

***In vitro* antimicrobial activity of *Rumex Dentatus L.* (Polygonaceae) plant extracts**Zainab<sup>1</sup>, Muhammad Shahab Khan<sup>2\*</sup>, Munair Ahmad<sup>3</sup>, Muhammad Aimal khan<sup>4</sup> and Sobia<sup>5</sup><sup>1</sup>Department of Botany, Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan<sup>2</sup>Department of Chemistry, University of Malakand, Khyber Pakhtunkhwa, Pakistan<sup>3</sup>Department of Zoology, Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan<sup>4</sup>Department of chemistry, Government postgraduate college, Dargai Malakand, KPK, Pakistan<sup>5</sup>Department of Botany, Abdul Wali khan University, Mardan, Khyber Pakhtunkhwa, Pakistan**Abstarct**

The realization of the treatment lies in the continuous exploration of new drugs to counteract the challenges pretended by the resilient kinds of different microorganisms. *Rumex dentatus L.* also entitled toothed dock or dentate dock typically utilized as an antimicrobial agent. Almost 150 different species of the genus *Rumex* are extensively found all over the world. Conventionally, *Rumex dentatus* is employed for the cure of countless disorders in the body of organisms. In the current research, we evaluated the phytochemical investigation of the existence of numerous antimicrobial activities of the whole plant extracts of distinct solvents namely ethanol, ethyl acetate, methanol, hexane and water. *Rumex dentatus* against various microbes such as gram-positive bacteria namely, *B. subtilis*, *S. aureus*, *B. atrophoeus*, gram-negative bacteria such as *S. typhi*, *K. pneumoniae* and *E. coli* and fungal strains including, *C. albicans*, *Aspergillus* and *Rhizopus* by in-vitro agar diffusion technique. The effectiveness order of different solvent extracts was ethanol > ethyl acetate > methanol > hexane > aqueous extract respectively. An Ethanolic extract of the plant under investigation exhibited the maximum inhibitory activity against fungus *C. albicans* with a zone of inhibition  $22 \pm 0.23$  mm. in case of Gram-positive and Gram-negative bacteria, noteworthy effect was expressed by ethanolic extract against *S. aureus* with a zone of inhibition  $18 \pm 0.16$  mm and *E. coli* with a zone of inhibition  $19 \pm 0.08$  mm. The present approach offers a scientific base for the noteworthy practice of this plant in the cure of numerous pathological diseases.

**Keywords:** Antimicrobial, *Rumex dentatus*, Disc diffusion, Gram negative bacteria and Gram positive bacteria**\*Corresponding Author's Email:** [Shahabalchemist@gmail.com](mailto:Shahabalchemist@gmail.com)

## 1. Introduction

Biologically active composites from Plant sources have constantly been a great attention for scientists working on various infectious diseases [1]. The objective of the usage of these medicinal plants is to accomplish a relationship with the biochemistry of organisms [2]. Because of the low possibility of toxicity and effectiveness, plants are worldwide used for the inhibition and cure of numerous diseases [3]. Plants with the medicinal importance are characteristically utilized in non-chronic situations [4]. Around four billion people worldwide today trust plants as a main source of remedies, even in advanced countries. Presently, at least 25% of typical medications suggested by a physician originate from traditional drugs [5]. Exploring healing potential in plants is an ancient concept. Plant based moieties have lately become of great importance, comprising to their adaptable applications [6]. Remedies of medicinal plants have been utilized for centuries for human infection diseases because they are the main bio-resources of potential drugs of traditional medicinal systems [7]. Research for novel antimicrobial means is essential due to the advancement of microbial opposition and adaptable fetal disorders [8]. Finding and developing novel antimicrobial drug is a continuing process [9]. The significant assortment of chemical substances in biological samples has remarkable power in the examination of newly antimicrobial agents. Almost every antifungal agent currently used is relatively costly and has toxic side effects [10].

In Pakistan rural areas people are utilizing medicinal plants for the treatment of various types of disorder [11].

