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Comparison of antibacterial activities of some selected wild cucurbits collected from Similipal Biosphere Reserve

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ABSTRACT

Similipal Biosphere Reserve (SBR) is situated in the district of Mayurbhanj, Odisha. It forms the major part of Eastern Ghats having rich floral diversity. SBR is inhabited by many tribal communities too. They are in habit of using the wild phytoresources against different microbial infections. Wild cucurbits are prime components of the phytoresources of SBR. Keeping this in view, four wild cucurbits (*Trichosanthes tricuspidata, Diplocyclos palmatus, Cucumis melo, Trichosanthes cucumerina*) were collected from SBR and experimented for their antibacterial activities against five selected bacterial strains (*Streptococcus mutans* - MTCC 497, *Streptococcus pyogenes* - MTCC 1926, *Vibrio cholerae* - MTCC 3906, *Shigella flexneri* - MTCC 1457 and *Salmonella typhi* - MTCC 1252). The MIC (minimum inhibitory concentration) of the extracts was determined using broth dilution assay. Results revealed that the methanol extract of *T. tricuspidata* fruits showed lowest MIC values against *S. pyogenes* whereas the acetone and methanol extract of *C. melo* fruits showed lowest MIC values against *S. mutans*. The paper highlights a comparative account of antibacterial potential of wild cucurbits collected from SBR and activity of the extracts against specific bacterial species.

1. Introduction

Similipal Biosphere Reserve (SBR) is situated in the central part of Mayurbhanj district in the state of Odisha (Bhakta *et al.,* 2014). It lies between 21° 10′ to 22° 12′ N latitude and 85° 58′ to 86° 42′ E longitude, ranging between 300 m to 1,180 m above sea level (Das and Das, 2008). The name "Similipal" has been derived from the pre-dominant floral species Semul, the red silk cotton (*Bombax ceiba* L., Malvaceae) which bloom abundantly in this area (Mishra *et al.,* 2008; Rout and Thatoi, 2009; Misra *et al.,* 2013). This biosphere reserve is unique for its varied topography, geologic formation, and excellent biodiversity along with many inhabited tribal communities (Rout and Panda, 2010; Panda *et al.,* 2010). It has mixed vegetation such as Orissa semi evergreen forest, Tropical moist broadleaf forest, Tropical moist deciduous forest, Dry deciduous hill forest, High level Sal forest with grassland and Savanna (Mishra, 2010; Misra *et al.,* 2011; Kumar *et al.,* 2012; Misra *et al.,* 2013; Tripathy *et al.,* 2014). The diverse vegetation here provides rich diversity of wild medicinal plants. The rural and tribal communities of SBR are in habit of using these medicinal plants for cure against microbial infections (Thatoi *et al.,* 2008; Rath *et al.,* 2009; Panda *et al.,* 2010; Padhi *et al.,* 2011; Panda *et al.,* 2012; Kumar *et al.,* 2013; Kumar *et al.,* 2014). Large number of medicinal plants belonging to different families are available in SBR which are used by the locals against various diseases. Among them, wild cucurbits are quite popular due to their easy availability in the forest edges of SBR. The most common wild cucurbits are *Cucumis melo, Trichosanthes cucumerina, Trichosanthes tricuspidata, Diplocyclos palmatus, Solena* spp, *Mukia maderaspatana* etc. These species posses wide ethnobotanical uses against microbial infections throughout the study area (Tripathy *et al.,* 2013). The available reports also support the use of these plants against some common

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diseases (Tang *et al.,* 2010; Jain *et al.,* 2012; Yuvarajan *et al.,* 2015; Castillon *et al.,* 2012; Giday and Teklehaymanot, 2013; Teklay *et al.,* 2013; Maroyi, 2013; Megersa *et al.,* 2013; Rai *et al.,* 2013; Natarajan and Dhas, 2013; Ma *et al.,* 2014; Yaseen *et al.,* 2015; Agarwal and Varma, 2015). Murthy *et al.* (2013) reported the traditional medicinal uses of some wild cucurbits from the Eastern Ghats of Odisha. The authors previously have reported the preliminary antibacterial activity (disc diffusion and agar well diffusion assay) of some common wild cucurbits (Tripathy *et al.,* 2014a; Tripathy *et al.,* 2014b; Tripathy *et al.,* 2014c) of SBR. Keeping all these in view, an attempt has been made in the present study to assess the antimicrobial activity of some common selected wild cucurbits (*Trichosanthes tricuspidata, Diplocyclos palmatus, Cucumis melo* and *Trichosanthes cucumerina* (Fig. 1) available in SBR and to compare their antibacterial potential through determination of MIC. It also aims at drawing the attention of pharmacological scientists / researchers for screening of new antimicrobial compounds present in these plant species, for their successful use in formulation of new antimicrobial drugs.

