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Research Article

# Antimicrobial, phytochemical and traditional studies of selected medicinal plant in Bajaur agency, Pakistan

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## ABSTRACT

Phytochemicals are mainly divided into groups like secondary and primary constituents on the basis of their metabolic function in plants. Primary constituents contain amino acid, proteins chlorophyll, and common sugars whereas secondary constituents contain saponins, alkaloids, terpenoids, phenolic compounds, flavonoids, tannins.The subsequent method was used for the determination of antimicrobial activity. *Oxalis corniculata* ethanolic extract were 17.5±2.5, 19 ± 0.5 and 15 ± 0.1 against the fungus, (*C. albican)* Gram positive bacterium (*S. aureus)* and negative bacterium (*E. coli*), respectively. *Rumex dentatus* extract in the same solvent made the 22±0.2, 18± 0.1 and 18±0.1 mm zones of inhibition against the fungus (*C. albicans*) Gram positive bacterium (*S. aureus)* and negative bacterium (*E. coli*), respectively. In addition to this, the extract *Tagetes minuta* made the 22±0.2, 20±0.2 and 18±0.2 mm zones of inhibition against the fungus (*C. albicans*) Gram positive bacterium (*B. subtilis*) and negative bacterium (*E. coli*), respectively. In other words, the order of inhibitory potential was *Cichorium intybus* > *Medicago sativa* > *Tagetes minuta* > *Rumex dentatus* >*Oxalis corniculata.* Ethanolic extract was more effective followed by methanolic, ethyl acetate, hexane and aqueous extracts. Ethanol was more effective followed by methanol, ethyl acetate, hexane and aqueous extracts. Gram positive bacteria were more resistant followed by Gram negative bacteria and fungi

***Key words:*** *Antimicrobial activities; Phytochemistry; Traditional medication; Pakistan*

## INTRODUCTION

Plants have been conventionally used in the handling of numerous diseases of human. Their therapeutic as well as pharmacological properties have been ascribed to different chemical constituents isolated with antioxidant activities[1]. Phytochemical are mainly divided into groups like secondary and primary constituents on the basis of their metabolic function in plants. Primary constituents contain amino acid, proteins chlorophyll, and common sugars whereas secondary constituents contain saponins, alkaloids, terpenoids, phenolic compounds, flavonoids, tannins etc [2]. They may have raw or processed ingredients from more or one plant which is useful for the human health [3]. Plants having medicinal properties are vital with respect to pharmaceutical industry and latest drugs. Plants were used in ancient time as the medicine. In recent times, the use of medicinal plants improved substantially [4]. A large number of plants are there in Pakistan which include medicinal properties and such plants are used for remedial purposes. Pakistan is a developing country and up to great extent, depends on plant assets for agricultural, food, fodder, shelter and herbal medicines [4]. In emergent countries like Pakistan, extracts of the plants are still the chief source of conventional handling. Recent study have determined that about 60-80%ofthe total world population, still use extracts of the plant as a conventional, medicinal and antimicrobial agents. In every part of the world. Plants having medicinal characteristics are broadly worn for the treatment of diseases [5]. World Health Organization estimated that almost80% population of the world is appealing attentioninhabitant as curative remedy [6]. Diarrhea,

infection of the skin, diabetes, respiratory disease, malaria, bacterial and fungal disease are serious recognizable health problems inrusticareas. In developing countries, a lot of medicinal plants are used conservatively which are remedial in opposition to such disease [7].

**1.2 *Oxalis corniculata* L.**

*Oxalis corniculata* L. (Oxalidaceae) is also called creeping wood, sorrel [8]. In Pakistan, this plant is distributed in Hazara, Chitral, Hunza, Peshawar, Rawalpindi, Attock, Jhelum, and Lahore and in the provinces of Sind and Baluchistan [9].

**1.2.1 Phytochemical investigation**

Phytochemical analysis of *Oxalis corniculata* shows the occurrence of flavonoids, phenol, tannins, phytosterol, glycoseides volatile oil and fatty acids. The leaves have vitexine-2- O- beta – D-glucopyrunosideiso-vitexine and flavonoids. It is high source of important fatty acids like oleic, linolenic palmitic acid, stearic acids and linoleic acid. It is high source of carotene, vitamin C and contain a high level of oxalates [10].

**1.2.2 Traditional Use**

This plant is a good appetizer, removes vata, kapha and piles, cures dysentery and diarrhoea, astringent for skin diseases and quarten fevers. Small leaves are externally used to eliminate opacities and warts of cornea. The leaves are refrigerant and anti-inflammatory [11].

**1.3 *Rumex dentatus* L.**

*Rumex dentatus* L. (Polygonaceae) is also called toothed dock dentate dock and Indian dock Conventionally, *Rumex* is used as a antibacterial. About 150 species of the genus *Rumex* are widely distributed around the world [12]. They are found from Kashmir to Kumaon, 8000-12000 feet from sea level all over the temperate Western Himalayas. This plant is also found in Pakistan in the differentparts of District Mansehra including Bafa, Dhodialand Shinkiyari [12].

**1.3.1 Phytochemical investigation**

Phytochemical examination of*Rumex dentatus* showed the occurrence of anthraquinones, phytosterols, phytoesteryl esters, free fatty acids, flavonoids, chromones and anthrones. Furthermore, some phenolics have been detected in *R. dentatus* [13].

**1.3.2 Traditional Use**

Medicinally, it **is** used asanti-inflammatory, bactericidal, anti-tumor, antidermatitis astringent, cholagogue, diuretic, tonic and laxative agent [13].

**1.4 *Tagetes minuta* L.**

*Tagetes minuta* is important member of the family Asteraceae. *Tagetes glandulifera*Schrank is the synonym of *Tagetes minuta* L. Its involucres are tiny, having particular odour and toxic flowers. It is a weed; and is capable to adapt themselves in approximately alltemperate region [14]. This plantis the inhabitant of mountainous regions of the South America and Temperate Grasslands, it is naturalized in Asia, Europe, Africa, Australia, United States and New Zealand such as Cape Verde, Hawaii, Madagascar and Madeira. *Tagetes minuta* isalso foundin Kenya, Ethiopia and other parts of the Northern India, waste places of Spain and East Africa [14]. In Pakistan, this plantcan survive in a broad range of environmental conditions opening from 3000-11000 ft height from the level of sea in the Northwestern and Northern regions of Pakistan. *Tagetes minuta* prefers cooler climates in Pakistan and is frequent in Hazara and Swat districts of KP, Pakistan.

**1.4.1 Phytochemical investigation**

Phytochemical study of the plant demonstrates the presence of saponins, tannins, terpenoids, flavonoids and alkaloids [15].

**1.4.2 Traditional use**

This plant is used as a condiment, diuretic, stomach strengthened, diaphoretic, purgative, menstrual stimulant, hysteria remedy and for flavouring to milk and cheese [16].

## MATERIAL AND METHOD

**2.1 Plant Material**

The Three Plant species *Targets minuta* (AWKUM-145), *Rumex dentates* (AWKUM-146) and *Oxalis corniculata* (AWKUM-147) were collected from Bajaur agency. The collected plant species were identified by Dr. Gul Jan (Assistant Professor Botany department) and submitted to Abdul Wali Khan University, Mardan, Pakistan Herbarium.

**2.2 Extraction**

The plants were dipped in tap water and kept at room temperature in shade, at Pakistan Council of Scientific and Industrial Research Centre Peshawar (PCSIR). The dried whole plants of the *Oxalis corniculata, Rumex dentatus* and*Targets minuta* were grinded with ordinary grinder. Three hundred grams of powder of whole plant of *Oxalis corniculata Rumex dentatus* and *Tagetes minuta* were dipped in ethanol in flask for one week [17, 18]. The extract was filtered by using Rotary evaporator and concentrated below reduced pressure. The extract was completely dried out to condense into liquid form. Extracts such as methanol, ethyl acetate, hexane and aqueous extracts were prepared by using separating funnel. The extracts were kept at 4C° in fridge. The plant extract i.e. methanol, ethanol and aqueous were tasted for the absence or presence of phytochemical constituents' like alkaloids, tannins ,Phlobatannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins, volatile oils, resins glycosides and terpenoids.

**2.3 Media**

In this research, nutrient broth medium was used for standardization shaking, incubation and inoculation of selected microbes. Nutrient agar medium was added in one liter D.W used for culturing and growth of selected microbes.

**2.4 Media Preparation**

1.3g of nutrient broth and 2.8g of nutrient agar were prepared in distilled water and transferred into flasks. Nutrient broth (about 20 ml) was transferred into the test tubes. The medial test tubes as well as flasks were plugged up by the cotton and then kept in autoclave at 121ºC under 15 pounds pressure for fifteen minutes for sterilization. The Nutrient medium (agar) was transferred into uncontaminated Petri plates in a biological safety cabinet. A germ free atmosphere was sustained for the period of pouring to keep away from impurity. The media were allowed to solidify in Petri plates for one hour and then such Petri plates were kept in inverted position (to keep away from vanishing of water from the medium within such plates) in incubator for 24 hrs at 37ºC. For the shaking incubation of microorganisms, about 20 ml per flask of nutrient broth was used. Whereas, for the standardization of culturing of microorganisms, nutrient broth was used in test tubes.

