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*Original Article*

# Evaluation of Anti-oxidant Activity and Flavonoid Content in *Macaranga peltata* Leaves

**Honnesh N H[1], Santanu Saha[2]***

## ABSTRACT

**Introduction:** Macaranga peltata is a woody shrub and tree found in forest of Western Ghats of India. The leaves of M. peltata are large so used to wrap sweets in kerala. The plant is used in folk medicine and siddha medicine to treat fever, cough, wound and ulcers. There is very limited research has been conducted on exploring this plant medicinal properties and phytochemical estimation. **Materials and Methods:** In the present study attempt was made to know the antioxidant activity of MPCF by DPPH assay, FRAP assay and $H_2O_2$ assay methods and Optimization of mobile phase by TLC method and supporting major flavonoid content quantification by HPTLC method **Results:** The result shown the Chloroform fraction has significant antioxidant activity of $IC_{50}$ value of 123.71(DPPH assay), 185.85(FRAP assay) and 204.31($H_2O_2$ assay). The TLC method optimum mobile phase was found to be Toluene: Ethyl acetate: Formic acid (5:4:0.2). The standard flavonoids used are Luteolin, Diosmin and ellagic acid. The HPTLC method results shown MPCF was found to contain 11.70% Luteolin.

**Key words:** DPPH, Fraction, FRAP, Luteolin, Macaranga peltata, MPCF.

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## INTRODUCTION

*Macaranga peltata* tree is traditionally called as uppalige in kannada and chandada in Malayalam language. The plant grows beside roads in mangalore and western ghat forests. In folklore medicine used for treatment of wounds. In previous research work we noticed bark extract of other species of Macaranga (*Macaranga barteri* stem bark extract) was found to possess wound healing activity[3] and antioxidant activity so an attempt was made to know the antioxidant activity and major flavonoid present in available M peltata species in karnataka. In previous article M Peltata found to contain phytocompounds such as bergenin, siaresinolic acid, lupeol, sitosterol, stigmasterol and oleanolic acid[1,2] Present study is to explore the medicinal use of M Peltata leaf ethanol extract and fractions for antioxidant activity and estimation and quantification of major flavonoids which may be responsible for medicinal properties of the M Peltata.

## MATERIALS AND METHODS

All the chemicals and reagents used in the studies were of analytical grade obtained from S.D. fine chemicals Pvt. Ltd, Bombay, India and Merck Pvt. Ltd Bombay, India. The Phytocompounds were purchased from yucca enterprises, Mumbai. The TLC plates were obtained from Vasa scientific Pvt. Ltd.

### Collection and Authentification of Plants

The *Macaranga peltata* leaves were collected from Paneer, Deralakatte areas in Mangalore. The collected plant materials were subjected to authentication at Foundation for Revitalization of Local Health Traditions (FRLHT), Bangalore and the voucher specimen (4717-18/28.0/.2021) was deposited

**Extraction:** The authentified leaves were dried in hot air oven, powder and sieved to obtain coarse powder. (5kg) The powders were subjected to maceration with Ethanol, and successively fractionated with Chloroform, Petroleum ether, Ethyl acetate and methanol respectively. Filtered and dried to obtain the extract and fraction which were preserved in desiccator.

### Fractionation of ethanol extract

The ethanol extracts of *Macaranga peltata*(1200g) was extracted successively with petroleum ether 60 – 80°C, (8×500ml), chloroform (8×500ml), ethyl acetate (8×500ml) and methanol (8×500ml). All the fractions were then washed with distilled water (30 ml), dried over anhydrous sodium sulphate and freed of solvent by distillation.

### Antioxidant activity

**Method 1:** Diphenyl Picryl Hydrazyl (DPPH) Radical Scavenging method[4]

**Procedure:**

This assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical (DPPH). The free radical DPPH is reduced to the corresponding hydrazine when it reacts with hydrogen donors. This ability is evaluated by more frequently used technique is the discoloration assay, which evaluates the absorbance decrease at 518-528 nm produced by the addition of the antioxidant to a DPPH solution in ethanol or methanol.

