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Investigation of phytochemicals & estimation of total phenolic and flavonoid content of *myxopyrum smilacifolium* (wall.) blume leaves

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ABSTRACT

Myxopyrum smilacifolium (Wall.) Blume is a large woody climbing shrub belonging to the family Oleaceae. It is used in the treatment of cough, asthma, neuropathy, rheumatism, cephalalgia and otopathy. The main objective of the study is to investigate the presence of various phytochemicals and estimate the total phenolic and flavonoid content in various fraction of *Myxopyrum smilacifolium* (Wall.) Blume leaves. The crude ethanol extract was subjected to fractionation using Petroleum ether, Chloroform and Ethyl acetate based on their polarity. The fractions were subjected to preliminary phytochemical analysis for the detection of various phytoconstituents. The total phenolic and flavonoid content in different fractions were estimated by Folin-Ciocalteau method and Aluminium chloride colorimetric assay respectively. The qualitative phytochemical analysis revealed the presence of carbohydrates, alkaloids, flavonoids and phenolics. Ethanol residue and petroleum ether fraction of *M. smilacifolium* was found to be rich in phenolics and flavonoids respectively.

Keywords: *M. smilacifolium*, Soxhlet extraction, Phenolics, Flavonoids, Folin-Ciocalteau method, Aluminium chloride colorimetric assay.

INTRODUCTION

Myxopyrum smilacifolium is a large woody twining shrub commonly called as Chaturamulla in Malayalam belonging to the family Oleaceae. The plant is mainly distributed in the forest of Bangladesh, India, Cambodia, Myanmar, Thailand, Laos and Vietnam. In India these plants are found in Western Ghats and it grows throughout Kerala and evergreen forests at altitudes of 600m-900m [1]. The plant is known to have several medicinal properties. Its root, stem, leaves are of much medicinally active and is employed in many traditional systems of medicine. The roots are used to treat various diseases like scabies, cough, rheumatism, fever, cuts and wounds [2]. The leaves are astringent, acrid, sweet, thermogenic, anodyne, febrifuge and tonic [3]. Previous studies have shown the presence of triterpenoid ursolic acid [4] and iridoid glycoside myxopyroside [5] in leaves.

Figure1: *Myxopyrum smilacifolium* (Wall.) Blume.

MATERIALS AND METHODS

Plant material

Fresh healthy disease free leaves of *Myxopyrum smilacifolium* (Wall.) Blume were collected from Department of Pharmaceutical Sciences, CPAS, Cheruvandoor, Kottayam district, Kerala in the month of November. The plant was authentified by Mr. Rogimon P Thomas, Assistant Professor, Department of Botany, CMS College Kottayam with a voucher number CMS 1305 dated 13/01/2018.

Extraction and fractionation

Fresh leaves of *M. smilacifolium* were shade dried, coarsely powdered and stored in air tight containers. It was packed (50g) in a Soxhlet apparatus and subjected to continuous hot extraction for 24-36 hours using 300mL of ethanol (95%) as solvent. The extraction was repeated twice. The combined extract was concentrated to dryness. The crude ethanol extract was a dark green colored solid sticky mass. The total ethanolic extract was fractionated successively with solvents of increasing polarities such as petroleum ether (3x100mL), chloroform (3x100mL) and ethyl acetate (3x100mL). This yielded four fractions namely, PEMS (petroleum ether fraction of *M. smilacifolium*), CMS (chloroform fraction of *M. smilacifolium*), EAMS (ethyl acetate fraction of *M. smilacifolium*) and ERMS (ethanol residue of *M. smilacifolium*). Each fraction was concentrated, weighed and stored in a refrigerator (2-8^{o}C). The yield of EAMS was negligible, thus it was not considered for further analysis [6].

Preliminary phytochemical screening

The fractions of ethanolic extract were subjected to preliminary phytochemical screening for the detection of various plant constituents like alkaloids, carbohydrates, flavonoids, glycosides, terpenoids, tannins and phenols [7, 8].

Estimation of total phenolic content

Phenolics are secondary plant metabolites reported to exhibit anti-carcinogenic, anti-inflammatory, antimicrobial, antiulcer, anti-thrombotic, anti-atherogenic, immunomodulating, vasodilatory and analgesic effects [9]. Total phenolic content was estimated by Folin-Ciocalteau method [10]. The assay is the simplest method available for the measurement of phenol content in plant extracts. The method relies on the transfer of electrons in alkaline medium from phenolic compounds to form a blue chromophore containing a phosphotungstic/phosphomolybdenum complex where the maximum absorption depends on the concentration of phenolic compounds. The reduced Folin-Ciocalteu reagent is detectable with a spectrophotometer in the range of 690 to 750 nm.

