



Quantitative Phytochemical and Heavy Metal Estimation of *Mesua ferrea* Flowers and *Argyrea speciosa* Leaves

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ABSTRACT

Mesua ferrea Linn. belongs to the family Clusiaceae. The flowers have been cream coloured, ebracteate, pedicellate, pedicel short and axillary. The plant *Argyrea speciosa* Sweet belongs to the family Convolvulaceae. It is a woody climber. Both the plant parts have been authenticated and identified taxonomically. Defatting of flower and leaves have been done by light petroleum ether 40-60°C and then extracted with ethanol by soxhlet extraction method. Gallic acid has been taken as reference standard for total phenolic estimation. The total phenolic in the ethanolic extract of MF and AS equivalent to gallic acid has been found to be 10.12 and 14.72 mg per gram of dry weight extract respectively. Tannic acid has been taken as reference standard for total tannin estimation. The total tannin in the ethanolic extract of MF and AS equivalent to tannic acid has been found to be 8.75 and 11.25 mg per gram of dry weight extract respectively. Rutin has been used as reference standard for flavonoid and flavonol estimation. The total flavonoid content have been found to be 23 ± 1.37 and 30 ± 0.52 mg / gm plant extract in rutin equivalent respectively. The total flavonol content have been found to be 0.49 ± 0.03 and 3.60 ± 0.25 mg / gm plant extract in rutin equivalent respectively. The saponin content of MF have been 98.75 mg/g and AS have been 7.70 mg/g. Heavy metal estimation has been done by inductively coupled plasma optical emission spectrometer. The heavy metals content of MF have been Cd – 0.001 mg/L, Cr - 0.021 mg/L, Ni - 0.012 mg/L, and Pb - 0.010 mg/L and of AS have been Cd – 0.000 mg/L, Cr - 0.013 mg/L, Ni - 0.021 mg/L, and Pb - 0.011 mg/L.

Keywords: Phytochemical, Heavy metal, *Mesua ferrea* flowers, *Argyrea speciosa* leaves.

INTRODUCTION

It has been estimated that out of 4, 22, 000 flowering plants reported from the world, more than 50,000 are used for medicinal purposes.¹ In India, more than 43% of the total flowering plants are reported to be of medicinal importance.² Utilization of plants for medicinal purpose has been used since long time back in ancient literature. Estimation of the quantity of phytoconstituents provides more information on how much quantity the compounds might be present for isolation.

Quantitative estimation of the phytoconstituents also helps in predicting the amount of those compounds that have the good therapeutic activity on the body. Contamination of medicinal plant with certain heavy metals like Arsenic, Lead, and Cadmium is unacceptable. Therefore, their limit should be specified so that no harm is encountered in the human body. Medicinal plants materials containing saponins have the ability to cause haemolysis of the blood.

Mesua ferrea Linn. belongs to the family Clusiaceae (Hindi – Nagkesara, English - Ceylon Ironwood). *Mesua ferrea* is found in moist or semi-evergreen forest, either scattered or in more or less pure patches or belt. It requires well drained, deep fertile soil with a neutral pH. It is a strong shade bearer, particularly when young.³ The flowers are cream coloured, ebracteate, pedicellate, pedicel short, axillary or terminal, solitary or in pairs (cluster) and 2.5-7.5 cm in diameter, bisexual, large, sub-sessile and buds are sub-globose. The plant *Argyrea speciosa* Sweet belongs to the family Convolvulaceae. It is a woody

climber. The plant is commonly known as Elephant creeper in English and in Hindi, it is known as 'Vryddhadaru'.

MATERIALS AND METHODS

The authentication and identification of *Mesua ferrea* flowers (MF) and *Argyrea speciosa* leaves (AS) was done by Prof. S. D. Dubey, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Science, Banaras Hindu University, Varanasi and a voucher specimen COG/H.No. – 025 and COG/H.No. – 024 respectively have been preserved for further references.

Chemicals

Gallic acid, Tannic acid and Folin Ciocalteu reagent were purchased from Merck Co. (Germany).

