folkloric medicine. However the specific antimicrobial principles inherent to the plants as well as the mode or mechanism of action of these active compounds need to be fully investigated.

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This article does not contain any studies with animals performed by any of the authors.

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