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PHYTOCHEMICAL (TPC) ANALYSIS OF FICUS *RELIGIOSA, AEGLE MARMELOS* AND *BUTEA MONOSPERMA PLANTS*

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ABSTRACT

The most important of these bioactive constituents of natural products or plants are phenols, flavonoids, alkaloids, terpenoids, tannins, glycoside and saponins. Phenols, flavonoids are known to possess wide range of biological activities like antimicrobial, antioxidant and anti-inflammatory properties. The present study was performed to evaluate the Comprative Phytochemical capacity of *Ficus religiosa, Aegle marmelos* and *Butea monosperma* against Phytochemical characterization of PEFR, MEFR, PEAM, MEAM, PEBM and MEBM was performed to identify various phyto-constituents namely alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins and tannins in different extracts, phytochemical screening was performed on the basis of method mentioned in the material and methods chapter. PEFR contained sterols and fats, whereas the MEFR extract contained alkaloids, flavonoids, phenolics, tannins, and glycosides. The PEAM contained terpenoids, tannins, fats, and MEAM contained carbohydrates, reducing sugars, tannins, phenolics, saponins, flavonoids and alkaloids. PEBM contained alkaloids, sterols, terpenoids, saponins and fats, whereas the MEBM contained carbohydrates, alkaloids, tannins, phenolics, flavonoids, saponins and glycosides. The results support local claims of their therapeutic uses in folklore medicine. In conclusion, all three i.e. *F. religiosa, A. marmelos* and *B.monosperma* plant extracts could serve as free radical scavengers, can be considered as potent antioxidants. Their activity may be attributed to high phenolic and flavonoid contents.

Key Word- Phytochemical,*Ficus religiosa, Aegle marmelos* and Butea *monosperma.*

INTRODUCTION

Plants, either as indigenous therapy or isolated active principles, have served as a common source of medicine (Farnsworth et al., 1985). To alleviate human suffering, plants have played a major role in traditional as well as in modern medicine (Akerele, 1993). Indigenous ethno pharmacology has been

considered as an important tool in the discovery of new drugs (Cox, 1990, Farnsworth, 1990, Fabricant and Farnsworth, 2001). Herbal medicines are a popular form of complementary and alternative medicine practiced throughout the world in the treatment of various types of ailments. Medical herbalism has been popularized because of its fewer side effects and reported efficacy against a number of diseases. Indigenous use of botanicals plays a crucial role in human and livestock healthcare in a wider part of the world especially in the underdeveloped and developing countries. Crude herbal preparations with or without additives are reported to have significant therapeutic value. Synergistic interaction between the phytochemicals of the crude drugs may play a major role in ethnomedicine. A significant number of herb based drugs have been evaluated clinically and are used these days even in the developed countries as a potent alternative system of medicine. Most of the drugs affect these tissues in a dose dependent manner; though, there are differences in susceptibility to individual members. Chemotherapy in cancer possess several adverse effects in recipient like use of alkylating agents (cyclophosphamide, chlorambucil, carmustine, lomustine, cisplatin etc.) causes bone marrow depression, gastrointestinal disturbances such as nausea and vomiting; anthracyclines (doxorubicin, epirubicin, mitoxantrone, bleomycin etc) causes myelosuppression (bone marrow suppression), increased risk of infection and bleeding, cardiac toxicity/arrhythmias, tissue necrosis/extravasation, secondary malignancies, radiation recall (the recurrence of skin damage from previous radiotherapy), alopecia (hair loss), nausea and vomiting, oral ulceration; taxanes (paclitaxel, docetaxal, estramustine) causes nausea and vomiting, diarrhoea, mouth sores, joint and muscle aches, alopecia (hair loss), paraesthesia (abnormal sensation), mild allergic reactions (flushing, shortness of breath, urticaria (hives), rash), anaphylactic reactions, injection site reactions. Vinca alkaloids (vinblastine, vincristine, vindesine, vinorelbine etc.) cause nausea and vomiting, allopacia (hair loss), mouth sores, headache, constipation. Various reports showed that plant parts of *F. religiosa* has been used as folk medicine like leaf juice has been used for the cough, asthma, diarrhoea, sexual diseases, haematuria, tooth-ache, migrane, eye disorders, GIT problems earache and scabies. Decoction of leaf is useful in toothache, as an analgesic. Fruits are useful in asthma and other tracheal disorders and scabies treatment. Stembark is useful in the management of gonorrhoea, diabetes, diarrhoea, bleeding, astringent, paralysis, bone-fracture and as anti-septic & anti-dote (Kunwar *et al.*, 2006). The root bark is aphrodisiac. Fruits are digestive and laxative at high dose. The powder of fruit is also used to improve fertility and in dysentery. In blood diseases, it is used to treat ulcers, uterine troubles, biliousness, and used as bitter tonic. Whole plant part is acrid and used in the treatment of blood disorders, UTIs, given in leucorrhoea, burning sensation, ulcers and biliousness. In vedic literature, *A. marmelos* is indicated for the treatment of jaundice, inflammations, constipation, asthma, chronic diarrhea, dysentery, stomach ache, stomachic, fever, febrile elirium, acute-bronchitis, snake-bite, abdominal distress, acidity, burning impression, epilepsy, in-digestion, leporsy,