*Rumex dentatus* medicinal plant (Fig 1) is also acknowledged as dentate dock and toothed dock. It is generally accustomed as an antibacterial-agent in the cure of numerous diseases [12]. Almost 150 different types of species of the *Rumex* genus are extensively apportioned so far. Mostly these species are found in temperate western regions of the world [13]. In Pakistan it is found in district Mansehra including Bafa, Dhodial and Shinkiyari as well [14]. Phytochemical inspection of *Rumex dentatus* plant exhibited the existence of different class of compounds, namely, phytoesteryl esters, anthraquinones, flavonoids, phytosterols and enthrones [15].

Pharmaceutically, it is utilized as antimicrobial, anti-inflammatory, anti-tumor, anti-diuretic and laxative agent respectively [16]. Plants contain various numbers of biologically important active compounds, for their examination, it is significant to have the essential techniques, comprise proper bio-activity assays and chemical exploring techniques [17].



**Fig 1.** Image of *Rumex dentatus* plant

## **2. Materials and methods**

### **2.1. Instruments and apparatus**

Analytical Balance, Memmert Incubator, Rotary evaporator, Heating Plate, Petri dishes, Autoclave, Agar media and Laminar flow hood.

### **2.2. Bacteria and reagents**

Gram-negative bacteria, stains namely *E. coli*, *K. pneumonia* and *S. typhi*, Gram-positive bacterial strains included in the study namely, *S. aureus*, *B. subtilis* and *B. atropoeus* and fungal strains such as *C. albicans*, *Rhizopus sp* and *Aspergillus sp*.

### 2.3. Extraction

The plant sample under investigation was immersed in water (distilled) and store in a shed at NTP. The dried entire *Rumex dentatus* plant was grinded with grinder. Grinded form of whole plant (300g) was immersed in ethanol for one week. The under observation plant extracts were refined by utilizing Rotary evaporator. In next step plant extracts were concentrated at low pressure. The extract was entirely dehydrated out to condense (liquid form). Ethanol, hexane, ethyl acetate, methanol and water extracts were set by mean of separating funnel and prepared solutions were kept at 4C° in the thermal block. In the current work, nutrient Agar was utilized for uniformity, inoculation and incubation of particular microorganisms. The agar nutrient was utilized for the growth of nominated microbes. *Rumex dentatus* whole plant extracts of solvents were evaluated for anti-microbial capabilities.

### 2.4. Disc Diffusion Method

*Rumex dentatus* antimicrobial activity was explored with the help of disc diffusion technique. To investigate the presence of the antibacterial compounds in the plant extracts, it is one of the best methods. The Petri dishes were dried for almost 15 minutes and then utilized for the antibiotic assay. The discs that had been submerged with the *Rumex dentatus* extracts were placed outwardly on Mueller-Hinton's agar. The standard antibiotic disc was clarithromycin (30 µg) was applied to these bacteria. The discs were then left for 18 to 24 hrs at 37°C. Depending on the bacterial types used in the samples. After incubation, the plate-inhibiting zone was investigated. The zone of inhibition was then calculated using callipers and then recorded. The trials were repeated four times to confirm that the activity was reliable.

### 2.5. Statistical analysis

The standard error of the means was calculated employing SPSS.V.16 software.

## 3. Results and discussion

### 3.1. Use of standard drug against different bacteria and fungi

Ciprofloxacin, a standard antibiotic drug was utilized against the Gram-negative bacteria, *E. coli*, *S. typhi* and *K. pneumonia* and Azithromycin another standard antibiotic drug beside the Gram-positive bacterial strains

namely, *S. aureus* (Fig.2), *B. subtilis* and *B. atropoeus* while Coltrimazole was utilized beside fungal strains, *C. albicans*, *Aspergillus sp.* and *Rhizopus sp.* with a dose rate of 50mg per 6  $\mu$ L concentration. Standard antibiotic drug confirmed a zone of inhibition of 50mm, 48mm and 65mm against *B. subtilis*, *S. aureus* and *B. atropheous*. The other antibiotic drug revealed a zone of inhibition of 40mm, 35mm and 45mm against *S. typhi*, *E. coli* and *K. pneumonia*, respectively. While standard anti-fungal drug exhibited 25mm, 65mm and 60mm zone of inhibition against *C. Albicans*, *Aspergillus sp.* and *Rhizopus sp.*