**2.5 Microbes**

The test microbes were 3 Gram positive bacteria *Bacillus subtilis*, *Styphyloccus aureus*, *Bacillus atrophoeus* and 3 Gram negative bacteria *Salmonella typhi*, *Klebsella pneumoniae* and *Escherichia coli*and as well as fungal strains, *Candida albicans*, *Rhizopussp*and *Aspergillus sp.*

**2.6 Plant extracts and preparation of stock solution**

Extracts of medicinal plants such as ethanol, methanol, ethyl acetate, hexane and aqueous were assessed for antimicrobial activity. The plant extracts were adjusted to 1 miligram/6 microliters in dimethyl sulfoxide (DMSO) and diluted. The solution of stock were prepared. (Each 6 microliters of the solution contains 1 miligram of the extract).

**2.7 Antimicrobial activities determination**

**2.7.1. Disc Diffusion Method**

Antimicrobial activity of *Oxalis corniculata Rumex dentatus* and *Tagetes minuta* were resulted by the disk diffusion process. For the antifungal activity, *Candida albicans, Rhizopus sp.* and *Aspergillus sp.* was adjusted to the 10cfu/milliliter concentration. *Candida albicans, Rhizopus sp.* and *Aspergillus sp.* were cultured in sterilized solution of normal saline (0.9%) and inoculated onto agar plates (Saboured dextrose agar medium). upto 0.5 turbidity Mcfarland principles and immunized onto agar plates (nutrient agar). Whattman No. 1 (sterilized filter paper) discs were soaked with extracts of plants in 1, 2 and 3 milligram and in the concentrations of 6, 12 and 18 microliter of extract were applied on the discs. Fungal and bacterial cultures were then left for 18 hrs at 37 ºC [21].

2**.7.2 ANTIMICROBIAL BIOASSAY**

The subsequent method was used for the determination of antimicrobial activity.

**a. Preparation and pouring of media**

The required amount of 1.3 gram/100 ml of nutrient broth and 2.8gram/100 ml of nutrient agar were taken in flasks and dissolved in distilled water. All the equipment's and media used in the experiment were sterilized at 121 ºC at 15 psi for 1h than sterilized media, were transferred into plates in biological safety cabinet. The media was allowed to solidify in Petri plates for about one hour and then such Petri plates were kept in inverted position in incubator for 24 hrs at 37 ºC.

**b. Streaking and incubation**

In biological safety cabinet, the microbes stock cultures were freshened by streaking with germ-free inoculating loop on the agar plate These cultures were heated for 24 hrs at 37 ºC in incubator

**c. Inoculation and shaking**

Uncontaminated agar (nutrient broth) streaked cultures were inoculated. For the shaking incubation of microorganisms. About 20 ml per flask of nutrient broth were used.

**d. Turbidity and Standardization**

For standardization, the microbes cultures were diluted (from flasks) in test tube containing uncontaminated nutrient broth. With the help of spreader, fifty micro liter of microbial standardized cultures were spread on agar (nutrient agar). For 15 minutes these plates were kept in a refrigerator.

**e. Applying test**

The plates containing microbial standardized inoculums, were again kept in biological safety cabinet after absorption. Whattman No. 1 (sterilized filter paper) disc soaked with extracts of plants in 1, 2 and 3 milligram discs in concentrations of 6, 12 and 18 micro liter were applied on the discs. Fungal cultures and bacterial cultures were kept for 18 hrs at 37 ºC. Antibiotics (Coltrimazole Ciprofloxacin, Azithromycin,) were applied at the doze of 6 micro liter/disc as a positive control against fungi, Gram negative and Gram positive bacteria, respectively.

**f. Measurement of zone of inhibition**

By measuring the zones of inhibition in each extract antimicrobial activity was recorded properly. By taking the mean of inhibitory zones, the results were compiled.

## Statistical Analysis

Standard error means were found with the help of SPSS.V.16.

## RESULTS AND DISCUSSION

### 3.1 Antibiotics

The standard antibiotic Ciprofloxacin was used against three Gram negative bacteria (*Escherichiacoli,Klebsella pneumonia* and *Salmonella typhi)* and Azithromycin against three Gram positive bacterial strain (*Styphyloccus aureus, Bacillus subtilis* and *Bacillus atropoeus)* while Coltrimazole was used against fungal strains *(Candida albicans,Aspergillus sp.* and*Rhizopussp.*) in fifty microgram per six microliter concentration. Azithromycin demonstrated (50mm, 48mm and 65mm zone of inhibition) against *Bacillus subtilis, Staphylococcus aureus* and *Bacillus atropheous*. Ciprofloxacin showed (40mm, 35mm and 45mm against *Salmonella typhi*, *Escherichia coli* and *Klebsella pneumoniae* respectively While Coltrimazole showed 25mm, 65mm and 60mm against *Candida.albicans, Aspergillussp. and Rhizopus sp.* respectively.(Table 4.1a. to table 4.1c. Graph 4.1.).

**Table: 1 Antibacterial activity of Azithromycin against Gram positive bacteria.**

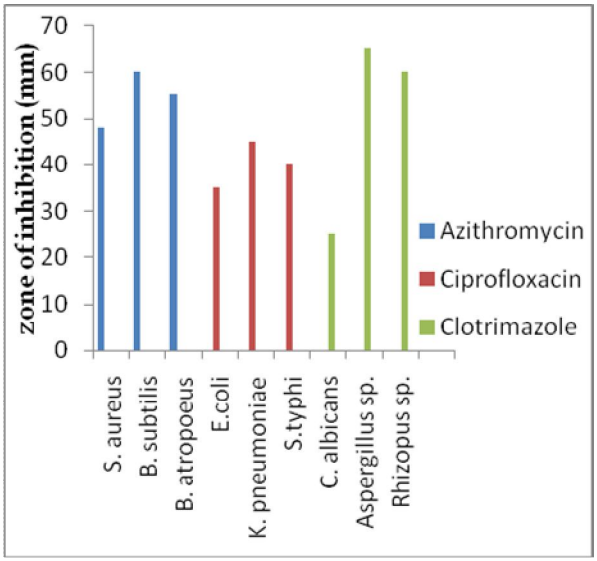
| Microbes | Azithromycin Concentration | Inhibition zone diameter(mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 50µg/6µl | 48±0.0 |
| *B. subtilis* | 50µg/6µl | 50±0.0 |
| *B. atropoeus* | 50µg/6µl | 65±0.0 |

**Table: 2Antibacterial activity of Ciprofloxacin against Gram negative bacteria.**

| Microbes | Ciprofloxacin Concentration | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *E.coli* | 50µg/6µl | 35±0.0 |
| *K.pneumoniae* | 50µg/6µl | 45±0.0 |
| *S.typhi* | 50µg/6µl | 40±0.0 |

**Table: 3 Antifungal activity of Coltrimazole against three strains**

| Microbes | Coltrimazole Concentration | Inhibition zone diameter(mm) (mean±standard deviation) |
|---|---|---|
| *Candida albicans* | 50µg/6µl | 25±0.0 |
| *Aspergillus sp.* | 50µg/6µl | 65±0.0 |
| *Rhizopus sp.* | 50µg/6µl | 60±0.0 |




**Graph:1 Antimicrobial activities of standard antibiotics against different bacteria and fungi**.

### 3.2 Oxalis *corniculata*

Five different solvent were used such as (ethanol, methanol, hexane, ethyl acetate, and aqueous). Various solvents were experienced against various species of fungi and bacteria.

#### 3.3.1 Ethanolic extract of *Oxalis corniculata*

The antimicrobial activity of the ethanolic extract of *Oxalis corniculata* is showed in (Table 4.4.1. Graph 4.4.1. And Fig.10). Ethanolic extract of *Oxalis corniculata* showed the maximum inhibitory activity againstthe Gram positive bacterium, *S. aureus* (19±0.5 mm zone of inhibition), against the Gram negative bacterium,*E. coli* (15±0.1 mm zone of inhibition) and fungus, *C. albicans* (17.5±2.5 mm zone of inhibition). While the controls results were 48±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.

**Table: 4 Antimicrobial activity of the ethanolic extract of *Oxalis corniculata***

| Microbes | Extract Concentration (mg/ µl) | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 6 mg/µl | 10±0.0 |
| | 12 mg/µl | 13.5±0.5 |
| | 18 mg/µl | 19±0.2 |
| *B. subtilis* | 6 mg/µl | 10.5±2.5 |
| | 12 mg/µl | 12.5±2.5 |
| | 18 mg/µl | 15±0.0 |
| *B. atropoeus* | 6 mg/µl | 11±0.1 |
| | 12 mg/µl | 12±0.2 |
| | 18 mg/µl | 14±0.0 |
| *E.coli* | 6 mg/µl | 10±0.1 |
| | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 15±0.1 |
| *S.typhi* | 6 mg/µl | 11.5±0.5 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 19±0.5 |
| *K.pneumoniae* | 6 mg/µl | 10±0.1 |
| | 12 mg/µl | 12.5±0.5 |
| | 18 mg/µl | 14±0.0 |
| *C. albicans* | 6 mg/µl | 11.5±0.5 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 17.5±2.5 |
| *Rhizopus sp.* | 6 mg/µl | 14±0.2 |
| | 12 mg/µl | 17±0.0 |
| | 18 mg/µl | 19±0.0 |
| *Aspergillus sp.* | 6 mg/µl | 11±0.1 |
| | 12 mg/µl | 15±0.1 |
| | 18 mg/µl | 20±0.0 |

