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RESEARCH ARTICLE

Molecular Structural Elucidation of Coloumn Secondary Fraction in *Malvaviscus penduliflorus* (*Malvaceae*)

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ABSTRACT

Malvaviscus penduliflorus (*Malvaceae*) is a popular erect under shrubs. It is garden ornamental widely grown across tropical and subtropical regions. The plant widely grown as both garden ornamentals and medicinal plants with varied ethnomedical uses, mostly for wounds, fever, hypertension, sore throat, bronchitis, gastritis, and liver and gall bladder problems. The pharmacognostic studies such as moisture content, ash values, extractive values, histology, and powder analysis were carried out. Successive solvent extraction and phytochemical screening were carried out. The extracts showed the presence of alkaloids, glycosides, phenols, steroids, terpenoids, carbohydrates, and saponins. The fraction was extracted from leaves and purified by column chromatography. The phytochemical studies gave conformation of the above said results. Molecular Formula: C20H18O10, Formula Weight: 418.354, Composition: C(57.42%) H(4.34%) O(38.24%) 16-(25,27-dihydroxyphenyl)-8-hydroxy-11,12-dihydro-15H-7-benzopyran-10-one)-5-(hydroxymethyl)-3-hydrofuran-2,4,6-triol.

Keywords: *Malvaceae*, *Malvaviscus penduliflorus*, Therapeutic uses

INTRODUCTION

Malvaviscus species flowers are complete, bisexual, that is, with functional male (androecium) and female (gynoecium), including stamens, carpels, and ovary. Pollination is entomophilous, that is, by insects, or cleistogamy, that is, by self or allogamy, that is, by cross pollination. Flowering/Fruiting: Throughout the year, *Malvaviscus arboreus* are also suitable for the preparation of jellies, salads, and herbal teas. The biotechnological development of plant products is importance to therapeutic, cosmetic, and pharmaceutical value in particular to chemotaxonomic value.

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MATERIALS AND METHODS

The *Malvaviscus penduliflorus* (*Malvaceae*)[1] was obtained in Kasaragod of area located with the latitude of, Kerala, India. The latitude is 12.507643 N, and the longitude is 74.988213 E. and subjected to Maceration procedure using Aqueous methanol as the solvent and the resulting *M. penduliflorus* (*Malvaceae*) extracts are concentrated under vacuum and preserved in a refrigerator and the percentage yield was studied and recorded.

Column chromatography of aqueous methanolic maceration extract of *M. penduliflorus* (*Malvaceae*)

Aim

The aim of this study was to isolate the active constituents from the Aqueous Methanolic

Maceration extract column chromatography was attempted. A preliminary column was set up and the collected fractions were monitored by TLC. Based on the TLC, the main column was started.

Requirements

- Stationary phase-Silica gel G (100–200 mesh)
- Mobile phase-Pet-ether (60–80), Hexane, Benzene, Chloroform, Methanol, and water
- Volume of each fraction-25 mL.

Procedure

- Pre-column preparation: The pre-column preparation included adsorption of the selected extract/fraction onto silica gel, charging, and saturation of the column.
- Adsorption of the extract: The extract was adsorbed on stationary phase in ratio 1:2 and dried at 60°C.

