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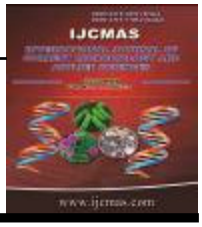

Original Research Article

Antibacterial Efficacy of *Moringa oleifera* and *Tabernaemontana divaricata* Flower Extracts on Occular Pathogens

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Keywords

Moringa oleifera, *Tabernaemontana divaricata*, Ocular pathogen, Antibacterial, Flower, Petal extract

A B S T R A C T

The potential presence of naturally occurring antimicrobials in petals of flowers of *Moringa oleifera* and *Tabernaemontana divaricata* was investigated against isolated eye pathogens. Owing to the usage of these flowers in Siddha medicine for eye ailments and common folklore medicine, the extracts of petals were screened for antibacterial activity against pathogenic microbes isolated from the eyes of eye infected persons. Bioactive compounds were extracted by cold extraction method, wherein Methanol, Dichloromethane (DCM) and Ethyl acetate were used as solvents. The antibacterial activity of the extracts was assessed by agar well diffusion method. The results of antibacterial activity were analyzed by using two – way analysis of variance (ANOVA). The study revealed that the extracts possessed antibacterial activity in a close dependent manner. Among the tested flowers, *T. divaricata* showed better activity. Of the three extracts, DCM extract exhibited significant antibacterial potential $P < (0.05)$ on most of the ocular pathogens tested. *S. aureus*, *S. agalactiae* and *Propionibacterium acnes* were resistant to all extracts of *M. oleifera* whereas *E. coli* is found to be resistant to *T. divaricata* extracts. The present study indicates that the DCM extracts of petals of the flowers of *M. oleifera* and *T. divaricata* can be used to discover antibacterial agent for developing new pharmaceuticals to treat eye infections.

Introduction

Plants are well known as a major source of modern medicines. From ancient times, humans have utilized plants for the treatment or prevention of diseases, leading to the dawn of traditional medicine. Many plant species produce a wide range of chemical products that are not involved in primary metabolism and called secondary metabolites (Rhodes, 1994). Alkaloid and terpenoids are main secondary metabolites

that have many physiological and pharmacological properties to living cells.

Secondary metabolites of plants are having good efficacy in controlling various pathological disorders While plant leaf, stem and root extracts have been widely evaluated for bioactive compounds, screening for plant flower has not been extensive. The flower petals which provide physical protection to the reproductive compounds can be expected to synthesize potent bioactive compounds. Interestingly, the symptoms of most plant diseases of bacterial and fungal origin have been reported mostly on the leaves, stems, roots and seldom on petals (Darokar *et al.,* 1998). In the present study the petals of flowers of *M. oleifera* and *T. divaricata* were chosen to investigate its effect on eye diseases as these flowers are analyzed in common folk medicine to treat eye patients. Growing scientific evidence supports that this plant has medicinal benefits and its extracts could possibly be used as pharmacological interventions in various diseases (Neimkhum *et al.,* 2010). Therefore, the aim of this study was to evaluate the biological properties of the Methanolic, DCM and Ethyl acetate extracts of *Moringa Oleifera* and *Tabernaemontana divaricata* petals on bacterial isolates from eye diseases.

M. oleifera (family: Moringaceae) is a medium- sized tree about 10m high, cultivated throughout India. It is known as drumstick in English, Saragvo in Gujarati, Nugge in Kannada, sigru in Malayalam, Shevga in Marathi, shobhanjana in Sanskrit, Munaga in Telugu and Murungai in Tamil. All parts of the tree are considered to possess medicinal properties. The flowers cure inflammations and muscle diseases. *Moringa* tree is cultivated for food and medicinal purposes (Olson, 2002) Moringa leaf is a natural antihelminthic, antibiotic, detoxifier, outstanding immune builder used in some countries for the treatment of malnutrition and malaria (Thilza *et al.,* 2010). According to Fuglie (2000), the many uses of *Moringa* include alley cropping, biogas, fertilizer, nutrient, gum, honey and sugar from flower nectar and medicine. However, much has not been reported on the antibacterial property of *M. oleifera* flowers. Hence, the present study was made to find out the antibacterial activity of *Moringa* flowers on bacterial isolates of ocular origin.