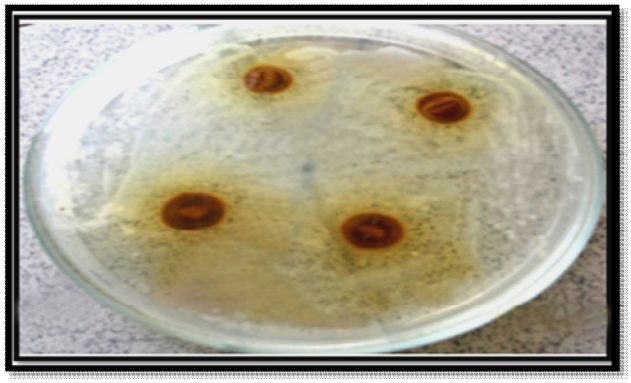
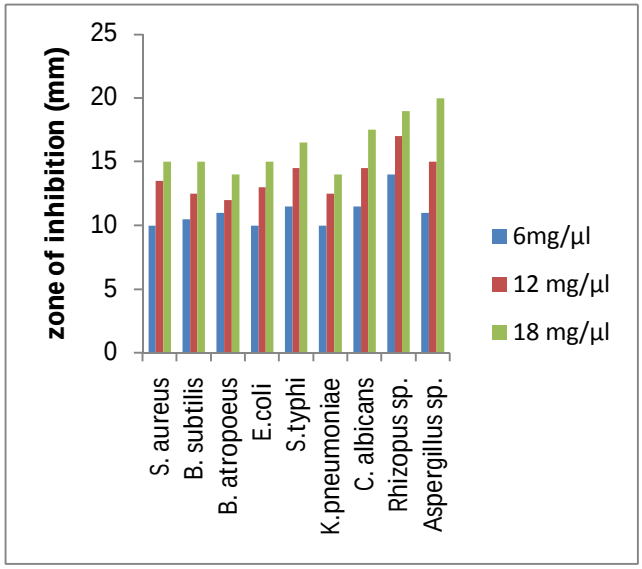


**Fig:1 Ethanolic extract of *Oxalis corniculata* against *E.coli***




**Graph: 2 Antimicrobial activity of the ethanolic extract of *Oxalis corniculata***

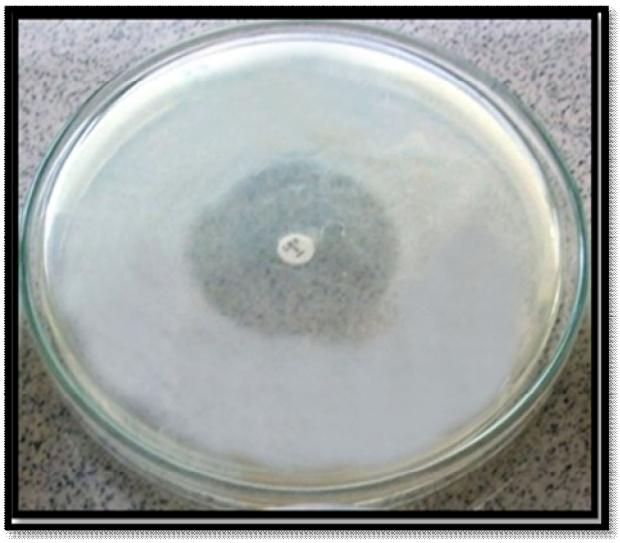


**Fig: 2 Use of Ciprofloxacin (standard) against *E.coli***

### 3.4.2 Methanolic extract of *Oxalis corniculata*

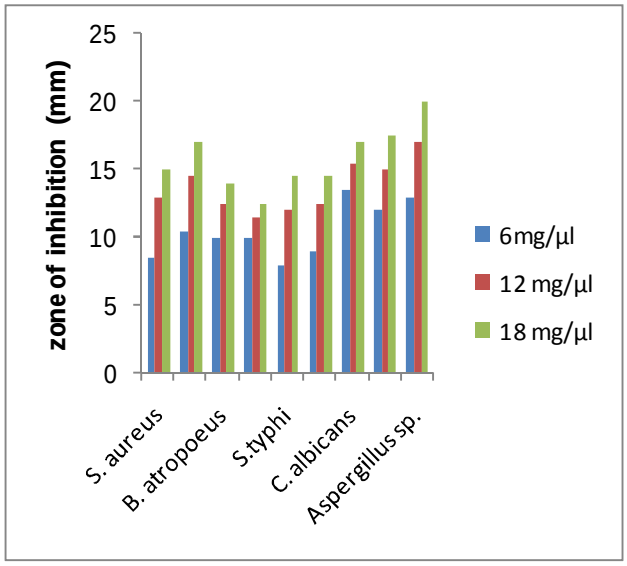
The antimicrobial activity of the methanolic extract of *Oxalis corniculata* is showed in (Table 4.4.2. and Graph 4.4.2). Methanolic extract of *Oxalis corniculata* showed the maximum inhibitory activity against the Gram positive bacterium, *B. subtilis* (17±0.0 mm zone of inhibition), against the Gram negative bacterium, *E.coli* (12±0.5 mm zone of inhibition) and fungus, *C. albicans* (17±0.0 mm zone of inhibition). While the controls results were 50±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.

**Table: 5 Antimicrobial activity of the methanolic extract of *Oxalis corniculata***

| Microbes | Extract Concentration (mg/ µl) | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 6 mg/µl | 8.5±0.5 |
| | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 15±0.2 |
| *B. subtilis* | 6 mg/µl | 10.5±1.5 |
| | 12 mg/µl | 14.5±1.5 |
| | 18 mg/µl | 17±0.0 |
| *B. atropoeus* | 6 mg/µl | 10±0.2 |
| | 12 mg/µl | 12.5±1.5 |
| | 18 mg/µl | 14±0.0 |
| *E.coli* | 6 mg/µl | 10±0.1 |
| | 12 mg/µl | 11.5±0.5 |
| | 18 mg/µl | 12.5±0.5 |
| *S.typhi* | 6 mg/µl | 8±0.1 |
| | 12 mg/µl | 12±0.0 |
| | 18 mg/µl | 14.5±0.5 |
| *K.pneumoniae* | 6 mg/µl | 9±0.2 |
| | 12 mg/µl | 12.5±2.5 |
| | 18 mg/µl | 14.5±0.5 |
| *C. albicans* | 6 mg/µl | 13.5±0.5 |
| | 12 mg/µl | 15.5±0.5 |
| | 18 mg/µl | 17±0.0 |
| *Rhizopus sp.* | 6 mg/µl | 12±0.2 |
| | 12 mg/µl | 15±0.2 |
| | 18 mg/µl | 17.5±0.5 |
| *Aspergillussp.* | 6 mg/µl | 13±0.0 |
| | 12 mg/µl | 17±0.3 |
| | 18 mg/µl | 20±0.0 |

### 3.5.3 Ethyl acetate extract of *Oxalis corniculata*

The antimicrobial activity of the ethyl acetate of *Oxalis corniculata* is showed in (Table 4.4.3. and Graph 4.4.3). Ethyl acetate extract of *Oxalis corniculata* showed the maximum inhibitory activity against the Gram positive bacterium, *S. aureus* (15.5±0.5 mm zone of inhibition), against the Gram negative bacterium, *E. coli* (18.5±0.5 mm zone of inhibition) and fungus, *C. albicans* (18±0.5 mm zone of inhibition). While the controls results were 48±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.

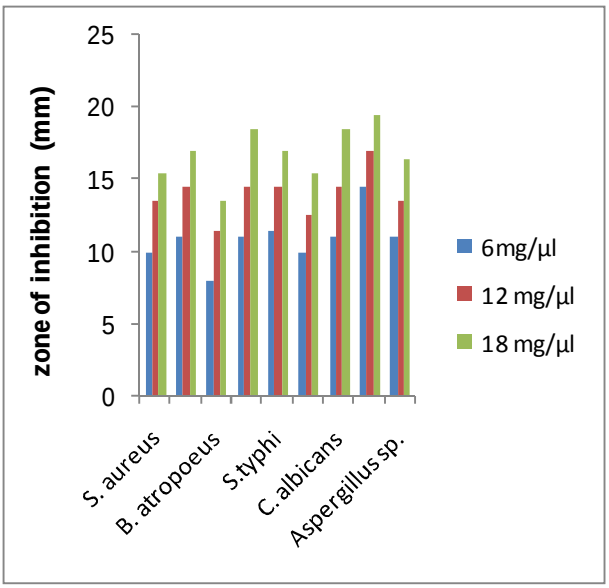



**Graph: 3 Antimicrobial activity of the methanolic extract of *Oxalis corniculata***

**Table:6 Antimicrobial activity of the ethyl acetate extract of *Oxalis corniculata***

| Microbes | Extract Concentration (mg/ µl) | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 6 mg/µl | 10±0.2 |
| | 12 mg/µl | 13.5±0.5 |
| | 18 mg/µl | 15.5±0.5 |
| *B. subtilis* | 6 mg/µl | 11±0.1 |
| | 12 mg/µl | 14.5±1.5 |
| | 18 mg/µl | 17±0.0 |
| *B. atropoeus* | 6 mg/µl | 8±0.2 |
| | 12 mg/µl | 11.5±0.0 |
| | 18 mg/µl | 13.5±0.5 |
| *E.coli* | 6 mg/µl | 11±0.1 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 18.5±0.5 |
| *S.typhi* | 6 mg/µl | 11.5±0.0 |
| | 12 mg/µl | 14.5±0.0 |
| | 18 mg/µl | 17±0.0 |
| *K.pneumoniae* | 6 mg/µl | 10±0.2 |
| | 12 mg/µl | 12.5±0.5 |
| | 18 mg/µl | 15.5±0.5 |
| *C. albicans* | 6 mg/µl | 11±0.1 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 18.5±0.5 |
| *Rhizopus sp.* | 6 mg/µl | 14.5±0.5 |
| | 12 mg/µl | 17±0.1 |

| | | |
|---|---|---|
| | 18 mg/µl | 19.5±0.0 |
| *Aspergillus sp.* | 6 mg/µl | 11±0.1 |
| | 12 mg/µl | 13.5±1.5 |
| | 18 mg/µl | 16.5±0.0 |