2. Materials and Methods

2.1. Collection of wild cucurbits for experimental work

The plant samples were collected from the Padampur, Sanuski and Kalikaparsad village of SBR during late autumn and early winter season and are kept in poly bags tagged with the botanical name. They were sorted out as per standard sampling procedure and passport description (Koppar, 1998). The collected germplasm of experimental plants were propagated and grown in the field gene bank of Department of Botany, Ravenshaw University, Cuttack.

2.2. Preparation of plant extracts

Soxhlet method was adopted to obtain the plant extracts (Tiwari *et al.,* 2011). The plant parts (leaf, fruit and root) of experimental plants were collected and dried at room temperature under shade and were powdered after drying using mechanical devices. The powdered material of the experimental plant was kept in thimble and extraction was carried out as 1:10 ratio (solute: solvent) with n-butanol, methanol, acetone and aqueous using the Soxhlet apparatus. The residues were collected and left for air drying and dried crude extracts were stored for further experimental work.

2.3. Determination of MIC

The extracts of experimental plant parts were screened for antibacterial activity against two Gram-positive bacteria *Streptococcus mutans* (MTCC 497) and *Streptococcus pyogenes* (MTCC 1926); three Gram-negative bacteria *Vibrio cholerae* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella typhi* (MTCC 1252). These microbes were taken for study since very less reports are available on the effect of plant extracts on these common human pathogens. All used MTCC (Microbial Type Culture Collection)

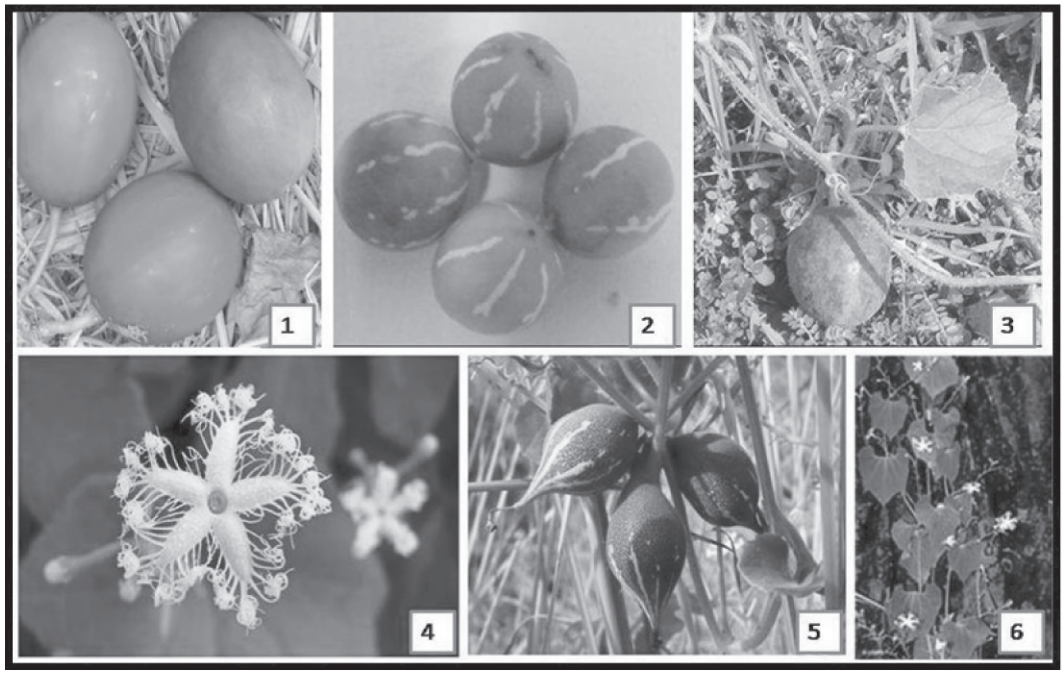

Fig.1. Morphological details of exprimented wild cucurbits, 1: Fruits of *T. tricuspidata*, 2: Fruits of *D. palmatus*, 3: Fruit of *C. melo*, 4: Flowers of *T. cucumerina*, 5: Fruits of *T. cucumerina*, 5: Vegetative parts of *T. cucumerina*

bacterial strains were collected from Institute of Microbial Technology (IMTECH), Chandigarh. Antibacterial activity was assessed by estimation of Minimum Inhibitory Concentration (MIC) (Rai *et al.,* 2010) by broth dilution assay with standard Kanamycin. For the estimation of MIC, 5.0 mg of each extract was dissolved in 10 ml of trypticase soya broth to get 500 µg/ml and for the standard 0.5 mg was taken to get 50 µg/ml.

