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ORIGINAL ARTICLE

Antibacterial and Antifungal Activities of *Acacia modesta*, *Achyranthe saspara* and *Solanum surattense* used in Folk Medicine

Nazia Sana[1], Rabia Shaheen[1], Aneela Sharif[2], Sehrish Kalsoom[2,*], Zahida Nasreen[2]

[1]Department of Botany, University of Mianwali, Mianwali, Pakistan
[2]Department of Zoology, University of Mianwali,Mianwali, Pakistan

ABSTRACT

Background: The antibiotic resistance is a grave concern across the globe due to inappropriate and over usage of antibiotics without prescription which could be fatal for the humans in the long run. Therefore, the need of the hour is to look for more effective and economical options for treatment worldwide to reduce antibiotic resistance.

Objective: This study was targeted to assess the antibacterial and antifungal properties of leaf extracts of three plants (*Achyranthes aspara, Acacia modesta* and *Solanum surattense*) against *Escherichia coli*, *Salmonella* and *Malassezia* species.

Methodology: The bioactive components were extracted using methanol and ethanol (70%) as solvents. The agar-well diffusion method was employed to assess the antibacterial and antifungal activities of leaf extract. The inhibitory zones were recorded. The bioassay studies of the crude extracts were undertaken at three different concentrations i.e., 30.0, 40.0, and 50.0 mg/ml.

Results and Conclusion: The ethanol extracts of *Achyranthes aspara* had shown the highest growth inhibitory effect at a concentration of 50.0 mg/ml with an inhibition zone of 13.2mm against *E. coli*. Ethanolic leaf extracts of *solanum surattense* had shown maximum inhibition effects at a concentration of 50.0 mg/ml with 12.5mm zones of inhibition against *Salmonella. Acacia modesta* did not show good results against any bacterial and fungal strains as compared to the other two plants.

Keywords	*Address of Correspondence	Article info.
Medicinal plants, ethanol extract, antimicrobial activity, A. saspara, S.surattense, A. modesta.	*Sehrish.kalsoom1820@gmail.com*	*Received: June 26, 2022* *Accepted: October 07, 2022*

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INTRODUCTION

According to World Health Organization, 80% of the world's population uses plant extracts and their constituents as folk medicine in traditional therapies. It has been estimated also that about 50% of all current theoretical drugs are from natural sources[1].

Natural compounds in plants have antimicrobial properties that have been confirmed through several *in vitro* studies. A remarkable number of medicinal plants are used in the making of various drugs and the production of other conventional medicines[2]. It is estimated that out of

422127 reported plant species, only 35,000 to 70,000 are involved in the antimicrobial activity[3].

Plants are employed in drug manufacturing due to the prevalence of different phytochemicals such as flavonoids, terpenoids, polyphenols, carotenoids, vitamins, tannins, alkaloids, saponins, pigments, minerals and enzymes that have both antioxidant and antimicrobial activity. As medicinal plants have fungicidal and bactericidal effects, there is a dire need to evaluate plants for drug manufacturing and drug discovery[4]. The use of medicinal plants has a long history in the treatment of a range of diseases, including infectious diseases, and these days hundreds of thousands of plant species have been tested for their medicinal effect[5]. However, the phytochemical and pharmacological activities of many more plants remain to be studied. Plant-derived substances are tolerated and accepted by patients and seem a reliable source of anti-microbial compounds[6].

The global impact of infectious diseases caused by fungi and bacteria is a major health problem worldwide. In recent years, antibiotic and multidrug resistance has become a grave concern in medical communities due to the increased incidence of infections. The world's leading cause of premature death is infectious diseases. Therefore, it is the need of the hour to explore new antimicrobial compounds to resolve this problem[7].

Malassezia, *Salmonella* and *Escherichia coli* (*E. coli)* species are the most common disease-causing pathogens for various infections, especially in those countries that have poor health care systems. The studies on these organisms and their antibiotic resistance pattern showed the quick spread of multidrug resistance in these pathogenic microbes. So, there is an urgent need for public education for healthcare providers and the general population regarding the careful utilization of antibiotics[8].