The fraction (1 mg/mL) was mixed with 5 mL Folin-Ciocalteu reagent diluted with water 1:10 v/v and 4 mL of 7.5% sodium carbonate. The mixture was vortexed for 15 s and allowed to stand for 30 min at 40^{o}C for color development. Absorbance was then measured at 750 nm using UV-Visible spectrophotometer (Agilent, Cary 60). A calibration curve was prepared using Gallic acid (100–1000 µg/mL) as standard and used for calculation of total phenolic compound [11].

The Folin-Ciocalteau (F-C) method of assay is the simplest method available for the measurement of phenolic content in products.

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Estimation of total flavonoid content

Flavonoids are well known for their beneficial effects on health. More than 4000 varieties have been identified. Flavonoids are used as antioxidants and possess anti-inflammatory, antiviral, antiallergic, and anticarcinogenic properties. Total flavonoid content was measured using Aluminium chloride colorimetric assay [12] using Quercetin as the standard. The principle involved is that $AlCl_3$ forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids [13].

In brief, 50 µL of fractions (1 mg/mL ethanol) were made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 mL with distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 415 nm. The total flavonoid content was calculated from a calibration curve [14].

RESULTS

Preliminary phytochemical screening

The qualitative phytochemical analysis of all fractions of *Myxopyrum smilacifolium* leaves showed the presence of various phytoconstituents like alkaloids, phenolics, flavonoids, carbohydrates, proteins, sterols and terpenoids. The results of phytochemical analysis of various fractions are tabulated in table 1. Phenolics and flavonoids were found to be rich in ethanol residue and petroleum ether fraction respectively.

Table 1: Preliminary phytochemical screening of various fractions of *M. smilacifolium* leaves.

Phytoconstituents	PEMS	CMS	ERMS
Alkaloids	++	+	+
Glycosides	+	++	-
Phenolics	++	+	+++
Flavonoids	+++	++	+
Carbohydrates	+	-	+
Proteins and amino acids	-	+	+
Terpenoids	++	-	-
Sterols	+	++	-
Saponins	+	+	-

(+), (-) presence and absence of the respective class of compounds

Estimation of total phenolic content

The standard graph was plotted using various concentrations of Gallic acid. The total phenolic content in various fractions of extract was determined and expressed as gram equivalents of Gallic acid per 10mg. The absorbance values obtained for different concentrations of standard Gallic acid is shown in table 2.

Table 2: Absorbance values obtained for different concentrations of standard

SI. No	Concentrations (µg/ml)	Absorbance (750nm)
1.	100	0.1808±0.0051
2.	200	0.2737±0.0056
3.	400	0.3600±0.0057

4.	800	0.6806±0.0054
5.	1000	0.8500±0.0115

Values expressed as Mean±SEM (n = 3)

A calibration curve of Gallic acid was plotted using absorbance on Y axis and concentration on X axis (figure 2).

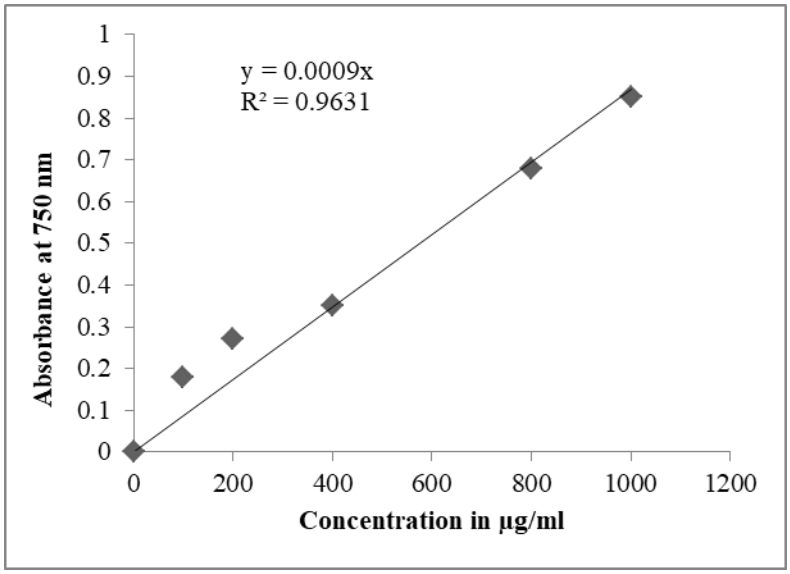

Figure 2: Calibration curve of Gallic acid

The total phenolic content of various fractions of *M. smilacifolium* were calculated as gram equivalents of Gallic acid per 10mg and is tabulated in table 3. The absorbance was measured at 750nm.

