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Isolation, quantification and structure of polysaccharides from *Manilkara hexandra* bark

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Abstract : Plants are the most important source of human survival. Plant polysaccharides are ideal candidates for therapeutics with immunomodulatory, antitumor and wound-healing action. *Manilkara hexandra* (*Mimusops hexandra)* is an evergreen tree belongs to family *Sapotaceae*. The present study deals with the isolation and quantification of the water soluble polysaccharides from the bark of *Manilkara hexandra*. The isolation of the polysaccharides (MHPS) was carried out using standard procedure and was tested for the presence of sugar compounds by both the test tube and TLC methods. Later, the quantification of the four sugar moieties, sucrose, maltose, xylose and lactose was performed by phenol sulphuric acid method. The amount and concentration of sucrose, xylose, maltose and lactose was found to be 0.48, 0.29, 0.42, and 0.425 % respectively. The individual sugar molecules were separated from MHPS by column chromatography. The structures of the isolated polysaccharides were confirmed by IR, NMR and mass spectroscopy.

Keywords : *Manilkara hexandra*; Bioactive polysaccharides; Isolation; Quantification.

INTRODUCTION

Manilkara hexandra (*Mimusops hexandra)* is an evergreen tree belong to family *Sapotaceae*. The *Manilkara* is a genus of trees in the family of *sapotaceae*. Collectively known as *Manilkara* trees, they occur throughout the tropics. Trees of this genus yield edible fruit, useful wood and latex. The best-known species are *M. bidentata* (*Balata*), *M. chicle* (*Chicle*) and *M. zapota* (*Sapodilla*)[1]. The bark is astringent, sweet refrigerant, aphrodisiac, alexipharmic and anthelmintic, it is useful in uiorrhagia, ulitis, odontopathy, fever, colic dyspepsia, helminthiasis, hyper dyspepsia, burning sensation and vitiated conditions of pitta, it retards the fermentation process in toddy[2]. In our previous investigation we found that the polysaccharides of *Manilkara hexandra* bark possesses immunomodulatory activity[3]. In the present investigation we have isolated the individual polysaccharides and the structures were elucidated by spectroscopic analysis.

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MATERIALS AND METHODS

Plant material

Manilkara hexandra bark was collected from Kakatiya University medicinal garden, Warangal district, Andhra Pradesh, India and taxonomically identified and authenticated by the Dr. Raju S. Vastvya, Professor, Department of Botany, Kakatiya University, Hanamkonda. A. P., India. A voucher specimen (PG/2011/01) was deposited in department of Pharmacognosy and Phytochemistry, Vaagdevi college of pharmacy, Hanamkonda, A.P., India for future reference. The collected bark was shade dried and powdered using a mechanical grinder. The powdered plant material was used for the extraction of the polysaccharides.

Extraction of polysaccharides[4]

960 g of the *Manilkara hexandra* bark powder was allowed to stand in 1 L of 0.1 N HCl for overnight at room temperature. The extract was filtered through a typical woman's nylon cloth. Then the filtrate was neutralized with 1 N NaOH, and polysaccharides were precipitated with 3 volumes of ethanol. After centrifugation for 30 min 4000 rpm, the precipitate was re-dissolved in distilled water. Then the pH of the suspension was adjusted to 2.0 with 1 N HCl and $CaCl_2$ was added to the final concentration of 2 M. The resulting precipitate was removed by centrifugation and the supernatant was treated with 3 volumes of ethanol. The ethanol precipitation was repeated twice and the precipitate was re-dissolved in distilled water, and evaporated to get crude polysaccharides designated as MHPS.

QUALITATIVE TESTS FOR EXTRACTED POLYSACCHARIDES[5]

About 50 mg of the MHPS was dissolved in 5 ml of distilled water and tested for the presence of carbohydrates.

Molish's test

2-3 ml of test solution was added few drops of alpha-naphthol solution in alcohol, shaken and added conc. H_2SO_4 from sides of the test tube and was observed for violet ring at the junction of two liquids.