2.4. Data Interpretation

After the incubation, the tubes showing no visible growth after 8 h till 24 h were considered to be inhibition of bacteria which represent MIC (minimum inhibitory concentration) values of a respective concentration. Inoculums control showed visible growth due to the absence of antimicrobial agents, where as the broth control showed no growth due to absence of bacteria. Triplicates were maintained and the experiment was repeated thrice, for each replicates. For the bactericidal and bacteriostatic studies, the sample tubes were kept under observation until 72 h after readings for MIC were taken.

3. Results and Discussion

All the four extracts (n-butanol, methanol, acetone and aqueous) of selected wild cucurbits were screened for their antibacterial activity. The extracts of selected wild cucurbits (plant parts) showed significant MIC (minimum inhibitory concentration) values against all used tested microbial strains. It was observed that non-edible cucurbits (*T. tricuspidata* and *D. palmatus*) were more effective as compared to the other two edible cucurbits (*C. melo* and *T. cucumerina*). The comparative results of the used wild cucurbits on selected microbial strains were analysed. It was noticed that, the *T. tricuspidata* showed lowest MIC values followed by *D. palmatus, C. melo* and *T. cucumerina* (Table 1-4). It was further observed that the fruit extracts of experimental plants exhibited higher antibacterial activity followed by leaves and root extracts. Among the used solvent extracts, methanol extract of experimental plant parts showed lowest MIC values followed by acetone, aqueous and n-butanol (Table 1-4). The growth of the experimental bacterial strains was inhibited significantly by the used extracts of selected wild cucurbits (Table 1-4). Among the tested strains, it was noticed that the growth inhibition of *S. pyogens* and *S. mutans* was more with the acetone and methanol extracts of experimental plant parts (fruits and leaves) (Fig. 2-3). The methanol extract of *T. tricuspidata* fruits showed highest inhibitory effect in low concentration (lowest MIC = 200 µg/ml) against *S. pyogens* (Fig. 2) while the acetone and methanol extracts of *C. melo* fruits showed lowest MIC (200 µg/ml) against *S. mutans* (Fig. 3). It was observed that the extracts of *T. tricuspidata* had significant inhibitory effect against all the tested microbial strains. It was further noticed that the methanol extract of fruits of this vine showed lowest MIC values against *S. pyogens* followed by leaf and root extract (Table 1).

When MIC values of *D. palmatus* (fruit, leaves and root) extracts were analysed, it was observed that leaves and fruits extracts showed significant activities. The methanol extract of fruit showed lowest MIC values (300 µg/ml) against all strains followed by acetone extract of fruits (Table 2). Experiment with the solvent extracts of *C. melo* plant parts exhibited that the acetone and methanol extracts of fruits had lowest MIC against *S. mutans* followed by *S. pyogenes*, *V. cholerae*, *S. typhi* and *S. flexneri* (Table 3). In a comparison experiment on the antibacterial activities of *T. cucumerina*, it was seen that only n-butanol, acetone and methanol extracts of leaves showed antibacterial activities (500 µg/ml) but the MIC was higher than the other vines indicating its lower efficiency in comparison to other (Table 4).