Table 3: Total phenolic content of different fractions of ethanolic extract of *Myxopyrum smilacifolium* leaves.

Sl. No	Extract fractions	Absorbance (750nm)	Gram equivalents of Gallic acid per 10mg
1	PEMS	0.0523±0.0057	0.0784
2	CMS	0.0626±0.0080	0.0940
3	ERMS	0.1707±0.0052	0.2554

Values expressed as Mean±SEM (n = 3)

In the present investigation, it was found that ethanolic residue of the *M. smilacifolium* (ERMS) leaves had the highest phenolic content followed by chloroform (CMS) and petroleum ether (PEMS) fraction. Total phenolic content of the ethanol residue of *M. smilacifolium* leaves was 0.2554 g Gallic acid equivalents (GAE)/10 mg extract.

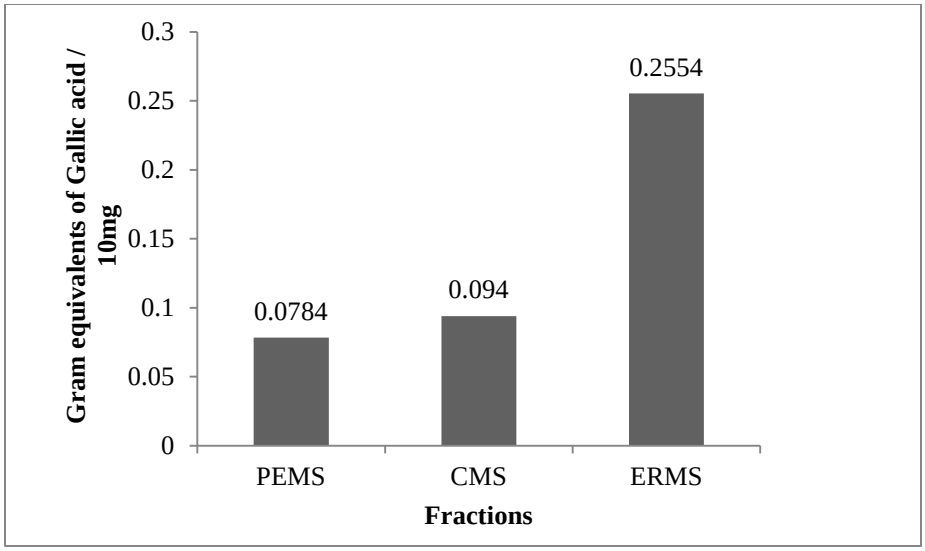

Figure 3: Comparative evaluation of total phenolic content of various fractions

Estimation of total flavonoid content

The total flavonoid content in PEMS, CMS and ERMS were determined spectrophotometrically by Aluminium chloride colorimetric method. The Quercetin is used as standard and the absorbance values obtained for different concentrations of Quercetin is tabulated in table 4.

Table 4: Absorbance values obtained for different concentrations of standard

SI. No.	Concentration (µg/ml)	Absorbance (415 nm)
1.	100	0.0271±0.0043
2.	200	0.0300±0.0057
3.	400	0.0722±0.0057
4.	800	0.1160±0.0069
5.	1000	0.146±0.0089

Values expressed as Mean±SEM (n = 3)

A standard graph of Quercetin was plotted using absorbance versus concentration (figure 3). The absorbance was measured at 415nm.

