

total flavonoid content (TFC) was determined as per the method of Aiyegroro and Okoh, (2010) with some modifications [18].

2.5. STATISTICAL ANALYSIS:

Pearson correlation coefficient test was performed between IC 50 values and TPC and between TPC and TFC by using PAST3 software. Standard deviation was also determined for inhibition zone and MIC values of plant extracts.

3. RESULTS AND DISCUSSION:

Alternanthera is a large genus of family amranthaceae with nearly 137 species [19]. Though, many species of this genus have been investigated for their medicinal properties [21-24], but scanty information is available in literature about species *pungens*. Hence efforts were made in the present investigation to understand its antimicrobial, antioxidant and phytochemical properties. The results obtained are described and discussed below.

3.1 ANTIMICROBIAL ACTIVITY:

All the four crude extracts prepared from the aerial parts of *A. pungens* possess promising antibacterial activity. Among the four concentrations of plant extracts, significant observations were made with 200 mg/ml concentration; therefore, results obtained with this concentration are compared among different solvents.

The zone of inhibition obtained with the different crude extract against bacterial strains used is given in Table 1. The crude extracts were specific in action against test bacterial strains, signifying the presence of antimicrobial compounds in this plant. On contrary to our results, Zongo et al., (2011) observed weak antimicrobial and antioxidant activity of this plant [20].

Mycobacterium smegmatis (18.60±1.5 mm), *Staphylococcus aureus* (9.60±0.57 mm) and *Bacillus subtilis* (7.60±0.50 mm) revealed susceptibility with acetone extract. All the three Gram negative strains were found to be resistant to this crude extract as no activity was obtained with these strains in disc diffusion assay.

Aqueous extract revealed maximum inhibition zone 15.66±2.08 mm against *Bacillus subtilis* trailed by *M.*

smegmatis (8.60±0.57 mm). Least activity was observed against *K. pneumoniae* and *P. aeruginosa* (7.0±0 mm) with this extract. Although the extract showed activity against both Gram positive and Gram negative bacteria, the extract was found to be more effective against Gram positive strains. Similarly, Kumari and Krishnan (2016) reported antibacterial activity of the aqueous extracts of two species of *Alternanthera* i.e. *sessilis* and *philoxeroides* against both Gram positive and Gram negative organisms [21].

Ethanol extract revealed maximum zone of inhibition (11.60±0.57 mm) against *M. smegmatis* followed by *C. violaceum* (10.60±0.57 mm) and *K. pneumoniae* (10.30±0.57 mm). The extract was almost ineffective against three bacterial strains i.e. *P. aeruginosa*, *S. aureus* and *B. subtilis* exhibited least inhibition zone (7.0±0 mm). Both Gram positive and Gram negative strains were found to be sensitive to ethanolic extract though to variable degrees as compared to aqueous extract. Similar to our results, ethanolic extract was reported to be ineffective against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* by Gupta et al., 2010 [22]. Studies carried out with *A. sessilis* also showed that *B. subtilis* and *K. pneumoniae* to be resistant to ethanolic and aqueous extracts [23]. Sivakumar and Sunmathi (2016) revealed that both Gram positive and Gram negative bacterial strains were susceptible to ethanolic extract of two species of *Alternanthera* i.e. *A. sessilis* and *A. philoxeroides* [24]. When the petroleum ether extract was evaluated for its antibacterial potential, maximum inhibition zone was observed against *Chromobacterium violaceum* (9.0±1.0 mm) followed by *Staphylococcus aureus* (8.30±0.57 mm). Minimum zone of inhibition was observed against *Pseudomonas aeruginosa* (7.0±0 mm) with this extract.

The positive control i.e. Ampicillin exhibited greater zone of inhibition when compared to all the plant extracts. This is probably due to the fact that crude plant extracts contains a number of impurities which may be inert and devoid of antibacterial activities. Ampicillin, is a pure chemical compound, and hence produces larger zone of inhibition even at a very low concentrations [25].

Table 1- Inhibition zone against six bacterial strains with extracts of *Alternanthera pungens* observed by disc diffusion assay.