T. divaricata (family: Apocynaceae) commonly known as Togor, Dedhphul in Bangladesh and wax flower, crepe flower, crepe jasmine in India, is an evergreen shrub to 6 feet (1.8 cm). It is used in Chinese, ayurvedic and Thai traditional medicine for the treatment of fever, pain and dysentery (Boonyaratanakornkit *et al.,* 2005). The phytochemistry and a number of chemical constituents from the leaves, stems and roots have been previously reported. Constituents studied include alkaloids and non alkaloids constituents such as terpenoids, steroids, flavonoids, phenyl propanoids, phenolic acids and enzymes (Ingkaninan *et al.,* 2006; Pratchayasakul *et al.,* 2008; Arambewela and Ranatunge, 1991).The most common medicinal use of crude *T. divaricata* extract involves its antimicrobial action against infectious diseases such as syphilis, leprosy and gonorrhoea as well as its antiparasitic action against worms, dysentery, diarrhoea and malaria (Van Beck *et al.,* 1984). The growing scientific evidence supports that this plant has medicinal benefits and its extracts could possibly be used as pharmacological interventions in various diseases. Therefore, the aim of this study was to evaluate antibacterial properties from extracts of *T. divaricata* flowers and to be employed for further development of health promotion pharmaceutical product.

Materials and Methods

Plant materials

Fresh flowers of *M. oleifera* and *T. divaricata* were plucked with sterilized forceps in the morning (6–8 am). The flowers were identified and authenticated with the herbarium the Botany Department of Rani Anna Government College (w), Tirunelveli, Tamilnadu. The collected flowers were used for extraction directly.

Extract preparation

Plant materials were successively extracted using solvents of increasing polarity (Arokiaraj *et al.*, 2009) solvents such as EA, DCM + M was used for extraction was done by cold extraction method. Stalk of flowers were removed to get petals alone. Each flower, 2 kg of petals was soaked in 5 liter of each solvent viz., Methanol, DCM and Ethyl acetate in a separate air tight containing. These were allowed to stand at room temperature for 5 days, with occasional manual agitation of the container using a sterile glass rod at every few hours. The extracts were separately filtered using sterile Whatman No.1 filter paper. The resulted filtrates were then concentrated in a rotary evaporator (Laborator 4000- efficient, heidolph Germany) at 400 rpm/50°c. Fifty ml of gummy extract were obtained upon evaporation for each extract. The gummy extract was stored at 4^0C for further studies.

Microorganisms

Ten bacterial strains isolated from eye infected cases examined in Agarwal Eye Hospital, Tirunelveli were used in the study. Of which nine were gram-positive, bacteria viz., *Staphylococcus aureus, S. epidermidis, Gardnerella vaginalis, Enterococcus faecalis, S. agalactiae, Propionibacterium acnes, Corynebacterium macbinleys, Bacillus serus, B. subtilis* and one gram-negative *viz., E. coli*. All the bacterial strains were from patients with eye diseases. The bacteria were initially identified by streak plate method in blood agar medium and specifically identified at Nellai Scans microbiological laboratory using enzyme assay method (VITEK 2 – COMPACT) and maintained on nutrient agar slants at 4^0C.

Antibacterial activity assay

Antibacterial activity of the methanol, DCM, Ethyl acetate extracts of petals of flowers *M. oleifera* and *T. divaricata* were assayed using agar-well diffusion method of Kirby Bauer. The concentrated gummy petal extracts of 200 µg were dissolved in 1 ml of di methyl sulfoxide (DMSO) and dilutions of 25µl, 50µl and 75µl were prepared for each extract respectively, to assess the antibacterial activity. Approximately 10 ml of sterile Muller-Hinton Agar (MHA) was poured into sterile culture plates and allowed to set. About 1ml of 24 h old culture of 10 bacterial isolates were maintained in nutrient broth and stored in an incubator. Sterile cotton swabs were dipped in the bacterial suspension and evenly streaked over the entire surface of the agar plate to obtain uniform inoculums. The seed medium was then allowed to dry at room temperature for about 30 minutes. With the aid of a sterile well-cutter, wells of about 6 mm in diameter were punched on the plates. About 0.5 ml of each dilution of the extracts was dispensed into the wells and the plates were incubated at 37^0C for 24 h. Triplicates were maintained for each sample of the extracts respectively. For each bacterial assay control with standard antibiotic streptomycin (75µl) was maintained. At the end of incubation period, inhibition zones formed on the medium were evaluated in mm.

Statistical analysis

The results of the antibacterial activity of the replicates were expressed as antibacterial activity of the replicates wee expressed as mean ± standard deviation (SD) and the data were subjected to examine by analysis of variance (ANOVA) ($p < 0.05$) by using a software, SPSS 13.0 for windows version.