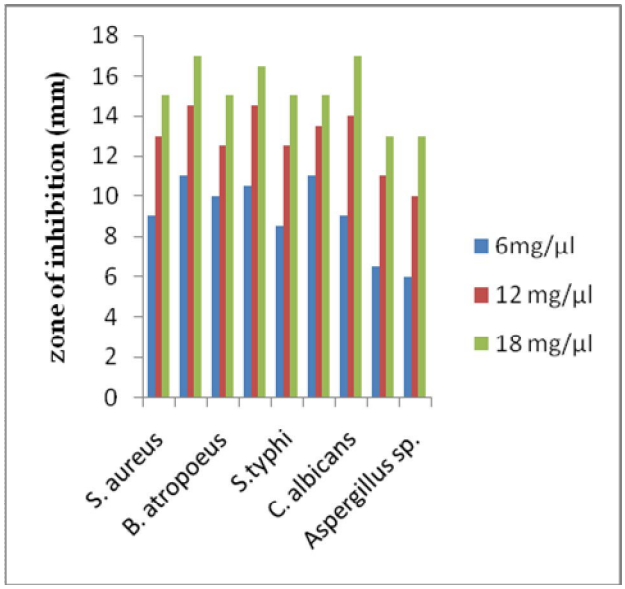

**Graph: 4 Antimicrobial activity of the ethyl acetate extract of *Oxalis corniculata***

### 3.6.4 Hexane extract of *Oxalis corniculat*

The antimicrobial activity of the hexane extract of *Oxalis corniculata* is showed in Table 4.4.4. And Graph 4.4.4). Hexane extract of *Oxalis corniculata* showed the maximum inhibitory activity against the Gram positive bacterium, *B. subtilis* (17±0.2 mm zone of inhibition), against the Gram negative bacterium, *E. coli* (16.5±0.5 mm zone of inhibition) and fungus, *C. albicans* (17±0.0 mm zone of inhibition). While the control were 50±0.0 mm, 35±0.0 mm and 25±0.0 mm respectively.

**Table # 7 Antimicrobial activity of the hexane extract of *Oxalis corniculata***

| **Microbes** | **Extract Concentration (mg/ µl)** | **Inhibition zone diameter (mm) (mean±standard deviation)** |
|---|---|---|
| *S. aureus* | 6 mg/µl | 9±0.1 |
| | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 15±0.0 |
| *B. subtilis* | 6 mg/µl | 11±0.3 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 17±0.2 |
| *B. atropoeus* | 6 mg/µl | 10±0.0 |
| | 12 mg/µl | 12.5±0.5 |
| | 18 mg/µl | 15±0.0 |
| *E.coli* | 6 mg/µl | 10.5±0.5 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 16.5±0.5 |
| *S.typhi* | 6 mg/µl | 8.5±0.5 |
| | 12 mg/µl | 12.5±0.5 |
| | 18 mg/µl | 15±0.0 |
| *K.pneumoniae* | 6 mg/µl | 11±0.1 |
| | 12 mg/µl | 13.5±1.5 |
| | 18 mg/µl | 15±0.0 |
| *C. albicans* | 6 mg/µl | 9±0.1 |
| | 12 mg/µl | 14±0.2 |
| | 18 mg/µl | 17±0.0 |
| *Rhizopus sp.* | 6 mg/µl | 6.5±0.5 |
| | 12 mg/µl | 11±0.1 |
| | 18 mg/µl | 13±0.1 |
| *Aspergillus sp.* | 6 mg/µl | 6±0.0 |
| | 12 mg/µl | 10±0.0 |
| | 18 mg/µl | 13±0.0 |




**Graph: 5 Antimicrobial activity of the hexane extract of *Oxalis corniculata***

### 3.7.5 Aqueous extract of *Oxalis corniculata*

The antimicrobial activity of the aqueous extract of *Oxalis corniculata* is showed in (Table 4.4.5. and Graph 4.4.5). Aqueous extract of *Oxalis corniculata* showed the maximum inhibitory activity againstthe Gram positive bacterium, *B. subtilis* (15±0.0 mm zone of inhibition), against the Gram negative bacterium, *E. coli* (15±0.0 mm zone of inhibition) and fungus, *C. albicans* (15.5±0.5 mm zone of inhibition). While the controls results were 50±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.

**Table: 8 Antimicrobial activity of the aqueous extract of *Oxalis corniculata***

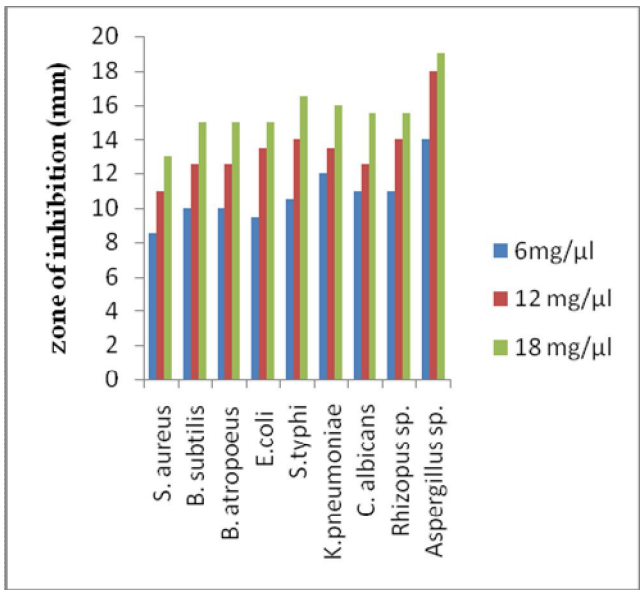
| Microbes | Extract Concentration (mg/ µl) | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 6 mg/µl | 8.5±0.5 |
| | 12 mg/µl | 11±0.0 |
| | 18 mg/µl | 13±0.1 |
| *B. subtilis* | 6 mg/µl | 10±0.1 |
| | 12 mg/µl | 12.5±0.5 |
| | 18 mg/µl | 15±0.0 |
| *B. atropoeus* | 6 mg/µl | 10±0.1 |
| | 12 mg/µl | 12.5±0.5 |
| | 18 mg/µl | 15±0.2 |
| *E.coli* | 6 mg/µl | 9.5±0.5 |
| | 12 mg/µl | 13.5±0.5 |
| | 18 mg/µl | 15±0.0 |
| *S.typhi* | 6 mg/µl | 12.5±1.5 |
| | 12 mg/µl | 14±0.0 |
| | 18 mg/µl | 16.5±0.0 |
| *K.pneumoniae* | 6 mg/µl | 12±0.2 |
| | 12 mg/µl | 13.5±0.5 |
| | 18 mg/µl | 16±0.0 |
| *C. albicans* | 6 mg/µl | 11±0.1 |
| | 12 mg/µl | 12.5±0.5 |
| | 18 mg/µl | 15.5±0.5 |
| *Rhizopus sp.* | 6 mg/µl | 11±0.0 |
| | 12 mg/µl | 14±0.0 |
| | 18 mg/µl | 15.5±0.5 |
| *Aspergillus sp.* | 6 mg/µl | 14±0.2 |
| | 12 mg/µl | 18±0.0 |
| | 18 mg/µl | 19±0.1 |

### 3.5 *Rumex dentatus*

Five different solvent were used such as (ethanol, methanol, hexane, ethyl acetate, and aqueous). Different solvents were tested against different species of bacteria and fungi.

#### 3.5.1 Ethanolic extract of *Rumex dentatus*

The antimicrobial activity of the ethanolic extract of*Rumex dentatus* is showed in (Table 4.5.1. Graph 4.5.1 and Fig.12). Ethanolic extract of*Rumex dentatus* showed the maximum inhibitory activity againstthe Gram positive bacterium, *S. aureus* (18±0.1 mm zone of inhibition), against the Gram negative bacterium, *E. coli* (19±0.0 mm zone of inhibition) and fungus, *C. albicans* (22±0.2 mm zone of inhibition). While the controls results were 48±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.