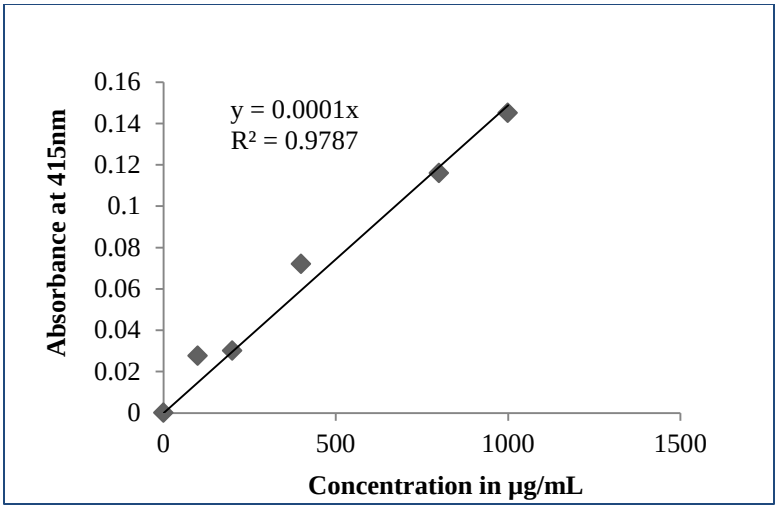

Figure 4: Calibration curve of Quercetin

The total flavonoid content was determined and expressed as gram equivalents of Quercetin per 10mg and the values obtained for different fractions of *M. smilacifolium* were tabulated in table 5.

Table 5: Estimation of total flavonoid content of the various fractions of *M. smilacifolium* leaves.

Sl. No.	Extract fraction	Absorbance (415nm)	Gram equivalents of Quercetin per 10mg
1.	PEMS	0.1943±0.0030	2.7145
2.	CMS	0.1927±0.0019	2.6866
3.	ERMS	0.1334±0.0039	1.8660

*Values expressed as Mean±SEM (n = 3)

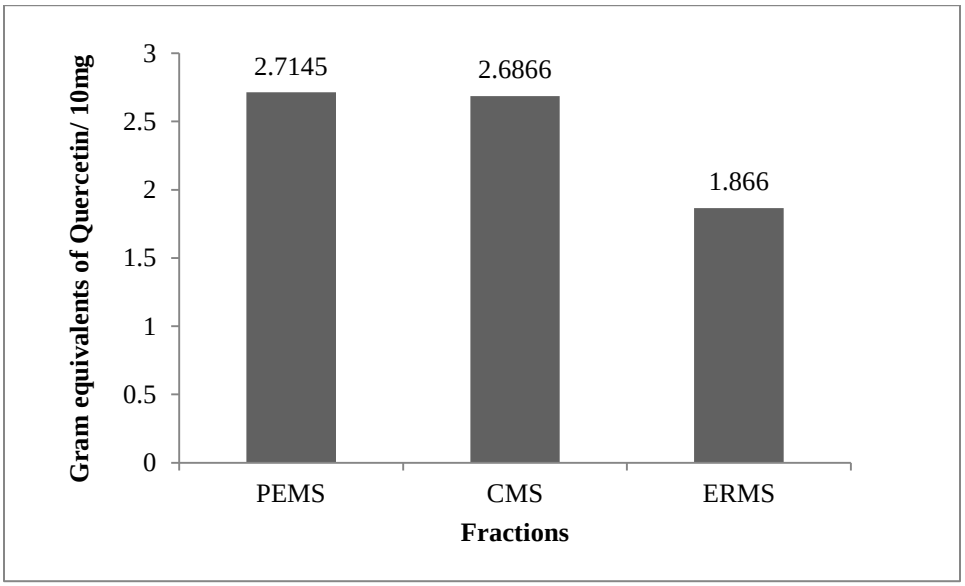

Figure 5: Comparative evaluation of total flavonoid content in various fractions of *M. smilacifolium*

The results showed that, petroleum ether fraction (PEMS) have higher flavonoid content when compared with other fractions.

DISCUSSION

The results of qualitative phytochemical screening of various fractions of *M. smilacifolium*

showed the presence of various phytoconstituents like alkaloids, flavonoids, phenolics, terpenoids, sterols, saponins, glycosides and carbohydrate. The estimation of total phenolic and flavonoid content showed that *M. smilacifolium* leaves are rich in phenolics and flavonoids. The phenolic compounds are secondary plant metabolites and are abundantly present in human diet. They possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic and have remarkable ability to modify the gene expression. The results showed that higher amount of phenolics are present in ethanol residue compared to other fractions. Flavonoids are secondary plant metabolites with polyphenolic structure and are well known natural antioxidants. The results revealed the presence of higher amount of flavonoid content in petroleum ether fraction.

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