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Antimicrobial Examination of Mucilage Obtained from Fruits of Tilkor [*Mamoradica monadelpha*]: A Potential Medicinal Plant

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ABSTRACT: The present study includes isolation and purification of mucilage from fruits of Tilkor and its chemical characterization using FTIR and TLC. The extraction of mucilage is standardized and is purified. FTIR of the pure freeze dried powder showed the presence of complex carbohydrates starch and this confirmed the film forming property of the mucilage. using n-ethanol: acetic acid: water (4:3:7v/v/v) as solvent system Rf values were found to be 0.96; 0.87; 0.95; 0.72; 0.46; 0.20. Also, these spots reflected color under UV light. A prominent Rf of 0.87 was observed in case of sample and standard. The film is effective on *Staphylococcus aureus* MTCC 738 and bacteria isolated from infected skin with 12.25±.03 and 10.03± 0.2mm zone diameter. It did not show any antifungal activity on *Asperfigllus foetidus* MTCC 2736.

Keywords: Fruit mucilage; Coccinia grandis; Ivy gourd and Carbohydrate polymer.

INTRODUCTION: Momoradica Monadelpha commonly called as Tilkor belongs to the family Cucurbitaceae. It is commonly found in India, Pakistan and Srilanka. It is also distributed in Tropical Africa. It is a climbing perennial herb with white and unisexual flowers. Fruits are ovoid to oblong berries green in unripe state and red in ripen state. Seeds are ovoid, compressed and grey in color. Leaves are consumed for traditional prepration. The plant parts such as roots, leaves and fruits are used for numerous medicinal purposes as reported by Vaids of Ayurveda.

Who use this plant in various herbal preprations. But it pharmalogical activities are not yet established by clinical trial. However, the pharmalogical activities of some other members of its family (such as Coccinia grandis etc.) are already reported in literatures.

The methanolic extract of leaves possess analgesic and antipyretic activity.[1] The methanolic extract of the fruits have anti-tussive property.[2]

The plant also possesses Blood-sugar lowering effect.[3] Its leaves and fruits also have Anti-Oxidant property.[4] Ethanol extract of leaves has Antidyslipidemic activity.[5] Ethanolic extract of its leaves possesses significant hepatoprotective activity.[6] The fruits show Anti-oxidant and Anti-inflammatory properties.[7] Aqueous extract of the leaves shows significant Anti-malarial activity.[8] Root extracts of Coccinia grandis possesses Anti-oxidant activity.[9] The leaves of this plant show Antiulcerogenic and antioxidant effect.[10] The leaf and stem extracts exhibit Anti-bacterial activity.[11]

There were studies on mucilage extraction from different parts of many plants and their applications as pharmaceutical excipients. Mucilages are most commonly used adjuvant in pharmaceutical preparations. Plant mucilages are pharmaceutically important polysaccharide with wide range of applications such as thickening agent, binding, disintegrating, suspending, and emulsifying, stabilizing and gelling agents. They have been also used as matrices for sustained and controlled release drugs. Naturally available mucilages are preferred to synthetic materials due to their non toxicity, low cost, emollient and non irritating nature. Acacia, tragacanth, gum ghati, gum karaya are popular examples of plant mucilages.

In the present work the method of isolating the mucilage from the fruit is standardized. The compounds in the mucilage are screened by chemical and analytical methods. It was found from literature that the mucilage of the fruit was not studied for its antimicrobial

property. Hence, antimicrobial activity of the film formed by the mucilage is studied on microbes isolated from skin infection and standard strains. A simple method of desiccation is used for film formation. As there are no other synthetic chemicals added as film forming agents the mucilage thus obtained can be of great importance in food and cosmetic industry which can serve as an alternative and cheep source.

MATERIALS AND METHODS: Tilkor fruits were taken from the *medicinal plant garden of* Shri Himanshu Shekhar Malik. It was identified by Prof. Shashi Shekhar N. Sinha. All the chemicals used were of analytical reagent-grade.

Extraction of mucilage: The fruit pulp was made and initially heated with steam at 70^0C for 5 min to inhibit enzymatic browning reaction. The fruit pulp was homogenized with five times its weight of water. The solution was heated at 50°C for 8 hrs and then filtered with muslin cloth. The filtrate was collected and the mucilage was precipitated with three times its volume of ethanol and washed with ethanol followed by acetone. The cream colored solid was dried under vacuum (less than 1 Torr at 20°C for 13 hrs) and gave a yield of 7 g mucilage/kg fruits. The yield was found to be more from ripened fruits under the same conditions.

Purification of the mucilages: The crude mucilage was homogenized with cold dilute trichloroacetic acid solution **(7**%). The solution was centrifuged (3700 g for 25 min), neutralized with sodium hydroxide by drop wise addition, and then dialyzed for 35 hrs against distilled water. The mucilage was re-precipitated with ethanol (three volumes), and washed successively with ethanol, acetone and diethyl ether. Then mucilage was dried by freeze dryer as well as crushed and sieved via 60 meshes. The mucilage powder was stored in desiccators for use in subsequent tests.

