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Comparative analysis of antibacterial activity of *Nigella sativa L* and *Rumex dentatus L.*

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ABSTRACT
The present study presents the effectiveness of Nigella sativa and Rumex dentatus against some selected microorganisms which are known to cause diseases in human beings and the comparative study of extent of antimicrobial properties of various extracts. Petroleum ether, Methanol and Aqueous extracts of seeds and whole plant respectively were prepared and then tested against pathogenic microorganisms such as Pseudomonas aeruginosa, Salmonella typhimurium, Klebsiella pneumonia and Escherichia coli using agar well diffusion method. The zone of inhibition for Nigella sativa was found maximum in petroleum ether extract of 30 mm at a dose of 250µg/ml against Salmonella typhimurium while as at the same concentration the highest inhibition zone diameter of 21mm was recorded against K. pneumoniae for methanol extract in case of Rumex dentatus.

Keywords:- *Microorganisms, Nigella sativa, Rumex dentatus, antimicrobial and Pathogen.*

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I. INTRODUCTION

Pharmaceutical and scientific communities have recently received the attention of medicinal plants and various publications have documented the therapeutic worth of natural compounds to validate the claims of their biological activity. Profuse use of commercial antibiotic and synthetic pesticides for human and crop protection is harmful to human health, ecosystem and environment. Attention has also been drawn to the antimicrobial properties of plants and their metabolites due to the growing incidences of drug-resistant pathogens of both clinical and agricultural importance. Medicinal plants have their intrinsic ability to resist pathogenic microorganisms and this has led the researchers to investigate their mechanisms of action. *Nigella sativa* is an economically important umbel growing wild in the dry temperature regions belonging to the botanical family of Ranunculaceae is an annual flowering plant, native to south and southwest Asia. It was first identified and described by Linnaeus in 1753 (Jansen *et al.,* 1981). The plant is an erect profusely branched herb that can attain heights of 40-70cm. It bears alternate leaves, terminal white flowers and capsule like fruits. The flowers are delicate, and usually colored pale blue and white, with five to ten petals. The fruit is a large and inflated capsule composed of three to seven united follicles, each containing numerous seeds. The latter are filled with black ovoid seeds attaining lengths and widths ranging from 2.5 to 3.5mm and widths from 1.5 to 2mm. respectively. The plant is known to all Arabian and Islamic countries and carries various colloquial names. It is known generally by the names Habbat Albarakah, Alhabahat Alsawda and Alkamoun Alaswad (Saad *et al.,* 2009). Of all the plant organs it is only the seeds which have attracted most of the researchers starting from Egypt and the Sudan in Africa and extending to Saudi Arabia, India and Pakistan in Asia and most recently those in Japan, France England, Canada and USA. Studies conducted over past 2 decades have revealed a multi-range of actions that covered almost all known ailments of mans various body systems. Traditionally, it is used as a natural remedy for a number of illnesses that include asthma, cough, hypertension, bronchitis, diabetes, headache, eczema, fever, infl ammations, and other diseases (Morsi, 2000; Saad *et al.,* 2009).While as the *Rumex dentatus L.*, a medicinal plant belonging to family Polygonaceae was selected for the present study. Preliminary phytochemical screening was carried out to detect the presence of phytochemicals that add to the medicinal value of the plant.

II. MATERIALS AND METHODS

PLANT MATERIAL

Both *Nigella sativa* seeds and *Rumex dentatus* plant were collected locally from Srinagar and identified at Department of Botany and University of Kashmir, Srinagar.

III. EXTRACTION OF PLANT MATERIAL

15 gram powdered sample of *Nigella sativa* seeds was extracted with Petroleum ether, Methanol and Aqueous by cold extraction method. The extracts were filtered through Whatman. No.1 filter paper, evaporated on water bath and stored at4ºC.for further use meanwhile in case of *Rumex dentatus* 50g of powdered plant material was loaded in Soxhlet extractor and defatted with petroleum ether. This solvent extract was collected, dried and later weighed and kept for further usage. The defatted material was also dried and then re-loaded in Soxhlet extractor for other solvent (methanol and aqueous) extraction. Extracts collected were dried and weighed and kept for further usage in sterilized caped vials at 4ºC.