Result and Discussion

Evaluation of the antibacterial potential of flower extracts

The data pertaining to the antibacterial potential of the petals of the selected flower extracts using methanol, DCM, Ethyl acetate extracts are presented in tables 1 and 2 and figures 1 and 2 respectively. The extracts from *M. oleifera* petals showed inhibitory activity against seven of the ten ocular bacterial isolates. The DCM extracts presented the highest activity and the extracts were able to inhibit six bacterial isolates viz., (*S. epidermidis, G. vaginalis, E. faecalis, C. macbinleys, B. subtilis* and *E. coli).* The highest activity rate was recorded against *G. vaginalis* (18 ±0.43mm). On the other hand, the methanol extracts, DCM and Ethyl acetate extracts also exhibited their highest activity against *C. macbinleys* (19 ±0.40mm, 16 ±0mm and 15 ±0 mm zone diameter respectively). Ethyl acetate extracts of the petals of the flowers of *M. oleifera* was able to inhibit three bacterial species including *G. vaginalis, C. macbinleys* and *B. cereus*. Methanol extracts were effective against five bacterial isolates viz., *S. epidermidis, E. faecalis, C. macbinleys, B. cereus* and *E. coli* (Table 1 and Figure 1). In an earlier study it was pointed out that *B. subtilis* was more sensitive to EA, ethanol and methanol extracts of Eucalyptus and *Ocimum*, but *E. coli* was less sensitive (Pathmanathan, 2010). Moyo Busani *et al.* (2012) reported that acetone extracts of leaves of M. oleifera had bactericidal properties against *E. coli*. It is known to be a multi – resistant bacteria (Afolayan, 2003).

Even at the highest concentration of 5 mg/ml, the acetone extract was able to kill *E. coli* Gram – negative bacteria have been reported to be resistant to antibiotics (Boussada *et al.,* 2008). According to Pintore *et al.* (2002) and Wilkinson *et al.* (2003), these bacteria are generally less sensitive to the activity of plant extracts. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Adwan and Abu – Hasan, 1998). Results of Kalpana *et al.* (2013), on Chloroform extracts of leaves of *M. Oleifera* against *E. coli* is found to be 8 mm to 11 mm, *Klebsiella pneumoniae* 6mm to 9mm, and S. aureus 7mm to 11mm from 200 to 800 mg concentration. In this study antimicrobial activity of chloroform extract of leaves of *M. Oleifera* was significantly effective against the gram positive and gram negative bacteria. Ethanolic extract of leaves *M. Oleifera* against *E. coli* and *Klebsiella pneumonia* organisms showed 7mm to 12mm zone of inhibition at 200mg to 800mg concentration respectively. Their result confirms that ethanolic and chloroform leaf extract had strong inhibitory effect against all the gram positive and gram negative bacteria. Our findings also had antibacterial activity against most of the gram positive and gram negative bacteria in all the three extracts of flowers of *M. Oleifera*. The results confirm the use of this plant in traditional medicine. This also indicates the presence of broad – spectrum antibiotic compounds in the plant (Siddhuraj and Becker, 2003). Moreover, Mohammed Abu Sayeed *et al.* (2013), in their investigation, found out antibacterial activity of the fruit

extract of *M. Oleifera* against nine pathogenic microorganisms, *S. aureus, B. subtilis, V. cholerae, B. cereus, S. typhi, S. dysenteriae, P. aeruginosa, Klebsiella species* and *Proteus sp,* with a maximum zone of inhibition of 22mm.

In the present study, the antibacterial activity of *M. oleifera* flower extract showed significant differences in their efficacy on isolated ocular pathogens. *S. epidermidis, G. vaginalis, P. acnes, C. macbinleys, B. subtilis* and *E. coli* showed significant differences ($P < 0.05$) between the variation in solvent system and concentration of the extract. *E. faecalis, Streptococci agalactiae, B. cereus* showed variation in significance between the two way analysis while *S. aureus* showed less significant variation ($P > 0.05$) (Table 3). This finding suggests that extracts of *M. oleifera* petals showed variation in potency according to their levels of concentration in six of the bacteria tested (Bakht *et al.* 2011), has found out that the type of solvent has an important role in the process of extraction.