% Antioxidant activity = {Absorbance of Control – Absorbance of Sample/

Absorbance of Control} x 100

**Method 2:** FRAP (Ferric Reducing Antioxidant Power) Assay method[4]

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**Procedure:**

It's recommended to use only fresh samples for the most accurate and reproducible assay. The extractions from plants can be done using various solvents like aqueous, methanol, ethanol, or acetone. The extraction method depends on the type of specific sample being extracted.

• The dilution series was made using plant materials.

• Phosphate buffer 2.5 ml (0.2 M, pH 6.6) was added to all the tubes.

• The contents in each tube were thoroughly mixed.

• Then, 2.5 ml of 1 % potassium ferricyanide K3F(CN)6 solution was added to all the samples.

• After this, each reaction mixture was vortexed well using a vortex shaker.

• The samples were incubated at 50°C for around 20 minutes.

• Once the incubation time was over, 2.5 ml of 10 % trichloroacetic acid (TCA) was added to each sample.

• The test tubes were centrifuged at 3,000 rpm for 10 minutes. From these centrifuged samples, 2.5 ml

supernatant was collected in separate test tubes.

• After this, in the same new separate test tubes, 0.5 ml of ferric chloride (FeCl3) was added.

• This gave us a bluish color formation.

• And then the absorbance was measured at 700nm.

• A sample having more concentration was shown higher absorbance and the opposite is true

% Antioxidant activity = {Absorbance of Control – Absorbance of Sample/

Absorbance of Control} x 100

**Method 3:** H2O2 (Hydrogen Peroxide) Free Radical Scavenging Activity Assay method[4]

**Procedure:**

The dilution series was made using plant extract powder.

• Then, 600 µL of H2O2 was added to the tubes (Control, sample, standard).

• 100 µl of plant extract or standards (Ascorbic acid) was added into respective test tubes.

• After that, the volume was made up to 4 mL with phosphate buffer (pH 7.4) in all test tubes.

• The identical Ascorbic acid series without sample served as the standard (phosphate buffer, H2O2, and Ascorbic acid).

• All the test tubes were incubated for 10 min at room temperature.

• The absorbance of the H2O2 solution was measured at 300 nm against the blank.

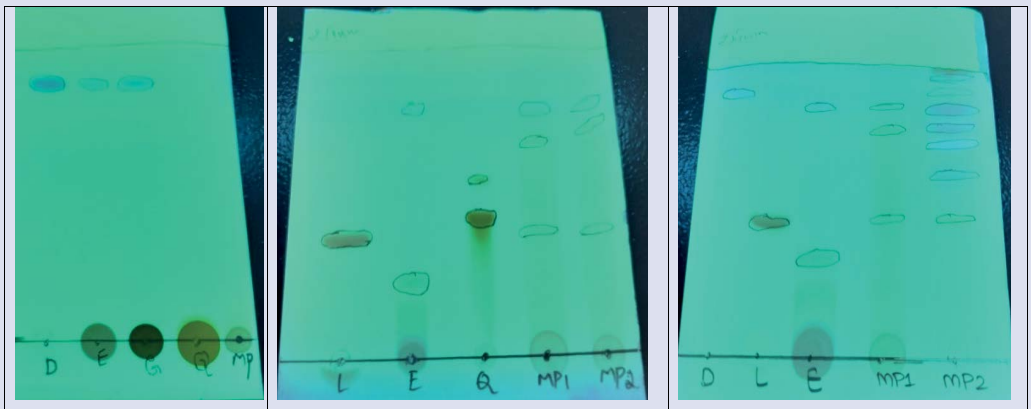
• H2O2 scavenging activity percentage and IC50 were calculated compared to the standard by the bellow

% Antioxidant activity = {Absorbance of Control – Absorbance of Sample/

Absorbance of Control} x 100

## Qualitative- Thin layer chromatography (TLC)

The plant fractions MPCF were subjected to thin layer chromatographic studies to optimize the mobile phase.(Figure 1). The Ellagic acid, Luteolin and Diosmin standards were used for standardization of Macaranga fraction. The different mobile phases were prepared by using different solvents Toluene, ethyl acetate, diethyl amine, formic acid. The standardized mobile phase is used for quantification of flavonoid by HPTLC at Manipal College of pharmaceutical sciences, Manipal, Karnataka.