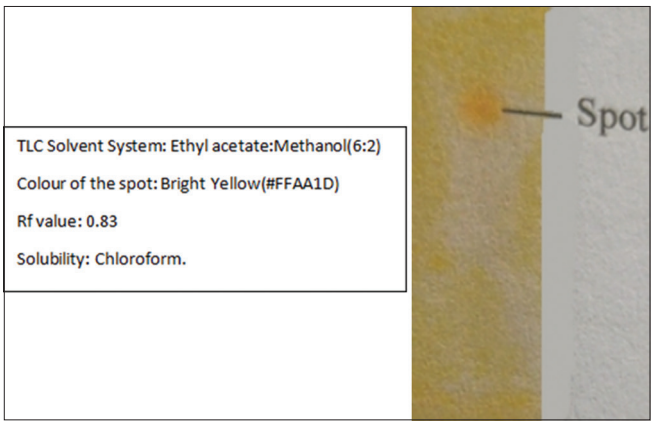


Figure 1: Thin layer chromatography

- Preparation of column: A glass column was selected and rinsed with the solvent. A piece of cotton was placed at the bottom of the column and the column was charged with the slurry mixture prepared from the adsorbent silica gel and the solvent in a ratio of 1:20. The column of silica gel was allowed to stabilize till a clear layer of solvent was observed above the packed column.
- Charging of column: The dried extract was adsorbed on silica gel and was charged into the column. Another layer of cotton was placed over the charged matter to prevent the disturbance of the extract bed while pouring the eluting solvent from the top.
- Saturation of the column: The charged column was left for 6 h for complete saturation and removal of air bubbles to make the bed static.
- Elution: The charged column chromatography of aqueous methanolic macerated extract of leaf of *M. penduliflorus* (*Malvaceae*) was eluted with mixture of two solvents whose proportions were varied to gradually increase the polarity. The column was first eluted with 100% hexane. The polarity of mobile phase hexane was increased with pet ether from 10 to 100% followed by chloroform, methanol, and water. Fractions each of 25 mL were collected and monitored by TLC. The fractions were collected and dried in rotary evaporator and weighed. All the fractions were subjected to TLC for the identification of the eluted compounds.