Literature survey revealed that there is no reports of comparative antibacterial activities in terms of MIC are available on selected wild cucurbits but researchers earlier have also documented the preliminary antibacterial activity of *T. tricuspidata, T. cucumerina, D. palmatus* and *C. melo* plant parts by disc diffusion (DD) and agar well diffusion methods (AWD) such as Kage *et al.* (2009) reported the antibacterial activity of *T. cucumerina* AWD assay against *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus*. Reddy *et al.* (2010) reported the antibacterial activity of *T. cucumerina* against gram-negative and gram-positive bacteria such as *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella paratyphi*, *S. aureus, E. coli, Streptococcus faecalis, Proteus vulgaris*, *Klebsiella pneumoniae, P. aeruginosa* and *Serratia marcescens*. Kavita *et al.* (2012) reported the antibacterial activity of *D. palmatus*. Vadnere *et al.* (2013) reported the antimicrobial activity of *D. palmatus* using AWD assay against *Salmonella typhimurium* and *B. cereus.* Saboo *et al.* (2013) reported the antibacterial activity of *T. tricuspidata* using AWD assay against *E. coli, S. aureus, B. subtilis and P. aeruginosa*. Patel and Kishnamurthy, (2013) reported the antibacterial activity of *D. palmatus*. Gupta and Wagh, (2014) reported the antibacterial activity of *D. palmatus* plant pars using AWD assay against *S. aureus, Micrococcus luteus, B. cereus* and *P. aeruginosa*. Gavrakar *et al.* (2014) reported the antibacterial activity of *C. melo* fruits against gram-negative bacteria using AWD assay against *E. coli, P. aeruginosa, B. cereus* and *S. aureus.* Siddeeg *et al.* (2014) reported the antibacterial activity of *C. melo* seed oil using AWD assay against Gram-negative bacteria.

Table 1
Estimation of MIC values of *Tricosanthes tricuspudata* extracts (µg/ml, n=3)

Plant Extract	MTCC 3906	MTCC 1252	MTCC 1457	MTCC 497	MTCC 1926
TTLNB	GC	GC	GC	GC	GC
TTLM	400 µg/ml	500 µg/ml	500 µg/ml	300 µg/ml	300 µg/ml
TTLA	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
TTLAQ	GC	GC	GC	GC	GC
TTFNB	GC	GC	GC	GC	GC
TTFM	300 µg/ml	300 µg/ml	300 µg/ml	300 µg/ml	200 µg/ml
TTFA	400 µg/ml	500 µg/ml	500 µg/ml	400 µg/ml	400 µg/ml
TTFAQ	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
TTRNB	GC	GC	GC	GC	GC
TTRM	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
TTRA	GC	GC	GC	GC	GC
TTRAQ	GC	GC	GC	GC	GC
Standard	25 µg/ml	12.5 µg/ml	25 µg/ml	12.5 µg/ml	12.5 µg/ml
IC	GC	GC	GC	GC	GC
Broth control	No Growth	No Growth	No Growth	No Growth	No Growth

(TT: *T. tricuspidata*, TC: *T. cucumerina*, DP: *D.palmatus*, CM: *C. melo*, L: Leaf. F: Fruits, R: Root, M: Methanol, AQ: aqueous, A: acetone, NB: n-butanol, GC: Growth in all concentrations, IC: Inoculums control; *Streptococcus mutans*: MTCC 497, *Streptococcus pyogenes*: MTCC 1926, *Vibrio cholerae* : MTCC 3906, *Shigella flexneri*: MTCC 1457, *Salmonella typhi:* MTCC 1252; Concentration: for Standard- 6.25 – 50 µg/ml; for extracts- 100 – 500 µg/ml)

Table 2
Estimation of MIC values of *Diplocyclos palmatus* extracts (µg/ml, n=3)

Plant Extract	MTCC 3906	MTCC 1252	MTCC 1457	MTCC 497	MTCC 1926
DPLNB	GC	GC	GC	GC	GC
DPLM	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
DPLA	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
DPLAQ	500 µg/ml	GC	GC	500 µg/ml	500 µg/ml
DPFNB	500 µg/ml	GC	GC	GC	GC
DPFM	300 µg/ml	300 µg/ml	300 µg/ml	300 µg/ml	300 µg/ml
DPFA	300 µg/ml	400 µg/ml	400 µg/ml	300 µg/ml	300 µg/ml
DPFAQ	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
DPRNB	GC	GC	GC	GC	GC
DPRM	GC	GC	500 µg/ml	500 µg/ml	500 µg/ml
DPRA	GC	GC	GC	GC	GC
DPRAQ	GC	GC	GC	GC	GC
Standard	25 µg/ml	12.5 µg/ml	25 µg/ml	12.5 µg/ml	12.5 µg/ml
IC	GC	GC	GC	GC	GC
Broth control	No Growth	No Growth	No Growth	No Growth	No Growth