Extraction

This step was conducted separately for MF and AS. The crude drugs were dried under shade for 4-6 days. They were then powdered in mixer grinder until a suitable sized was achieved and passed through sieve number 22/60. The coarse crude drug was then placed in a soxhlet extractor for the extraction purpose and 10% of the fines were added. The powder was then initially defatted with 40-60°C petroleum ether for 15 hours until the fatty material had been removed and finally followed with a 24 hours ethanolic extraction. The ethanolic extracts of MF and AS were dried and stored in desiccator and further used for various estimation.

Estimation of total phenolic content

It was done based on the Folin-Coicalteau calorimetric methods of determination proposed by Hagerman, A., *et al*, 2000, with some modifications.⁴ Here gallic acid in ethanol was taken as standard for preparation of standard curve. Absorbances were recorded at 765 nm against blank using UV spectrometer and the total phenolic contents were expressed in terms of gallic acid equivalent (mg/gm of dry mass).

Estimation of total tannin content

The estimation of total tannin content was also done as per the method proposed by Hagerman, A., *et al*, 2000, with some modifications.⁴ Here tannic acid dissolved in triple distilled water was taken as standard for preparation of standard curve. Absorbances were recorded at 775 nm against blank using UV spectrometer and the total tannin contents were expressed in terms of tannic acid equivalent (mg / gm of dry mass).

Estimation of total flavonoid and flavonol content

It was done as per the method proposed by Kumaran, A., *et al*, 2006 using rutin as the standard reference compound.⁵ The method was based on the formation of a flavonoid / flavonol-aluminum complex having the absorbitivity maximum at 415 nm and 440 nm for flavonoid and flavonol respectively. The amount of flavonoids and flavonol in plant extracts in Rutin equivalents (RE) was calculated by using the formula: $X = (A \cdot m_0) / (A_0 \cdot m)$, where X is the flavonoid or flavonol content, mg/g plant extract in RE, A is the absorbance of plant extract solution, A_0 is the absorption of standard Rutin solution, m is the weight of plant extract in mg and m_0 is the weight of Rutin in the solution in mg.

Determinations of total saponin content

It was conducted as per the method of Ren *et al.*, 2005.⁶ Diosgenin was taken as reference standard and absorbance was taken at 408 nm.

Determination of heavy metal content

Preparation of the sample by acid digestion was done as per the method given in 'Quality control methods for medicinal plant materials', WHO, Geneva, 1998.⁷ Inductively coupled plasma optical emission spectrometer (ICP-OES) was used for multi elemental analysis of major, minor & trace elements. Make and Model: Perkin Elmer, USA; Optical 2100DV ICP-OES.

Detectors: UV sensitive dual backside illuminated CCD array detector, Spectral Range: 160 nm – 900 nm, Spectral Resolution: 0.009 nm at 200 nm and Speed: 20-25 elements in < 5 min.

RESULTS AND DISCUSSION

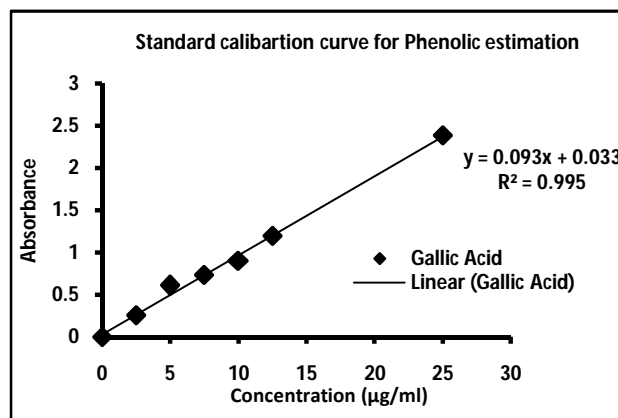
Total phenolic content

Standard calibration curve Graph-1 was plotted by taking absorbances of different concentrations of gallic acid. Table-1 shows absorbances for MF and AS. The

absorbance for MF and AS was 0.991 and 1.325 respectively. The total phenolic in the ethanolic extract of MF and AS equivalent to gallic acid was found to be 10.12 and 14.72 mg per gram of dry weight extract respectively. AS contains more polyphenolics than MF.