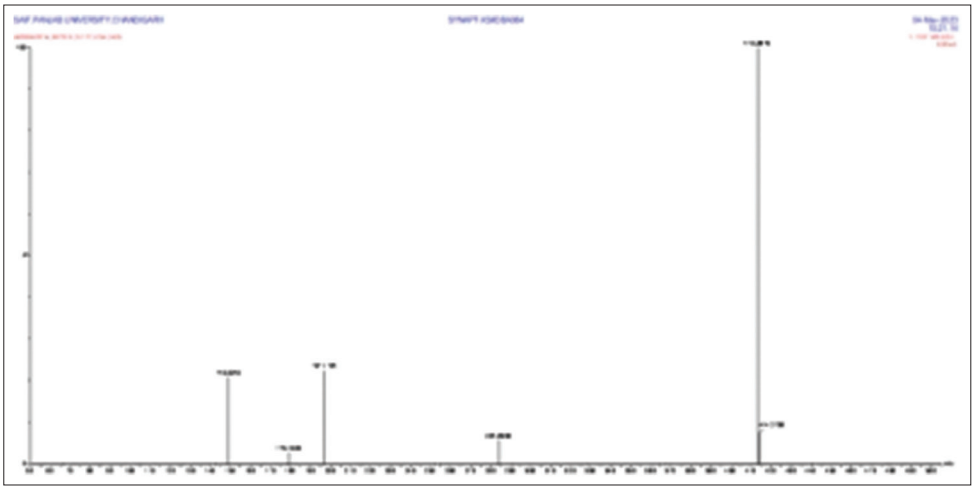


Figure 2: Carbon nuclear magnetic resonance/figure

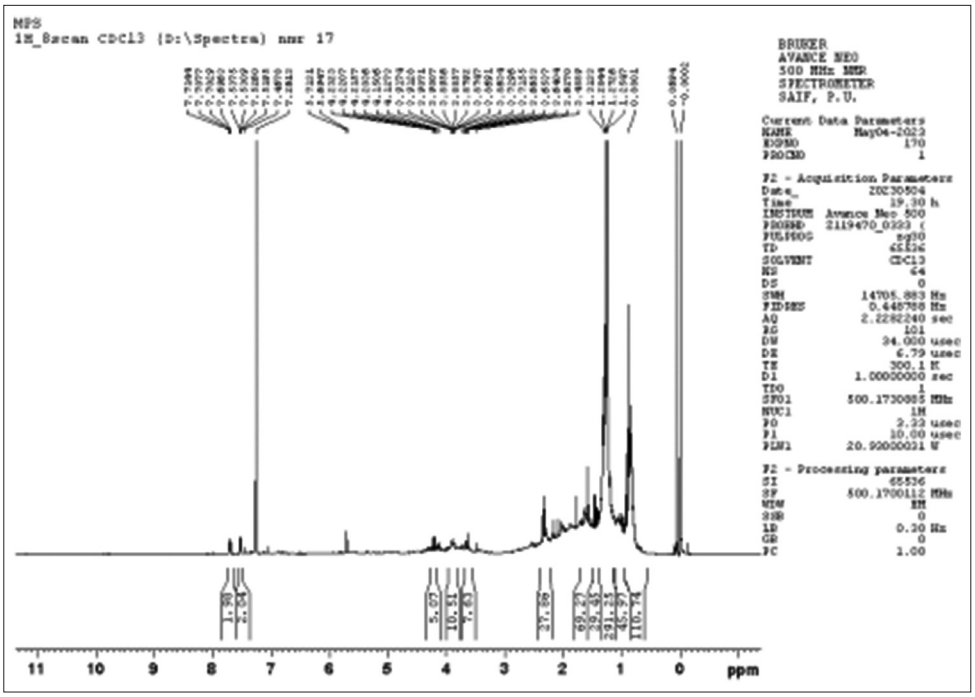


Figure 3: Hydrogen nuclear magnetic resonance

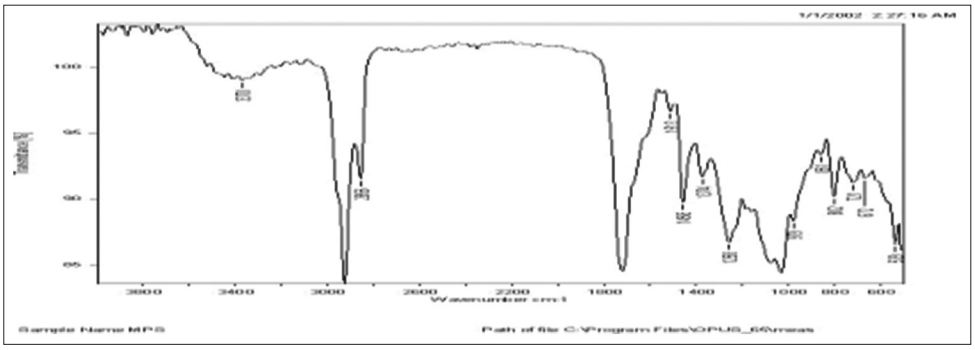


Figure 4: Fourier transmission infrared resonance

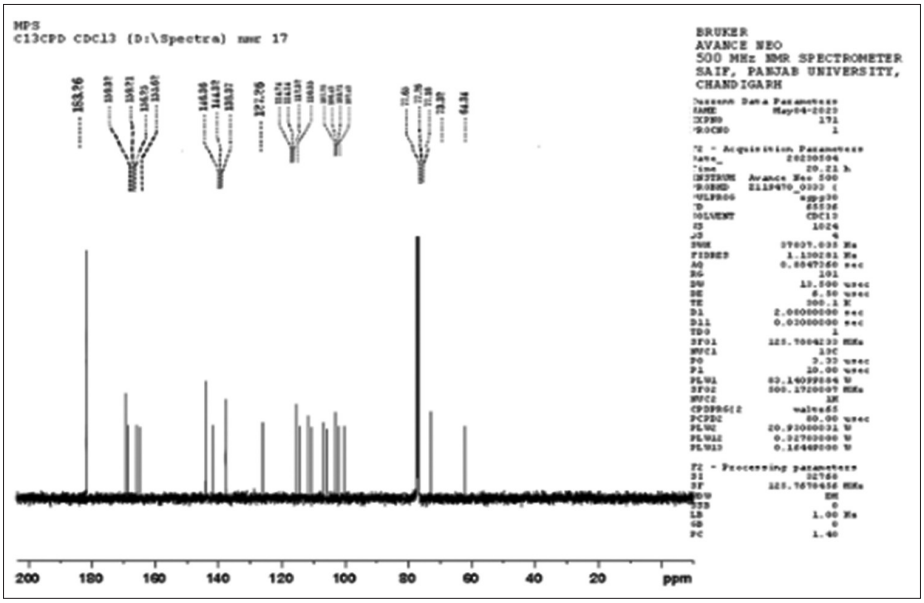


Figure 5: Mass spectrometry

Table 1: Instrumental Reading of FTIR / H-NMR VALUES / H-NMR NUCLEUS VALUES / INTERPRETATION / C-NMR VALUES / INTERPRETATION / MASS

Ir	H-nmr	H-nmr nucleus values	Interpretation	C-nmr values	Interpretation	Mass
3370	0.8801	45.97	-CH3	0.00		413.2670
2925*	1.2547	291.25	-CH3	14.12	R-CH3	
2856	1.2726	291.25	-CH3	22.70	R-CH3	
1725*	1.2844	291.25	-CH3	29.37	R-CH2-R	
1458	1.3133	291.25	-CH3	29.71	R-CH2-R	
1374	3.4889	27.86	-OH	31.94	R-CH2-R	
1258	3.6270	27.86	-OH	76.76	R-CΞC-R	
997	3.6404	27.86	-OH	77.02	R-CΞC-R	
861	3.6537	27.86	-OH	77.27	R-CΞC-R	
802	3.6653	27.86	-OH			
721	3.7155	7.63	-OH			
672	3.7296	7.63	-OH			
539	3.8634	10.51	-OH			
	3.8691	10.51	-OH			
	3.8792	10.51	-OH			
	3.8857	10.51	-OH			
	3.8956	10.51	-OH			
	3.9007	10.51	-OH			