### 3.2. Different solvents extract of *Tagetes minuta* plant against different microbes

*Rumex dentatus* ethanolic extract exhibited the extraordinary inhibitory efficacy beside the Gram-positive bacterium, *S. aureus* with a zone of inhibition  $18 \pm 0.1$  mm (Fig. 3), beside the Gram-negative bacterium, *E. coli* with  $19 \pm 0.06$  mm zone of inhibition and fungus, *C. albicans* with a zone of inhibition  $22 \pm 0.26$  mm and that of the standard was  $48 \pm 0.04$  mm,  $35 \pm 0.03$  mm and  $25 \pm 0.07$  mm zone of inhibition (Graph.1). *Rumex dentatus* Methanolic extract explored the maximum inhibitory potency beside the Gram-positive bacterium, *S. aureus* with a zone of inhibition  $15 \pm 0.52$  mm, against the Gram-negative bacterium, *E. coli* with a zone of inhibition  $18.5 \pm 0.56$  mm and fungus. *C. albicans* with a zone of inhibition  $19 \pm 0.05$  mm and that of the standard was  $48 \pm 0.06$  mm,  $35 \pm 0.08$  mm and  $25 \pm 0.05$  mm zone of inhibition respectively (Graph.2). *Rumex dentatus* ethyl acetate extract explored the maximum inhibitory efficiency beside the Gram-positive bacterium, *S. aureus* with a zone of inhibition  $16 \pm 0.07$  mm, against the Gram-negative bacterium, *E. coli* with a zone of inhibition  $18 \pm 0.08$  mm and fungus, *C. albicans* with a zone of inhibition  $21 \pm 0.09$  mm and that of the standard was  $48 \pm 0.05$  mm,  $35 \pm 0.07$  mm and  $25 \pm 0.05$  mm zone of inhibition (Graph.3). *Rumex dentatus* hexane extract explored the determined inhibitory efficiency beside the Gram-positive bacterium, *B. subtilis* with a zone of inhibition  $16.5 \pm 0.52$  mm, beside the Gram-negative bacterium, *E. coli* with a zone of inhibition  $16 \pm 0.04$  mm and fungus, *C. albicans* with a zone of inhibition  $18 \pm 0.05$  mm and that of the standard was  $50 \pm 0.03$  mm,  $35 \pm 0.07$  mm and  $25 \pm 0.02$  mm zone of inhibition (Graph.4). *Rumex dentatus* aqueous extract