IV. TEST ORGANISMS

The test microorganisms used in this study *Klebsiella pneumoniae, Escherichia coli, seudomonas aeruginosa* and *Salmonella typhimurium* were obtained from Bacteriological and Mycological section of Department of Microbiology, SKIMS, Soura, Srinagar.

Result:-

Antibacterial activity:-

The antibacterial activities of different concentrations ranging from 125g/ml to 250µg/ml of three extracts of *Nigella sativa* (table 1)and *Rumex dentus* (table 2) were determined against different bacterial strains respectively and their activity recorded as inhibition zone diameter (IZD), measured in 'mm' methanol as negative control, gentamicin(G) and Streptomycin(S) as positive control. The results of the antibacterial activity of the investigated extract are shown in Tables 1 and 2. In this study, petroleum ether, methanol and aqueous extracts showed no inhibition against all the bacteria tested at lower concentrations (125 µg/ml). Generally, the petroleum ether and methanol extract of *Nigella sativa* exhibited higher antibacterial effect compared with aqueous extracts. The petroleum ether showed inhibitory effects against all the tested bacterial strains except *Klebsiella pneumonia* with highest inhibition zone diameter of 30mm for *Salmonella typhimurium* at 250µg/ml concentration and the lowest 20mm against *P. aeruginosa* at 250µg/ml concentration. The inhibition zone of the tested strains at different concentrations ranged between 20-30mm. Methanol extract of the plant showed highest inhibitory activity against *K. pneumonia* and *Salmonella typhimurium* 20mm at 250µg/ml concentrations and no effect was seen on *Escherichia coli*. However, aqueous extract of the seeds showed highest inhibitory activity against *Klebsiella pneumoniae* 28mm at 250µg/ml whereas *Salmonella typhimurium* showed resistance while as The methanol extract of *R. dentatus* showed highest inhibitory activity against *K. pneumoniae* with IZD of 14mm and 21mm at lower and higher concentrations respectively against the control (gentamycin 35 mm). The methanolic extract at lower concentration did not show any inhibitory activity against *E. coli, P. aeruginosa.* However, *S. typhimurium* was found to be resistant for this extract. The aqueous extract of *Rumex dentatus* did not exhibit any inhibitory activity towards any of the tested bacterial strains as shown in Tables 2.

Table 1

Bacterial strains	Inhibition zone diameter (IZD) mm						
	Petroleum ether		Methanol		Aqueous		Antibiotic
	125µg/ml	250 µg/ml	125 µg/ml	250 µg/ml	125 µg/ml	250 µg/ml	250 µg/ml
Pseudomonas aeruginosa	-	-	-	20	-	10	S (25)
Salmonella typhimurium	-	-	-	30	-	20	G (20)
Klebsilla pneumonia	-	-	-	-	-	20	G(35)
Escherichia coli	-	-	-	26	-	-	G(30)

Table 2:-

Bacterial strains	Inhibition zone diameter (IZD) mm						
	Petroleum ether		Methanol		Aqueous		Antibiotic
	125µg/ml	250 µg/ml	125 µg/ml	250 µg/ml	125 µg/ml	250 µg/ml	250 µg/ml
Pseudomonas aeruginosa	-	-	-	17	-	-	S (25)
Salmonella typhimurium	-	-	-	-	-	-	G (20)
Klebsilla pneumonia	-	-	14	21	-	-	G (35)
Escherichia coli	-	-	-	12	-	-	G (30)