	3.9071	10.51	-OH			
	3.9128	10.51	-OH			
	3.9174	10.51	-OH			
	4.1273	5.07	H-C-O			
	4.1506	5.07	H-C-O			
	4.2036	5.07	H-C-O			
	4.2157	5.07	H-C-O			
	4.2207	5.07	H-C-O			
	4.2323	5.07	H-C-O			
	7.4678	2.04	C6H6-CH			
	7.5195	2.04	C6H6-CH			
	7.5260	1.98	C6H6-CH			
	7.5309	1.98	C6H6-CH			
	7.5375	1.98	C6H6-CH			

RESULTS

Compound 1

Compound 1 was aqueous methanolic column fraction obtained (Yield 10.6 g) when the column was eluted with solvent system ethyl acetate: Methanol (6:2) Rf value of 0.8 is given in Figure 1.

One Spot were isolated and found homogenous by TLC.

One spot was isolated and found homogenous by TLC. By instrumental analysis, one compound is identified with a molecular weight of 584.56634 and had a molecular formula of $C_{27}H_{36}O_{14}$ and analyzed for C (55.48%), H (6.21%), and O (38.32%) revealed, In the ^{1}H-NMR, C-NMR, FTIR observation from Figures 2-5, revealed in Table 2, that, the Structure 1.

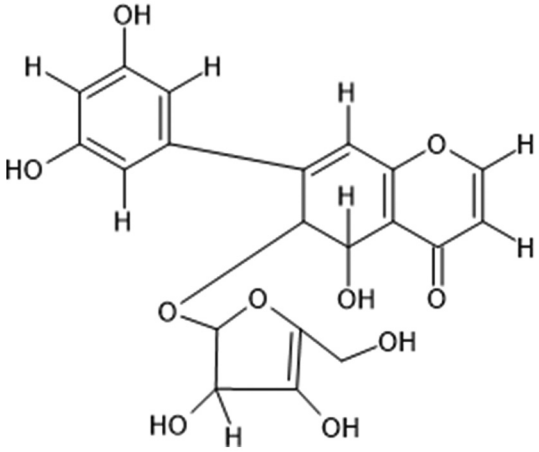
Ring

- medium C-H stretching alkane × 2
- medium C-H bending alkane × 1
- strong C-H bending 1,2,4-trisubstituted × 1(option)

