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ISOLATION, PURIFICATION AND CHARACTERIZATION OF THE GUM EXUDATES FROM MARDI (TERMINALIA TOMENTOSA)

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ABSTRACT

Mardi (Terminalia tomentosa) is Important gum producing tree of Gadchiroli district and having considerable, commercial and industrial importance therefore the study undertaken for isolation of gum exudates obtained from Mardi and their characterization and evaluation. The Gum was dried, pulverized and drawn out using distilled water and isolated by acetone. Various physicochemical tests and other parameters like micromeritic properties, swelling index and viscosity were evaluated for characterizing the isolated and purified gum. The result revealed that water-based extraction of gum has excellent flow properties. It has a good swelling index of 44 ± 0.58%. The gum was examined for purity by carrying out various phytochemical tests and showed that carbohydrates, sugar and amino acids and gum were found to be present. The pH of 1% solution of gum was found to be 5.6 ± 0.01 and the total ash value was found to be 3.20 ± 0.01. Bulk density, tapped densities were found to be 0.73 ± 0.01 g/cm3 and 0.91 ± 0.01 g/cm3, respectively. Bulkiness was found to be 1.37 ± 0.02 cm3/g. Hausner's ratio and carr's index were found to be 1.25 ± 0.01 and 19.78 ± 0.12%. Extracted gum wasswells and forms a gel with cold water, form a viscous colloidal solution in hot water and this property can be utilized for sustained drug delivery. The results of evaluated parameters showed that mardi gum has satisfactory pH and physicochemical properties, which can be used as pharmaceutical adjuvant in formulating various dosage forms.

KEY WORDS

Adjuvant, Characterization Gum, Isolation, Mardi (Terminalia tomentosa).

INTRODUCTION

Mardi (Terminalia tomentosa Roxb (ex DC) Wight &Arn, Synonyms: Terminalia alata Heyneex. Roth, Terminalia crenulata Roth, Terminalia elliptica Willd.) is member of family Combretaceae. It is a large deciduous tree, 20-35m high & 1m in diameter [1]. The plant is commonly known in Sanskrit as Asana, in English as Black murdah, in Hindi as Asan, Saj, Sain and in Marathi as Ain [2], in Gondi Language plant is known as mardi.

The plant is commonly found in the forests, especially in the Indian humid regions, including the sub Himalayan tracts of North West provinces, Sikkim and Nepal, also Peninsula Southwards [3]. The plant is uses for many pharmacological properties like antioxidant [4], antifungal [5], anti-hyperglycaemic [6], anti-diarrhoeal& anti leucorrheal [7].

The Plant bark is useful in conditions of pitta, dysentery, ulcers, vata, fractures, leucorrhoea, haemorrhages, bronchitis cardiopathy, strangury, haemoptysis, wounds, cough, verminosis, gonorrhea & burning sensation (Ayurveda)[8,9]. Phytoconstituents reported such as tannins like arjunic acid, arjunolic acid, arjunetin, ellagic acid, gallic acid, and triterpenoids like oleanolic acid, betulinic acid and steroid like β-sitosterol in T.tomentosa [10, 11, 12, 13, 14].

From the literature survey, it was learnt that no substantial work has been carried out on the gum Exudates of mardi. Hence an attempt was made to investigate gum exudates for physicochemical

parameters, Micromeritic properties, organoleptic properties and physical properties.

MATERIAL AND METHODS:

Materials

Mardi exudates were collected from Muska region of district Gadchiroli, Maharashtra, India, in the month of January –March. All chemicals used were of analytical grade.

Methods

Collection and purification of exudate gum

Plant exudate Isolation and collection

For exudate extraction, the selected Mardi plants were subjected to the stress by making injury on the trunk in first week of January and collected in February and month of March. Collected gum exudates were treated with petroleum ether and chloroform (to remove pigments and chlorophyll) and then carefully washed with distilled water16, dried under shade for 24 h, further dried at 30–40°C until constant weight was obtained. Size was reduced through grinder. Powdered gum passed through sieve no. #22 and stored it in air tight container for further use.

Purification of exudate: Purification of the exudate gum was carried out using the combination of methods reported by UzmaFarooq [15] and Girish K Jania [16], with minor modifications.

Procedure includes two steps.