myalgia, small-pox, spermatorrhoea, leuco-derma, opthelmic-disorders, upper respiratory tract infections, ulcer, mental-illnesses, nausea, thirsts, sores, thyroid-disorder, tumor and ulcer (Sekar *et al.*, 2011). *B. monosperma* is widely used in all the medicinal systems viz. Ayurveda, Unani, Homeopathic and Modern medicine. Genus butea is famous for colouring materials; plant parts are used as tonic, astringent, aphrodisiac and diuretics. Various parts of *B. monosperma* are used for various purpose, some are: Roots are used in filariasis, night-blindness, helminthiasis, piles, ulcer, tumors; flowers are used in dermetological disorders, diarrhoea, astringent, gout; stem bark is used for dysentery, peptic-ulcer, throat-sore and snake-bite; leaves are used for making plates, cups and bowls; bark fibers are used for making cordage; wood is used for well curbs & water scoop; wood pulp is used for newsprint manufacturing (Kirtikar, 1935; Ambasta, 1994).

MATERIALS AND METHODS

Reagents and laboratory wares

All reagents used were analytical grade were purchased from Hi-Media and Merck.

Collection of Plant Material

Leaves of *Ficus religiosa, Aegle marmelos* and *Butea monosperma* were collected in August-October locally from Meerut (U.P.).Special precautions were taken to collect healthy plants avoiding foreign materials. Herbarium of both the plants were prepared and submitted for authentication to GBPATU PantNagar(Department of Horticulture),UttraKhand, India. Authentication was done by Dr. Anju Pal Associate Professor (Department of Horticulture). Authentication voucher numbers were 5027/Horticulture/GBPATU/13,4047/ Horticulture/GBPATU /13 and 4048/ Horticulture/GBPATU /13 for *F. religiosa, A.marmelos* and *B. monosperma* respectively.

Quantitative phytochemical estimation

Total phenolic content estimation

Reference standard gallic acid was used for plotting calibration curve by preparing dilutions of 20-100µg/ml gallic acid mixed with 2ml folin-ciocalteu reagent and 4ml sodium carbonate solution and incubated for 30 minutes at room temperature. For all the extracts and polyherbal formulations (100), 1 ml of each extract was mixed separately with the same reagents, as previously used in gallic acid calibration curve. Absorbance was measured at the wavelength of 765 nm utilizing methanol as blank. Total phenolic content in the test sample was of galic acid using gallic acid standard curve (**Ainsworth 2007; Alhakmani, 2013**).

Total flavonoid content estimation

An Aliquot of diluted sample around 100µg/ml was added to 75µl of $NaNO_2$ solution, and mixed for 6 min, before adding 0.15 mL of 100g/l AlCl3. After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Similarly, calibration curve of rutin was prepared by preparing dilution of rutin in 20- 100µg/ml of methanol and all procedure was followed as

performed in extract. Absorbance of both extracts & polyherbal formulations and rutin was determined at 510 nm against the same mixture, without the sample as a blank.Total flavonoid content was expressed as µg rutin/mg dryweight, through the calibration curve of rutin. All samples were analysed in triplicates (**Zhishen *et al.*, 1999**).