The data obtained for the extracts of *T. divaricata* petals are presented in table 2 and figure 2. The results revealed variability in the inhibitory concentrations for each extract when tested against isolated ocular pathogens. The inhibitory effect of these flower extracts on ocular pathogens differed significantly ($P< 0.05$). The bacteria, *E. coli* was resistant to the petal extracts of flowers of *T. divaricata*. All the other nine bacterial isolates were sensitive to the Methanol, DCM and Ethyl acetate extracts of *T. divaricata* petals. Such results are very interesting. DCM extracts registered a maximum activity against all the tested ocular bacterial isolates except *E. coli*. *G. vaginalis* showed the highest inhibitory activity (18 ±0.30mm). Followed by DCM extract, ethyl acetate extract registered a maximum activity against seven tested isolates except *G. vaginalis, S. agalactiae* and *B. subtilis*. But methanol extracts showed less activity against only on three tested pathogens (*S. aureus, S. epidermidis* and *P. acnes*). The results were compared with standard antibiotic. *T. divaricata* is a thoroughly studied medicinal plant, possessing an array of medicinal properties, especially in their flowers. Studies of Bijeshmun and Shibu George (2014), revealed that flower extracts of *T. divaricata* in methanol is effective to inhibit the growth of *S. aureus and E. coli* (15mm zone of inhibition) than petroleum ether and aqueous extracts.

Similarly, Gopinath *et al.* (2011), in their studies have revealed that ethanol, methanol and aqueous extracts of *T. divaricata* leaves possess maximum potency against infectious pathogens such as *S. aureus, Streptococcus agalactiae, E. coli and Streptococci uberis*, with maximum zone of 26mm in *S. aureus* in methanolic extract. To this point, it emanates that the different extracts of *T. divaricata* flowers contain a vast array of bioactive constituents, which can be a potential candidate for drug development. Table 4 shows the effect of three solvents on the petals of *T. divaricata* tested against ten bacterial isolates. Both variation in solvent system and concentration of the extract gave significantly ($P < 0.05$) high inhibitions on nine of the bacteria tested except *E. coli* where no significant difference ($P > 0.05$) between the efficacies of petal extracts against the microbe was noted.

In the two way analysis of variance for the data on anti – bacterial activity in zone of inhibition (mm) of flower extracts against ocular bacterial isolates, as a function of concentration and solvent systems (Table 3 and 4) *T. divaricata* flower extracts had significantly ($P < 0.05$) higher

inhibition on DCM extracts compared to other extracts (Ethyl acetate and methanol) of same plant part, also it is found to be comparatively more significant than *M. oleifera* flower extracts of DCM, Ethyl Acetate and methanol.

Extracts of petals of flowers of *T. divaricata* showed a higher antibacterial activity than that of *M. oleifera's* petal extracts. Among the three solvents used, DCM is found to extract more potential compounds from the petals than ethyl acetate and methanol. The order of priority in solvents used ranges as

DCM > Ethyl acetate > Methanol

The results of the present study revealed that the antibacterial activity of the extracts of petals of flowers of *M. oleifera* inhibited seven of the ten bacteria tested (*S. epidermidis, G. vaginalis, E. faecalis, C. macbinleys, B. cereus, B. subtilis* and *E. coli),* whereas, the extracts of petals of flowers of *T. divaricata* inhibited nine ocular pathogens out of ten organisms tested (*S. aureus, S. epidermidis, G. vaginalis, E. faecalis, S. agalactiae, P. acnes, C. macbinleys, B. cereus* and *B. subtilis).* The antibacterial activity of the crude extract might be due to the presence of lipophilic compounds that might bind within or internal to the cytoplasmic membrane of bacterial cell wall (Boyd and Beveridge, 1979, 1981). John de Britto *et al.* (2013) reported that methanolic leaf extracts of *Wrightia tinctoria* have significant microbial activity against *staphylococcus aureus, S. typhi* and *S. dysenteriae.*

The extracts of the petals worked in a dose dependent manner, when the concentration of the extract was decreased the activity was also decreased. This was due to susceptibility of the species towards concentration of the extracts (Ordonez *et al.,* 2006). It has been reported that different solvents have different extraction capabilities and spectrum of solubility for the phyto-constituents (Majorie, 1999; Srinivasan *et al.,* 2001). Our findings corroborate with the above reports that in the present study, DCM extract of *M. oleifera* and *T. divaricata* possess more effective anti-bacterial activity than, ethyl acetate extracts and methanol extracts, thus signaling its broad spectrum of antibacterial activity. However, studies of Jeyaseelan *et al.* (2011), reported that flower extracts of *Allium Sativum* showed better inhibition on phytopathogens in the ethyl acetate, methanol and ethanol extracts than DCM and aqueous extracts.

In the present study the isolated ocular pathogen *C. macbinleys* was found to be more sensitive to all the three extracts of *M. oleifera* petals and also to the DCM and ethyl acetate extracts of *T. divaricata*. These results are in close agreement with most research findings that reports that most plant extracts have more activity against gram positive bacteria (Aiyegoro *et al.,* 2008; Boussaada *et al.,* 2008; Ashafa and Afolayan 2009; Koday *et al.,* 2010). Rajendrhan *et al.* (1998) also reported *E. coli* to be resistant to *Moringa* extracts.