Different plant parts (root, stem, leaves, and fruits) are useful for therapeutic and medicinal purposes. Medicinal plants contain several chemical components which can be opted for the treatment of different diseases and to assess their inhibitory activity against different well-known pathogens[9].

The leaf extract of *Achyranthes aspera* showed hepatoprotective activity in rifampicin. It is an antibiotic used to treat albino rats caused by *Salmonella. E. coli* and *Cryptosporidium*[10]. However, it is also investigated for *invitro* antimicrobial activity against *Escherichia coli* and *Salmonella typhi* by disc diffusion method. *Achyranthe saspera* leaves extract inhibited the growth of *Salmonella typhi* and *E. coli*[11].

Acacia modesta leaves extract are used for the treatment of skin cancer as an astringent, antispasmodic and aphrodisiac. Leaves contain carbohydrates, glycosides, phytosterols and another major compound that inhibit the growth of *E. coli* and fungal pathogens[12]. To show more positive results phytochemical screening (flavonoids, tannins, sterols, alkaloids and carbohydrates) was done by different extracts. These extracts inhibit the growth of *E. coli*[13].

MATERIALS AND METHODS

Collection of medicinal plants

Three selected plants i.e., *Acacia modesta, Solanum surattense, and Achyranthe saspara* have been used in the present study. These plants were collected from the surroundings of Musa Khel and Swans in the district of Mianwali.

Processing of medicinal plants

Collected plant leaves were washed, cleaned, and then dried under shade for two weeks. Leaves were ground in a grinder to obtain a fine powder and stored in a container.

Culture of pathogens

E. coli, Salmonella, and *Malassezia spp* were studied in the present work. The bacterial sample was taken from sewage water as it contains abundant pathogenic microbes and the fungal samples were obtained from the head of a student. These were cultured in the laboratory. Biochemical tests and staining were used for the identification of *E. coli, Salmonella* and *Malassezia spp.*

Preparation of leaf extract

70% ethanol and 70% methanol were taken in 3 separate flasks (250 ml capacity each). 30.0 grams of plant material was poured into each of the flasks and mixed well to prepare methanolic & ethanolic extracts. To avoid contamination, these flasks were plugged into cotton and covered with aluminum foil. Then these solutions were shaken from time to time via using an orbital shaker for 7

days to get a homogenized mixture. Extracts were filtered with Whitman's filter paper no. 3. Then filtrate was dried in a fume hood at room temperature. After 3 days crude leaves extracts were obtained. For dilution DMSO (Dimethyl sulfoxide) was added in crude extracts and then finally these extracts were stored in the refrigerator at 4^0C for further investigation.

Antimicrobial activity of plants extracts

Antimicrobial activities of selected ethanolic and methanolic leaf extracts of *Acacia modesta, Solanum surattense,* and *Achyranthes aspara* were investigated by using the agar well diffusion method. For this purpose, cultured media was prepared. This media was autoclaved at121^0C for 60 minutes. Autoclaved media was poured into sterilized Petri plates. When agar was solidified, 10.0 µl *E. coli*, *Salmonella and Malassezia spp* samples were taken with the help of sterilized micropipettes from culture broth and spread on Petri plates. Wells were formed with sterilized tips then these wells were filled with selected plant leaves crude extract. These plates were incubated for 24 hours at 37^0C. After the 24-hours zone of inhibition was formed, observed and measured.

RESULTS

The present study was designed to analyze the antimicrobial effect of different medicinal plants against *Salmonella, E. coli*, and *Malassezia spp*. Overall results showed the most significant antimicrobial activity by using methanol extract of *Achyranthes aspara* against *E.coli* and ethanol extract of *Solanum suratense* against *Salmonella*. Methanol extract of *Solanum surattense* shows the highest antimicrobial activity against *Malassezia spp*.

