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The antimicrobial Potentiality Of *Tetranthera macrophylla (*ROXB.*)* flower

Saqib Ullah[1], Khushnood Ur Rehman[1*], Sayyar Muhammad[2], Muhammad Noor[3], Saleena Khan[1], Tabassum Yaseen[4], Sumbal Khan[5] and Murad Muhammad[5]

[1]Department of Botany, Islamia College Peshawar, **Pakistan**
[2]Department of Chemistry, Islamia College Peshawar, **Pakistan**
[3]Department of Agriculture, Hazara University, Mansehra, **Pakistan**
[4]Department of Botany, Bacha Khan University Charsadda, **Pakistan**
[5]Department of Botany, University of Peshawar, **Pakistan**

***Correspondence:** drkhushnood@icp.edu.pk **Received 19-04-2021, Revised: 22-06-2021, Accepted: 24-06-2021 e-Published: 29-06-2021**

In this study, the *Tetranthera macrophylla (Roxb.)* crude extract with two solvent systems including methanol and n-hexane was investigated for its antibacterial and antifungal activities. The antibacterial activity was tested against 6 bacterial strains including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtillis*, *Clavibacter michiganensis.*, *Xanthomonas axonopodis.* and *Proteus vulgaris*., whereas antifungal activity was tested against *Aceromonium cucurbitacerum*, *Aspergillus flavus.*, *Aspergillus niger,* and *Rhizopus stolonifers* with well diffusion method. The crude of *T. macrophylla* showed a significant biological activity effect against these bacterial and fungal strains. Results showed that the antibacterial activity against the tested bacteria species of *T. macrophylla* methanol and n-hexane extracts at both levels i.e. 500 and 1000 ug/ml were recorded in the range from 14mm of ZOI to 26mm of ZOI. The plant Flower extract showed antifungal activity, ranging from 8mm ZOI to 16mm ZOI, and then against *Aceromonium spp.* the plant methanol extract at 500 ug/ml showed 18mm ZOI, and at 1000 ug/ml showed 22mm ZOI. The study concluded significant antimicrobial activities recommend further exploitation of plant stem-Flower extract for other different bioassays.

Keywords: *Tetranthera macrophylla* , antibacterial, antifungal activity, methanol, n-hexane

INTRODUCTION

T. macrophylla (Roxb.) belonging to the family *Lauraceae* have been considered to originate from Burma, Nepal, India, Bangladesh, and another sub-Himalayan tract (Troup, 1986). This plant is grown at an altitude of 1400 m in the Himalayas (Dev, 2006). *T. macrophylla* harboring ovate-shaped leaves is a medium-sized species (Lalhmingliani, 2015). The plant Flower is visualized as light steaks with rough, blaze bugs and deep irregular cracks (Fretz, 2017). This plant bears yellow flowers which are enclosed in 9-12 stamens and 6 petals, with short racemes. The Colour of the fruit of *T. macrophylla* is black with ellipsoid to an oblong shape of 0.7 to 1.2 cm long. These fruits are seated on perianth and dispersed by bats. This plant was considered as a medicinal plant and same like other plants, its crude was used traditionally in the treatment of various human infections such as boil skin disease and other (Tasdemir et al. 2020); (Zhang et al. 2009);(Baul et al. 2006);(Alviano and Alviano, 2009);(Kumar et al. 2013).

Plants comprise a large group of phytochemicals such as phenolic compounds, terpenoids, tannins, flavonoids, alkaloids, etc., which are also called natural products or secondary metabolites. These natural products

exhibit various biological activities (Sher, 2009). (Bulbul et al. 2020) reported that the *T. macrophylla* plant leaves are potent sources of reducing sugars, tannins, steroids, flavonoids, and alkaloids compounds. Traditionally the leaves of *T. macrophylla* were used in the treatment of arthritis, anti-hyperglycemic, antimicrobial activity, anti-inflammatory, and clot lysis activity (Khair et al. 2014); (Sharmin and Sarkar, 2017); (Anisuzzaman et al. 2014)). The Flower of the plant has been exploited for beta-sitosterol and actinodaphinine compounds. Further, *T. macrophylla* Flower has been found to contain mild astringent activity, stomachic, stimulant, and used for the treatment of dysentery, diarrhea, bruises, contusions, pains, and fractures (Wang et al. 2016). Thus, keeping in view the traditional importance of *T. macrophylla*, the present study was conducted from *T. macrophylla* Flower that was collected from the district Buner tribal area for its antibacterial and antifungal activities.

MATERIALS AND METHODS

Sample collection

The plant *T. macrophylla* sample was collected from the district Buner tribal area.

Sample Identification

The deposited sample plant with its specific voucher number was then identified at the Botany Department, Islamia College Peshawar.

Preparation of crude extract

First, from the plant the Flower was separated which was then washed thoroughly with simple tap water and then stored for 3 months to let it get dried. After that then the dried stem Flower samples with a mechanical grinder were ground. From the resultant powder from Flower, approximately 100g powder was weighted and then soaked in methanol and n-hexane of 600ml each. The sample was then stored for 21 days and then filtered; the extracts were then with a rotary evaporator at 45ºC and were further dried. Additionally, the crude was dried by keeping for 1 hour and then stored in vile in the refrigerator at -4ºC, until further required for antibacterial and antifungal activity (Zahran, 2015).