V. DISCUSSION

N. sativa seed extracts have been found to possess remarkable antibacterial activity. In the present study three different extracts (petroleum ether, methanol and aqueous) of *N. sativa* showed pronounced activity against *Klebsiella pneumoniae* and *Escherichia coli* and low activity against *Salmonella typhimurium*. Similarly, in a study conducted on the extract of *N. sativa* it was found to be effective on Gram negative bacteria. The Gram positive bacterial strains were resistant due to the fact that they possess an outer membrane which acts as a barrier to many environmental substances including antibiotics (Jigna et al., 2007). The antibacterial effects of the extracts could be explained by disturbance of the permeability barrier of the bacterial membrane structure (Cowan et al., 1999). The results of the antimicrobial activity of the investigated extract are shown in which the petroleum ether, methanol and aqueous extracts showed no inhibition against all the bacteria tested at lower concentrations (125 µg/ml). Generally, the petroleum ether and methanol extract of *Nigella sativa* exhibited higher antibacterial effect compared with aqueous extracts as suggested by (Hasan et al., 2013), in which the two methanol and aqueous extracts were tested against bacterial and fungal strain and it was found that both methanol and aqueous extracts showed no inhibition against all the bacteria tested at lower concentrations (<50 mg/mL). Generally, the methanol extract of *Nigella sativa* exhibited higher antibacterial effect compared with aqueous extracts. Another recent study conducted by Shahid et al., (2013) on antibacterial activity in vitro of medicinal plants which revealed that the plant extracts inhibited bacterial growth but their effectiveness varied. Ethyl acetate extract of selected plants showed higher inhibition against tested bacteria at high concentration. While methanol and ethanol extract of selected plants had more significant effect on various tested bacteria as compared to ethyl acetate extract. The antibacterial activity has been attributed to the presence of some active constituents in the extracts. The demonstration of broad spectrum of antimicrobial activities by the plants used in this study may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of gastrointestinal, wound infections and typhoid fever. Similarly (Abdelmalek et al., 2013) studied the antibacterial activity of honey alone and in combination with *Nigella sativa* seeds against *Pseudomonas aeruginosa* infection which proved fruitful. in which in vitro activities, of three honeys sample, and *Nigella sativa* against *Pseudomonas aeruginosa* alone and in combination and an additive effect between honey and *N. sativa* as regards of their three varieties of honeys studied against *P. aeruginosa* showed that the MIC for the three varieties of honeys were by decreasing order of effect.; 3% (v/v) and 12% (w/v), 2% (v/v) and 14% (w/v), and 2% (v/v) and 10% (w/v). The methanol, butanol, ethanol and ethyl acetate extracts of *Rumex dentatus* displayed promising antimicrobial activity against a wide range of the bacteria and fungi that were tested. The methanol extract inhibited maximum bacterial strains whereas the aqueous extract and petroleum ether inhibited none of the tested bacterial strains. The results indicate that the methanol yielded more potent extracts with higher antimicrobial activity thus inhibiting the highest number of bacterial strains as is evident. Rabe and Van Staden, 1997 and Vlachos *et al*. (1996) reported similar findings on the high antibacterial activity displayed by the methanol extract in comparison to other extracts. This may also be attributed to the presence of soluble phenolic and polyphenolic compounds (Kowalski and Kedzia, 2007). The results are also in confirmation with a recent study of Bandh *et al*. (2011) in which it was shown that the methanol extract of *Nepeta cataria* inhibited the growth of all the bacterial and fungal test organisms, with maximum inhibitory effects on *S. aureus*, *P. multocida* and *E. coli* and a minimum effect on *A. flavus*. Thus suggesting that the antimicrobial activity of the extract may be related to the mono-terpenoid component i.e., nepeta-lactone. The lack of antibacterial activity in some of the concentrations of the extract is not surprising as a number of plant extracts have been found ineffective against certain test organisms at lower concentrations and may be attributed to the presence of lesser amounts of the antimicrobial compounds. The antibacterial effects of the extracts could be explained by disturbance of the permeability barrier of the bacterial membrane structure (Cowan, 1999). The bacterial isolate,

S. typhimurium was inhibited by the chloroform extract only and none other extract had an inhibitory effect on this bacterial strain. The aqueous extract of the plant inhibited none of the bacterial strains in comparison to the other extracts. This is in consonance with the results of Shale *et al*. (1999) that reported on the water being less effective than other alcoholic solvents at extracting the active compounds from plants. The results are also confirmed by the findings of Igbinosa *et al*. (2009) showing the aqueous extract of *Jatropha curcus* as inactive against all the bacteria at all the concentration tested. Saponins and tannins which are reported to be in abundance could be extracted by methanol and acetone (Masoko and Eloff, 2005). Non-polar solvents yield more lipophilic components, while alcoholic solvents give a larger spectrum of polar material.

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