

**Chapter-6 Discussion on results**

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CHAPTER 6
DISCUSSION ON RESULTS

In the present investigation two medicinal plants *Merremia turpethum* and *Grewia tenax* were selected for the Ananthapuramu district A.P. These plants were collected, extracted, screened for preliminary phytochemical analysis; antioxidant, antimicrobial (antibacterial and antifungal), anthelmintic activity and isolation of compound from the most potent extracts are discussed in this chapter.

6.1. Preliminary phytochemical analysis

From the preliminary phytochemical analysis it was evident that *Merremia turpethum* and *Grewia tenax* n-hexane extract showed the presence of tannins and steroidal compound. The *Merremia turpethum* ethanolic extract showed the presence of alkaloids, amino acids, carbohydrates, anthraquinon, saponin, tannin, flavonoids and triterpenoids. The Hydro alcoholic extract of *Merremia turpethum* showed the presence of phytoconstitution similar to that of the ethanolic extract except amino acids.

The *Grewia tenax* ethanolic extract showed the presence of alkaloids, amino acids, carbohydrates, glycosides, anthraquinon, saponin, tannin, and triterpenoids. The *Grewia tenax* hydro alcoholic extract showed the presence of phytoconstitution similar to that of ethanolic extract expect amino acid, glycoside and anthraquinone.
6.2. Antioxidant activity

Antioxidant activity for various crude extract of *Merremia turpethum* and *Grewia tenax* was subjected for DPPH and hydrogen peroxide model for the identification of the antioxidant activity of the crude extracts. The reactive oxygen species (ROS) are important biochemical process which involves in activation of the diseased condition such as cancer, alzheimers, cardiac problems and Parkinson. The living biosystem, cell prevents this ROS formation by producing biochemical changes to form antioxidants which in turn inactivity the reactive oxygen species, but the formation of antioxidant is an not an simple task for the biological cell since the production of antioxidant is diminished by many factor.

Many of the antioxidants are generated for the natural products from plants and marine sources.

6.2.1. DPPH free radical scavenging activity

Crude extracts *Merremia turpethum* showed a potent antioxidant activity for the ethanolic extracts with the IC$_{50}$- 26.507other extract a moderate activity. For crude extract of *Grewia tenax* the ethanolic extract showed a potent DPPH radical scavenging activity the IC$_{50}$-56.477 and other extract showed a moderated antioxidant. All the extracts were reported to the standard ascorbic acid which produces an IC$_{50}$ value of 19.933.
6.2.2 Hydrogen peroxide scavenging activity

Oxidative stress is an important mechanism which involves in the generation of hydroxyl radical which causes and irreversible damages. This reaction is best explain by the Fenton reaction

\[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{HO}^{-} + \text{HO}^{+} \]

The hydrogen peroxide radical scavenging activity was determined for the crude extract of *Merremia turpethum* and *Grewia tenax* among all the extract the ethanolic extract of both the crude extract showed an a potent activity on hydroxyl. The ethanolic extraction of *Merremia turpethum* and *Grewia tenax* was found to be 167.424 and 245.206. The hydro- alcoholic extract of both the plants showed a moderate and n-hexane extract showed and less activity when compared with the other extract and the standard compound gallic acid (IC\textsubscript{50} - 197.087).

From the antioxidant activity of the crude extract of *Merremia turpethum* and *Grewia tenax* were clearly show that the ethanolic extract of both the plants showed an potent antioxidant activity for the both the models (DPPH and hydrogen peroxide) since these crude extract were consist of some common phytococonstitution such as saponin, tannin, and triterpenoids. These phytocostitution have shown a strong antioxidant activity. Hence for antioxidant activity was the ethanolic activity was
found to be potent when compared with the respective reference standard ascorbic acid and gallic acid.

6.3. Antimicrobial activity

The antimicrobial activity was performed for all the crude extract and ethanolic fraction of *Merremia turpethum* and *Grewia tenax*. The antimicrobial screening was performed for antibacterial (both gram positive and gram negative microorganism) and antifungal activity was performed as per the procedure discussed in chapter 3.7 and 4.4.

6.3.1. Antibacterial activity

Crude extracts of *Merremia turpethum* were screened for the antibacterial activity for both gram positive and gram negative microorganism by disc diffusion and cup and plate diffusion methods was adopted for the antibacterial activity. Among all the extract the ethanolic extract showed a potent activity followed by hydro-alcoholic and n-hexane extracts when compared with the standard ampicillin. The disc diffusion method was comparatively showed better zone of inhibition as compared with the cup and plate method.