Comparison of Inhibition zone of three plant extracts against *E. coli*

Table **1** shows, an overall comparison of the antimicrobial activity of ethanol and methanol leaves extracts of selected plants. Statistical analysis exhibit that the antimicrobial activity of *Achyranthes aspara* was found to be very high as compared to *Acacia modesta* and *Solanum surattense*. The dilution of 1.0ml, ethanol and methanol extracts of *Achyranthes aspara* shows the maximum zone of inhibition at the concentration of 50.0mg/ml against *E. coli* and a minimum zone of inhibition at 30.0mg/ml. Ethanol extract have remarkable antimicrobial activity (Fig **1)** than methanol extract shown in series of: Ethanol extract: 1.0ml=13.2mm> Methanol extract: 1.0ml=12.6mm. The inhibition zone of *Solanum surattense* shows a moderate effect against *E. coli*. Ethanol extract of *solanum surattense* showed a maximum zone of inhibition that was 11.6mm at a concentration of 50.0mg/ml. Table **1** show that *Acacia modesta* leaves extracts show a minimal effect on *E. coli growth* as compared to the other two plants. The inhibitory zone of ethanol and methanol leaves extract of *Acacia modesta* was 10.9mm at a concentration of 50mg/ml. Fig 2 shows inhibition zones labelled 1,2,3,4 against *E coli.*

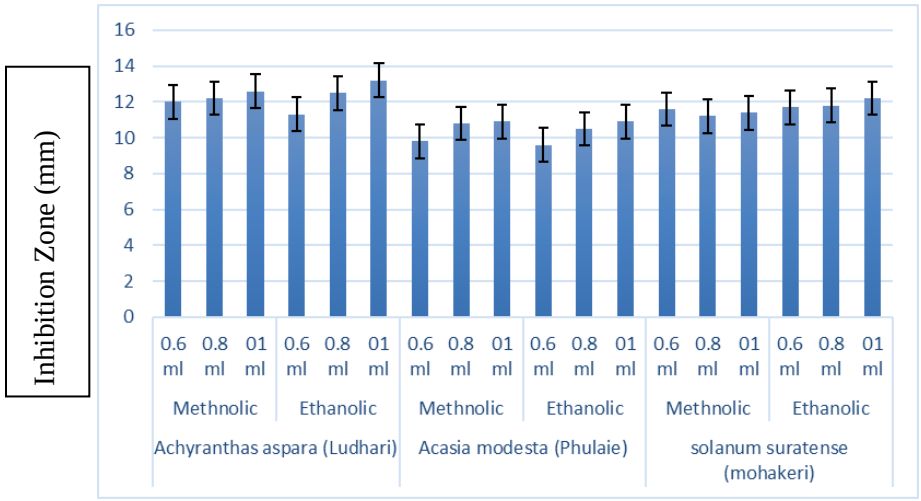

Figure 1. Effect of different concentrations of ethanolic and methanolic leaves extracts of *Achyranthes aspara*, *Acacia modesta and Solanum surattense* on growth of *E. coli*

Table 1. Inhibition Zone of Three Plant Extracts against *E.Coli*.

Plant	Extract	Concentration (mg/ml)	Inhibition zone (mm)
Achyranthe saspara	Methanolic	30	12.0
		40	12.2
		50	12.6
	Ethanolic	30	11.3
		40	12.5
		50	12.2
Solanum surattense	Methanolic	30	11.3
		40	11.2
		50	11.4
	Ethanolic	30	11.7
		40	11.8
		50	12.2
Acacia modesta	Methanolic	30	9.8
		40	10.8
		50	10.9
	Ethanolic	30	9.6
		40	10.5
		50	10.9