RESULTS AND DISCUSSION

Quantitative phytochemical estimation

Total phenolic content (TPC) estimation

Total phenolic content (TPC) of all the extracts (PEFR, PEAM, PEBM, MEFR, MEAM & MEBM) was determined by Folin–Ciocalteu's (FC) method using gallic acid as standard. Results were expressed as mg of gallic acid equivalent weight (GAE). The FC reagent is formed from a mixture of phosphotungstic acid and phosphomolybdic acid. This mixture causes oxidation of phenols and is reduced to blue color solution of tungsten and molybdenum which is measured by spectrophotometer having absorption maxima at 750 nm. The blue color is proportional to the total quantity of phenolics present (**Kamtekar *et al.*, 2014**). The TPC was determined using the calibration curve for gallic acid shown in figure 5.1. The regression co-efficient was found to be R2= 0.975. The plot has a slope of 0.005 and intercept of 0.065. The total phenolic contents of MEFR, MEAM and MEBM were calculated with a regression equation based on a standard curve using gallic acid (10-100µg/ml) as standard, represented in table 5.4. The extract MEFR had the highest phenolic content, 213.0±0.712mg GAE/g extract. The mid value obtained for MEAM extract (191.6±1.058 mg GAE/g extract) and the lowest value obtained for MEBM extract, 174.7±1.007 mg GAE/g extract. TPC of the methanolic extract of the plants follows the order; MEFR > MEAM > MEBM.

Table 5.4: TPC of MEFR, MEAM and MEBM

	TPC (mg/gm) extract GAE		
Equation	**MEFR**	**MEAM**	**MEBM**
y=0.005x+0.065 R2=0.975	213.0±0.721	191.6±1.058	174.7±1.007

NOTE- *F. religiosa (LFR), A. marmelos (LAM)* and *B. monosperma (LBM)*

Total flavonoid contents (TFC)

The total flavonoid content (TFC) of extracts was measured with the aluminium chloride colorimetric assay using rutin as standard. Aluminium chloride forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxide group of flavones and flavonols. They also form labile complexes with

ortho-dihydroxidegroups in A/B rings of flavonoids. The absorbance was measured 510 nm using water as blank in UV Spectrophotometer (**Kamtekar *et al.*, 2014**). The TFC was determined using the calibration curve for rutin shown in figure 5.3. The regression co-efficient was found R2= 0.988. The plot has a slope of 0.001 and intercept of 0.120. The total flavonoid contents of MEFR, MEAM and MEBM were calculated with a regression equation based on a standard curve using rutin (10-100µg/ml) as standard, represented in table 5.5 and figure 5.4. The extract MEFR had the highest flavonoid content, 148.7±2.517 mg/g RE. The mid value obtained for MEAM extract (115.3±1.155 mg/g RE) and the lowest value obtained for MEBM extract, 92.3±3.055 mg/g RE. TFC of the methanolic extract of plants follows the order; MEFR > MEAM > MEBM.

Table 5.5: TFC of MEFR, MEAM and MEBM

	TFC (mg/gm) extract RE		
Equation	**MEFR**	**MEAM**	**MEBM**
$y = 0.001x + 0.120$ $R^2 = 0.988$	148.7±2.517	115.3±1.155	92.3±3.055

NOTE- *F. religiosa (LFR), A. marmelos (LAM)* and *B. monosperma (LBM)*

CONCLUSION

The present study concludes that the crude methanolic extract of *Ficus religiosa*, *Aegle marmelos* and *Butea monosperma* exhibited a significant antioxidant activity which may be relevant in the treatment of oxidative stress. Estimation of total phenolic content and total flavonoids content wasperformed by the methods reported earlier. These determinations were carried out during the stability studies of formulations, which has been an integral part of this thesis. There was no significant difference in the amount of TPC and TFC during the course of stability study indicating that the extracts were chemically stable. The result reveals that all the extracts have the scavenging character in accordance with the standards. The further work has been developed for the isolation of particular phenolic compound for this activity, and also can be used for the new formulation development.

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