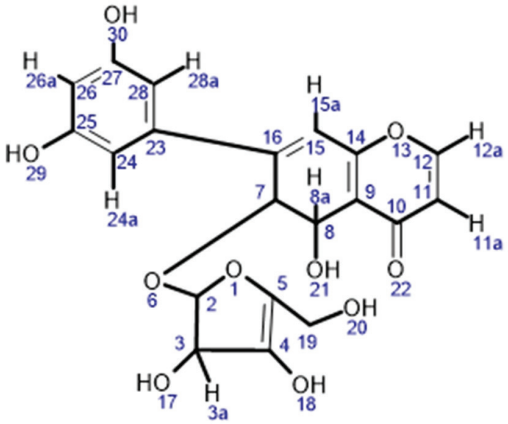
Phytochemistry

S. No	Qualitative test	Pet. Ether	Chloroform	Methanol	Aqueous
1	**Alkaloids**				
a.	Mayer's test	-	-	-	-
b.	Wagner's test	-	-	+	+
c.	Hager's test	-	+	+	+
d.	Dragendorff's test	+	+	+	+
2.	Glycosides				
a.	Legal's test	-	-	-	+
b.	Baljet's test	+	+	-	+
c.	Libermann Buchard test	-	-	-	-
d.	Borntrager's test	-	-	-	-
e.	Modified Borntragers' test	-	-	+	-
3.	Phenolics				
a.	Ferric chloride test	-	-	-	-
b.	lead acetate test	-	-	-	+
c.	Decolorization	-	-	+	++
4.	Flavones and Flavonoids				
a.	Aqueous NaOH test	-	-	-	-
b.	Ammonia test	-	-	-	-
5.	Carbohydrates				
a	Molisch's test	-	+	+	++
b.	Benedict's test	-	+	+	-
c.	Fehling's test	-	+	-	+++
6.	Proteins and Amino acid				
a.	Millon's test	-	-	+	-
b.	Biuret test	-	-	-	+
c.	Ninhydrin test	-	-	+	++
7.	Terpenoids				
a.	Salkowski's test	-	+	+	+
8.	Sterols				
a.	Libermann buchard test	-	-	-	-
b.	Salkowski's test	-	+	+	+
9.	Saponins				
a.	Foams test/ Froth test	-	-	+	++
10.	Gum and mucilages	-	-	-	+

- strong C-H bending 1,3-disubstituted × 1(option)
- medium C=C bending alkene × 1
- strong C=C bending alkene × 3
- strong C-O stretching aromatic ester × 1
- C=O × 1
- strong, broad O-H stretching × 1
- medium O-H bending alcohol × 1



Formula Weight: 418.354



Structure 1: 16-(25,27-dihydroxyphenyl)-8-hydroxy-11,12-dihydro-15H-7-benzopyran-10-one)-5-(hydroxymethyl)-3-hydrofuran-2,4,6-triol

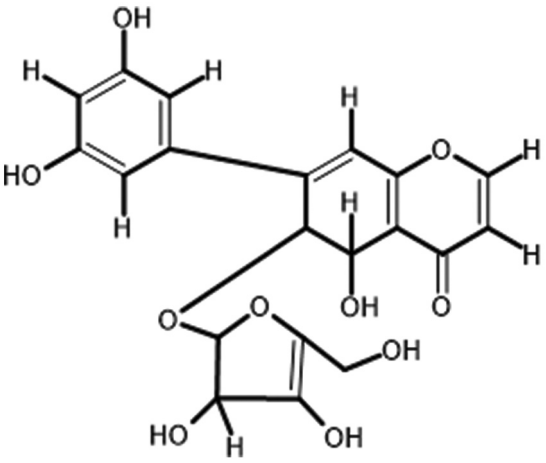
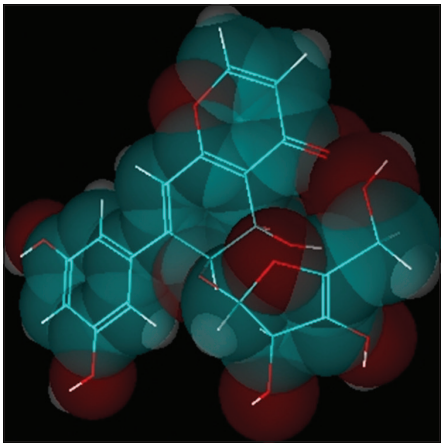