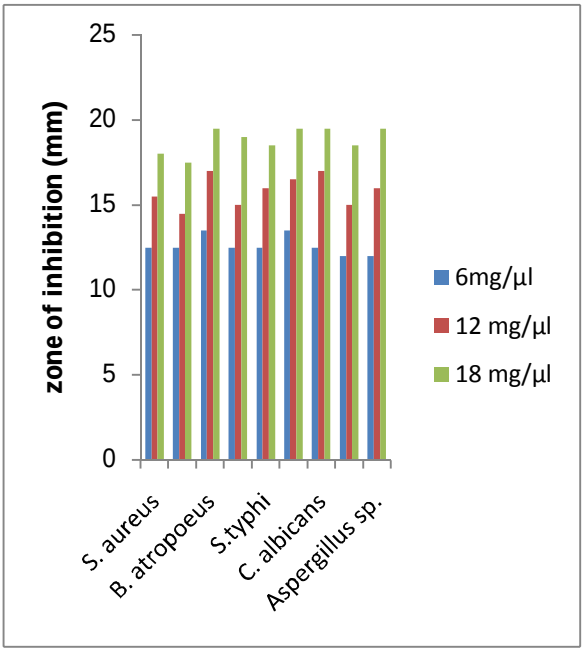



**Graph: 6 Antimicrobial activity of the aqueous extract of *Oxalis corniculata***

**Table: 9 Antimicrobial activity of the ethanolic extract of *Rumex dentatus***

| Microbes | Extract Concentration (mg/ µl) | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 6 mg/µl | 12.5±0.5 |
| | 12 mg/µl | 15.5±0.0 |
| | 18 mg/µl | 18±0.1 |
| *B. subtilis* | 6 mg/µl | 12.5±1.5 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 17.5±0.5 |
| *B. atropoeus* | 6 mg/µl | 13.5±0.5 |
| | 12 mg/µl | 17±0.1 |
| | 18 mg/µl | 19.5±0.5 |
| *E.coli* | 6 mg/µl | 12.5±0.0 |
| | 12 mg/µl | 15±0.1 |
| | 18 mg/µl | 19±0.0 |
| *S.typhi* | 6 mg/µl | 12.5±0.5 |
| | 12 mg/µl | 16±0.1 |
| | 18 mg/µl | 18.5±0.0 |
| *K.pneumoniae* | 6 mg/µl | 13.5±0.0 |
| | 12 mg/µl | 16.5±0.5 |
| | 18 mg/µl | 19.5±0.5 |
| *C. albicans* | 6 mg/µl | 12.5±0.5 |
| | 12 mg/µl | 17±0.2 |
| | 18 mg/µl | 22±0.2 |
| *Rhizopus sp.* | 6 mg/µl | 12±0.0 |
| | 12 mg/µl | 15±0.1 |

| | | |
|---|---|---|
| | 18 mg/µl | 18.5±0.5 |
| *Aspergillus sp.* | 6 mg/µl | 12±0.2 |
| | 12 mg/µl | 16±0.2 |
| | 18 mg/µl | 20±0.0 |




**Graph : 7 Antimicrobial activity of the ethanolic extract of *Rumex dentatus***

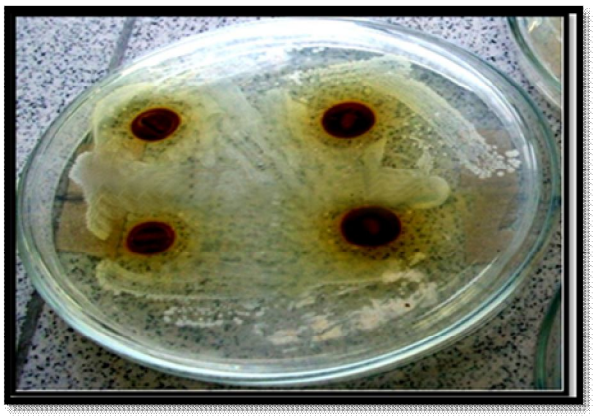


**Fig : 3 Ethanolic extract of *Rumex dentatus*against *S.aureus***

**3.5.2 Methanolic extract of *Rumex dentatus***

The antimicrobial activity of the methanolic extract of*Rumex dentatus* is showed in (Table 4.6.2 and Graph 4.6.2). Methanolic extract of*Rumex dentatus* showed the maximum inhibitory activity againstthe Gram positive bacterium, *S. aureus* (15±0.5 mm zone of inhibition), against the Gram negative bacterium, *E. coli* (18.5±0.5 mm zone of inhibition) and fungus. *C. albicans* (19±0.0 mm zone of inhibition). While the controls results were 48±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.

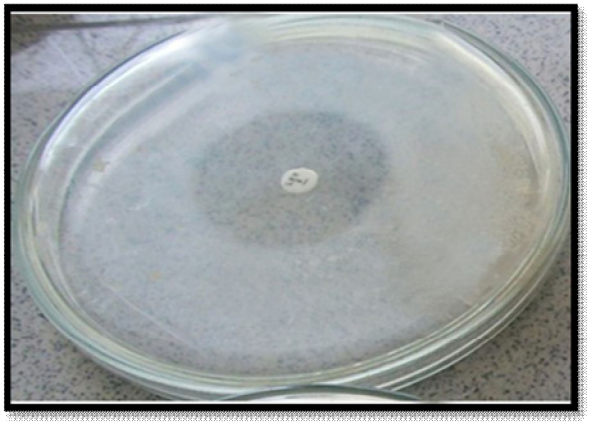
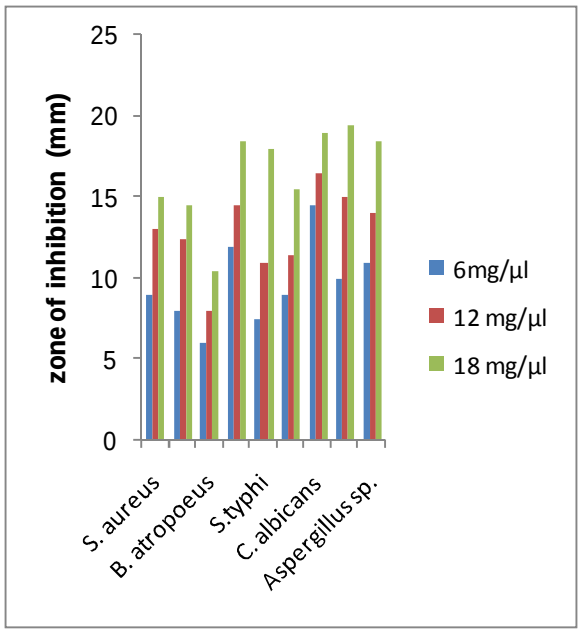


**Fig: 4 Use of Azithromycin (standard) against *S.aureus***

**Table: 10 Antimicrobial activity of the methanolic extract of *Rumex dentatus***

| **Microbes** | **Extract Concentration (mg/ µl)** | **Inhibition zone diameter (mm) (mean±standard deviation)** |
|---|---|---|
| *S. aureus* | 6 mg/µl | 9±0.1 |
| | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 15±0.0 |
| *B. subtilis* | 6 mg/µl | 8±0.2 |
| | 12 mg/µl | 12.5±0.5 |
| | 18 mg/µl | 14.5±0.0 |
| *B. atropoeus* | 6 mg/µl | 6±0.1 |
| | 12 mg/µl | 8±0.2 |
| | 18 mg/µl | 10.5±0.5 |
| *E.coli* | 6 mg/µl | 12±0.2 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 18.5±0.5 |
| *S.typhi* | 6 mg/µl | 7.5±0.0 |
| | 12 mg/µl | 11±0.0 |
| | 18 mg/µl | 18±0.2 |
| *K.pneumoniae* | 6 mg/µl | 9±0.1 |
| | 12 mg/µl | 11.5±1.5 |
| | 18 mg/µl | 15.5±0.0 |
| *C. albicans* | 6 mg/µl | 14.5±1.5 |
| | 12 mg/µl | 16.5±0.5 |
| | 18 mg/µl | 19±0.0 |
| *Rhizopussp.* | 6 mg/µl | 10±0.0 |
| | 12 mg/µl | 15±0.0 |
| | 18 mg/µl | 19.5±0.2 |
| *Aspergillus sp.* | 6 mg/µl | 11±0.1 |
| | 12 mg/µl | 14±0.1 |
| | 18 mg/µl | 18.5±0.5 |



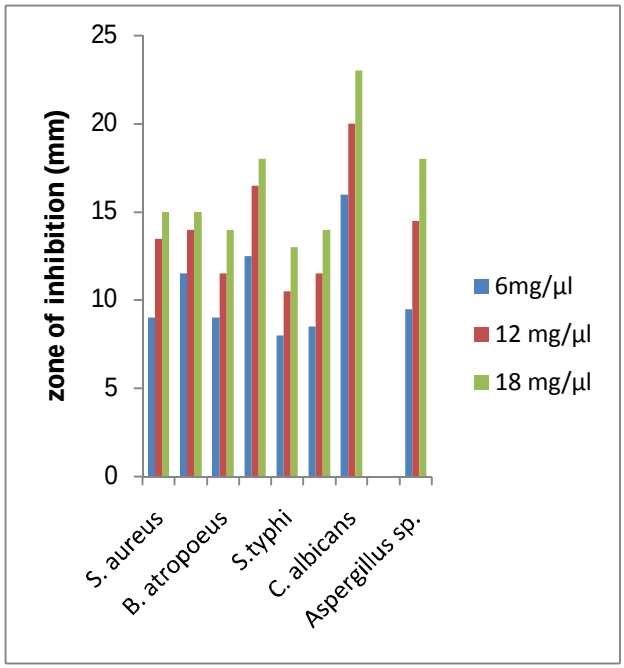

**Graph: 8 Antimicrobial activity of the methanolic extract of *Rumex dentatus***

### 3.5.3 Ethyl acetate Extract of *Rumex dentatus*

The antimicrobial activity of the ethyl acetate extract of*Rumex dentatus* is showed in (Table 4.6.3. and Graph 4.6.3). Ethyl acetate extract of*Rumex dentatus* showed the maximum inhibitory activity againstthe Gram positive bacterium, *S. aureus* (16±0.0 mm zone of inhibition), against the Gram negative bacterium, *E. coli* (18±0.0 mm zone of inhibition) and fungus, *C. albicans* (21±0.0 mm zone of inhibition). While the controls results were 48±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.