The results of the present investigation clearly indicated that the antibacterial activity vary with the solvents used for the extraction and the species of plant material used. The higher antibacterial activities present in the extracts *T. divaricata* when compared with *M. oleifera* justify its uses in folk medicine. The present study ascertains the value of plants used in ayurvedic medicine and it creates a considerable interest for the development of new drugs. Further studies are being carried out to isolate and characterize the active compounds and to determine the toxicity and the optimum dose for treatment.

Table.1 Antibacterial activity of the flower extracts of *M. oleifera* in diameter of zone of inhibition (mm)

SL NO	OCULAR BACTERIAL ISOLATES	GRAM REACTION	CONCENTRA-TION	METHANOL EXTRACT	DCM EXTRACT	ETHYLACETATE EXTRACT	STANDARD ANTIBIOTIC (STREPPTOMYCIN 50 mg)
1	*Staphylococcus aureus*	**G+**	25µg	10±0	10±0	10±0	**16**
			50µg	10±0	10±0	10±0.33	
			75µg	10±0	10±0.33	11±0	
2	*Staphylococcus epidermidis*	**G+**	25µg	10±0	11±0	10±0	**17**
			50µg	11±0	12±0	11±0	
			75µg	14±0	14±0	11±0.33	
3	*Gardnerella vaginalis*	**G+**	25µg	10±0	12±0.33	10±0	**16**
			50µg	11±0	14±0	12±0	
			75µg	12±0	16±0	15±0	
4	*Enterococcus faecalis*	**G+**	25µg	10±0	10±0	10±0	**18**
			50µg	11±0	11±0	11±0	
			75µg	15±0.33	16±0	12±0.33	
5	*Staphylococcus agalactiae*	**G+**	25µg	10±0	10±0	10±0	**16**
			50µg	11±0	11±0	11±0	
			75µg	12±0	12±0	12±0	
6	*Propionibacterium acnes*	**G+**	25µg	10±0	10±0	10±0	*18*
			50µg	11±0	10±0.33	11±0	
			75µg	12±0	11±0.33	12±0	
7	*Corynebacterium macbinleys*	**G+**	25µg	11±0	11±0	10±0	**20**
			50µg	14±0	12±0	12±0	
			75µg	19±0	16±0	14±0.33	
8	*Bacillus cereus*	**G+**	25µg	10±0	10±0	10±0	**16**
			50µg	12±0	10±0	12±0	
			75µg	16±0.33	11±0	14±0	
9	*Bacillus subtilis*	**G+**	25µg	10±0	11±0	10±0	**16**
			50µg	11±0	12±0	11±0	
			75µg	12±0	15±0	11±0	
10	*Escherichia Coli*	**G-**	25µg	12±0	11±0	10±0	**16**
			50µg	14±0	12±0	11±0	
			75µg	17±0	13±0	12±0	

Table.2 Antibacterial activity of the flower extracts of *T. divaricata* in diameter of zone of inhibition (mm)

S. NO	OCULAR BACTERIAL ISOLATES	GRAM REACTION	CONCENTRA-TION	METHANOL EXTRACT	DCM EXTRACT	ETHYLACETATE EXTRACT	STANDARD ANTIBIOTIC (STREPPTOMYCIN 50 mg)
1	*Staphylococcus aureus*	**G+**	25µg	10±0	12±0	12±0.33	**18**
			50µg	11±0	14±0.33	14±0.33	
			75µg	13±0.33	18±0.33	15±0.33	
2	*Staphylococcus epidermidis*	**G+**	25µg	10±0	11±0	12±0	**17**
			50µg	11±0	13±0.33	14±0.33	
			75µg	14±0	17±0.33	16±0.33	
3	*Gardnerella vaginalis*	**G+**	25µg	10±0	11±0	11±0	**16**
			50µg	11±0	15±0.33	12±0	
			75µg	12±0	18±0.33	14±0	
4	*Enterococcus faecalis*	**G+**	25µg	10±0	11±0	11±0	**12**
			50µg	11±0	13±0.33	12±0.33	
			75µg	11±0.33	17±0.33	15±0.33	
5	*Staphylococcus agalactiae*	**G+**	25µg	10±0	10±0	11±0	**18**
			50µg	10±0	13±0	12±0.33	
			75µg	11±0	16±0	13±0.33	
6	*Propionibacterium acnes*	**G+**	25µg	12±0	10±0	111±0	**16**
			50µg	13±0.33	11±0	13±0	
			75µg	14±0.33	13±0	15±0	
7	*Corynebacterium macbinleys*	**G+**	25µg	10±0	12±0	11±0	**17**
			50µg	11±0	14±0.33	12±0	
			75µg	11±0.33	16±0.33	15±0.33	
8	*Bacillus cereus*	**G+**	25µg	10±0	12±0	11±0	**16**
			50µg	11±0	14±0.33	11±0	
			75µg	11±0.33	15±0.33	14±0	
9	*Bacillus subtilis*	**G+**	25µg	10±0	12±0	11±0	**18**
			50µg	10±0	13±0.33	12±0	
			75µg	11±0	17±0.33	13±0	
10	*Escherichia Coli*	**G-**	25µg	11±0	10±0	12±0	**16**
			50µg	11±0	12±0	13±0	
			75µg	11±0	14±0	14±0	