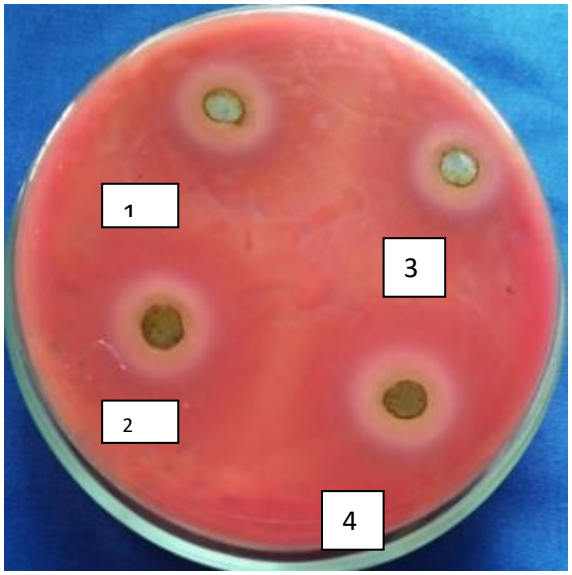

Figure 2. Ethanolic leaves Extract of *Achyranthes aspara* against *E. coli.* Concentration of ethanolic extracts: (1) 30 mg/ml (2) 50mg/ml (3) 30mg/ml (4) 40mg/ml

Over all comparison of ethanol and methanol leaves extracts of three plants shown in table **2**. Each plant, leaves extract show different activity against *Salmonella* species. The inhibitory effects of leaves extracts were directly proportional to concentration of extracts. Ethanol leaves' extract of *Solanum surattense* and *Achyranthes aspara* showed excellent result as compared to Methanol leaves' extract against *Salmonella spp.* 1.0ml, 0.8ml and 0.6ml dilution of different extracts showed varying degree of inhibitory effect. Maximum zone of inhibition was obtained (12.5mm) at 50mg/ml concentration in ethanol extract of *Solanum surattense* of 1.0ml dilution.

Table 2. Inhibition Zone of Three Plant Extracts against *Salmonella spp.*

Plant	Extract	Concentration(mg/ml)	Inhibition zone(mm)
Achyranthe saspara	Methanolic	30	8.5
		40	9.7
		50	11.4
	Ethanolic	30	10.0
		40	10.9
		50	12.5
Solanum surattense	Methanolic	30	9.9
		40	10.3
		50	11.9
	Ethanolic	30	9.0
		40	9.8
		50	12.5
Acacia modesta	Methanolic	30	8.6
		40	9.4
		50	11.9
	Ethanolic	30	8.8
		40	10.2
		50	12.1