**Table: 11 Antimicrobial activity of the ethyl acetate extract of *Rumex dentatus***

| Microbes | Extract Concentration (mg/ µl) | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 6 mg/µl | 9±0.1 |
| | 12 mg/µl | 13.5±0.5 |
| | 18 mg/µl | 15±0.0 |
| *B. subtilis* | 6 mg/µl | 11.5±0.5 |
| | 12 mg/µl | 14±0.2 |
| | 18 mg/µl | 15±0.0 |
| *B. atropoeus* | 6 mg/µl | 9±0.1 |
| | 12 mg/µl | 11.5±0.0 |
| | 18 mg/µl | 14±0.1 |
| *E.coli* | 6 mg/µl | 12.5±0.5 |
| | 12 mg/µl | 16.5±0.5 |
| | 18 mg/µl | 18±0.0 |
| *S.typhi* | 6 mg/µl | 8±0.2 |
| | 12 mg/µl | 10.5±0.5 |
| | 18 mg/µl | 13±0.3 |
| *K.pneumoniae* | 6 mg/µl | 8.5±0.0 |
| | 12 mg/µl | 11.5±0.5 |
| | 18 mg/µl | 14±0.1 |
| *C. albicans* | 6 mg/µl | 16±0.2 |
| | 12 mg/µl | 20±0.1 |
| | 18 mg/µl | 23±0.0 |
| *Rhizopus sp.* | 6 mg/µl | 0±0.0 |
| | 12 mg/µl | 0±0.0 |
| | 18 mg/µl | 0±0.0 |
| *Aspergillus sp.* | 6 mg/µl | 9.5±0.5 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 18±0.2 |




**Graph: 9 Antimicrobial activity of the ethyl acetate extract of *Rumex dentatus***

### 3.5.4 Hexane extract of *Rumex dentatus*

The antimicrobial activity of the hexane extract of*Rumex dentatus* is showed in (Table 4.6.4 and Graph 4.6.4). Hexane extract of*Rumex dentatus* showed the maximum inhibitory activity againstthe Gram positive bacterium, *B. subtilis* (16.5±0.5 mm zone of inhibition), against the Gram negative bacterium, *E. coli* (16±0.0 mm zone of inhibition) and fungus, *C. albicans* (18±0.0 mm zone of inhibition). While the controls results were 50±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.

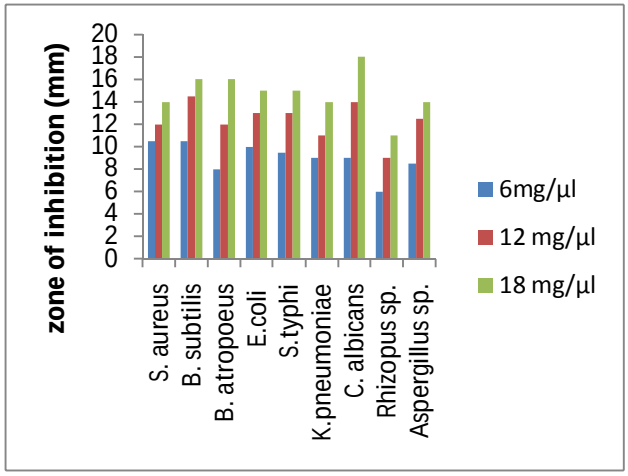
**Table:12 Antimicrobial activity of the hexane extract of *Rumex dentatus***

| Microbes | Extract Concentration (mg/ µl) | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 6 mg/µl | 10.5±0.5 |
| | 12 mg/µl | 12±0.2 |
| | 18 mg/µl | 14±0.0 |
| *B. subtilis* | 6 mg/µl | 10.5±0.5 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 16.5±0.5 |
| *B. atropoeus* | 6 mg/µl | 8±0.2 |
| | 12 mg/µl | 12±0.2 |
| | 18 mg/µl | 16±0.1 |
| *E.coli* | 6 mg/µl | 10±0.0 |
| | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 15±0.1 |
| *S.typhi* | 6 mg/µl | 9.5±0.1 |
| | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 15±1 |
| *K.pneumoniae* | 6 mg/µl | 9±0.0 |
| | 12 mg/µl | 11±0.0 |
| | 18 mg/µl | 14±0.1 |
| *C. albicans* | 6 mg/µl | 9±0.1 |
| | 12 mg/µl | 14±0.2 |
| | 18 mg/µl | 18±0.0 |
| *Rhizopus sp.* | 6 mg/µl | 6±0.0 |
| | 12 mg/µl | 9±0.1 |
| | 18 mg/µl | 11±0.0 |
| *Aspergillus sp.* | 6 mg/µl | 8.5±0.5 |
| | 12 mg/µl | 12.5±0.5 |
| | 18 mg/µl | 14±0.0 |

### 3.5.5 Aqueous extract of *Rumex dentatus*

The antimicrobial activity of the aqueous extract of*Rumex dentatus* is showed in (Table 4.6.5 and Graph 4.6.5). Aqueous extract of*Rumex dentatus* showed the maximum inhibitory activity againstthe Gram positive bacterium, *S. aureus* (16±0.1 mm zone of inhibition), against the Gram negative bacterium, *E. coli* (14±0.0 mm zone of inhibition) and fungus, *C. albicans* (10.5±0.5 mm zone of inhibition).

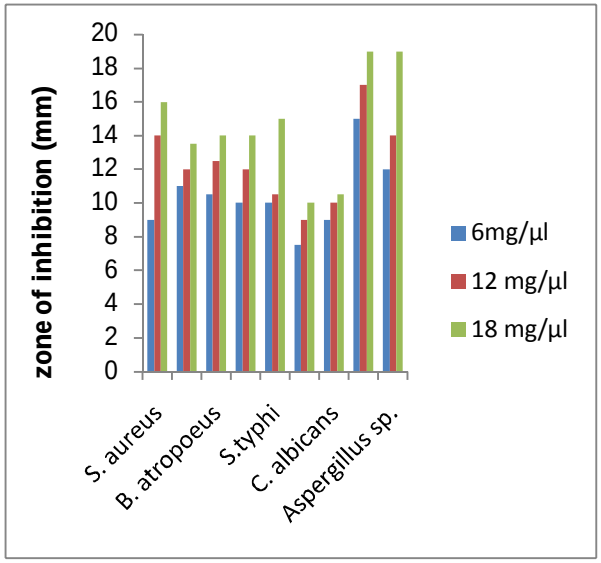
While the controls results were 48±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.




**Graph: 10 Antimicrobial activity of the hexane extract of *Rumex dentatus***

**Table: 13 Antimicrobial activity of the aqueous extract of *Rumex dentatus***

| Microbes | Extract Concentration (mg/ µl) | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 6 mg/µl | 9±0.1 |
| | 12 mg/µl | 14±0.0 |
| | 18 mg/µl | 16±0.1 |
| *B. subtilis* | 6 mg/µl | 11±0.1 |
| | 12 mg/µl | 12±0.0 |
| | 18 mg/µl | 13.5±0.5 |
| *B. atropoeus* | 6 mg/µl | 10.5±0.5 |
| | 12 mg/µl | 12.5±0.0 |
| | 18 mg/µl | 14±0.1 |
| *E.coli* | 6 mg/µl | 10±0.1 |
| | 12 mg/µl | 12±0.2 |
| | 18 mg/µl | 14±0.0 |
| *S.typhi* | 6 mg/µl | 9±0.0 |
| | 12 mg/µl | 10.5±0.5 |
| | 18 mg/µl | 15±0.1 |
| *K.pneumoniae* | 6 mg/µl | 7.5±0.5 |
| | 12 mg/µl | 9±0.1 |
| | 18 mg/µl | 10±0.0 |
| *C. albicans* | 6 mg/µl | 9±0.0 |
| | 12 mg/µl | 10±0.0 |
| | 18 mg/µl | 10.5±0.5 |
| *Rhizopus sp.* | 6 mg/µl | 15±0.1 |
| | 12 mg/µl | 17±0.1 |
| | 18 mg/µl | 19±0.1 |
| *Aspergillus sp.* | 6 mg/µl | 12±0.0 |
| | 12 mg/µl | 14±0.0 |
| | 18 mg/µl | 19±0.1 |




**Graph: 11 Antimicrobial activity of the aqueous extract of *Rumex dentatus***

**3.6*Tagetes minuta***

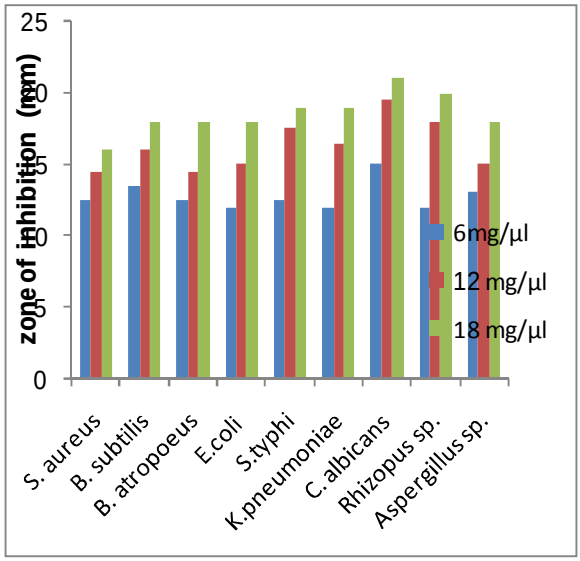
Five different solvent were used such as (ethanol, methanol, hexane, ethyl acetate, and aqueous). Different solvents were tested against different species of bacteria and fungi.

**3.6.1. Ethanolic extract of *Tagetes minuta***

The antimicrobial activity of the ethanolic extract of*Tagetes minuta*is showed in (Table 4.7.1 Graph and Fig.14). Ethanolic extract of*Tagetes minuta* showed the maximum inhibitory activity againstthe Gram positive bacterium, *B. subtilis* (20±0.2 mm zone of inhibition), against the Gram negative bacterium, *E. coli* (18±0.0 mm zone of inhibition) and fungus, *C. albicans* (22±0.2 mm zone of inhibition). While the controls results were 50±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.