Fehling's test

About 5 ml of test solution was hydrolyzed with 10 ml of dilute hydrochloric acid and neutralized with alkali. To the test tube added 1 ml Fehling's A and 1 ml Fehling's B, boiled for one minute and added equal volume of test solution. Heated in boiling water bath for 5-10 min and observed for brick red precipitate.

Benedict's test

To 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes and observed for precipitate and observed for red precipitate.

Barfoed's test

0.5 ml of Barfoed's reagent and 0.5 ml of test solution were mixed. Heated for 1-2 min in boiling water bath and cooled, observe for precipitate and observed for red precipitate.

Iodine test

To MHPS solution, iodine solution was added and observed for formation of blue colour.

QUANTITATIVE TESTS FOR EXTRACTED POLYSACCHARIDES

The extracted polysaccharides were evaluated quantitatively, using phenol sulphuric acid assay, to determine the content of sugars present in MHPS.

Phenol sulphuric acid assay

Calibration standards (1mg/ml) of sucrose, xylose, maltose and lactose were prepared individually in distilled water. They were transferred to 10 ml test tubes in 5 µl increment ranging from 5-50 µl with an accurate pipette. 10mg of extracted polysaccharides was taken in another 10 ml test tube. All the tubes were added with 500 µl 4% phenol and 2.5 ml sulphuric acid. Absorbance was measured at 490 nm for the sugar standards and the unknown. A graph was plotted against A_{490} Vs sugar weight (5-50 µgm). The intercept of the A_{490} of the unknown sample with the calibration line represents the amount of the sugar present in the sample[6,7].

The concentration and percentage of the sugar component present in the sample can be determined by the

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following formulas,

Concentration (mol/gm) = x gm/Mol. wt (gm/mol) X weight (g)

Percentage of sugar = x gm/ weight (g) X100

Where 'x' is the mass of the sugar deduced from the graph and 'Mol. wt.' represents the molecular weight of the sugar present in the sample and weight is the amount of the polysaccharides taken (0.01 gm).

ISOLATION

The extracted MHPS was subjected to column chromatography to separate the individual compounds. 8 gm of extracted polysaccharides was loaded to a silica gel column of 42cm length and 3mm diameter and eluted with solvent system Ethyl acetate: Pyridine: water (40:20:20 v/v upper phase). Fractions of about 50 ml each were collected at flow rate of 40 drops per minute. Fractions 6-27 with similar TLC profile were pooled and subjected to preparative TLC to recover pure compounds. The purified compounds were designated as I (Rf 0.65) and II (Rf 0.42) and their structures were confirmed using IR, NMR and Mass spectroscopic analysis.

RESULTS AND DISCUSSION

Extraction of polysaccharides from *Manilkara hexandra* (MHPS) were carried out using chemical method and 16.66 gm of crude polysaccharides was obtained and the percentage yield was found to be 1.73% w/w. MHPS gave positive reaction for Molish's test, Iodine test which indicates the presence of polysaccharides.

In quantitative tests four sugars were estimated using phenol sulphuric acid method and the amount and concentration of each sugar was depicted in the form of tables and figures.

1. **Sucrose:** Concentration and percent of sucrose was found to be 0.000014mol /gm and 0.48% respectively. The results were depicted in TABLE 1 and figure 1.
2. **Xylose:** Concentration and percent of xylose was found to be 0.0000193mol/gm and 0.29% respectively. The results were depicted in TABLE 2 and figure 2.
3. **Maltose:** Concentration and percent of maltose was found to be 0.0000122mol/gm 0.42% respectively. The results were depicted in TABLE 3 and figure 3.
4. **Lactose:** Concentration and percent of lactose was found to be 0.0000118mol/gm 0.425% respectively. The results were depicted in TABLE 4 and figure 4.

The amount of each sugar in MHPS is presented in TABLE 5.

Chromatographic techniques were used to isolate individual polysaccharides from MHPS (solvent system Ethyl acetate: Pyridine: Water (40:20:20 V/V) (Upper phase)). The two isolated compounds were designated as I and II and were analysed using IR, NMR and Mass spectroscopy.