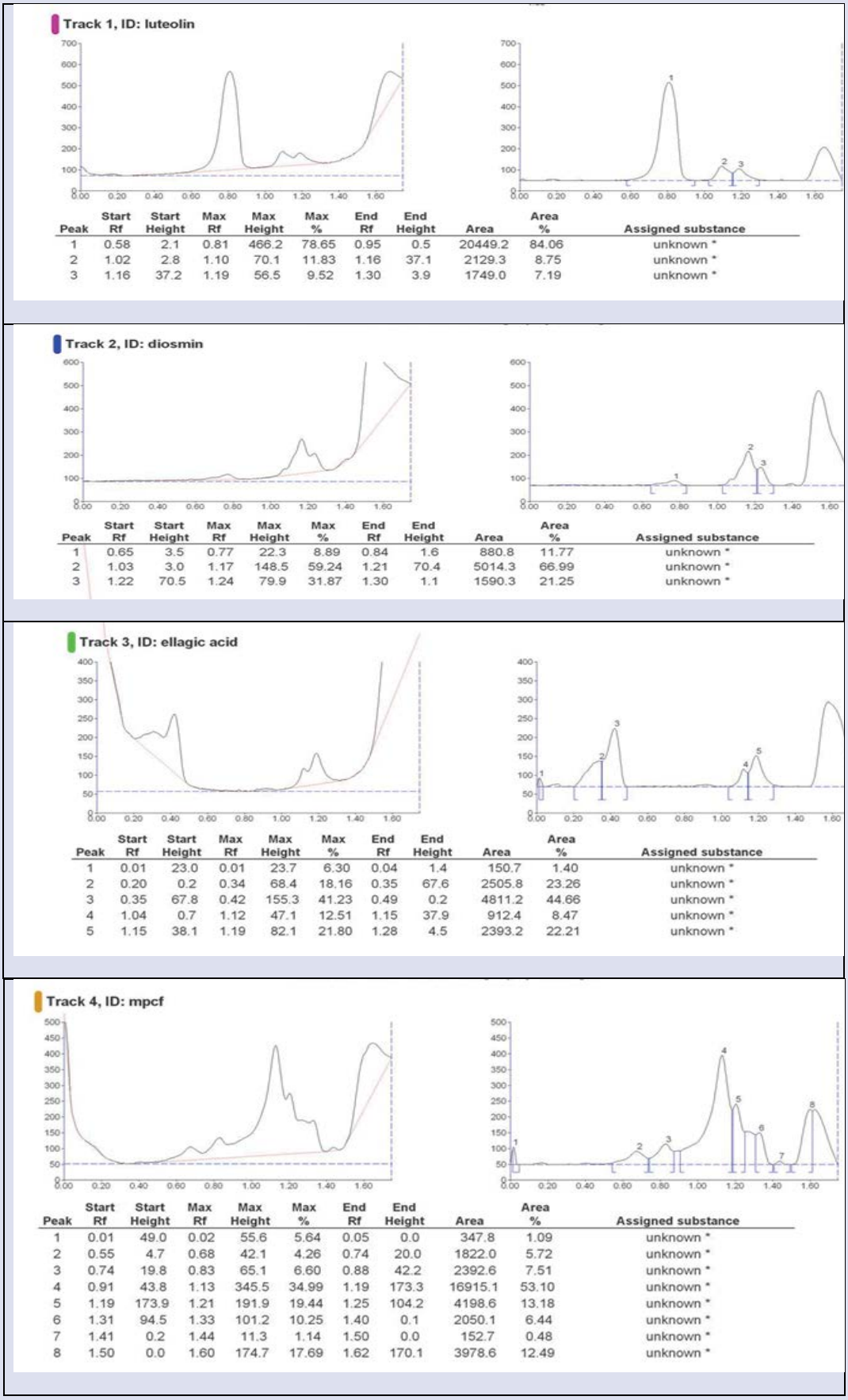

| Toluene: Ethyl acetate: Diethyl amine(7:2:1) | Toluene: Ethyl acetate: Formic acid (6:4:0.3)[5] | Toluene: Ethyl acetate: Formic acid (5:4:0.2)[6] |
|---|---|---|