Bioassays

Antibacterial activity

Preparation of solution dilution

The first stock solution of 1000ug/ml was prepared from which 500 ug/ml was taken as working dilution. The crude extracts of both methanol and n-hexane were taken 20 mg and dissolved in dimethyl sulfoxide (DMSO) thoroughly with a vortex mixer. The positive control contained Ciprofloxacin 0.05 % (Ciprofloxacin 25ml/cc) which contained 75ml/cc DMSO. The negative control contained a 500 ul DMSO solution. All the equipment was sterilized using an autoclave (121ºC for 15-20 minutes) before being used in a laminar flow hood that was sterilized with ethanol solution (75%).

Preparation of Media and inoculation

For bacteria growth the nutrient broth (oxide, UK) MS media was prepared (3.25 g/100ml). Also, nutrient agar media (PDA) were prepared for culturing bacteria. The bacteria strains including Gram-negative strains *Xanthomonas*, *E. coli*, and Gram-positive strains *B. subtillis*, *Proteus*, *Clavibacter,* and *S. aureus* which were all with help of cotton swab-streaked on the surface of the media. These samples were collected from KTC (Khyber Teaching Hospital, Peshawar) and HMC (Hayatabad Medical Complex, Peshawar).

Antifungal activity

Preparation of crude extract solution dilution

The stock solution and working dilution for antifungal activity were similarly prepared as for antibacterial activity. For positive control of antifungal activity, Nystatin 0.05% (25ml/cc) along with DMSO (75ml/cc) was taken, whereas for negative control only DMSO 500ul was used. The fungal strains including *Trichoderma, Rhizophus, Aspergillus niger, Acromonium spp.* were streaked on PDA media. These strains were collected from the Department of Botany, Islamia College Peshawar.

The two solvent solutions extract from the *T. macrophylla* plant were evaluated for their antibacterial and antifungal activities by following the (Arfan et al. 2008) method. The bacterial culture and the crude extracts were incubated at 37ºC for 24 hours after which the zone of inhibition was measured whereas the fungal cultures were incubated for 1 week. The tested extracts solution for both methanol and n-hexane were 500 and 1000 ug/ml.

Statistical analysis

For both antibacterial and antifungal activity, the data was collected and the statistical (p-value) difference was analyzed.

RESULTS AND DISCUSSION

The present research work was conducted to examine the antibacterial and antifungal activity of stem-Flower of *T. macrophylla* extract from two solvents system including methanol and n-hexane.

Antibacterial activity against Gram-negative Bacteria

Xanthomonas axonopodis.

Results indicating the *T. macrophylla* methanol extract (table 1) at 500 ug/ml showed 23.33±0.94mm ZOI and at 1000ug/ml the resultant ZOI was 26.33±0.47mm. In the case of n-hexane, at 500 ug/ml extract showed 16±0.81mm ZOI and with 1000 ug/ml there was 22.33±0.94 mm ZOI. The positive control Ciprofloxacin was recorded to be 30mm.

E. coli

The *T. macrophylla* stem-Flower methanol extract at 500 and 1000 ug/ml had shown 16.33±0.47mm and 24.33±0.47mm ZOI, respectively. The n-hexane extract of the plant stem-Flower had shown results similar to methanol 500 ug/ml i.e. 15.66±0.47mm, whereas the n-hexane extract antibacterial activity had a mean measurement of 20.33±0.47mm. The positive control Ciprofloxacin had shown 30 mm ZOI for *E. coli* (Table 2).

Antibacterial activity against Gram-positive Bacteria

B. subtillis

The highest ZOI was recorded for the 1000 ug/ml application of *T. macrophylla* methanol and n-hexane extract with resultant 25.33±0.94mm and 21.67±0.94mm ZOI, respectively (table 3). The 500 ug/ml of plant stem-Flower extract showed 14±0.81mm ZOI with methanol extract solvent solution and 17.33±0.47mm with n-hexane extract solvent solution. The ZOI for positive control Ciprofloxacin against *B. subtillis* was recorded 28mm.

Proteus vulgaris.

Results showed (Table 4) that the methanol plant extract at 500 ug/ml was calculated 18±0.81mm ZOI against *Proteus vulgaris*. while the 1000 ug/ml methanol extract had been calculated 24.33±0.47mm ZOI. The ZOI for n-hexane extract at 500 and 1000 ug/ml measured 17.66±0.47mm and 20.33±0.47mm, respectively. The ZOI for positive control Ciprofloxacin against *Proteus vulgaris*. was recorded 18mm ZOI.

Clavibacter michiganensis.

The methanol extract applied at 500 and 1000 ug/ml against *Clavibacter michiganensis.* had shown 15.67±0.47mm and 20.67±0.47mm ZOI (table 5). The n-hexane extract concentration 500 and 1000 ug/ml had shown 15.67±0.94mm and 20.33±0.47mm ZOI, respectively. The ZOI for positive control Ciprofloxacin against *Clavibacter michiganensis.* was recorded 26mm ZOI.

S. aureus

Results of the antibacterial activity of methanol and n-hexane extract against *S. aureus* are shown in table 6, which indicates that the 500 ug/ml extracts had lower ZOI compared to 1000 ug/ml application of extract. The methanol extract of *T. macrophylla* had shown 15.66±0.47mm and 21.66±0.94mm ZOI against S. aureus at 500 and 1000 ug/ml application. The results of the n-hexane extract of plant stem-Flower at 500 and 1000 ug/ml had shown 16.33±0.47mm and 20.66±0.47mm ZOI, respectively. The positive control Ciprofloxacin against *S. aureus* was recorded 26mm ZOI.

Antifungal activity

Aspergillus flavus.