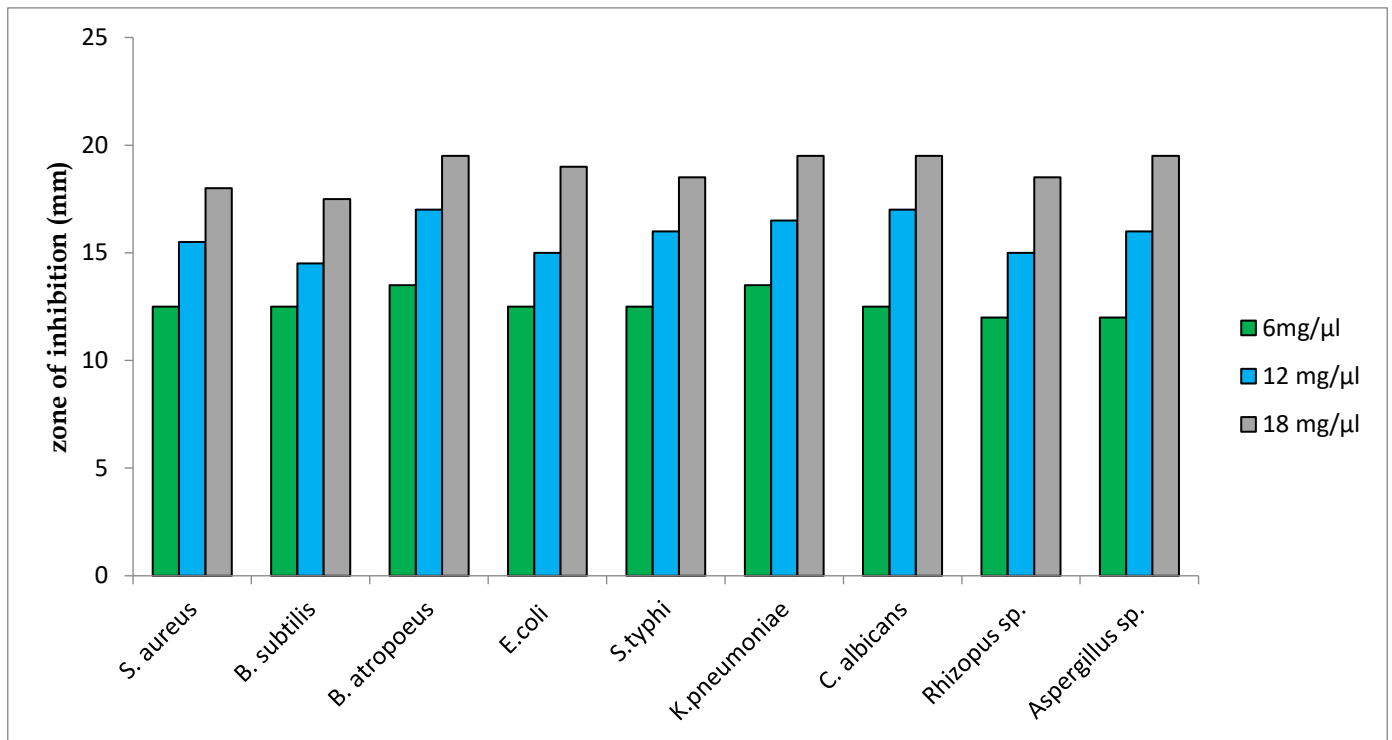
indicated the maximum inhibitory potential beside the Gram-positive bacterium, *S. aureus* with a zone of inhibition  $16\pm 0.13$  mm, against the Gram negative bacterium, *E. coli* with a zone of inhibition  $14\pm 0.06$  mm and fungus, *C. albicans* with a zone of inhibition  $10.5\pm 0.57$  mm and that of the standard was  $48\pm 0.0$  mm,  $35\pm 0.03$  mm and  $25\pm 0.07$  mm zone of inhibition (Graph.5).



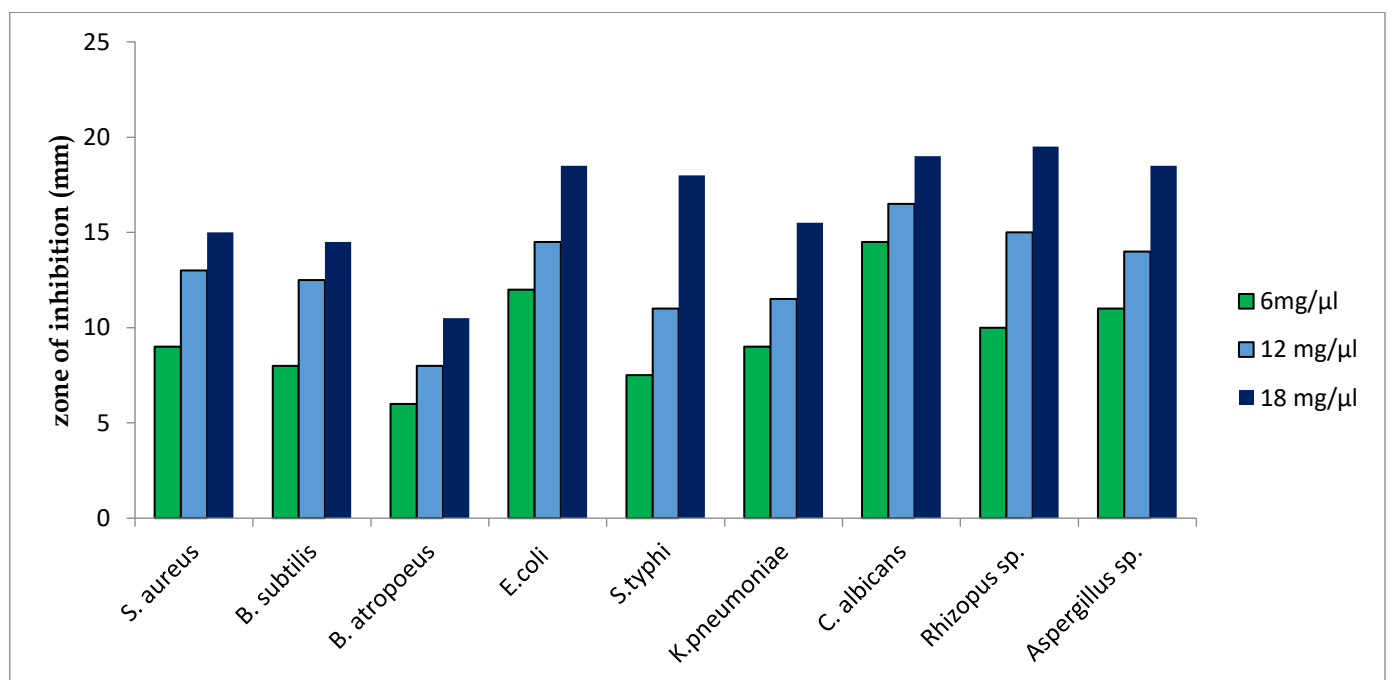
**Fig 2.** Image (standard antibiotic against *S. aureus*)