**Optimized Mobile phase:** Toluene: Ethyl acetate: Formic acid (5:4:0.2)

**Figure 1:** Results of TLC studies of *Macaranga peltata* chloroform fraction for phytochemicals.

**Samples**- MP-Macaranga Peltata

**Standards-** L-Luteolin, D-Diosmin, E-Ellagic Acid, Q-Quercetin, G-Gallic acid




**Track 1, ID: luteolin**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.58 | 2.1 | 0.81 | 466.2 | 78.65 | 0.95 | 0.5 | 20449.2 | 84.06 | unknown * |
| 2 | 1.02 | 2.8 | 1.10 | 70.1 | 11.83 | 1.16 | 37.1 | 2129.3 | 8.75 | unknown * |
| 3 | 1.16 | 37.2 | 1.19 | 56.5 | 9.52 | 1.30 | 3.9 | 1749.0 | 7.19 | unknown * |

**Track 2, ID: diosmin**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.65 | 3.5 | 0.77 | 22.3 | 8.89 | 0.84 | 1.6 | 880.8 | 11.77 | unknown * |
| 2 | 1.03 | 3.0 | 1.17 | 148.5 | 59.24 | 1.21 | 70.4 | 5014.3 | 66.99 | unknown * |
| 3 | 1.22 | 70.5 | 1.24 | 79.9 | 31.87 | 1.30 | 1.1 | 1590.3 | 21.25 | unknown * |

**Track 3, ID: ellagic acid**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 | 23.0 | 0.01 | 23.7 | 6.30 | 0.04 | 1.4 | 150.7 | 1.40 | unknown * |
| 2 | 0.20 | 0.2 | 0.34 | 68.4 | 18.16 | 0.35 | 67.6 | 2505.8 | 23.26 | unknown * |
| 3 | 0.35 | 67.8 | 0.42 | 155.3 | 41.23 | 0.49 | 0.2 | 4811.2 | 44.66 | unknown * |
| 4 | 1.04 | 0.7 | 1.12 | 47.1 | 12.51 | 1.15 | 37.9 | 912.4 | 8.47 | unknown * |
| 5 | 1.15 | 38.1 | 1.19 | 82.1 | 21.80 | 1.28 | 4.5 | 2393.2 | 22.21 | unknown * |

**Track 4, ID: mpcf**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 | 49.0 | 0.02 | 55.6 | 5.64 | 0.05 | 0.0 | 347.8 | 1.09 | unknown * |
| 2 | 0.55 | 4.7 | 0.68 | 42.1 | 4.26 | 0.74 | 20.0 | 1822.0 | 5.72 | unknown * |
| 3 | 0.74 | 19.8 | 0.83 | 65.1 | 6.60 | 0.88 | 42.2 | 2392.6 | 7.51 | unknown * |
| 4 | 0.91 | 43.8 | 1.13 | 345.5 | 34.99 | 1.19 | 173.3 | 16915.1 | 53.10 | unknown * |
| 5 | 1.19 | 173.9 | 1.21 | 191.9 | 19.44 | 1.25 | 104.2 | 4198.6 | 13.18 | unknown * |
| 6 | 1.31 | 94.5 | 1.33 | 101.2 | 10.25 | 1.40 | 0.1 | 2050.1 | 6.44 | unknown * |
| 7 | 1.41 | 0.2 | 1.44 | 11.3 | 1.14 | 1.50 | 0.0 | 152.7 | 0.48 | unknown * |
| 8 | 1.50 | 0.0 | 1.60 | 174.7 | 17.69 | 1.62 | 170.1 | 3978.6 | 12.49 | unknown * |

**Figure 2:** HPTLC study of *Macaranga peltata* chloroform fraction and selected phytocompounds.

**Table 1: Results of Antioxidant activity of *Macaranga peltata* chloroform fraction.**

| SL NO | Concentration (µg/ml) | DPPH free radical scavenging activity | FRAP free radical scavenging activity | $H_2O_2$ free radical scavenging activity |
|---|---|---|---|---|
| | 5 | 16.52 | 15.68 | 10.52 |
| | 10 | 20.22 | 17.25 | 15.34 |
| | 25 | 29.38 | 24.12 | 23.04 |
| | 50 | 42.94 | 31.68 | 31.26 |
| | 100 | 68.34 | 46.42 | 44.34 |
| | 250 | 89.50 | 72.42 | 68.61 |
| | 500 | 94.62 | 88.64 | 85.24 |
| | **IC50** | **123.71** | **185.85** | **204.31** |