Compound I

IR (KBr, cm^{-1}): 669.31 (-CH_2 stretch), 772.5 (Endocyclic -C-O stretch), 1046 (-C-O-C- bridge of glycosidic linkage), 1215.17 (–C-CH stretch), 3583.14

TABLE 1 : Phenol sulphuric acid method for sucrose

S.NO.	CONCENTRATION (µgm)	ABSORBANCE
1.	5	0.386
2.	10	0.391
3.	15	0.402
4.	20	0.413
5.	25	0.423
6.	30	0.433
7.	35	0.442
8.	40	0.454
9.	45	0.464
10.	50	0.475
11	MHPS	0.470

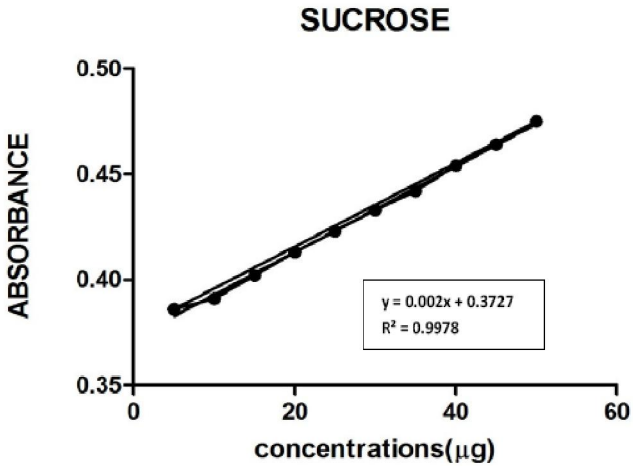

Figure 1 : Calibration curve for sucrose

TABLE 2 : Phenol sulphuric acid method for xylose

S.NO.	CONCENTRATION (µgm)	ABSORBANCE
1.	5	0.395
2.	10	0.413
3.	15	0.427
4.	20	0.443
5.	25	0.458
6.	30	0.474
7.	35	0.489
8.	40	0.503
9.	45	0.519
10.	50	0.535
11	MHPS	0.470

TABLE 3 : Phenol sulphuric acid method for maltose

S.NO.	CONCENTRATION (µgm)	ABSORBANCE
1.	5	0.358
2.	10	0.369
3.	15	0.386
4.	20	0.402
5.	25	0.418
6.	30	0.433
7.	35	0.450
8.	40	0.464
9.	45	0.480
10.	50	0.495
11	MHPS	0.470

TABLE 4 : Phenol sulphuric acid method for lactose

S.NO.	CONCENTRATION (µgm)	ABSORBANCE
1.	5	0.371
2.	10	0.385
3.	15	0.395
4.	20	0.412
5.	25	0.425
6.	30	0.438
7.	35	0.449
8.	40	0.464
9.	45	0.476
10.	50	0.490
11.	MHPS	0.470

TABLE 5 : Composition of different sugars and their concentration in extracted polysaccharides

Sr. No.	Sugar	Amount Present (µg/ml)	Amount Present (%)	Concentration (mol/gm)
1.	Sucrose	48.13	0.48	0.0000140
2.	Xylose	28.93	0.29	0.0000193
3.	Maltose	42.12	0.42	0.0000122
4.	Lactose	42.48	0.425	0.0000118