Plant extracts (200 mg/ml) /Antibiotic (100 µg/ml)	Zone of inhibition diameter (mm)					
	Bacterial strains used					
	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Mycobacterium smegmatis</i>	<i>Bacillus subtilis</i>	<i>Chromobacterium violaceum</i>
Acetone extract	-	-	9.60±0.57	18.60±1.50	7.60±0.50	-
Aqueous extract	7.0±0	7.0±0	-	8.60±0.57	15.66±2.08	-
Ethanol extract	10.30±0.57	7.0±0	7.0±0	11.60±0.57	7.0±0	10.60±0.57
Petroleum ether extract	-	7.0±0	8.30±0.57	-	-	9.0±1.0
Ampicillin	24.33±2.51	22.0±4.0	15.33±1.52	28.33±1.52	24.33±1.52	22.33±1.52

All values are mean±SD

MIC was performed by microbroth dilution assay only for those extracts that revealed inhibition zone with disc diffusion assay. Inhibition zone and MIC values obtained were contrarily related to each other which showed crude extracts with highest inhibition zone has least MIC value. The results are given below in table 2.

Table 2- MIC values against six bacterial strains with crude extracts of *A. pungens*.

Bacterial strains	Extract	MIC Value (mg)
<i>Klebsiella pneumoniae</i>	Aqueous	5.0±0
	Ethanol	1.60±0.72
<i>Pseudomonas aeruginosa</i>	Aqueous	5.0±0
	Ethanol	3.33±1.40
	Petroleum ether	5.0±0
<i>Staphylococcus aureus</i>	Acetone	0.51±0.17
	Ethanol	5.0±0
	Petroleum ether	1.66±0.72
<i>Mycobacterium smegmatis</i>	Acetone	0.41±0.17
	Aqueous	1.66±0.72
	Ethanol	0.51±0.17
<i>Bacillus subtilis</i>	Acetone	3.33±1.40
	Aqueous	1.04±0.36
	Ethanol	5.0±0
<i>Chromobacterium violaceum</i>	Ethanol	0.83±0.36
	Petroleum Ether	1.04±0.36

The MIC value for different extracts ranged between 0.41±0.17 mg to 5.0±0 mg. For *Klebsiella pneumoniae* minimum MIC value was observed with ethanolic extract (1.60±0.72 mg) followed by aqueous extract (5.0±0 mg).

MIC observed for *Pseudomonas aeruginosa* with ethanol extract was minimum (3.33±1.40 mg). Similar MIC value was observed with aqueous and petroleum ether extract (5.0±0 mg). For *Staphylococcus aureus*, MIC value (0.51±0.17 mg) acetone extract was found to be lower than the petroleum ether extract (1.04±0.36 mg) and ethanolic extract (5.0±0 mg). Acetone extract also revealed least MIC value against *Mycobacterium smegmatis* (0.41±0.17 mg) as compared to the ethanol extract (0.51±0.17 mg) and aqueous (0.83±0.36 mg) extract. MIC values of aqueous extract, acetone extract and ethanolic extract with *Bacillus subtilis* were 1.04±0.36, 3.33±1.40 and 5.0±0 mg respectively. Minimum MIC value 0.20±0.09 mg was reported against *Chromobacterium violaceum* with acetone extract followed by ethanol extract (0.62±0 mg) and petroleum ether extract (1.25±0 mg). Ethanol extract exhibited MIC value of 0.83±0.36 mg for *Chromobacterium violaceum* followed by petroleum ether extract (1.04±0.36 mg).

All crude extracts prepared from the aerial parts of *A. pungens* possess weak antifungal activity and the results obtained are given in Table 3. *A. niger*, *F. oxysporum* and *P. expansum* were found to be resistant against all the four crude extracts. *A. fumigatus* was inhibited by acetone extract and aqueous extract with zone of inhibition 9.66±0.57 mm and 7.66±0.57 mm respectively. Ethanolic and petroleum ether extract were found to be ineffective against *A. fumigatus*. Sivakumar and Sunmathi in 2016 reported antifungal activity of two species of *Alternanthera* i.e. *sessilis* and *philoxeroides* against *Candida albicans* (24). Antifungal activity of *Alternanthera philoxeroides* were also found to be against *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma viridae* (26). The MIC value obtained for *Aspergillus fumigatus* with acetone and aqueous extracts were 1.45±0.95 and 1.66±0.72 mg respectively. The results are shown in Table 4.

Table 3- Inhibition zone against four fungal strains with extracts of *Alternanthera pungens* observed by disc diffusion assay.