Table.3 Summary of Two-way analysis of variance for the data on antibacterial activity in zone of inhibition (mm) of M. Oleifera flower extract against ocular bacterial isolates as a function of concentration and solvent systems

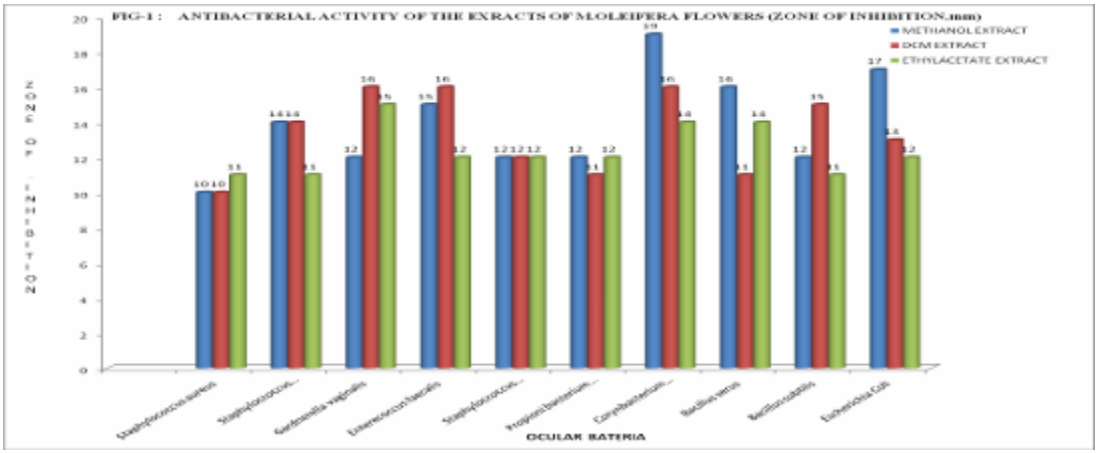
Ocular Bacterial isolates	Source of variation	Factor Df / Error Df	Sum of square	Mean Sum square	Frats	P. Value
Staphylococcus aureus	Variation due to solvent system Variation due to concentration of extract	2/4 2/4	0.222 0.222	0.111 0.111	1.0 1.0	>0.05 >0.05
Staphylococcus epidermidis	Variation due to solvent system Variation due to concentration of extract	2/4 2/4	10.88 4.22	5.44 2.11	7.00 2.71	<0.05 >0.05
Gardnerella Vaginalis	Variation due to solvent system. Variation due to concentration to extract	2/4 2/4	20.22 13.56	10.11 6.78	16.55 11.09	<0.01 <0.01
Enterococcus faecalis	Variation due to solvent system. Variation due to concentration of extract.	2/4 2/4	30.89 2.89	15.44 1.44	10.69 1.0	<0.01 >0.05
Staphylococcus agalactiae	Variation due to solvent system. Variation due to concentration of extract	2/4 2/4	0.667 4.67	0.33 2.33	0.50 3.50	>0.05 <0.05
Propionibacterium acnes	Variation due to solvent system Variation due to concentration of extract	2/4 2/4	4.22 0.889	2.11 0.44	19.0 4.0	<0.01 <0.05
Corynebacterium macbinleys	Variation due to solvent system. Variation due to concentration of extract	2/4 2/4	49.56 10.89	24.78 5.44	19.39 4.26	<0.01 <0.05
Bacillus cereus	Variation due to solvent system. Variation due to concentration of extract	2/4 2/4	20.67 8.67	10.33 4.33	6.20 2.60	<0.05 >0.05
Bacillus subtitles	Variation due to solvent system. Variation due to concentration of extract	2/4 2/4	8.22 6.89	4.11 3.44	5.29 4.43	<0.05 <0.05
Escherichia coli	Variation due to solvent system. Variation due to concentration of extract	2/4 2/4	13.56 17.56	6.78 8.78	8.71 11.29	<0.05 <0.01