Table 1: Absorbance for total phenolic content (At 765 nm)

Ethanolic extracts	Absorbance
MF	0.991
AS	1.325



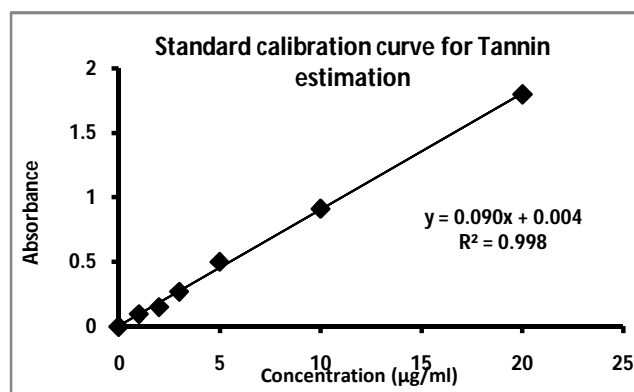
Graph 1: Standard calibration curve for total phenolic content (Gallic acid as standard)

Total tannin content

Standard calibration curve Graph-2 was plotted by taking absorbances of different concentrations of tannic acid. Table-2 shows absorbances for MF and AS. The absorbance for MF and AS was 0.788 and 1.013 respectively. The total tannin in the ethanolic extract of MF and AS equivalent to tannic acid was found to be 8.75 and 11.25 mg per gram of dry weight extract respectively. AS contains more tannins than MF.

Table 2: Absorbance for total tannin content (At 775 nm)

Ethanolic extracts	Absorbance
MF	0.788
AS	1.013



Graph 2: Standard calibration curve for total tannin content (Tannic acid as standard)

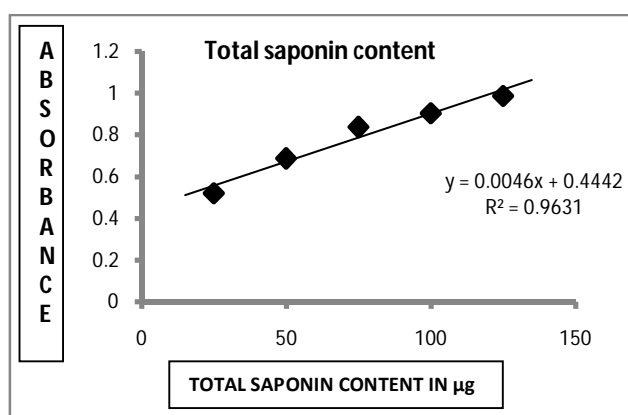
Total flavonoid and flavonol content

The absorbances at 415 nm for MF and AS were 0.228 and 0.309 respectively. Hence the total flavonoid content was found to be 23 ± 1.37 and 30 ± 0.52 mg / gm plant extract in rutin equivalent respectively. The absorbances at 440 nm for MF and AS were 1.314 and 2.683 respectively. Hence the total flavonol content was found to be 0.49 ± 0.03 and 3.60 ± 0.25 mg / gm plant extract in rutin equivalent respectively. The flavonol content in both the crude drug was very less.

Total saponin content

The absorbances for MF and AS were 0.523 and 0.450 respectively. Hence the saponin content of MF and AS was 98.75 mg/g and 7.70 mg/g respectively.

Graph 3: Standard calibration curve for total saponin content (Diosgenin as standard)



Heavy metal content

The heavy metals content of MF were Cd – 0.001 mg/L, Cr - 0.021 mg/L, Ni - 0.012 mg/L, and Pb - 0.010 mg/L and of AS were Cd – 0.000 mg/L, Cr - 0.013 mg/L, Ni - 0.021 mg/L, and Pb - 0.011 mg/L. In both MF and AS heavy metals content were found to be negligible.

CONCLUSION

The quantitative phytochemical information about MF and AS will help these plants to explore about pharmacological and therapeutical potential. As heavy metal contents are negligible hence they can be directly used for medicinal purpose.

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