Results indicated the *T. macrophylla* methanol extract (table 7) at 500 ug/ml showed 12.33±0.47mm ZOI and at 1000 ug/ml the resultant ZOI was 16.33±0.94mm. In the case of n-hexane, at 500 ug/ml extract showed 8.67±1.24mm ZOI and with 1000 ug/ml there was 12.67±0.47mm ZOI. The positive control Nystatin was recorded to be 20mm

Rhizopus stolonifer.

The *T. macrophylla* stem-Flower methanol extract at 500 and 1000 ug/ml had shown 9.67±0.47mm and 15±0.82mm ZOI, respectively. The n-hexane extract of the plant stem-Flower had shown results similar to methanol 500 ug/ml i.e. 8±0.82mm, whereas the n-hexane extract antibacterial activity had a mean measurement of 12.33±0.47mm. The positive control Nystatin had shown 22mm ZOI against *Rhizopus spp.* (table 8).

A. niger

The highest ZOI was recorded for the 1000 ug/ml application of *T. macrophylla* methanol and n-hexane extract with resultant 14.67±0.94mm and 12.33±0.47mm ZOI, respectively (table 9). The 500 ug/ml of plant stem-Flower extract showed 10.67±0.47mm ZOI with methanol extract solvent solution and 9.67±0.47mm with n-hexane extract solvent solution. The ZOI for positive control Nystatin against *A. niger* was recorded 22mm.

Table 1: ANOVA of Antibacterial activity of *T. macrophylla* Flower extract against *Xanthomonas axonopodis*.

Anova: Two-Factor With Replication						
SUMMARY	**Conc**	**Zone of inhibition**	**Positive control ciprofloxacin**	**Total**		
Methanol						
Count	2	2	2	6		
Sum	1500	49.66	60	1609.66		
Average	750	24.83	30	268.2767		
Variance	125000	4.5	0	164240.7		
n-hexane						
Count	2	2	2	6		
Sum	1500	38.33	60	1598.33		
Average	750	19.165	30	266.3883		
Variance	125000	20.03445	0	165355.6		
Total						
Count	4	4	4			
Sum	3000	87.99	120			
Average	750	21.9975	30			
Variance	83333.33	18.87556	0			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	10.69741	1	10.69741	0.000257	0.987736	5.987378
Columns	1397936	2	698967.8	16.77358	0.003492	5.143253
Interaction	21.39482	2	10.69741	0.000257	0.999743	5.143253
Within	250024.5	6	41670.76			
Total	1647992	11				

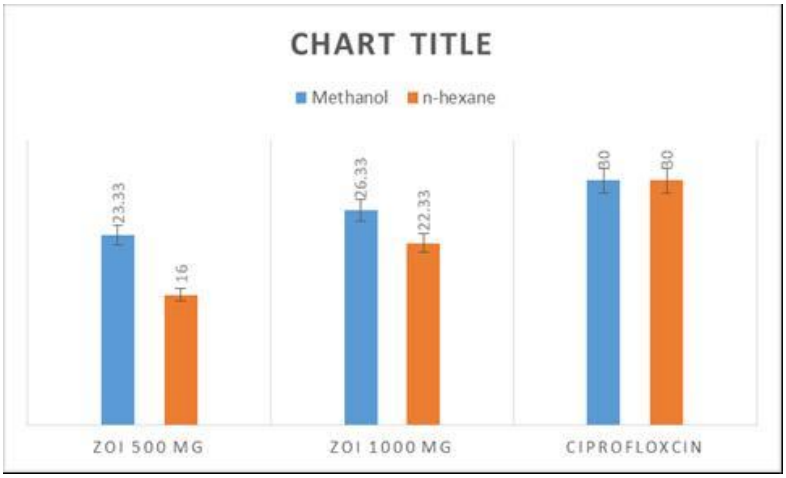

Figure 1: Antibacterial activity of *T. macrophylla* against *Xanthomonas axonopodis*.

Table 2: ANOVA of Antibacterial activity of *T. macrophylla* Flower extract against *E. Colli*

Anova: Two-Factor With Replication						
SUMMARY	**Conc**	**Zone of inhibition**	**Positive control**	**Total**		
Methanol						
Count	2	2	2	6		
Sum	1500	40.66	60	1600.66		
Average	750	20.33	30	266.7767		
Variance	125000	32	0	165128		
n-hexane						
Count	2	2	2	6		
Sum	1500	35.99	60	1595.99		
Average	750	17.995	30	265.9983		
Variance	125000	10.90445	0	165585.6		
Total						
Count	4	4	4			
Sum	3000	76.65	120			
Average	750	19.1625	30			
Variance	83333.33	16.11889	0			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1.817408	1	1.817408	4.36E-05	0.994945	5.987378
Columns	1403521	2	701760.6	16.83936	0.003458	5.143253
Interaction	3.634817	2	1.817408	4.36E-05	0.999956	5.143253
Within	250042.9	6	41673.82			
Total	1653570	11				