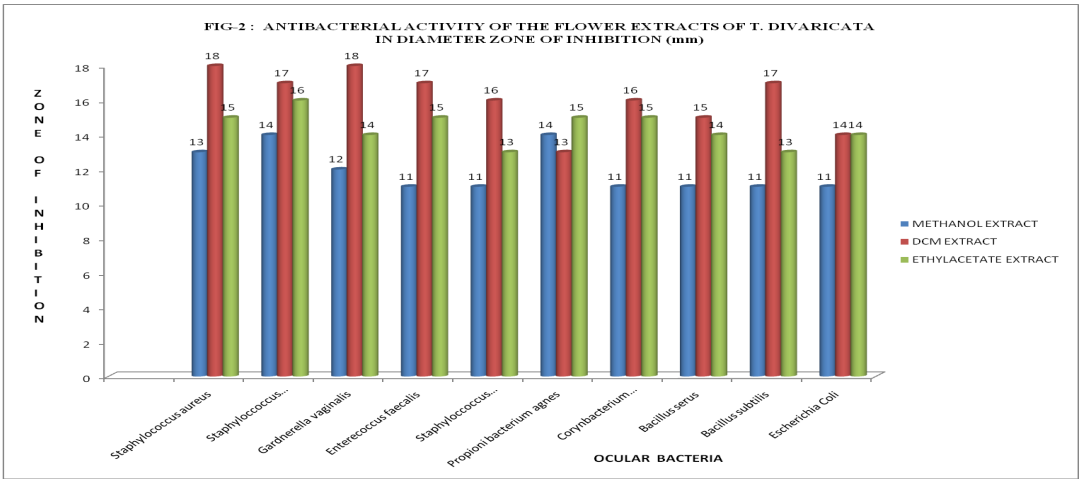
Note: P<0.05 is statistically significant at 5% level.

Table.4 Summary of Two-way analysis of variance for the data on antibacterial activity in zone of inhibition (mm) of T. divaricata flower extract against ocular bacterial isolates as a function of concentration of extract and solvent systems

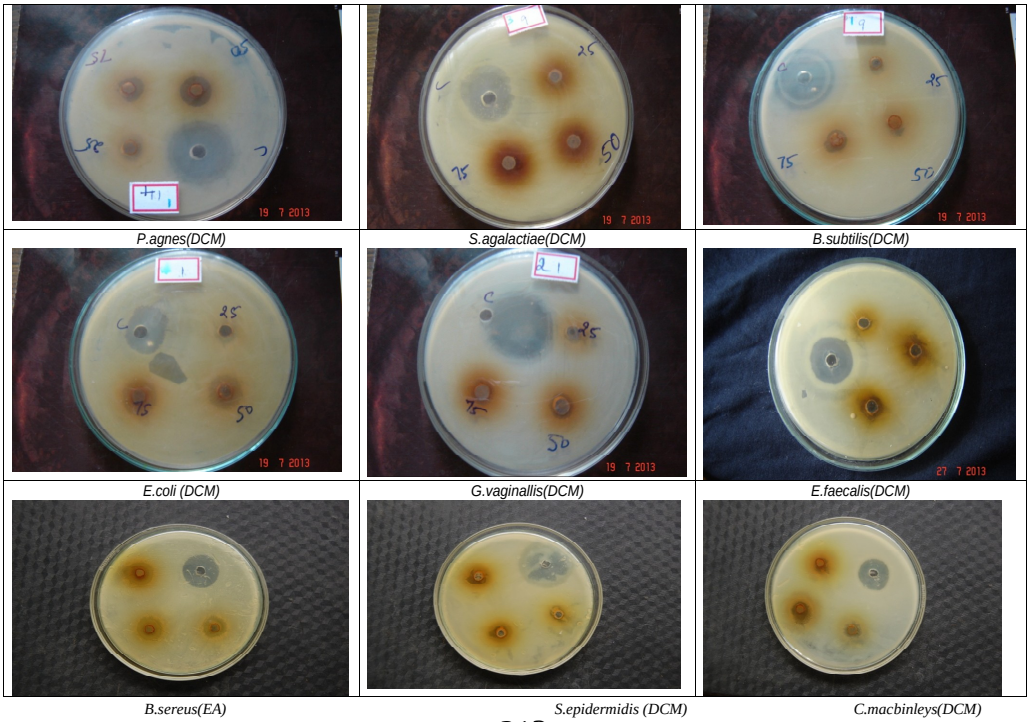
Ocular Bacterial isolates	Source of variation	Factor Dt / Error Dt	Sum of square	Mean Sum square	Frats	P. Value
Staphylococcus aureus (G+)	Variation due to solvent system	2/4	24.22	12.11	12.82	<0.01
	Variation due to concentration of extract	2/4	17.56	8.78	9.29	<0.05
Staphylococcus epidermidis (G+)	Variation due to solvent system	2/4	33.56	16.78	37.75	<0.001
	Variation due to concentration of extract	2/4	9.56	4.78	10.74	<0.01
Gardnerella vaginalis	Variation due to solvent system	2/4	24.0	12.00	6.54	<0.05
	Variation due to concentration of extract	2/4	20.67	10.33	5.63	<0.05
Enterococcus faecalis	Variation due to solvent system	2/4	20.67	10.33	5.63	<0.05
	Variation due to concentration of extract	2/4	14.00	7.00	3.81	<0.05
Staphylococcus agalactiae	Variation due to solvent system	2/4	13.55	6.78	3.81	<0.05
	Variation due to concentration of extract	2/4	10.89	5.44	3.06	<0.05
Propionibacterium acnes	Variation due to solvent system	2/4	13.56	6.78	24.40	<0.001
	Variation due to concentration of extract	2/4	5.56	2.78	10.10	<0.01
Corynebacterium macbinlays	Variation due to solvent system	2/4	13.56	6.78	7.17	<0.05
	Variation due to concentration of extract	2/4	16.84	8.44	8.94	<0.05
Bacillus cereus	Variation due to solvent system	2/4	8.22	4.11	5.29	<0.05
	Variation due to concentration of extract	2/4	13.56	6.78	8.71	<0.05
Bacillus subtilis	Variation due to solvent system	2/4	11.56	5.78	4.52	<0.05
	Variation due to concentration of extract	2/4	20.22	10.11	7.91	<0.05
Escherichia coli	Variation due to solvent system	2/4	6.89	3.44	2.38	>0.05
	Variation due to concentration of extract	2/4	2.89	1.44	1.00	>0.05