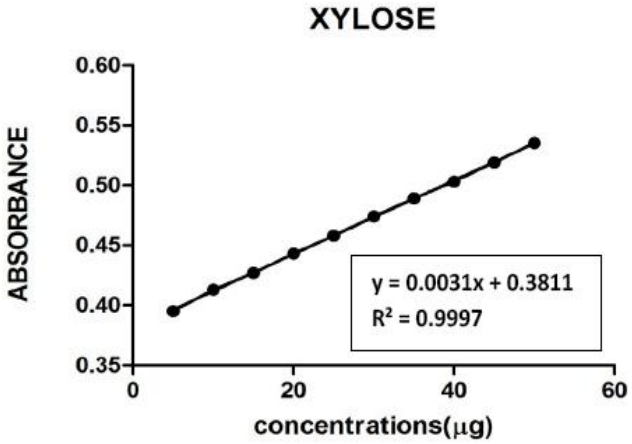

Figure 2 : Calibration curve for xylose

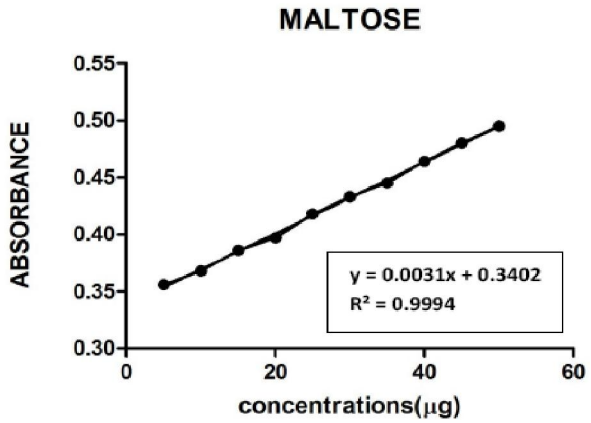

Figure 3 : Calibration curve for maltose

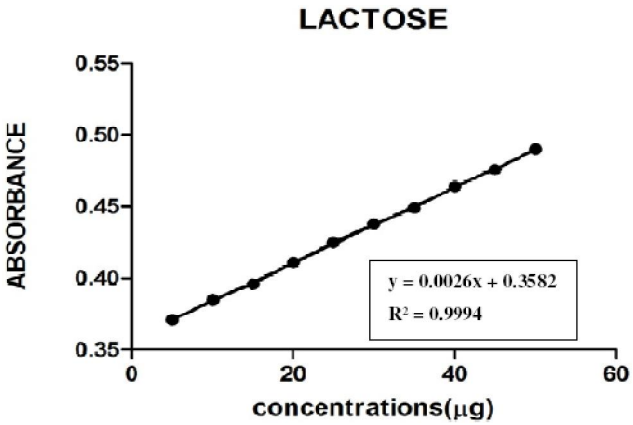

Figure 4 : Calibration curve for lactose

(O-H Stretch), 3672.38 (Free –O-H- vibration); ^{1}H NMR ($CDCl_3$; δ ppm): 3.8-4.5 (10 CH), 3.7-3.8 (4 CH_2), 4.4-4.5 (2 CH_2-OH), 4.7-4.8 (6 CH-OH). MS (EI-MS): 341.1 m/z. From the above IR, NMR and Mass data, compound I was identified as Lactose.

Compound II

IR (KBr, cm^{-1}): 848.8 (-C-O- stretch), 1025.08 (-

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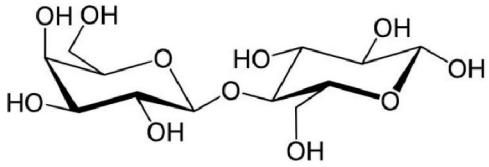
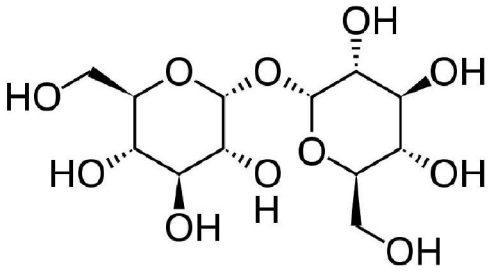
C-O-C- bridge of glycosidic linkage), 1206 (–C-CH strecth), 3298.3 (-O-H stretch); $_1$H NMR ($CDCl_3$, δ ppm): 3.5-3.7 (10 CH), 4.2-4.5 (4 CH_2), 4.5-4.8 (2 CH_2-OH), 5.0-5.5 (6 CH-OH). MS (EI-MS): 379.1 m/e. From the above IR, NMR and Mass data, compound II was identified as Trehalose.



Lactose



Trehalose

CONCLUSION

From the present investigation the different polysaccharides were isolated from *Manilkara hexandra* bark and two of them were confirmed as lactose and trehalose using spectroscopic analysis.

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