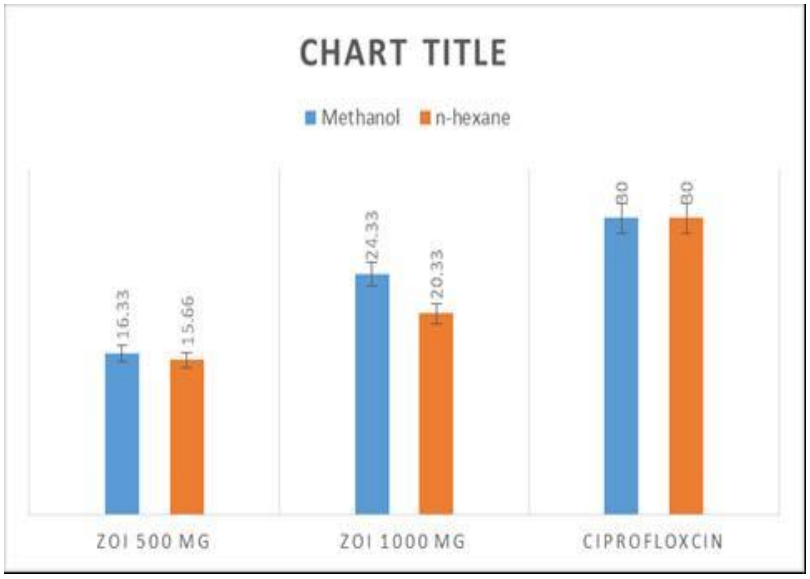

Figure 2: Antibacterial activity of *T. macrophylla* against *E. coli*.

Table 3: ANOVA of Antibacterial activity of *T. macrophylla* Flower extract against *B. subtillis*

Anova: Two-Factor With Replication						
SUMMARY	**Conc**	**Zone of inhibition**	**Positive control**	**Total**		
Methanol						
Count	2	2	2	6		
Sum	1500	39.33	56	1595.33		
Average	750	19.665	28	265.8883		
Variance	125000	64.18445	0	165645.2		
n-hexane						
Count	2	2	2	6		
Sum	1500	39	56	1595		
Average	750	19.5	28	265.8333		
Variance	125000	9.4178	0	165666.8		
Total						
Count	4	4	4			
Sum	3000	78.33	112			
Average	750	19.5825	28			
Variance	83333.33	24.54316	0			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.009075	1	0.009075	2.18E-07	0.999643	5.987378
Columns	1406486	2	703243.1	16.87287	0.00344	5.143253
Interaction	0.01815	2	0.009075	2.18E-07	1	5.143253
Within	250073.6	6	41678.93			
Total	1656560	11				

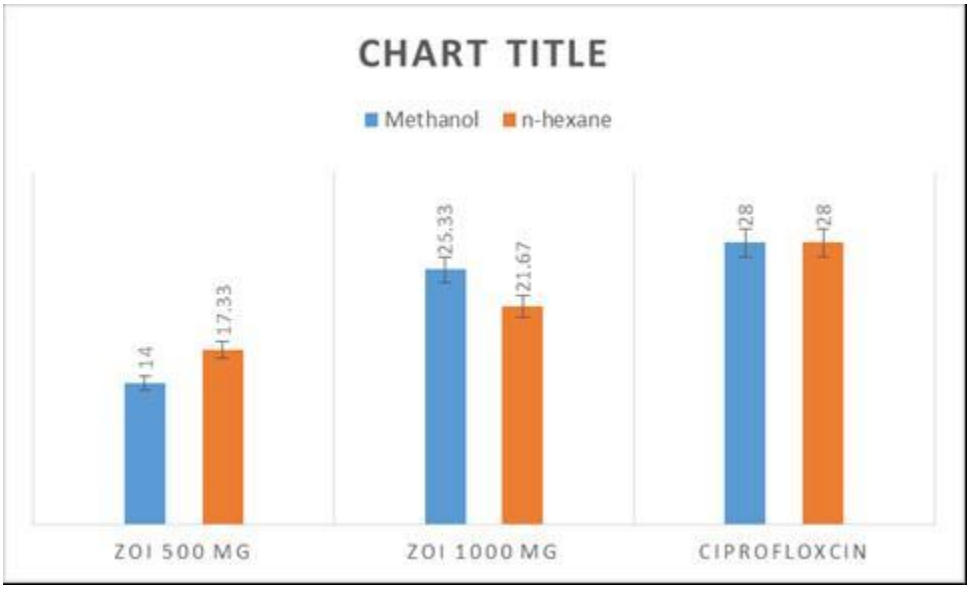

Figure 3: Antibacterial activity of *T. macrophylla* against *B. subtillis*.

Table 4: ANOVA of Antibacterial activity of *T. macrophylla* Flower extract against *Proteus vulgaris.*

Anova: Two-Factor With Replication						
SUMMARY	**Conc**	**Zone of inhibition**	**Positive control ciprofloxacin**	**Total**		
Methanol						
Count	2	2	2	6		
Sum	1500	42.33	36	1578.33		
Average	750	21.165	18	263.055		
Variance	125000	20.03445	0	167275.3		
n-hexane						
Count	2	2	2	6		
Sum	1500	37.99	36	1573.99		
Average	750	18.995	18	262.3317		
Variance	125000	3.56445	0	167693.2		
Total						
Count	4	4	4			
Sum	3000	80.32	72			
Average	750	20.08	18			
Variance	83333.33	9.435933	0			
ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	1.569633	1	1.569633	3.77E-05	0.995302	5.987378
Columns	1424815	2	712407.7	17.09617	0.003327	5.143253
Interaction	3.139267	2	1.569633	3.77E-05	0.999962	5.143253
Within	250023.6	6	41670.6			
Total	1674844	11				