Note: P20.05 is statistically significant at 5% level.

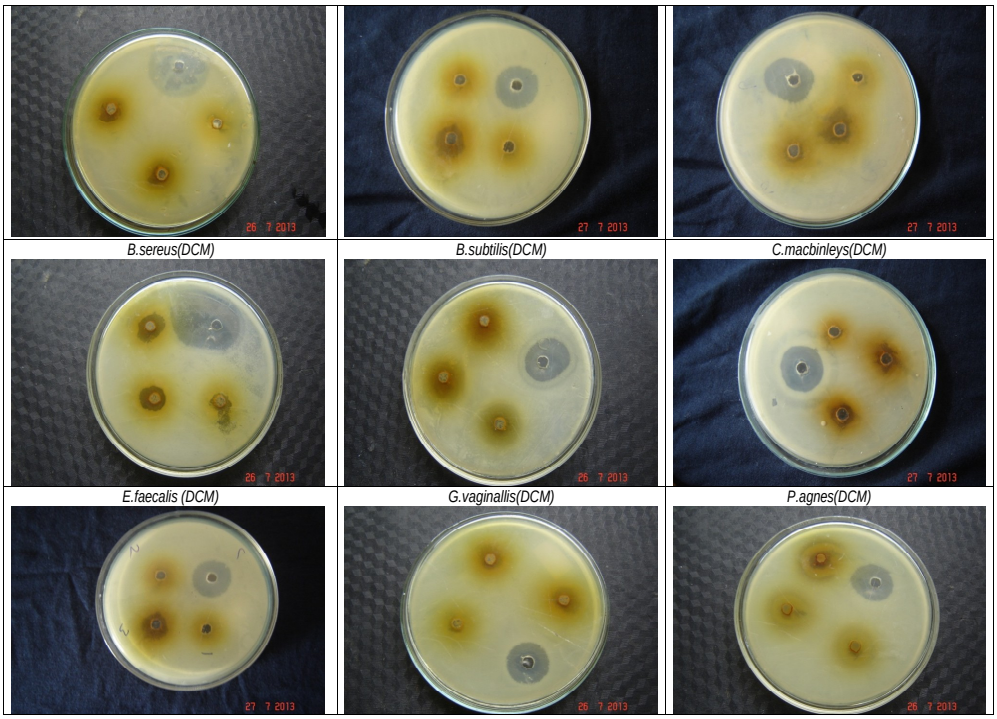




Antibacterial activity of petal extracts of *M. Oleifera*



Antibacterial activity of petal extracts of *T. divericata*



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