(TT: *T. tricuspidata*, TC: *T. cucumerina*, DP: *D.palmatus*, CM: *C. melo*, L: Leaf. F: Fruits, R: Root, M: Methanol, AQ: aqueous, A: acetone, NB: n-butanol, GC: Growth in all concentrations, IC: Inoculums control; *Streptococcus mutans*: MTCC 497, *Streptococcus pyogenes*: MTCC 1926, *Vibrio cholerae* : MTCC 3906, *Shigella flexneri*: MTCC 1457, *Salmonella typhi:* MTCC 1252, Concentration: for Standard- 6.25 – 50 µg/ml; for extracts- 100 – 500 µg/ml)

Table 3
Estimation of MIC values of *Cucumis melo* extracts (µg/ml, n=3)

Plant Extract	MTCC 3906	MTCC 1252	MTCC 1457	MTCC 497	MTCC 1926
CMLNB	GC	GC	GC	GC	GC
CMLM	GC	GC	GC	GC	GC
CMLA	GC	GC	GC	GC	GC
CMLAQ	GC	GC	GC	GC	GC
CMFNB	GC	GC	GC	GC	GC
CMFM	500 µg/ml	500 µg/ml	500 µg/ml	200 µg/ml	500 µg/ml
CMFA	500 µg/ml	500 µg/ml	500 µg/ml	200 µg/ml	500 µg/ml
CMFAQ	GC	GC	GC	500 µg/ml	500 µg/ml
CMRNB	GC	GC	GC	GC	GC
CMRM	GC	GC	GC	GC	GC
CMRA	GC	GC	GC	GC	GC
CMRAQ	GC	GC	GC	GC	GC
Standard	25 µg/ml	12.5 µg/ml	25 µg/ml	12.5 µg/ml	12.5 µg/ml
IC	GC	GC	GC	GC	GC
Broth control	No Growth	No Growth	No Growth	No Growth	No Growth

(TT: *T. tricuspidata*, TC: *T. cucumerina*, DP: *D.palmatus*, CM: *C. melo*, L: Leaf. F: Fruits, R: Root, M: Methanol, AQ: aqueous, A: acetone, NB: n-butanol, GC: Growth in all concentrations, IC: Inoculums control; *Streptococcus mutans*: MTCC 497, *Streptococcus pyogenes*: MTCC 1926, *Vibrio cholerae* : MTCC 3906, *Shigella flexneri*: MTCC 1457, *Salmonella typhi:* MTCC 1252, Concentration: for Standard- 6.25 – 50 µg/ml; for extracts- 100 – 500 µg/ml)

Table 4
Estimation of MIC values of *Trichosanthes cucumerina* extracts (µg/ml, n=3)

Plant Extract	MTCC 3906	MTCC 1252	MTCC 1457	MTCC 497	MTCC 1926
TCLNB	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
TCLM	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
TCLA	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
TCLAQ	GC	GC	GC	GC	GC
TCFNB	GC	GC	GC	GC	GC
TCFM	GC	GC	GC	GC	GC
TCFA	GC	GC	GC	GC	GC
TCFAQ	GC	GC	GC	GC	GC
TCRNB	GC	GC	GC	GC	GC
TCRM	GC	GC	GC	GC	GC
TCRA	GC	GC	GC	GC	GC
TCRAQ	GC	GC	GC	GC	GC
Standard	25 µg/ml	12.5 µg/ml	25 µg/ml	12.5 µg/ml	12.5 µg/ml
IC	GC	GC	GC	GC	GC
Broth control	No Growth	No Growth	No Growth	No Growth	No Growth

(TT: *T. tricuspidata*, TC: *T. cucumerina*, DP: *D. palmatus*, CM: *C. melo*, L: Leaf. F: Fruits, R: Root, M: Methanol, AQ: aqueous, A: acetone, NB: n-butanol, GC: Growth in all concentrations, IC: Inoculums control; *Streptococcus mutans*: MTCC 497, *Streptococcus pyogenes*: MTCC 1926, *Vibrio cholerae* : MTCC 3906, *Shigella flexneri*: MTCC 1457, *Salmonella typhi:* MTCC 1252, Concentration: for Standard- 6.25 – 50 µg/ml; for extracts- 100 – 500 µg/ml)