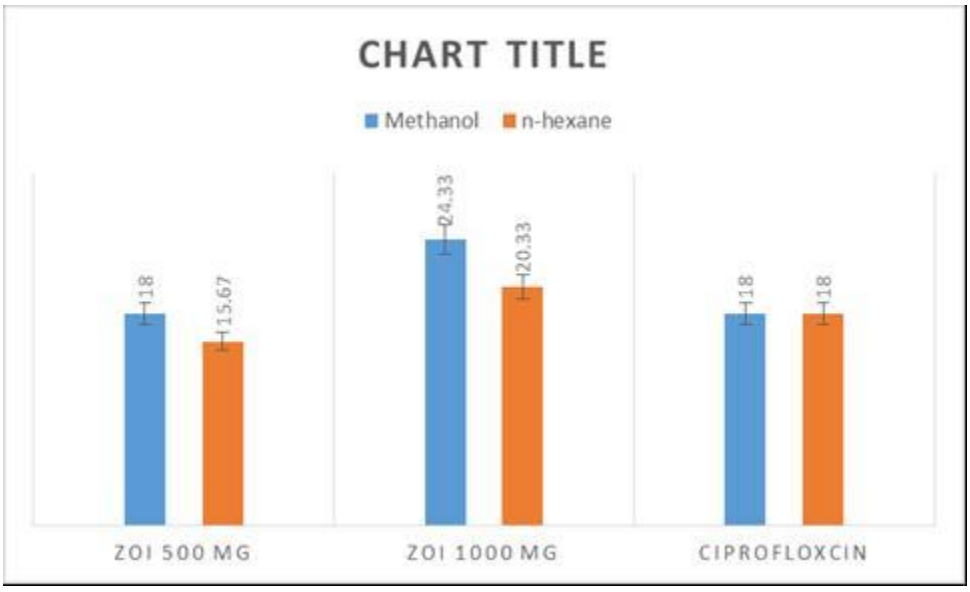

Figure 4: Antibacterial activity of *T. macrophylla* against *Proteus vulgaris*.

Table 5: ANOVA of Antibacterial activity of *T. macrophylla* Flower extract against *Clavibacter michiganensis.*

Anova: Two-Factor With Replication						
SUMMARY	**Conc**	**Zone of inhibition**	**Positive control**	**Total**		
Methanol						
Count	2	2	2	6		
Sum	1500	36.34	52	1588.34		
Average	750	18.17	26	264.7233		
Variance	125000	12.5	0	166310.8		
n-hexane						
Count	2	2	2	6		
Sum	1500	36	52	1588		
Average	750	18	26	264.6667		
Variance	125000	10.8578	0	166344		
Total						
Count	4	4	4			
Sum	3000	72.34	104			
Average	750	18.085	26			
Variance	83333.33	7.795567	0			
ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.009633	1	0.009633	2.31E-07	0.999632	5.987378
Columns	1413251	2	706625.5	16.95743	0.003397	5.143253
Interaction	0.019267	2	0.009633	2.31E-07	1	5.143253
Within	250023.4	6	41670.56			
Total	1663274	11				

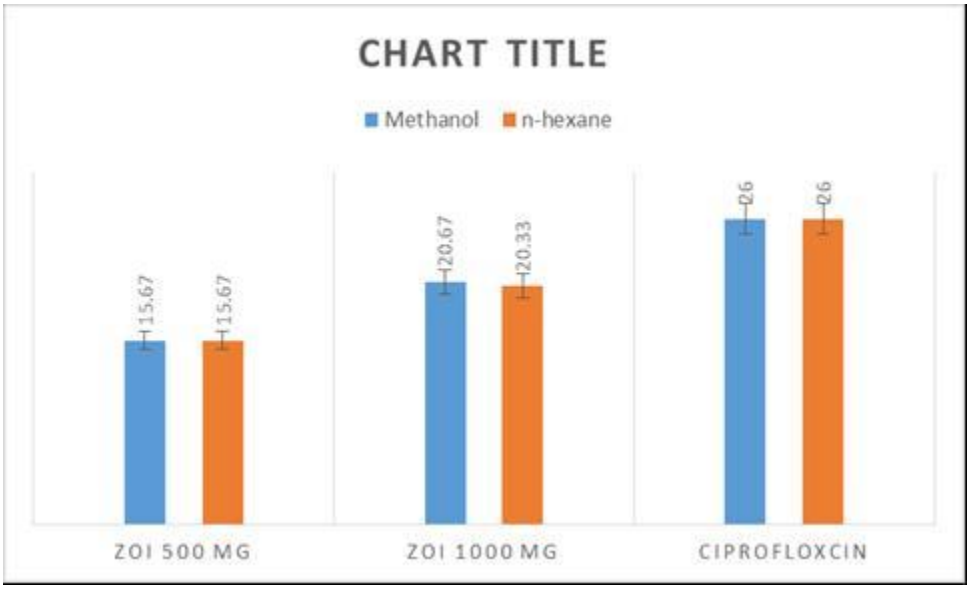

Figure 5: Antibacterial activity of *T. macrophylla* against *Clavibacter michiganensis*.

Table 6: ANOVA of Antibacterial activity of *T. macrophylla* Flower extract against *S. aureus*

Anova: Two-Factor With Replication						
SUMMARY	**Conc.**	**Zone of inhibition**	**Positive control**	**Total**		
Methanol						
Count	2	2	2	6		
Sum	1500	37.32	52	1589.32		
Average	750	18.66	26	264.8867		
Variance	125000	18	0	166215.3		
n-hexane						
Count	2	2	2	6		
Sum	1500	36.99	52	1588.99		
Average	750	18.495	26	264.8317		
Variance	125000	9.37445	0	166246.1		
Total						
Count	4	4	4			
Sum	3000	74.31	104			
Average	750	18.5775	26			
Variance	83333.33	9.133892	0			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.009075	1	0.009075	2.18E-07	0.999643	5.987378
Columns	1412280	2	706140	16.9455	0.003403	5.143253
Interaction	0.01815	2	0.009075	2.18E-07	1	5.143253
Within	250027.4	6	41671.23			
Total	1662307	11				