Plant extracts (200 mg/ml) /Antibiotic (100 µg/ml)	Zone of inhibition diameter (mm)			
	Fungal strains used			
	<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>	<i>Fusarium oxysporum</i>	<i>Penicillium expansum</i>
Acetone extract	9.66±0.57	-	-	-
Aqueous extract	7.66±0.57	-	-	-
Ethanolic extract	-	-	-	-
Petroleum ether extract	-	-	-	-
Miconazole	18.33±1.52	15.0±4.58	8.33±0.57	24.33±2.51

All values are mean±SD

Table 4- MIC values against *Aspergillus fumigatus* with crude extracts of *A. pungens*.

Fungal strains	Extract	MIC Value (mg)
<i>Aspergillus fumigatus</i>	Acetone extract	1.45±0.95
<i>Aspergillus fumigatus</i>	Aqueous extract	1.66±0.72

3.2 ANTIOXIDANT ACTIVITY OF PLANT EXTRACTS

The crude extracts of *A. pungens* were investigated for their free radical scavenging activity by DPPH assay. The antioxidant potential of the extracts was found to be concentration dependent (Fig. 2). The results obtained are displayed as IC₅₀ values which ranged between 100.79 µg/ml to 931.63 µg/ml (Fig. 3). Minimum IC₅₀ value (100.79 µg/ml) was observed with ethanolic extract followed by acetone (IC₅₀ value 203.56 µg/ml), aqueous (IC₅₀ value 324.43 µg/ml) and petroleum ether extracts (IC₅₀ value 931.63 µg/ml). The study assured free radical scavenging efficiency of *A. pungens* to a firm level. Though, the free radical scavenging efficacy varied with solvents used for extraction. Based on the results obtained, we observed that polar solvents are more effective antioxidants as compared to the non polar petroleum ether crude extract. This is because secondary metabolites are more soluble in polar solvents.

3.3 PHYTOCHEMICAL ANALYSIS

Plant acts as a natural repository of a wide spectrum of bioactive compounds which are responsible for their biological properties. Moreover these compounds are unique sources for food additives, flavors, and other industrial materials [27]. These metabolites have enormous therapeutic potential and are effective in treatment of various diseases also mitigate the side effects often associated with the synthetic drugs (28). The extracts of *A. pungens* prepared in four different solvents were screened for the presence of different phytochemical. The results obtained are given in Table 5. Crude extracts of *A. pungens* contains phenols, flavonoids, alkaloids, glycosides, steroids, saponins and tannins. Several studies have linked the biological activities of plants to phenolic compounds that they contain. There is now a strong consensus that phenolics and flavonoids are responsible for much of the antioxidant activity of plants [29-31].

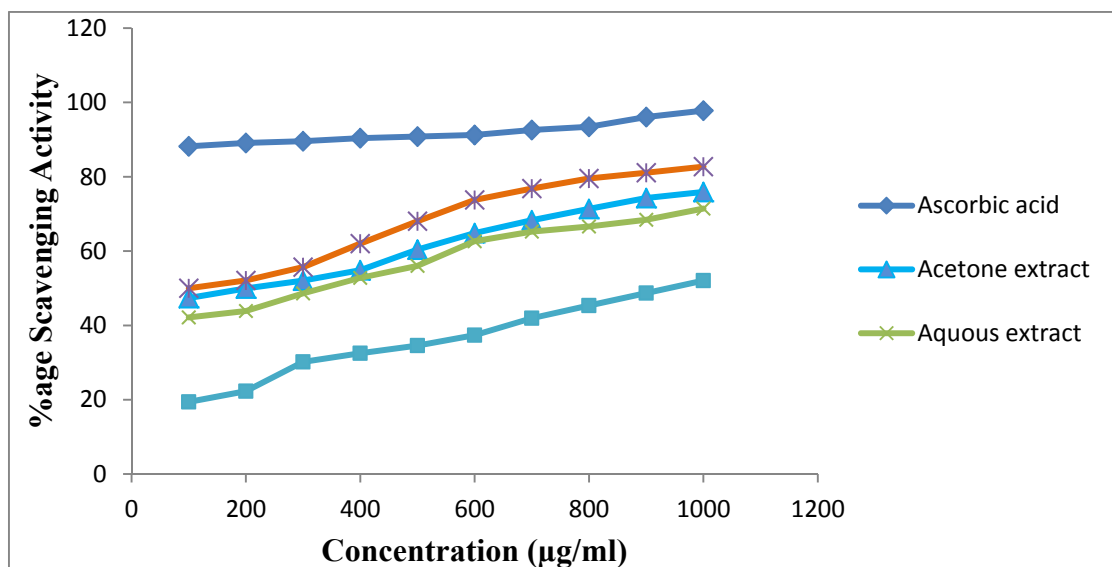


Fig. 2. %age scavenging activity obtained against different concentrations of *A. pungens* extracts prepared in different solvents and Ascorbic acid.