Table 2: Phytochemistry observation by IR

IR	VUES IN RANGE	BOND PREDICTION	APPROPIATE CHEMICAL GROUP	RANGE IN GROUPS
3370	3550–3200	strong, broad	O-H stretching	
2925*	3000–2840	medium	C-H stretching	alkane
2856	3000–2840	medium	C-H stretching	alkane
1725*	1750–1700		C=O	
1458	1465	medium	C-H bending	alkane
1374	1420–1330	medium	O-H bending	alcohol
1258	1310–1250	strong	C-O stretching	aromatic ester
997	995–985	strong	C=C bending	alkene
861	880±20	strong	C-H bending	1,2,4-trisubstituted
OR	880±20	strong	C-H bending	1,3-disubstituted
802	840–790	medium	C=C bending	alkene
721	730–665	strong	C=C bending	alkene
672	730–665	strong	C=C bending	Alkene/benzene derivative
539			δ(OUT OF PLANE)	solvent



- Molecular Formula: C20H18O10
- Formula Weight: 418.354
- Composition: C(57.42%) H(4.34%) O(38.24%)
- Molar Refractivity: 98.40 ± 0.4 cm^3
- Molar Volume: 236.2 ± 5.0 cm^3
- Parachor: 764.4 ± 6.0 cm^3
- Index of Refraction: 1.772 ± 0.03
- Surface Tension: 109.6 ± 5.0 dyne/cm
- Density: 1.77 ± 0.1 g/cm^3
- Dielectric Constant: Not available
- Polarizability: 39.01 ± 0.5 10–24 cm^3
- RDBE: 12
- Monoisotopic Mass: 418.089997 Da
- Nominal Mass: 418 Da
- Average Mass: 418.354 Da
- M+: 418.089448 Da
- M-: 418.090545 Da
- [M+H]+: 419.097273 Da
- [M+H]-: 419.09837 Da
- [M-H]+: 417.081623 Da
- [M-H]-: 417.08272 Da

CONCLUSION

M. penduliflorus Malvaceae belongs to the *Malvaceae* family and was collected and dried under shade. It was then powdered and is taken for extraction by maceration technique using aqueous methanol as solvent. After maceration, the collected extract was taken for the column chromatography. Solvent was selected and their proportions were varied to gradually increase the polarity.

The polarity of mobile phase hexane was increased with pet ether from 10 to 100% followed by chloroform, methanol, and water. Fractions each of 25 mL were collected. The fractions were collected and dried in a rotary evaporator and weighed. From all the fractions, two fractions were selected; they are 100% methanol fraction and 75% methanol, 25% aqueous fraction. These two fractions were taken for H NMR, C NMR, FTIR, and Mass instrumental analysis. These fractions were also subjected to TLC for the identification of the eluted compounds and the Rf values were calculated.

The main active component found by new research are Methanolic fractions of Rf, FTIR, H&C NMR, MS as.

ACKNOWLEDGMENT

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REFERENCE

1. Hafez OH, Refaat J, Abdelmohsen UR, Desoukey SY. Botanical studies of leaves of *Malvaviscus arboreus* Cav. family: *Malvaceae*, cultivated in Egypt. J Pharmacogn Phytochem 2017;6:149-53.