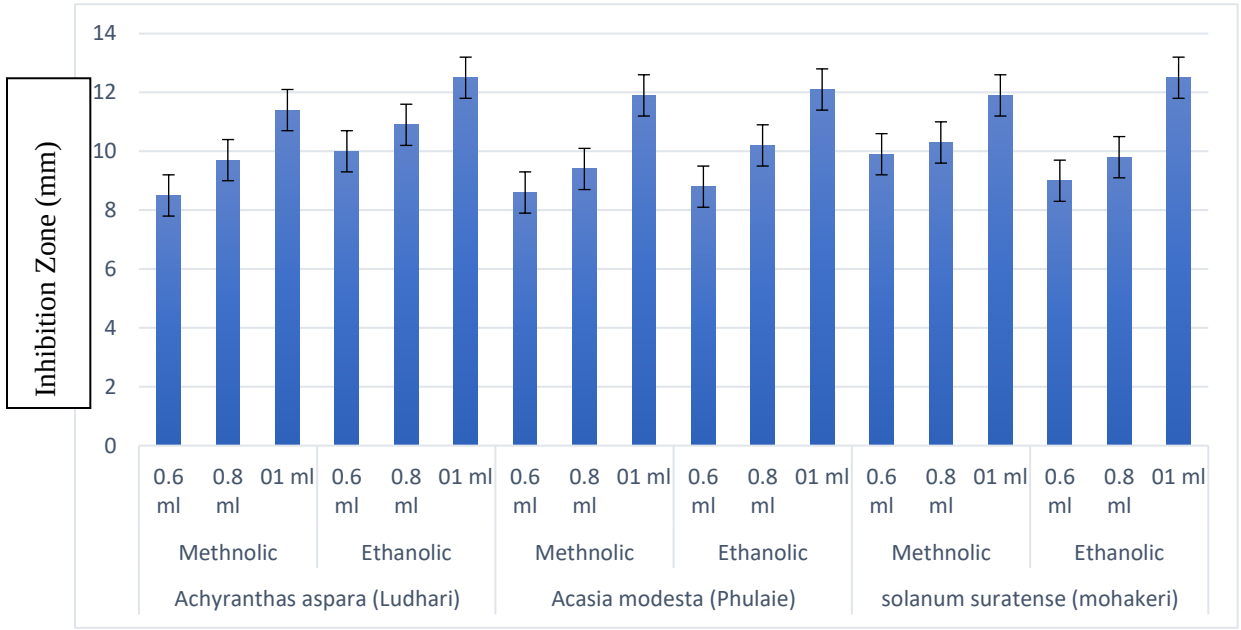

Figure 3. Effect of different concentrations of ethanolic and methanolic leaves extracts of *Achyranthe saspara*, *Acacia modesta and Solanum surattense* on growth of Salmonella spp.

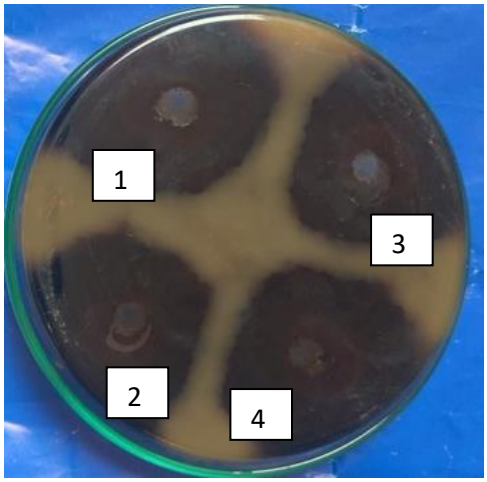

Figure 4. Ethanolic Leaves Extract of *Solanum surattense* Against *Salmonella spp.* Concentration of ethanolic extracts: (1) 30 mg/ml (2) 40mg/ml (3) 30mg/ml (4) 50mg/ml

Table 3. Inhibition Zone of Three Plant Extracts against *Malassezia spp.*

Plant	Extract	Concentration(mg/ml)	Inhibition zone(mm)
Achyranthe saspara	Methanolic	30	12.0
		40	9.0
		50	7.7
	Ethanolic	30	11.3
		40	12.5
		50	10.8
Solanum surattense	Methanolic	30	7.7
		40	8.3
		50	12.1
	Ethanolic	30	8.5
		40	10.6
		50	12.0
Acacia modesta	Methanolic	30	10.1
		40	10.8
		50	12.0
	Ethanolic	30	9.3
		40	9.7
		50	10.8

The overall comparison of ethanolic and methanolic leaves extracts of three plants is shown in Table **2**. Each plant leaves extract show different activity against *Salmonella* species. The inhibitory effects of leaf extracts were directly proportional to the concentration of extracts Fig **3**. Ethanol leaves extract of *Solanum surattense* and *Achyranthe saspara* shows excellent results as compared to Methanol leaves' extract against *Salmonella spp.* 1.0ml, 0.8ml, and 0.6ml dilution of different extracts showed varying degrees of inhibitory effect. The maximum zone of inhibition (Fig **4.)** was obtained (12.5mm) at 50mg/ml concentration in ethanolic extract of *Solanum surattense* of 1.0ml dilution.