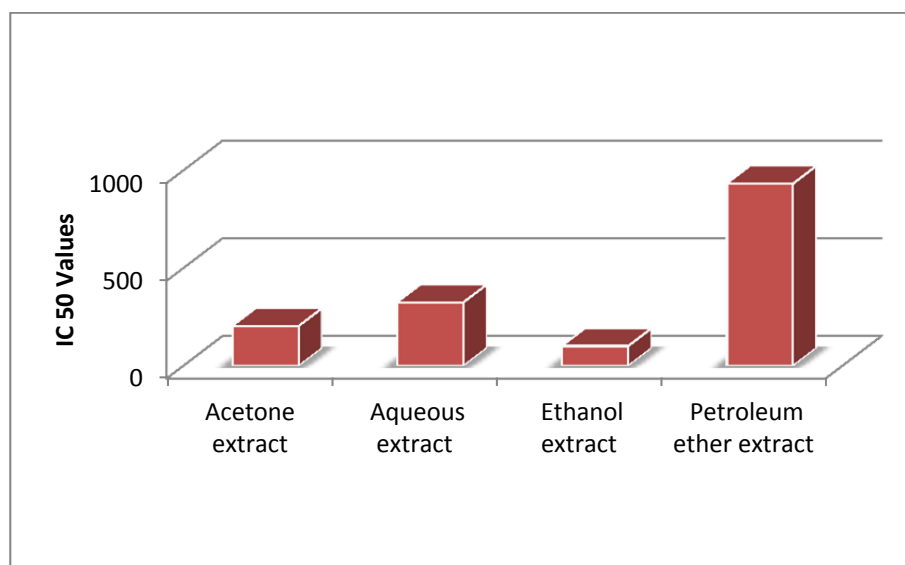


Fig. 3. IC₅₀ values obtained with different extracts of *A. pungens*

Table 5- Phytochemical analysis of different extracts of *A. pungens*

Phytochemical	Crude plant extracts			
	Acetone	Aqueous	Ethanol	Petroleum ether
Alkaloids	-	+	+	-
Phenols	++	+	+	+
Flavonoids	++	++	++	+
Glycosides	+	-	+	-
Tannins	+	+	-	+
Saponins	+	-	+	-
Steroids	+	+	+	-

Presence: +, absence: -

Total Phenolic Content reported as Gallic acid equivalents varied widely, ranging from 12.11 mgGAE/g obtained with ethanolic extract to 2.55 mgGAE/g obtained with petroleum ether extract (Fig. 4).

Similar to phenolic content, flavonoid content was also highest in ethanolic extract i.e. 9.23 mgQE/g and minimum in petroleum ether extract (2.07 mgQE/g) (Fig. 5). All values were Quercetin equivalents.

Correlation coefficient between IC₅₀ values and total phenolic content of different extracts was recorded as -0.844 which revealed a negative correlation between them.

On the other hand, correlation coefficient between total phenolic content and total flavonoids content (0.967) showed that these two variables are positively co-related.

Similar to *pungens* other species of *Alternanthera* have been reported to possess antioxidant properties and this is mainly due to the presence of phenolic and flavonoids. Ethanolic extract was found to be exhibited highest percentage scavenging activity and total phenolic and flavonoid content of the ethanolic extract was significantly high and this is correlated with highest percentage scavenging activity. As not much information is available in literature with regards to the *A. pungens* results are discussed and compared with the other species of *Alternanthera*. Similarly, Borah et al (2011) investigated total phenolic and flavonoid content and correlated it with the antioxidant potential of *Alternanthera sessilis* and concluded that these secondary metabolites are liable for its antioxidant potential [32]. Murugan et al (2013) also reported antioxidant activity of *Alternanthera sessilis* [33]. *Alternanthera bettzickiana* has also been shown to possess good antioxidant activity [34]. Hence other plant species of genus *Alternanthera*, *A. pungens* also attributed with various biological properties including antibacterial and antioxidant potential.