Ethanolic extract of *Grewia tenax* showed and potent inhibition against both gram positive and gram negative microorganism when compared with the standard ampicillin and the hydro-alcoholic showed a moderate activity when compared with the n-hexane extract. The results
are shown in table 5.3 and graphical presentations are shown in the Fig 5.1 and 5.2.

The ethanolic crude extract of *Merremia turpethum* and *Grewia tenax* were subjected for the column chromatography with solvent increasing in their polarity (chloroform, ethyl acetate and methanol). The fractions were collected for the dried under vacuum evaporation to a concentrated product of chloroform, ethyl acetate and methanolic fractions. The entire fraction was subjected for the antibacterial activity by disc plate agar diffusion method using prevailing strain of microorganisms. The methanolic fraction of both the plant showed the better activity followed by ethyl acetate. The chloroform fractions did not come to the window any activity. The results are presented in table 5.4 and the graphical representations are shown in fig.5.5 and 5.6.

### 6.3.2. Antifungal Activity

Antifungal activity was done by adopting the method described in the chapter 3.7 and chapter 4.4. The assay was performed by using the disc and cup-plate agar diffusion method against the standard fungal strains. Crude extracts of *Merremia turpethum* and *Grewia tenax*, the ethanolic extracts showed the better activity than the other extracts when compared with the reference standard clotimazole. The hydro-alcoholic extract showed a moderate activity than the n-hexane extracts.
The results are listed in table 5.3 and the graphical representation are illustrated in fig.5.3 and 5.4.

The ethanolic crude extract of *Merremia turpethum* and *Grewia tenax* were submitted for the column chromatography with solvent increasing in their polarity (chloroform, ethyl acetate and methanol) to obtain the chloroform, ethyl acetate and methanolic fractions. These fractions were subjected for the antifungal activity by disc-plate agar diffusion method. The methanolic fraction of both plant showed the better activity when compared with the other fraction. An moderate activity was seen in the ethyl acetate extract and followed by chloroform. Among all the, extract the methanolic extract of *Merremia turpethum* showed and potent antifungal activity than the other fractions. The results are shown in table 5.4 and graphical representations are shown in fig.5.7.

### 6.4. In-vitro anthelmintic activity

In-vitro anthelmintic activity was performed for the crude extract of *Merremia turpethum* and *Grewia tenax*. The ethanolic extract of both the extracts were showing potent anthelmintic activity when compared with the standard reference compound albendazole.

The ethanolic extract of *Merremia turpethum* showed the paralysis (P) time and death (D) time with increasing in the dose dependent ranging from (25 to 200 mg/ml). The ethanolic extract at a
concentration ranging from 200mg/ml showed 10.467 ± 1.825 and 19.53 ± 1.04 and the ethanolic extract of *Grewia tenax* showed the paralysis and death time at 200mg/ml was 11.567 ± 1.507 and 13.53 ± 3.90. Hydro-alcoholic extract showed a potent activity at highest concentration for both plant crude extract. All the results were compared with the standard reference albendazole (10mg/ml) was 12.967 ± 0.734 and 15.53 ± 1.22.

The ethanolic crude extract fractions of both the plants were subjected for the in-vitro anthelmintic activity among all the chromatography fractions the methanolic fraction of both the plants was showing potent anthelmintic activity. The paralysis and the death time of *Merremia turpethum* methanolic fraction was observed to show the dose dependent activity in the concentration ranging from (10, 25 and 50 mg/ml) and the potent activity was seen for the 50mg/ml was 08.503 ± 0.121 and 13.56 ± 1.97 and for the methanolic fraction of *Grewia tenax* showed a potent activity at 50mg/ml 22.18 ± 0.78 and 11.321 ± 2.873. The ethyl acetate fraction of both the plants showed a less activity and the chloroform fraction of both the plants showed a long paralysis and death time of earthworm.

From the studies it can be concluded the ethanolic crude extracts and the methanolic fraction of ethanolic crude extract, were possessed a potent anthelmintic activity. From the past literatures various authors
have reported that tannins, saponin, alkaloidal, flavonoids and phenolic compound proven to produce a potent anthelmintic activity. It is evident from the preliminary phytoconstitution analysis of the crude ethanolic extract of both plant possessed the above mentioned phytoconstitution.

The possible mechanism of action of the crude extract and the methanolic fraction can be taken from the standard albendazole since both the plant ethanolic and fraction of ethanolic extract of both the plant possessed an potent activity to that of albendazole, which works by accumulating the chloride ion in muscle membrane of worm to produces hyperpolarisation and reduced nervousness that leads to muscle relaxation and flaccid paralysis1-2.

The isolated compound 1(Lupeol) and compound 2 (caffeine) were reported as novel for their antimicrobial activity which was conformed by the references.