ABSTRACT

The research work highlights the “Investigation on phytochemical screening, analytical, microbiological and pharmacological activity of different Ficus species”. The selected Ficus benghalensis aerial roots and Ficus religiosa leaves were collected from in and around rajampet in the month of Sep’ 09 and authenticated. Plant materials were dried, powdered and extracted by continuous hot percolation method using various solvents in the order of increasing polarity. The constituent present in the extrtacts were screened by phyto chemical test. Ficus benghalensis aerial roots extracts shows the presence of triterpenoids, alkaloids, flavonoids and Ficus religiosa leaves extract shows the presence of terpenoids, flavonoids, alkaloids, phenolic compounds and tannins.

The flavonoid rich extracts were evaluated for microbiological activity. The methanolic and ethylacetate extracts of Ficus benghalensis aerial roots (MEFB & EEFB) and the methanolic and ethylacetate extracts of Ficus religiosa leaves (MEFR & EEFR) were performed for antimicrobial activity respectively by cup–plate method on Muller Hington agar medium against standard drugs (Tetracycline, Erythromycin). The MEFB shows active against P.vulgaris, S.typhi, S.epidermidis, S.griseus, & E.coli and EEFB was active against S.aureus, S.typhi, S.epidermidis, Lacto bacillus, C. diphtheriae, & E.coli. On other hand the MEFR shows active against S. aureus, S. typhi, S. griseus & E. coli and EEFR active against S. aureus, S. typhi, S. griseus, P. vulgaris & E. coli. The zone of inhibition of methanolic extracts shown very high inhibition when compare with ethyl acetate extracts. The minimum inhibitory concentrations (MIC) for methanol extract of both species i.e MEFB, MEFR were found to be 50µg/ml and 100 µg/ ml respectively. Antifungal activity was done by diffusion plate method using Sabouraud dextrose agar (SDA) medium. Three different
(Candida albicans, Monilinia fruticola, Auricularia polytricha) fungal organisms were tested against the methanolic extract of Ficus benghalensis linn and Ficus religiosa linn. The methanolic extract of aerial roots of Ficus benghalensis and leaves of Ficus religiosa shows effective action in the concentration of 1mg/ml against Candida albicans, Monilinia fruticola, and Auricularia polytricha. Amphotericin-B was used as reference standard to compare the activities of respective extracts.

All the extracts of Ficus benghalensis and Ficus religiosa subjected to in-vitro antioxidant activity by 1,1 diphenyl-2-picryl hydrazyl (DPPH) method. All the extracts and (reference standard) butylated hydroxy anisole (0.5 ml) containing different concentration of 10 µg, 50 µg, 100 µg and 500µg were prepared by using methanol. The methanolic solutions were mixed with 1,1 diphenyl-2-picryl hydrazyl reagent and absorbance measured at 517 nm. MEBF and MEFR were showed significant inhibition activity when compared with other extracts.

The hepatoprotective activity of MEFR and MEFB were performed by using two models like paracetamol induced hepatotoxicity and Isoniazide-Rifampicin (INH+RIF) induced hepatotoxicity. Male Wistar rats (150-200 g) were used for the enitre study. Rats were divided into six different groups. Group 1 served as a control, group 2 received INH+RIF (100 mg/kg, i.p.) and paracetamol in sterile water, groups 3, 4 and 5 received 100, 200 & 300 mg/kg bw, p.o. of MEFR and MEFB repsectively. Group 6 received Liv 52. All the treatment protocols were performed 21 days for INH-RIF induced hepatotoxicity and 7 days for paracetamol induced hepatotoxicity, and then the rats were sacrificed. Blood and liver samples were collected and examined for biochemical and histological studies, respectively. Administration of INH+RIF and paracetamol caused a significant elevation in the levels of liver marker enzymes (p<0.05 and p<0.01) and thiobarbituric acid reactive substances (p<0.001) in
experimental rats. Administration of MEFB and MEFR significantly prevented INH+RIF and paracetamol induced elevation in the levels of serum diagnostic liver marker enzymes and TBARS level in experimental groups of rats. Moreover, total protein and reduced glutathione levels were significantly (p<0.001) increased in treatment group. The effect of extract was compared with a standard drug, Liv 52. The changes in biochemical parameters were supported by histological profile. From the study it was concluded that the MEFB and MEFR protects against INH+RIF and paracetamol-induced oxidative liver injury in rats.

The isolation and identification of major phyto constituent of MEFB and MEFR were carried out by chromatographic techniques. The mobile phase for Ficus benghalensis aerial roots and Ficus religiosa leaves extracts selected with ratio of Ethyl acetate: Formic acid: Glacial acetic acid: Water (10:1.1:1.1:2.6) and Benzene: Chloroform (7:3) respectively. The flavonoid spots were identified as a blue fluorescence with 0.86 $R_f$ value in ethylacetate, methanolic, aqueous extracts of Ficus benghalensis Linn and the pink flouresence was found with 0.2, 0.36, 0.58 $R_f$ values in methanolic extracts of Ficus religiosa leaves. The active constituents were quantified by HPTLC using same mobile phase. The HPTLC analysis reveals that more percentage of the compound was found in methanolic extracts of both species with individual peaks.

The methanolic extracts of Ficus benghalensis aerial roots were allowed to column chromatography for the isolation of the major phyto constituent. The petroleum ether, hexane, ethylacetate were used as mobile phase with different gradual proportions. From among that the single compound was found at 22$^{nd}$ to 53$^{rd}$ fraction. The methanolic extract of Ficus religiosa leaves were subjected to fractionating method for the isolation of the active constituent by using diethyl ether
and n-butanol. The isolate I and II were subjected to UV, IR, NMR and Mass spectroscopy for the structure elucidation. $\lambda_{\text{max}}$ of Isolate I was 254nm which gives confirmation of flavonoids. IR reports given evidence for aromatic compound (2937) contains carbonyl atom (1224) and hydroxyl group (3392). The proton NMR shows $\delta$ values of 2.304 for Hydroxyl group on glycosidal linkage and $^{13}$C NMR shows possible value for isoflavone ring structure (154.9, 166.4, 163.9, 139.69, 146.5, and 124.4). The molecular weight of the isolated compound was found to be 464.37. So, elucidated structure might be Quercetin -3 –glycoside. The $\lambda_{\text{max}}$ of isolate II was 272nm confirmed as saturated fatty acids. IR reports given evidence for aliphatic compound contains carboxylic acid group (2921 & 1709 cm$^{-1}$). The characteristics chemical shifts of hydroxyl proton were noticeable at $\delta$ 4.05 and 4.07. $^{13}$C NMR given evidence for ester linked methyl group ($\delta$ 22.68) $^{154}$ and aliphatic carboxylic acid $\delta$ 170. The molecular weight of the compound was m/e 285 it’s confirmed as methyl ester of hexadecanoic acid.

The isolate I & II were shows better hepato protective activity on isoniazid – rifampicin induced hepatotoxicity. Hence *Ficus benghalensis* aerial roots and *Ficus religiosa* leaves shows significant hepatoprotective activity might be due to its quarcetin -3- glycoside and methyl ester of hexa decanoic acid.