Phytochemical analysis: Phytochemical analysis was done following the method of Trease and Evans & Kokate. The aqueous extract and mucilage powder of fruit samples were subjected to chemical tests for identification of its active constituents.

Proximate Analysis: The water extract and mucilage powder were also analyzed for chemical properties such as contents of fat, protein, amino acids, moisture, ash, carbohydrate, soluble and insoluble dietary fibers. Moisture and ash were determined by AOAC methods. Presence of metals in the mucilage was determined by atomic absorption spectroscopy (Techtron Model AA 100) on the digested mucilages.

Flouorescence: The fluorescence characteristics of aqueous extract and powdered mucilage under UV light after treating with different chemical reagents were studied using transilluminator and reported.

FTIR Analysis of Mucilage powder of Tilkor fruits: The amount of information that can be obtained from the infra red spectrum of polysaccharides is rather limited. The spectra are reported principally as a fingerprint for any future identification, since the absorption bands for such complex compounds are usually broad and diffuse. FTIR spectrometer (Nicolet, Thermo Electron) and software (Opus 6.5) were used in the test. Sample holder was cleaned with acetone prior to analysis. Background peak was obtained before putting sample and sample peak was obtained later. The spectra obtained was smoothened and saved.

Thin Layer Chromatography of Tilkor Mucilage: The TLC glass plates coated with silica gel G (20gms/50ml of water) were dried at room temperature. The plates are then activated by heating at 120^oC for 50min in an oven. The mucilage and standard quercetin are applied on to the plates 1cm above the edge of the plate. These plates were developed in an airtight chromatographic chamber containing 250ml of solvent mixture. Nine different solvent systems used were checked for separation.

Acetone: water (25: 75 v/v); 1-propanol: ethylacetate : water (8: 3: 2 v/v/v) ; dichloromethane: Methanol: 99.5% Acetic acid: water (59: 60: 35: 20 v/v/v/v); 2-propanol: 0.85 % water solution of boric acid : 99.7% acetic acid (45: 5: 2 v/v/v) ; Acetonitrile water : 95% HCl (18: 4: 2 v/v/v) ; 1-butanol : 1-propanol : Acetic acid : water (35: 15: 15: 20 v/v/v/v) ; Acetonitrile : water (8: 5 v/v); 8) 1-propanol : ethylacetate : water (3: 0.7: 0.8 v/v/v); n-butanol: glacial acetic acid : water (3:2:1v/v/v). Among them n-butanol: glacial acetic acid: water (3:5:2v/v/v) was found to be good for separation. The spots were visualized by exposing the plates to iodine vapor and to ammonia. Dried plates were also subjected for fluorescence under UV.

Polymeric property of the Mucilage: The mucilage powder is dispersed in a little quantity of distilled water at a minimum concentration of 7.5% and freeze dried for 15 hrs and drying was continued in desiccators to obtain a film of mucilage.

Antimicrobial activity: Antimicrobial activity is performed by using agar diffusion method against *Staphylococcus aureus* MTCC 737, *Asperfigllus foetidus* MTCC 2737 train and skin isolates collected by using sterile cotton swab from the puss of infected

patients. Film measuring around 1cm (l × b) and a thickness of 1mm is used for the test. The skin isolate.

RESULTS AND DISCUSSION:

Phytochemical characterization: Aqueous extracts of the fruit was found to contain Alkaloids, Flavanoids, Proteins, Carbohydrates, Tannins, phytosterols, saponins and fixed oils but the mucilage was composed of only carbohydrates, glycoprotein, flavonoids, fixed oils, and fats. The purified mucilage is devoid of alkaloids, tannins, phytosterols and saponins which are found to be present in the crude extract as presented in table 1.

Table 1: Preliminary Phytochemical Tests.

S. No.	Test	For Aqueous Extract	For mucilage
		(without precipitation)	(after precipitation)
1	Flavanoids	Present	Absent
2	Mucilage	present	Present
3	Carbohydrates	Present	Present
4	Tannins	Present	Absent
5	Alkaloids	present	Absent
6	Phytosterols	Present	Present
7	Glycosides	Absent	Absent
8	Proteins	present	Present
9	Fixed oils & fats	Present	Present
10	Saponins	Absent	Absent
11	Lignin	Absent	Absent

Proximate Analysis and Fluorescence: Metals were found to be absent in the mucilage. In the water soluble extract the fat content is found to be 0.36±0.05 g, 1.06±0.03g of protein, 87.23±0.02g of moisture, 1.43±0.05 g of ash, 4.04±0.02 g of carbohydrate, 2.85±0.03g and 3.07±0.01g of soluble and insoluble dietary fiber. Mucilage is found to contain 0.15±0.05g of fat, 0.25±0.03 g of protein, 1.07±0.08 g of ash, 8.85±0.05g of carbohydrate and soluble dietary fiber 88.83±0.07g. The mucilage is devoid of moisture and insoluble dietary fiber and the results are presented in table 2.