**Table: 14 Antimicrobial activity of the ethanolic extract of *Tagetes minuta***

| **Microbes** | **Extract Concentration (mg/ µl)** | **Inhibition zone diameter (mm) (mean±standard deviation)** |
|---|---|---|
| *S. aureus* | 6 mg/µl | 12.5±0.5 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 16±0.0 |
| *B. subtilis* | 6 mg/µl | 13.5±0.0 |
| | 12 mg/µl | 16±0.1 |
| | 18 mg/µl | 18±0.1 |
| *B. atropoeus* | 6 mg/µl | 12.5±0.5 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 20±0.2 |
| *E.coli* | 6 mg/µl | 12±0.2 |
| | 12 mg/µl | 15±0.0 |
| | 18 mg/µl | 18±0.0 |
| *S.typhi* | 6 mg/µl | 12.5±0.5 |
| | 12 mg/µl | 17.5±0.0 |
| | 18 mg/µl | 19.5±0.1 |
| *K.pneumoniae* | 6 mg/µl | 12±0.2 |
| | 12 mg/µl | 16.5±0.5 |
| | 18 mg/µl | 19±0.0 |
| *C. albicans* | 6 mg/µl | 15±0.1 |
| | 12 mg/µl | 19.5±0.5 |
| | 18 mg/µl | 22±0.0 |
| *Rhizopussp.* | 6 mg/µl | 12±0.1 |
| | 12 mg/µl | 18±0.0 |
| | 18 mg/µl | 20±0.1 |
| *Aspergillus sp.* | 6 mg/µl | 13±0.0 |
| | 12 mg/µl | 15±0.2 |
| | 18 mg/µl | 18±0.1 |




**Graph:12 Antimicrobial activity of the ethanolic extract of *Tagetes minuta***

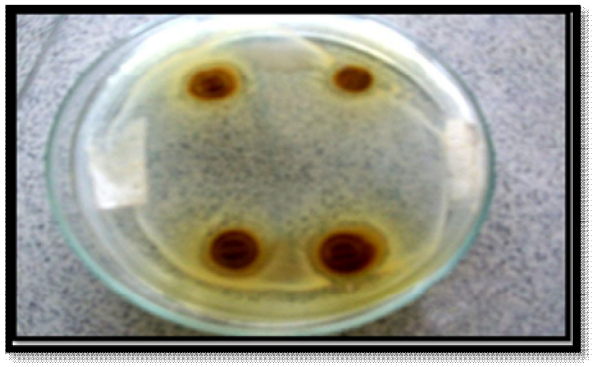


**Fig: 5 Ethanolic extract of *Tagetes minuta* against *S.aureus***

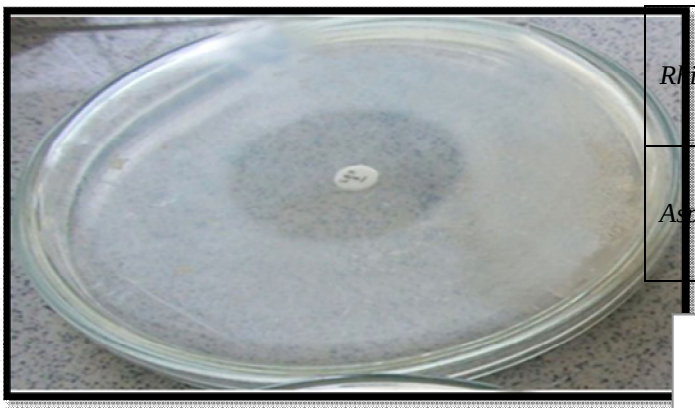


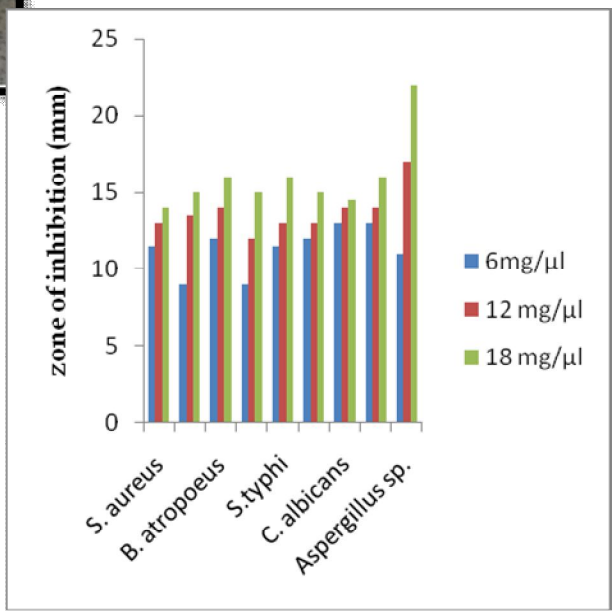
**Fig: 6 Use of Azithromycin (standard) against *S.aureus***

### 3.6.2 Methanolic extract of *Tagetes minuta*

The antimicrobial activity of the Methanolic extract of*Tagetes minuta*is showed in (Table 4.7.2. and Graph 4.7.2). Methanolic extract of*Tagetes minuta* showed the maximum inhibitory activity against the Gram positive bacterium, *S. aureus* (14±0.1 mm zone of inhibition), against the Gram negative bacterium, *E. coli* (15±0.0 mm zone of inhibition) and fungus, *C. albicans* (14.5±0.5 mm zone of inhibition). While the controls results were 48±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.

**Table: 15 Antimicrobial activity of the Methanolic extract of *Tagetes minuta***

| Microorganisms | Extract Concentration (mg/ µl) | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 6 mg/µl | 11.5±0.5 |
| | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 14±0.1 |
| *B. subtilis* | 6 mg/µl | 9±0.0 |
| | 12 mg/µl | 13.5±0.5 |
| | 18 mg/µl | 15±0.1 |
| *B. atropoeus* | 6 mg/µl | 12±0.1 |
| | 12 mg/µl | 14±0.0 |
| | 18 mg/µl | 16±0.0 |
| *E.coli* | 6 mg/µl | 9±0.1 |
| | 12 mg/µl | 12±0.2 |
| | 18 mg/µl | 15±0.0 |
| *S.typhi* | 6 mg/µl | 11.5±0.5 |
| | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 16±0.2 |
| *K.pneumoniae* | 6 mg/µl | 12±1.5 |
| | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 15±0.0 |
| *C. albicans* | 6 mg/µl | 13±0.2 |
| | 12 mg/µl | 14±0.0 |
| | 18 mg/µl | 14.5±0.5 |
| *Rhizopussp.* | 6 mg/µl | 13±0.1 |
| | 12 mg/µl | 14±0.1 |
| | 18 mg/µl | 16±0.1 |
| *Aspergillus sp.* | 6 mg/µl | 11±0.0 |
| | 12 mg/µl | 17±0.0 |
| | 18 mg/µl | 21±0.0 |




**Graph: 13 Antimicrobial activity of the Methanolic extract of *Tagetes minuta.***

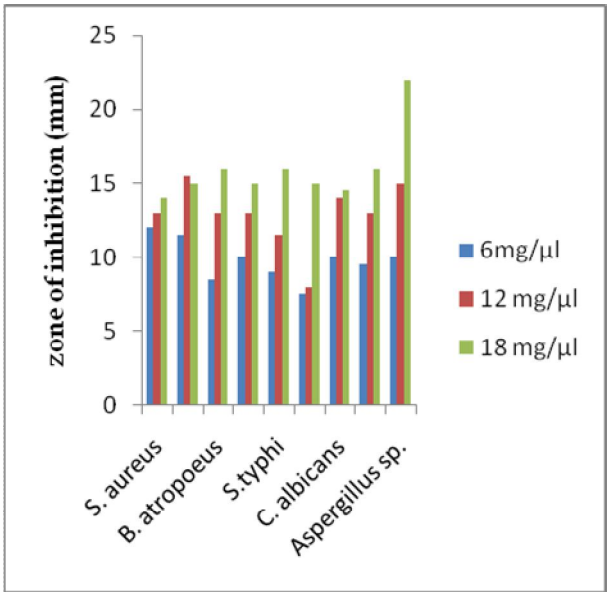
### 3.6.3 Ethyl acetate extract of *Tagetes minuta*

The antimicrobial activity of the ethyl acetate extract of*Tagetes minuta*is showed in (Table 4.7.3 and Graph 4.7.3). Ethyl acetate extract of*Tagetes minuta*showed the maximum inhibitory activity against Gram positive bacterium, *S. aureus* (14±0.1 mm zone of inhibition), against the Gram negative bacterium, *E. coli* (15±0.0 mm zone of inhibition) and fungus, *C. albicans* (14.5±0.5 mm zone of inhibition). While the controls results were 48±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.