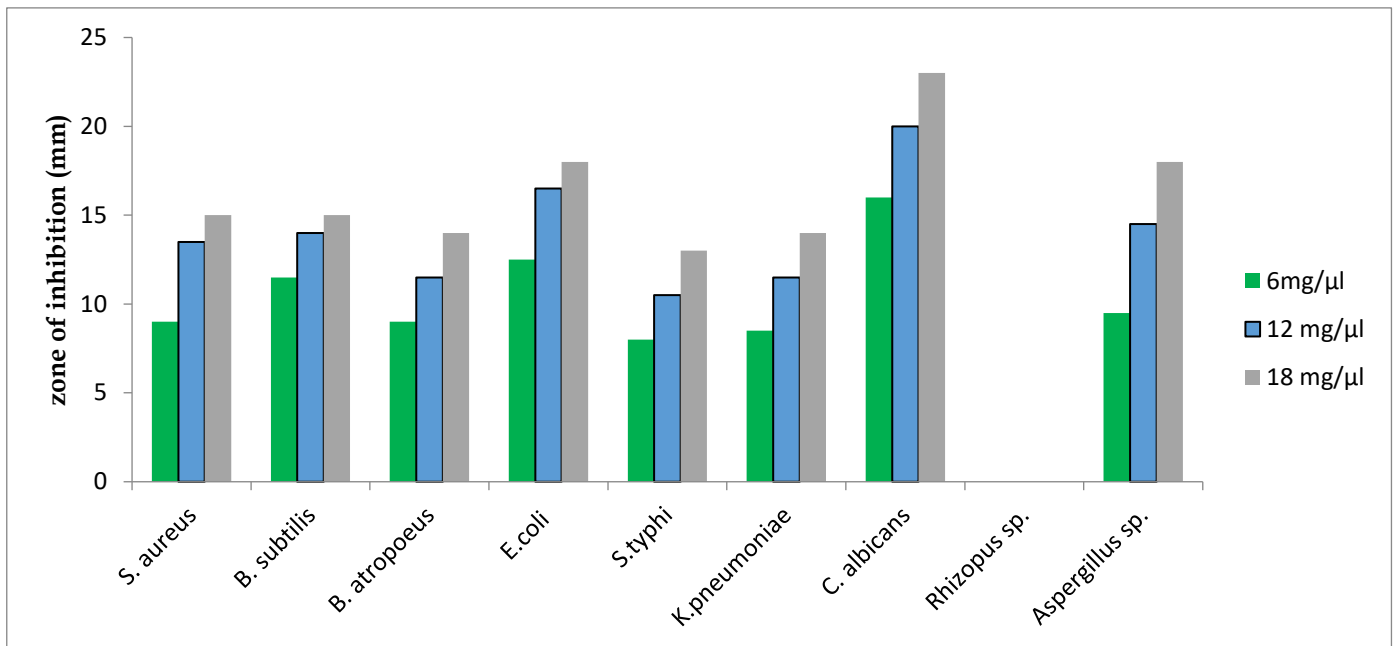
**Fig 3.** Image (Ethanolic extracts against *S. aureus*)