*Standard Ascorbic acid has shown IC50 value of 12.17, 12.64, 14.68 in DPPH, FRAP and H2O2 assay methods respectively

**Table 2: HPTLC results of *Macaranga peltata* chloroform fraction for phytochemicals.**

| Standard/Sample | Initial Concentration | Rf (Retardation factor) | AUC(Area under curve) at 254nm |
|---|---|---|---|
| Luteolin | 1mg/ml | 0.82 | 20449.2 |
| Diosmin | 1mg/ml | 0.77 | 880.8 |
| Ellagic acid | 1mg/ml | 0.42 | 4811.2 |
| MPCF | 1mg/ml | 0.83 | 2392.6 |
| **% of Luteolin** | | | **11.70%** |

**Quantitative- High performance thin layer chromatography (HPTLC)**

**HPTLC Instrumentation**[7,8]

The HPTLC system (Camag, Muttanz, Switzerland) consisted of

i) TLC scanner connected to a PC running WinCATS software under MS DOS;

ii) Linomat IV sample applicator using 100µl syringes and connected to a nitrogen tank. Each plate accommodated 1 tracks of sample and different standards, applied according to following settings: band width 6 mm; distance between bands 10 mm; application volume 10 µl; gas flow 150 nl/s. The plates were developed in a twin trough glass chamber presaturated with mobile phase. After development scanning was done by densitometer scanner. The data generated was captured by WinCATS Planar chromatography version 1.1.5.0 software (Figure 2).

## RESULTS

The *Macaranga peltata* leaves were found to contain 24.16 % ethanol extract. The fractions obtained from ethanol extract are Petroleum ether fraction(8.28%), Chloroform fraction(22.28%), Ethyl acetate fraction(22.14%) and methanol fraction(36.14%) respectively. The antioxidant activity results shown that MPCF has IC50 value of 123.71, 185.85 and 204.31 by DPPH free radical scavenging method, FRAP free radical scavenging method and $H_2O_2$ free radical scavenging method respectively.(Table 1). The IC50 values for standard ascorbic acid in the DPPH, FRAP, and H2O2 test techniques are 12.17, 12.64, and 14.68, respectively. The optimized mobile phase for Macaranga peltata chloroform fraction by TLC is Toluene: Ethyl acetate: Formic acid (5:4:0.2). The main flavonoid in *Macaranga peltata* chloroform fraction was determined and estimated using HPTLC analysis. The optimized mobile phase was used to separate flavonoids from the 10µL spots that were applied and developed in the TLC chamber. For the estimation of flavonoids in M peltata the standards Luteolin, Diosmin and Ellagic acid were used. The sample and standard spots were developed using the mobile phase Toluene: Ethyl acetate: Formic acid (5:4:0.2). Luteolin spot was clearly obtained in sample MPCF at retardation factor 0.81. No Diosmn and ellagic acid spots observed in sample for the selected mobile phase. The area under the curve (AUC) of luteolin in sample MPCF was 2392.6 and Standard Luteolin was 20449.2 at 254nm respectively. By calculation determined that MPCF was found to contain 11.70% Luteolin. (Table 2)

## CONCLUSION

The Macaranga peltata leaves contain polar phytocompounds so the significant ethanol extract (24.16%) was obtained. The major fractions reported from ethanol extract are methanol fraction (36.14%) and chloroform fraction (22.28%). The yield obtained by petroleum ether is very less.(8.28%). The MPCF was reported to possess significant antioxidant activity (IC50 value 123.71) The optimised mobile phase for estimation of flavonoids by HPTLC by preliminary TLC method is Toluene: Ethyl acetate: Formic acid (5:4:0.2). Finally the major flavonoid reported is Luteolin(11.70%) which may be responsible for medicinal use of M Peltata leaf. As per previous review article the luteolin 1 %(1028.75mg/100gm) reported in oregano and 0.06 % in juniper berries[9] many other fruits such as celery, parsley, peppermint, cauliflower, cabbage, rosmary and sage found to contain low luteolin content. Thus, it demonstrates that *Macaranga peltata* has a high luteolin concentration and the potential to separate luteolin from natural sources for use in industrial applications.

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