**Table:16 Antimicrobial activity of the ethyl acetate extract of *Tagetes minuta***

| Microbes | Extract Concentration (mg/ µl) | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 6 mg/µl | 12±0.0 |
| | 12 mg/µl | 13±0.0 |
| | 18 mg/µl | 14±0.1 |
| *B. subtilis* | 6 mg/µl | 11.5±0.5 |
| | 12 mg/µl | 15.5±0.5 |
| | 18 mg/µl | 15±0.0 |
| | 6 mg/µl | 8.5±0.5 |

| | | |
|---|---|---|
| *B. atropoeus* | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 16±0.1 |
| *E.coli* | 6 mg/µl | 10±0.0 |
| | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 15±0.0 |
| *S.typhi* | 6 mg/µl | 9±0.0 |
| | 12 mg/µl | 11.5±0.0 |
| | 18 mg/µl | 16±0.1 |
| *K.pneumoniae* | 6 mg/µl | 7.5±0.5 |
| | 12 mg/µl | 8±0.1 |
| | 18 mg/µl | 15±0.0 |
| *C. albicans* | 6 mg/µl | 10±0.1 |
| | 12 mg/µl | 14±0.0 |
| | 18 mg/µl | 14.5±0.5 |
| *Rhizopussp.* | 6 mg/µl | 9.5±0.5 |
| | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 16±0.0 |
| *Aspergillussp.* | 6 mg/µl | 10±0.2 |
| | 12 mg/µl | 15±0.0 |
| | 18 mg/µl | 21±0.0 |



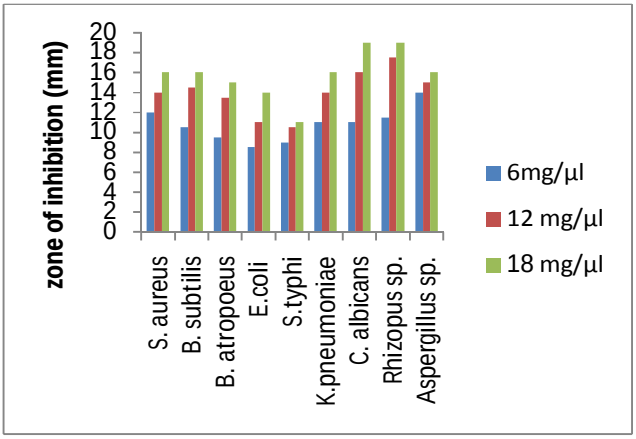

**Graph: 14 Antimicrobial activity of the ethyl acetate extract of *Tagetes minuta***

### 3.6.4 Hexane Extract of *Tagetes minuta*

The antimicrobial activity of the hexane extract of*Tagetes minuta*is showed in (Table 4.7.4 and Graph 4.7.4). Hexane extract of*Tagetes minuta*showed the maximum inhibitory activity against the Gram positive bacterium, *S. aureus* (16±0.0 mm zone of inhibition), against the Gram negative bacterium, *E. coli* (14±0.0 mm zone of inhibition) and fungus, *C. albicans* (19±0.0 mm zone of inhibition). While the controls results were 48±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively

**Table:17 Antimicrobial activity of the hexane extract of *Tagetes minuta***

| Microbes | Extract Concentration (mg/ µl) | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 6 mg/µl | 12±0.1 |
| | 12 mg/µl | 14±0.1 |
| | 18 mg/µl | 16±0.0 |
| *B. subtilis* | 6 mg/µl | 10.5±0.5 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 16±0.0 |
| *B. atropoeus* | 6 mg/µl | 9.5±0.5 |
| | 12 mg/µl | 13.5±0.5 |
| | 18 mg/µl | 15±0.1 |
| *E.coli* | 6 mg/µl | 8.5±0.5 |
| | 12 mg/µl | 11±0.1 |
| | 18 mg/µl | 14±0.0 |
| *S.typhi* | 6 mg/µl | 9±0.0 |
| | 12 mg/µl | 10.5±0.0 |
| | 18 mg/µl | 11±0.1 |
| *K.pneumoniae* | 6 mg/µl | 11±0.1 |
| | 12 mg/µl | 14±0.2 |
| | 18 mg/µl | 16±0.2 |
| *C. albicans* | 6 mg/µl | 11±0.1 |
| | 12 mg/µl | 16±0.1 |
| | 18 mg/µl | 19±0.0 |
| *Rhizopussp.* | 6 mg/µl | 11.5±0.0 |
| | 12 mg/µl | 17.5±0.5 |
| | 18 mg/µl | 19±0.1 |
| *Aspergillussp.* | 6 mg/µl | 14±0.1 |
| | 12 mg/µl | 15±0.0 |
| | 18 mg/µl | 16±0.0 |



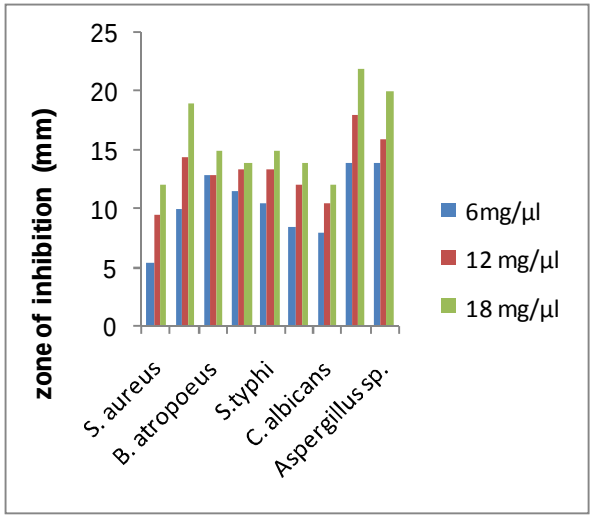

**Graph: 15 Antimicrobial activity of the hexane extract of *Tagetes minuta***

**4.6.5 Aqueous extract of *Tagetes minuta***

The antimicrobial activity of the aqueous extract of*Tagetes minuta*is showed in (Table 4.7.5 and Graph 4.7.5). Aqueous extract of*Tagetes minuta* showed the maximum inhibitory activity againstthe Gram positive bacterium, *B. subtilis* (19±0.0 mm zone of inhibition), against the Gram negative bacterium, *E.coli* (14±0.0 mm zone of inhibition) and fungus, *C. albicans* (12±0.1 mm zone of inhibition). While the controls results were 50±0.0 mm, 35±0.0 mm and 25±0.0 mm, respectively.

**Table: 18 Antimicrobial activity of the aqueous extract of *Tagetes minuta***

| Microbes | Extract Concentration (mg/ µl) | Inhibition zone diameter (mm) (mean±standard deviation) |
|---|---|---|
| *S. aureus* | 6 mg/µl | 5.5±0.0 |
| | 12 mg/µl | 9.5±0.5 |
| | 18 mg/µl | 12±0.1 |
| *B. subtilis* | 6 mg/µl | 10±0.1 |
| | 12 mg/µl | 14.5±0.5 |
| | 18 mg/µl | 19±0.0 |
| *B. atropoeus* | 6 mg/µl | 10±0.0 |
| | 12 mg/µl | 13±0.1 |
| | 18 mg/µl | 15±0.1 |
| *E.coli* | 6 mg/µl | 11.5±0.5 |
| | 12 mg/µl | 13.5±0.5 |
| | 18 mg/µl | 14±0.0 |
| *S.typhi* | 6 mg/µl | 10.5±0.0 |
| | 12 mg/µl | 13.5±0.1 |
| | 18 mg/µl | 15±0.0 |
| *K.pneumoniae* | 6 mg/µl | 8.5±0.5 |
| | 12 mg/µl | 12±0.0 |
| | 18 mg/µl | 14±0.1 |
| *C. albicans* | 6 mg/µl | 8±0.1 |
| | 12 mg/µl | 10.5±0.5 |
| | 18 mg/µl | 12±0.1 |
| *Rhizopussp.* | 6 mg/µl | 14±0.1 |
| | 12 mg/µl | 18±0.5-0 |
| | 18 mg/µl | 22±0.1 |
| *Aspergillussp.* | 6 mg/µl | 14±0.1 |
| | 12 mg/µl | 16±0.0 |
| | 18 mg/µl | 20±0.0 |




**Graph:16 Antimicrobial activity of the aqueous extract of *Tagetes minuta***

## DISCUSSION

There is a need to search their uses and to perform pharmacological and phytochemical studies to determine their therapeutic properties the basic nutritional importance of plants is assessed by their protein and carbohydrate contents. Oils, fats vitamins, minerals and water which are responsible for the development and growth in man and animals**.**In the present investigation, Out of Three studied plants during the present studies*Tagetes minuta*, showed the highest inhibitory activity followed by, *Rumex dentatus* and *Oxalis corniculata.* Ethanolic extract showed the best efficacy followed by methanolic, ethyl acetate, hexane and aqueous extracts. The inhibition ratio was more against fungi as compared Gram negative bacteria, and Gram positive bacteria respectively.

Methanolic extract of *Medicago sativa* showed the maximum inhibitory activity against*Rhizopussp* And *S.typhi*(16±0.1 and 16±0.2 mm zone of inhibition) a Gram negative bacterium. Similarly, against the Gram positive bacterium, the most significant results were in the case of methanolic extract against *B. subtilis* (15±0.1 mm zone of inhibition) In addition to this, the growth inhibition was more in the case of methanolic extract of*Tagetes minuta*against *C. albican*(14.5±0.5 mm zone of inhibition). Previously Doss *et al.*, 2011, worked on the antimicrobial activities of the methanolic extract of *Tagetes minuta*and found the significant results against Gram positive and Gram negative bacteria and fungi. Similar finding were reported by [19, 20].

## CONCLUSION

- The order of effectiveness of extracts (plant-wise) is *Cichorium intybus* > *Medicago sativa* > *Tagetes minuta* > *Rumex dentatus* >*Oxalis corniculata.*
- The order of effectiveness of extracts (solvent -wise) is ethanol> methanol > ethyl acetate > hexane > aqueous extract
- The order of inhibition according to the different groups of organisms was fungi > Gram negative bacteria> Gram positive bacteria
- The most resistant Gram positive bacterium was *Bacillusatropoeus,* Gram negative bacterium was Salmonella *typhi*and fungus was *Rhizophussp.*
- The most sensitive Gram positive bacterium was *Bacillus subtilise,*Gram negative bacterium was *Escherichiacoli*and fungus was*Candidaalbicans.*

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