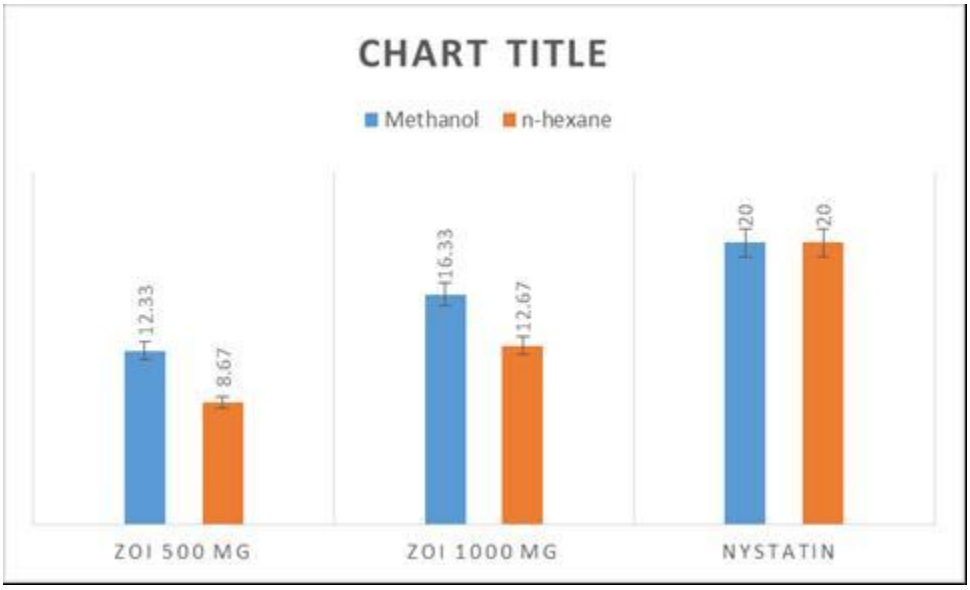

Figure 6: Antibacterial activity of *T. macrophylla* against *S. aureus*.

Table 7: ANOVA of Antifungal activity of *T. macrophylla* Flower extracts against *Aspergillus flavus*.

Anova: Two-Factor With Replication						
SUMMARY	**Conc**	**Zone of inhibition**	**Positive control**	**Total**		
Methanol						
Count	2	2	2	6		
Sum	1500	28.66	40	1568.66		
Average	750	14.33	20	261.4433		
Variance	125000	8	0	168220.6		
n-hexane						
Count	2	2	2	6		
Sum	1500	21.34	40	1561.34		
Average	750	10.67	20	260.2233		
Variance	125000	8	0	168947.7		
Total						
Count	4	4	4			
Sum	3000	50	80			
Average	750	12.5	20			
Variance	83333.33	9.798533	0			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	4.4652	1	4.4652	0.000107	0.992076	5.987378
Columns	1435817	2	717908.3	17.2287	0.003262	5.143253
Interaction	8.9304	2	4.4652	0.000107	0.999893	5.143253
Within	250016	6	41669.33			
Total	1685846	11				

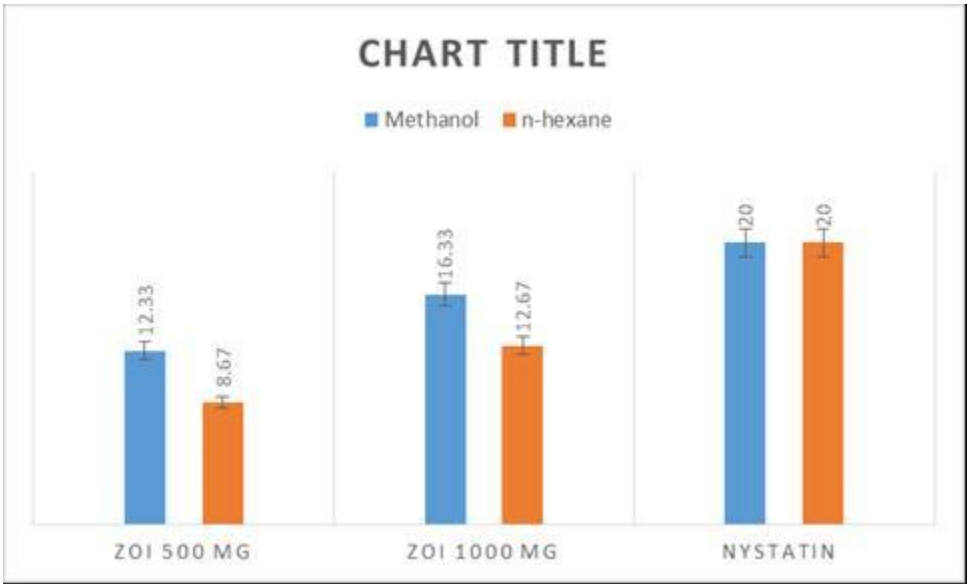

Figure 7: Antifungal activity of *T. macrophylla* Flower extracts against *Aspergillus flavus*.