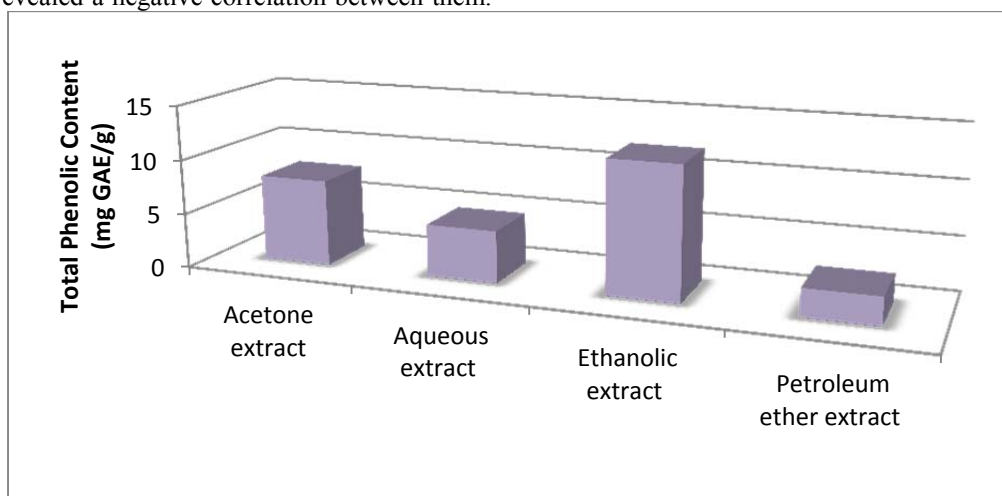


Fig. 4: Total Phenolic Content obtained for different extracts of *A. pungens*

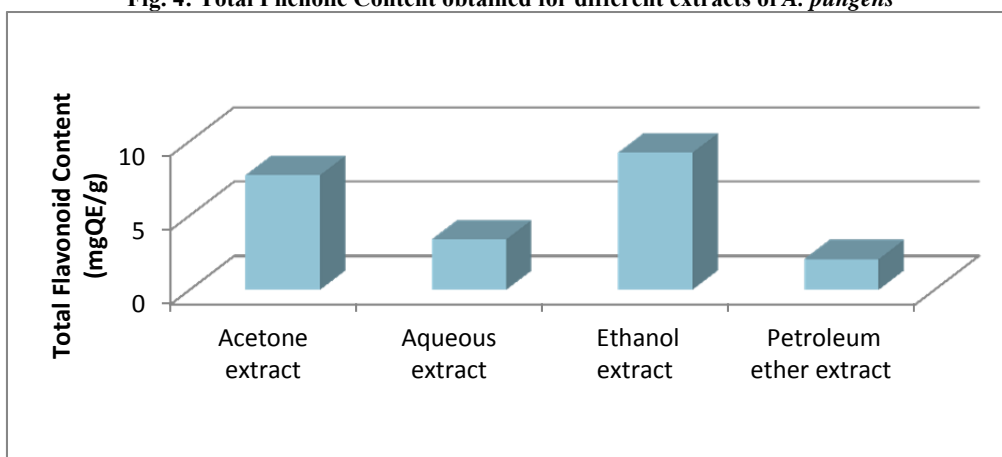


Fig. 5. Total Flavonoid Content obtained from different extracts of *A. pungens*

4. CONCLUSION

The present in vitro study revealed that crude extracts of *Alternanthera pungens* inhibits some particular bacterial strains effectively. The zone of inhibition with diameter of more than 10 mm was observed against *Klebsiella pneumoniae* (with ethanolic extract), *Mycobacterium smegmatis* (with acetone and ethanolic extract), *Bacillus subtilis* (with aqueous extract) and *Chromobacterium violaceum* (with acetone extract). Plant showed poor antifungal activity as reported only against *Aspergillus fumigatus* with acetone and aqueous extracts. Ethanolic extract was found to possess better antioxidant activity by DPPH radical scavenging activity followed by petroleum ether and aqueous extracts. Total phenolic content and total flavonoids content of different extracts, exhibits a significant relation with scavenging activity of respective extracts. The present study revealed the medicinal potential of plant and ascertains the value of this plant in development of new herbal drugs. However the plant needs more detailed investigation with ethno-pharmacological approach are required for its better use in therapeutics.

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