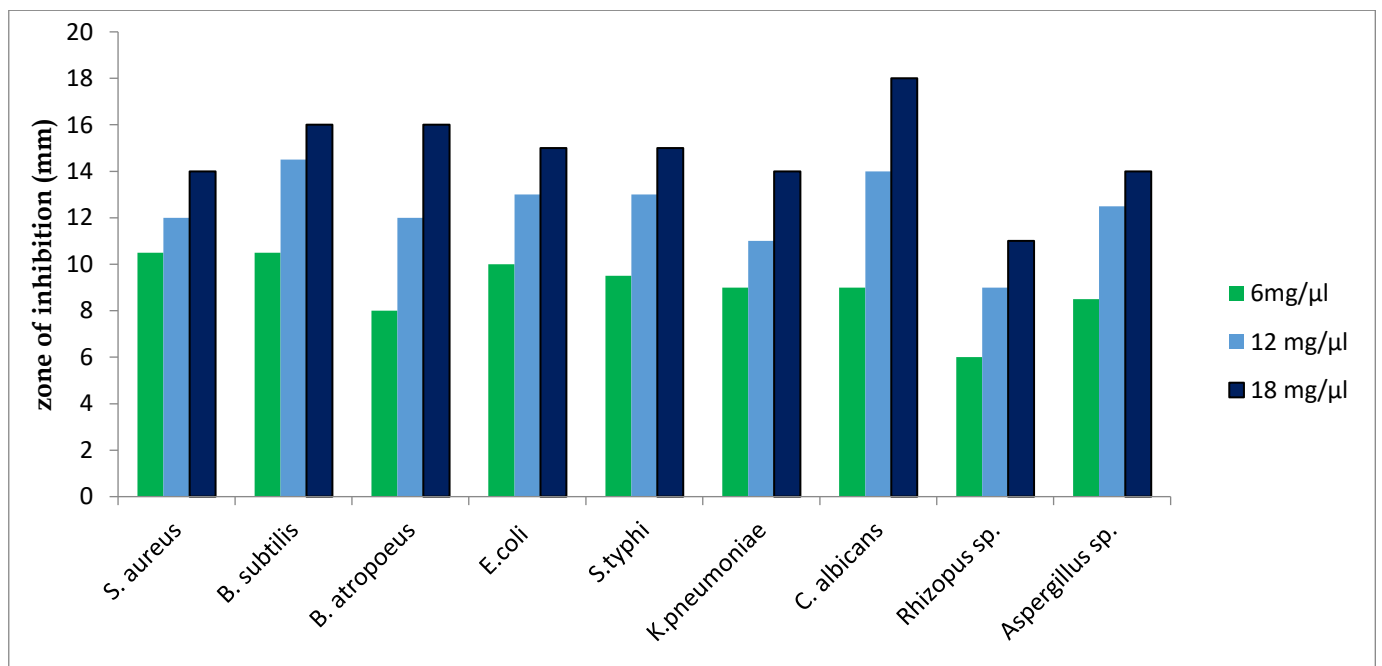
**Graph.1.** Antimicrobial activity of the ethanolic extract of *Rumex dentatus*