Table 8: ANOVA of Antifungal activity of *T. macrophylla* Flower extract against *Rizopus stolonifer.*

Anova: Two-Factor With Replication						
SUMMARY	**Conc**	**Zone of inhibition**	**Positive control**	**Total**		
Methanol						
Count	2	2	2	6		
Sum	1500	24.67	44	1568.67		
Average	750	12.335	22	261.445		
Variance	125000	14.20445	0	168233.1		
n-hexane						
Count	2	2	2	6		
Sum	1500	20.33	44	1564.33		
Average	750	10.165	22	260.7217		
Variance	125000	9.37445	0	168665.9		
Total						
Count	4	4	4			
Sum	3000	45	88			
Average	750	11.25	22			
Variance	83333.33	9.429267	0			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1.569633	1	1.569633	3.77E-05	0.995302	5.987378
Columns	1434468	2	717234.1	17.21199	0.00327	5.143253
Interaction	3.139267	2	1.569633	3.77E-05	0.999962	5.143253
Within	250023.6	6	41670.6			
Total	1684496	11				

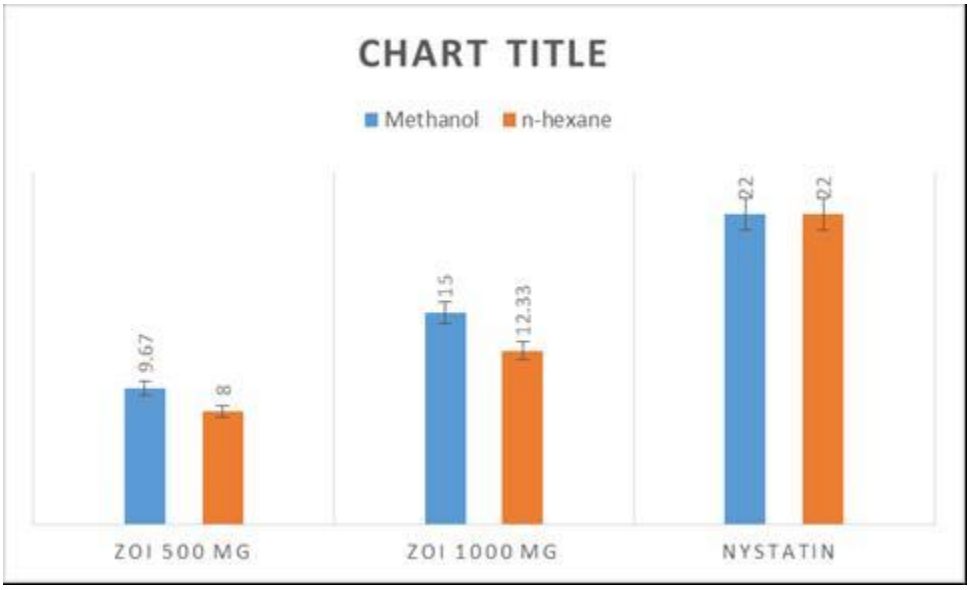

Figure 8: Antifungal activity of *T. macrophylla* Flower extracts against *Rizopus stolonifera.*

Table 9: ANOVA of Antifungal activity of *T. macrophylla* Flower extract against *A. niger*

Anova: Two-Factor With Replication						
SUMMARY	**Conc**	**Zone of inhibition**	**Positive control**	**Total**		
Methanol						
Count	2	2	2	6		
Sum	1500	25.34	44	1569.34		
Average	750	12.67	22	261.5567		
Variance	125000	8	0	168165.1		
n-hexane						
Count	2	2	2	6		
Sum	1500	22	44	1566		
Average	750	11	22	261		
Variance	125000	3.5378	0	168497.5		
Total						
Count	4	4	4			
Sum	3000	47.34	88			
Average	750	11.835	22			
Variance	83333.33	4.775567	0			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.929633	1	0.929633	2.23E-05	0.996384	5.987378
Columns	1433300	2	716649.9	17.1988	0.003276	5.143253
Interaction	1.859267	2	0.929633	2.23E-05	0.999978	5.143253
Within	250011.5	6	41668.59			
Total	1683314	11				

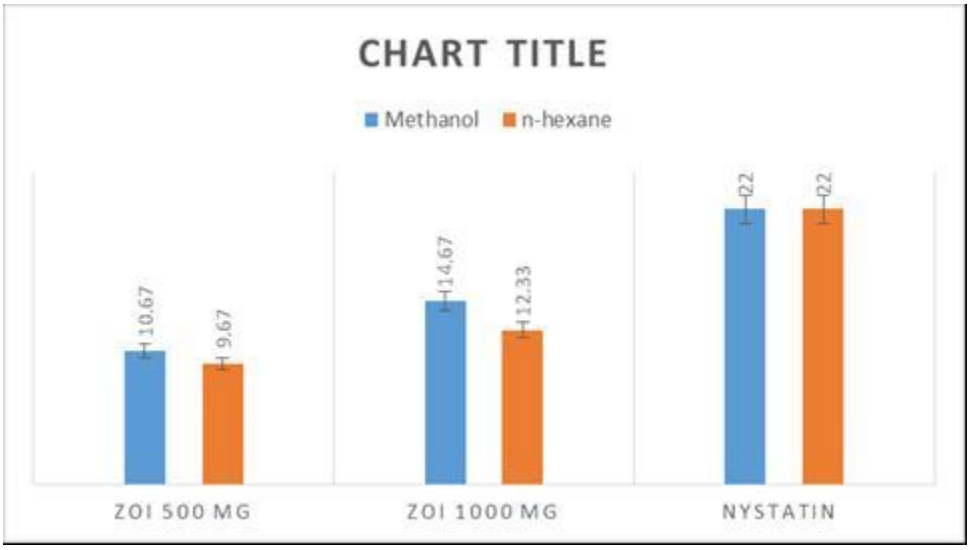

Figure 9: Antifungal activity of *T. macrophylla* Flower extracts against *A. niger.*