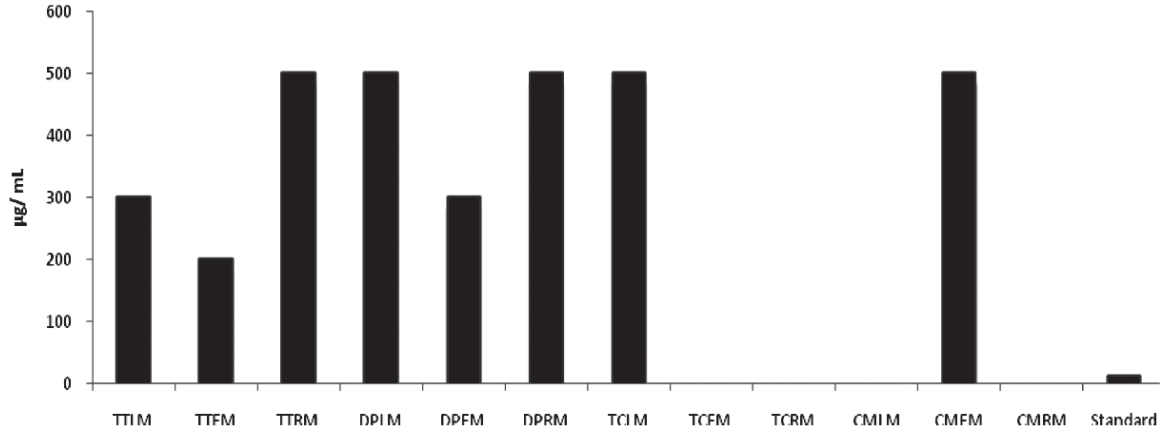

Fig. 2. Comparative antibacterial potential of methanol extract of selected plant parts against *S. pyogenes* (TT: *T. tricuspidata*, TC: *T. cucumerina*, DP: *D. palmatus*, CM: *C. melo*, L: Leaf. F: Fruits, R: Root, M: Methanol)

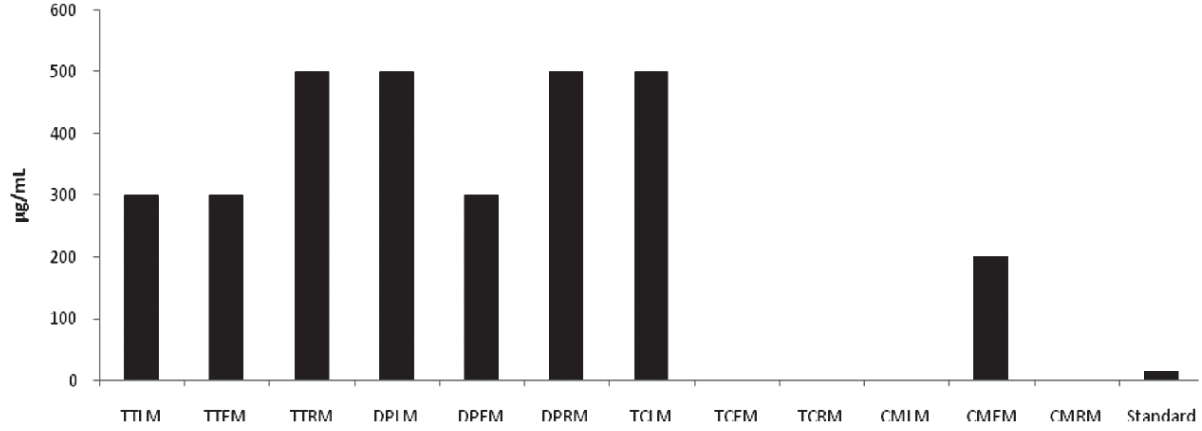

Fig. 3. Comparative antibacterial potential of methanol extract of selected plant parts against *S. mutans* (TT: *T. tricuspidata*, TC: *T. cucumerina*, DP: *D. palmatus*, CM: *C. melo*, L: Leaf. F: Fruits, R: Root, M: Methanol)

4. Conclusion

The present study highlights comprative antimicrobial potential of wild cucurbits available in Similipal Biosphere Reserve. The results obtained from the exprimental wild cucurbits like *T. tricuspidata*, *D. palmatus*, *T. cucumerina* and *C. melo* showed significant antibacterial activity against selected microbial pathogens. Among the said cucurbits, the methanol extract of *T. tricuspidata* fruits showed lowest MIC values against *S. pyogenes* (MTCC 1926) and methanol and acetone extract of *C. melo* root against *S. mutans* (MTCC 497). The bacteriostatic effects of the extracts were also indicated. The exprimented wild cucurbits posses significant antimicrobial activity against some selected bacterial strains. Further research can explore the bioactive compounds present in these plants responsible for such activities and successful utilisation of these compounds for formulation of new antimicrobial drugs which not only could check microbial infections but also might fight against AMR.

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