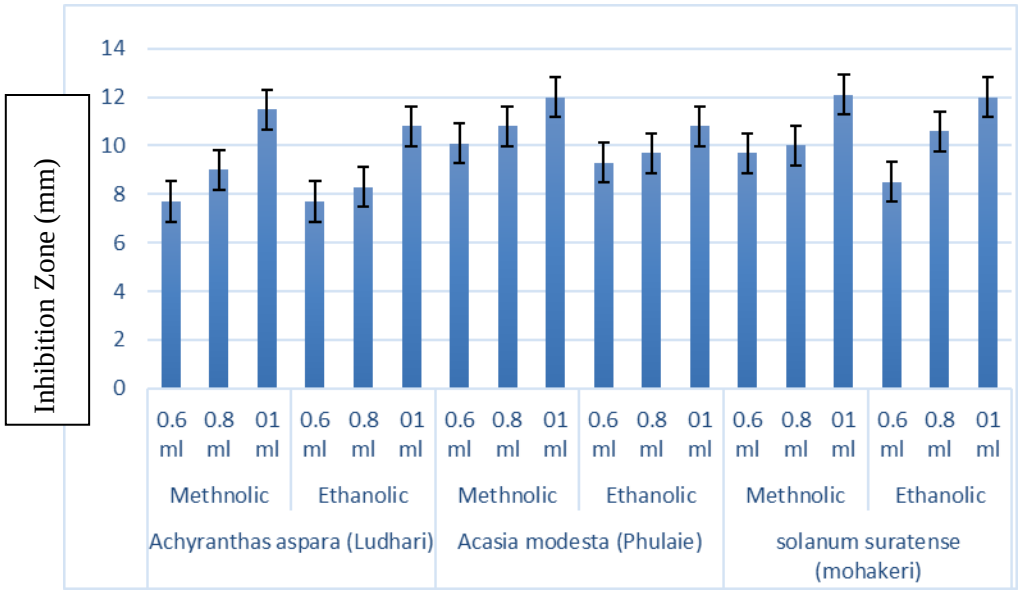

Figure 5. Effect of different concentrations of ethanolic and methanolic leaves extracts of *Achyranthe saspara*, *Acacia modesta and Solanum surattense* on growth of Malassezia spp.

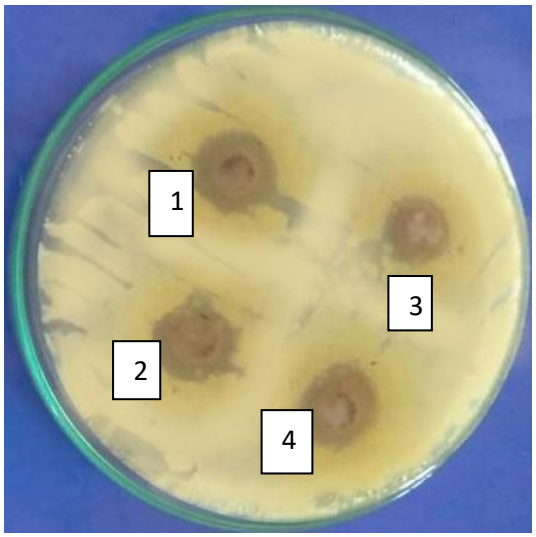

Figure 6. Methanolic leaves 'extract of *Solanum surattense* against *Malassezia spp.* Concentration of ethanolic extracts: (1) 50 mg/ml (2) 30mg/ml (3) 40mg/ml (4) 40mg/ml

The overall comparison of ethanol and methanol leaves extracts of three plants is shown in Fig **5.** and Table **3**. Each plant leaves extract shows different activity against *Malassezia* species. The inhibitory effects of leaf extracts were directly proportional to the concentration of extracts. Methanolic leaves extract of *Solanum surattense* and Ethanolic extract of *Achyranthe saspara* showed excellent results against *Malassezia spp.* 1.0ml, 0.8ml, and 0.6ml dilutions of different extracts showed varying degrees of inhibitory effect. The maximum zone of inhibition labelled 1 in Fig **6.** was obtained (12.1mm) at 50mg/ml concentration in methanol extract of *Solanum surattense* of 1.0ml dilution

DISCUSSION

The present study was aimed at checking the antimicrobial activity of *Achyranthe saspara, Acacia modesta,* and *Solanum surattense* against *Salmonella, E. coli,* and *Malassezia* species using the agar wells diffusion method.