Table 10: ANOVA of Antifungal activity of *T. macrophylla* Flower extract against *Aceromonium cucurbetacearum.*

Anova: Two-Factor With Replication						
SUMMARY	**Conc**	**Zone of inhibition**	**Positive control**	**Total**		
Methanol						
Count	2	2	2	6		
Sum	1500	40.33	56	1596.33		
Average	750	20.165	28	266.055		
Variance	125000	6.73445	0	165535.3		
n-hexane						
Count	2	2	2	6		
Sum	1500	27	56	1583		
Average	750	13.5	28	263.8333		
Variance	125000	1.3778	0	166857.1		
Total						
Count	4	4	4			
Sum	3000	67.33	112			
Average	750	16.8325	28			
Variance	83333.33	17.51149	0			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	14.80741	1	14.80741	0.000355	0.985571	5.987378
Columns	1411924	2	705962.2	16.94254	0.003404	5.143253
Interaction	29.61482	2	14.80741	0.000355	0.999645	5.143253
Within	250008.1	6	41668.02			
Total	1661977	11				

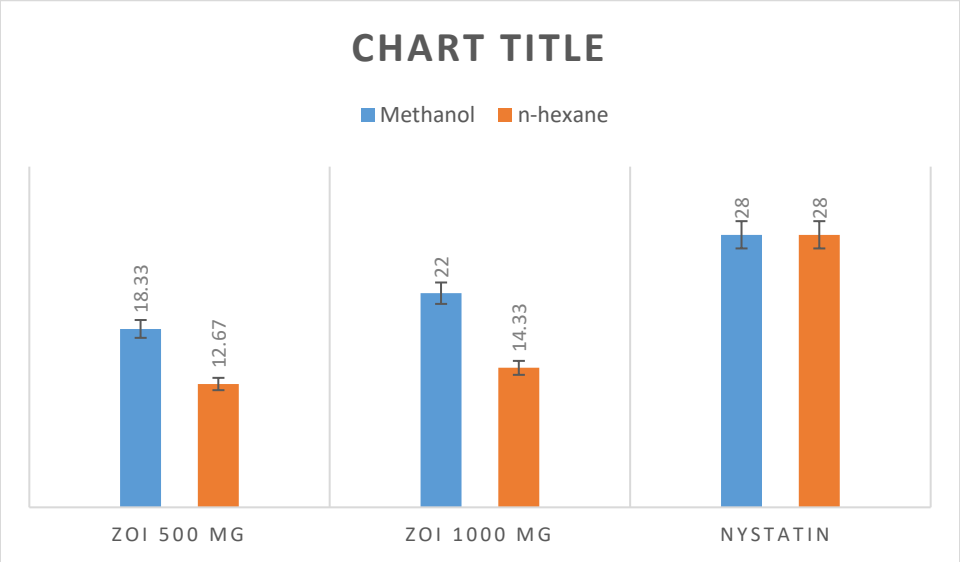

Figure 10: Antifungal activity of *T. macrophylla* Flower extracts against *Aceromonium cucurbetacearum*

Aceromonium cucurbetacearum. Results showed (Table 10) that the methanol

plant extract at 500 ug/ml was calculated 18.33±0.47mm ZOI against *Acromonium* fungal spp., while the 1000 ug/ml methanol extract had been calculated 22.00±0.82mm ZOI. The ZOI for n-hexane extract at 500 and 1000 ug/ml measured 12.67±0.47mm and 14.33±0.47mm, respectively. The ZOI for positive control Nystatin against *Acromonium* spp. was recorded 28mm ZOI.

DISCUSSION

The infectious disease that is caused by microbes is globally a serious threat to public health (Eggleston et al. 2010). Antibiotics are considered the alternate choice in treating these infectious microbes disease; however, their mutation in toxicity becoming antibiotic-resistant to many drugs is of main concern (Davies and Davies, 2010) (Li and Webster, 2018). The safety and antibiotics efficacy-related limitation have enhanced researchers to find other novel plant natural products that can be more effective against these infectious microbes (Doughari et al. 2009). Pakistan has a diverse medicinal and nutritional flora which has grown naturally due to the location of the country to be in a highly diverse ecological zone of the world (Ahmad et al. 2014); (Khan et al. 2012).

In the present research, the plant stem-Flower extract of both methanol and n-hexane had revealed that *T. macrophylla* extracts have potential inhibitory effects against all the tested bacteria. Furthermore, the methanol extract had been observed to have more significant results than n-hexane plant stem-Flower extract. (Hasan et al. 2016) reported that *T. macrophylla* leaves methanol extract against *B. subtillis* and *S. aurus* had shown 14mm ZOI. (López-Romero et al. 2018) reported that the plants had significant antibacterial activity against S. aureus. Similarly, the biological activity of *T. macrophylla* extract had been found slightly lower against fungal species than bacterial species. The present research antifungal activity results were supported by the findings of (Hasan and Sikdar, 2019) who recorded 5 to 13mm ZOI against *A. niger* and other fungal strains in *T. macrophylla* leaves methanol extract.

CONCLUSION

The current study revealed that *Tetranthera macrophylla* Flowers have significant antibacterial and antifungal potential due to the presence of various secondary metabolites and might be helpful in the production of antibiotics in the future.

CONFLICT OF INTEREST

The authors declared that the present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

SU and KR designed and performed the experiments and also wrote the manuscript. SM, MM, and SK performed data analysis. SU, MN, TY and KR prepared and reviewed the manuscript. All authors read and approved the final version.

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