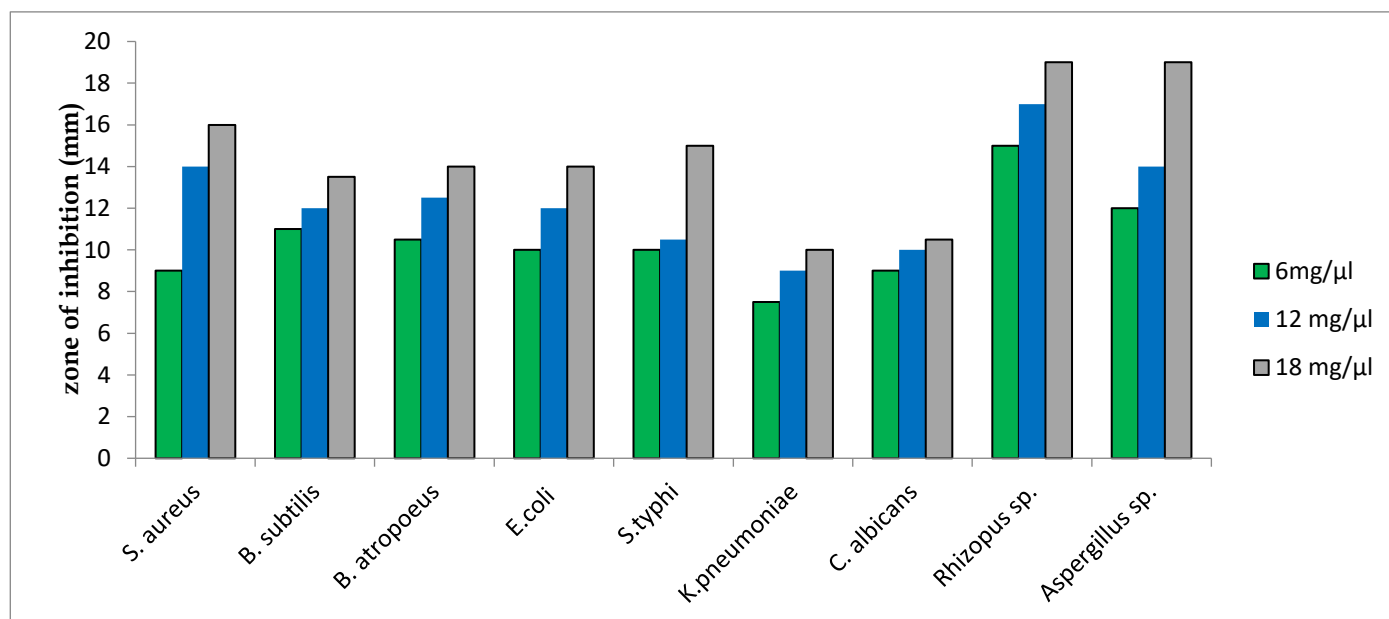
**Graph.2.** Antimicrobial activity of the Methanolic extract of *Rumex dentatus*



**Graph.3.** Antimicrobial activity of the ethyl acetate extract of *Rumex dentatus*



**Graph.4.** Antimicrobial activity of the hexane extract of *Rumex dentatus*



**Graph.5.** Antimicrobial activity of the aqueous extract of *Rumex dentatus*

### 3.3. Discussion

The mechanisms of action of natural products based on traditional medicinal plants have been focused on the advantage of their antioxidant activities. Lessons learned from nature could help overcome the toxicity problems of synthetic antioxidants. Efficacy order of different solvent extracts was ethanol>ethyl acetate>methanol>hexane>aqueous extract. *Rumex dentatus* ethanolic extract presented the remarkable inhibitory activity beside fungus *C. albicans* with a zone of inhibition  $22\pm 0.2$  mm. In case of Gram-positive and Gram-negative bacteria, noteworthy effect was expressed by an ethanolic extract against *S. aureus* with a zone of inhibition  $18\pm 0.16$  mm and *E. coli* with a zone of inhibition  $19\pm 0.08$  mm. *Rumex dentatus* includes flavonoids, saponins and anthraquinones in significant amount.

### 4. Conclusion

Medicinal Plants comprise various components which are valuable means of novel and biologically active molecules possessing antimicrobial potential. Based on the obtained result, we have concluded that the *Rumex dentatus* whole plant extracts have bioactive compounds having a good antibacterial and anti-fungal activities

against different microbes. Furthermore, the zone of inhibition order according to the numerous strains of organisms was fungi > Gram positive bacteria > positive-bacteria. Further study should be made for the finding of new anti-microbial medications.

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### Recommendation

The present study recommends additional in-vitro and in-vivo studies and clinical trials to improve novel antimicrobial agents in this era of antimicrobial resistance.

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### Conflict of interest

The authors declare that they have no potential competing interest.

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