Experiments were carried out in triplicates. Zones of inhibition were analyzed using statistical terms. Comparing mean ± standard deviation and statistical significance was calculated. A value of $p < 0.05$ was considered more significant statistically.

Achyranthes aspara leaves' extract has been investigated for *invitro* antimicrobial activity against *E. coli, Salmonella*, and *Malassezia* species. The different concentrations (1.0ml, 0.8ml, and 0.6ml) of ethanolic and methanolic extract were used against selected bacteria and fungi. 1.0 ml dilution showed the highest antimicrobial activity against *E. coli* with a zone of inhibition of 12.6 mm at the highest 50.0 mg/ml concentration (Table**1**, Fig **1**).

Naidu et al[11]also studied whole plant (leaves, stem, root, and inflorescence) extracts of *Achyranthe saspara* by disc diffusion method. About 200.0 mg leaves powder was dissolved in 40.0 ml of solvents *viz* Acetonitrile, methanol, chloroform, and n-hexane. Leaves extract was used against *E. coli* and *Salmonella.* They observed that methanolic extracts of whole plant powder inhibited the growth of *Salmonella typhi* with an inhibition zone of 7.0mm at 50.0 mg/ml concentration but no activity showed against *E. coli.* Only n-hexane inflorescences exhibit an inhibitory growth zone of 8.0mm against *E. coli*. When we matched our result (Table **1**, Fig **1.**) with Naidu's research, we noted that due to the use of different solvents and plant parts, our result is more significant and better than their experiment.

Solanum surattense displayed the highest antibacterial and antifungal activity against *Salmonella* and *Malasseziaspp*. The ethanolic leaves extract showed a maximum zone of inhibition of 12.5 mm at a concentration of 50 mg/ml against *Salmonella spp*. While methanolic leaves extract displayed a maximum zone of inhibition of 12.1mm at a concentration of 50 mg/ml against *Malassezia spp* (Table **2**,**3.** Fig **3**,**5.**).

The data was compared with Sheeba et al who also studied ethanolic leaves extract of *Solanum surattens*e, using 20.0 g to 30.0 g of leaf powder[15]. The antimicrobial activity of leaves was checked against *Pseudomonas aeruginosa*, *Salmonella* and *E. coli*. The leaf extracts showed highest antibacterial activity against *Salmonella spp* with a zone of inhibition of 16.0 mm at 500.0µg concentration.

But our experiment showed a minimum zone of inhibition of 12.5mm at the concentration of 50mg/ml against *Salmonella spp* (Table **2** Fig **3.**). Due to the difference in the concentration of crude extract, our leaves extract showed less activity as compared to research presented by Sheeba et al[14]. Ethanol was found to be a suitable solvent for the extraction against *E. coli* and *Salmonella*[15]. Similarly are results showed that ethanol extract displayed maximum activity against *Salmonella spp* (Table **2**, Fig **3.**).

However, Sieberi BM et al[16] studied leaves and stem extracts of *Acacia modesta*. The methanolic and water extracts were used against *Salmonella typhii* and *Bacillus subtilis*. They found that methanolic leaves and stem extract showed inhibitory growth zone of 8.0 mm and 10.0 mm. we observed that our results of methanolic leaf extract are highly effective against *Salmonella spp* with a zone of inhibition was 10.2 mm at 50.0mg/ml concentration.

CONCLUSION

The plant extracts under study showed antimicrobial activity when used against the test strains *E. coli, Salmonella*, and *Malassezia* species. These plant leaf extracts can be used in the formulation of new drugs used against bacterial and fungal strains.

ETHICAL APPROVAL

None.

CONFLICT OF INTEREST

None.

FUNDING SOURCE

None.

ACKNOWLEDGEMENTS

None.

LIST OF ABBREVIATIONS

DMSO Dimethyl sulfoxide

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