FTIR Analysis: FTIR spectroscopy is used to investigate the vibrations of molecules and polar bonds between the different atoms. The carbohydrate region lies between 1200 and 900 cm^{-1} while the fingerprint or anomeric region of carbohydrates lies between 900-700 cm^{-1} (Table 3). By referring to wavelengths of mono saccharides from previous research, the result showed that Tilkor fruit mucilage contained starch as a major component (indicated by the prominent peak at 1018cm^{-1}). It was also confirmed by FTIR of reference starch. Result also indicated that mucilage was composed of non-polar chemical groups such as alkyl groups like methyl and some hydrophilic groups that contributed to the hydrophilic characteristics of mucilage like hydroxyl group, carbonyl group and amino group. Peak at 1623 cm^{-1} and 2922 cm^{-1} confirms the presence of proteins and amino acids. The data was represented in Table 3.

Table 2: Chemical components of Water extract.

Chemical component	Content(g/ 100)	
	Extract	Mucilage powder
Fat	0.15 ± 0.05	0.36 ± 0.05
Protein	0.25 ± 0.03	1.06 ± 0.03
Ash	1.07 ± 0.08	1.43 ± 0.05
Moisture	ND	87.23 ± 0.02
Carbohydrate	8.85 ± 0.05	4.04 ± 0.02
Soluble dietary fiber	88.83 ± 0.07	2.83 ± 0.03
Insoluble dietary fiber	ND	3.07 ± 0.04

Results are expressed as the mean ± SD for three replications.

Table 3: FTIR Analysis of Mucilage powder.

S. No.	Band wave number	Type of bond/chemical
1	665 cm^{-1} – 685 cm^{-1}	C-H bending of Alkynes
2	900 cm^{-1} – 625cm^{-1}	C-H bending in aromatic rings
3	822 cm^{-1}, 755 cm^{-1}	C-C Stretching vibrations
4	1018 cm^{-1}	C-O-H deformations (Alcoholic C-O Stretch) / Starch
5	1376 cm^{-1}	Symmetrical C-H bending in methyl groups
6	1455 cm^{-1}	Asymmetrical C-H bending in methyl groups
7	1535 cm^{-1}	N-H group bending
8	1623 cm^{-1}	C=N bond Indicates proteins/Aminoacids
9	2922 cm^{-1}	-CH_2 group stretching
10	2965 cm^{-1}	Saturated hydrocarbon containing methyl group
11	3000-2800 cm^{-1}	C-H stretching in alkanes
12	>3000 cm^{-1}	C-H stretching in aromatic and heteroaromatic rings
13	3322-3265 cm^{-1}	C-H stretching of monosubstituted alkynes
14	3710-3513 cm^{-1}	Vibrations of free O-H groups

TLC analysis: The Rf values were found to be 0.96; 0.87; 0.95; 0.72; 0.46; 0.20 for the mucilage and 0.87 for quercetin. Also, these spots reflected color under UV light. From the prominent Rf values it can be concluded that the extract contains quercetin. The results are mentioned in table 4.

Table 4: Result of TLC Analysis of mucilage powder.

S. No.	Prominent	Colour Observed	Constituent
	Rf Value	**Under UV Light**	
1	0.87	blue	Quercitin
2	0.85	Purplish blue	extract

Antimicrobial property: Gram positive and gram negative rods are observed in the skin isolates. The film had shown good antimicrobial activity on these bacterial isolates. Zone diameter of about 12.22± 0.03 mm for staphylococcus and 14.01±0 .1 mm for the skin isolate is observed. The film had no activity on Aspergillus foetidus. Good antimicrobial property of the mucilage on skin isolates can thus be attributed to the presence of quercetin. This property of the mucilage can make it an important ingredient of many skin preparations and also in cosmetic industry.

CONCLUSION: From the results obtained, it can be concluded that the procedure for isolating mucilage from Tilkor is standardized and mucilage isolated contains starch which may be responsible for the film forming property of the mucilage. A good film can be formed at a concentration of 6.7%. This film forming property can be explored for its use as a sustained or controlled release polymer in the formulation of different pharmaceutical dosage forms. Also, the results of TLC shows that n-ethanol: acetic acid: water (4:3:7 v/v/v) can be good solvent systems for the separation of components of mucilage. From the Rf values presence of quercetin in the mucilage can be confirmed.The film formed is effective on *Staphyloccoccus aureus* and skin isolates. It is ineffective on fungi. This important property can be further used in the preparation of skin protectants. As the mucilage is found to be rich in carbohydrates it can form an alternative for food preparations which can be further established.

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