Step 1: As the authors described elsewhere, the powdered gum was put in 500 mL of distilled water in a 1000 mL beaker, then heated and stirred continuously for approximately 4 h. The concentrated solution was filtered through muslin cloth and cooled at 4°C-6°C

Step 2: Isolation of gum: - To the extract, three-fold quantity of acetone (1:3) was added for precipitation of gum to occur. The precipitated gum was washed with acetone and then collected through filtration by muslin cloth. Gum was further dried in hot air oven at a temperature less than 40°C. The obtained dried gum was grinded and passed through sieve #60 and finally stored in air tight container until further use.

Gum was dissolved into sufficient quantity of water for 48 hrs

↓

Colloidal solution was obtained

↓

Colloidal solution was filtered

↓

Acetone was added into filtrate (1:3)

↓

Precipitate was obtained

↓

Gum was collected and dried

Physicochemical characterization of gum

Identification tests for carbohydrates, proteins, tannins and gums:

1% aqueous solution of extracted gum was used for chemical characterization. Test for carbohydrates, proteins, polysaccharide, Volatile oil, alkaloids, fats, tannins, amino acids and gums were perform according to standard procedure. [17,18]

Organoleptic evaluation of isolated gum: The isolated gum was characterized for organoleptic properties such as color, odor, taste, fracture and texture.

Solubilitybehavior gum: One part of dry gum powder was shaken with different solvents and the solubility was determined.

pH of gum: The gum was weighed and dissolved in water separately to get a 1% w/v solution. The pH of solution was determined using digital pH meter [18].

Swellingindex: The swelling index is the volume (in ml) taken up by the swelling of 1 g of test material under specified conditions. The swelling index of the gum was determined by accurately weighing 1g of gum, which was further introduced into a 25ml glass-Stoppard measuring cylinder. 25ml of water was added and mixture was shaken thoroughly every 10 min for 1 h. It was then allowed to stand for 24h at room temperature. Then the volume occupied by gum, was measured. The same procedure was repeated thrice and the mean value was calculated [18].

Swelling Index = $\frac{\text{Final Volume – Initial Volume}}{\text{Initial Volume}} \times 100$

Micromeritic properties

Bulk density and bulkiness: The inverse of bulk density is called as bulkiness. Accurately weighed quantity of (50 g) was introduced into a graduated measuring cylinder. The cylinder was fixed on the bulk density apparatus and the volume occupied by the powder was noted. Then, the powder was subjected to tapping in a bulk density apparatus until constant volume was obtained. The final volume (bulk volume) was noted [19, 20].

Powder Flow Property: The flow characteristics were measured by angle of repose. The experiment was repeated thrice. Using the readings and the formula, the angle of repose was calculated [19,20].

Powder Compressibility: This property is also known as compressibility. The finely powdered gum (5g) was transferred into a measuring cylinder and calculations were done using bulk density apparatus [19,20].

Viscosity of Gum: Viscosity of 1 % w/v solutionof gum was measured using an Ostwald's viscometer [21].

Loss on Drying: 500 mg of gum was weighed and placed in a clean and neat china dish. It was kept in hot air oven at 105°C until a constant weight was obtained. The china dish was removed from the oven and again the weight of the gum powder was determined. The moisture content was then determined as the ratio of weight of moisture loss to weight of sample expressed as a percentage [22].

Ash values [23]

Determination of total ash: 2g of the powdered material was accurately weighed into a previously, ignited and tarred silica crucible. The material was then spread in an even layer in the crucible, ignited by gradually increasing the heat to 500-6000C until free from carbon, cooled in a desiccator and weighed. The percentage of total ash was calculated with reference to the air-dried drug.

Determination of acid insoluble ash: To the crucible containing the total ash, 25 ml of hydrochloric acid (approx. 70g/l) test solution was added, covered with a watch glass and boiled gently for 5min. The watch glass was rinsed with 5ml of hot water, which was then added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was then transferred to theoriginal crucible, dried on hot plate and ignited to constant weight. The residue was allowed to cool in suitable desiccator for 30 min and weighed without delay. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Determination of water-soluble ash: To the crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected on an ashless filter paper and washed with hot water & ignited for 15 minutes, at a temperature not exceeding 450°C. Subtract the weight of the residue obtained from the weight of total ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

FTIR analysis: FTIR analyses of the gums were carried out using a Scimadzu FTIR-8400S Fourier Transform Infra-red Spectrophotometer. The sample was prepared using KBr and the analysis was done by scanning the sample through a wave number range of 400 to 4,000 cm^{1}.

RESULT AND DISCUSSION

After isolating the gum from Mardi (terminalia tomentosa) using acetone, the phytochemical investigation showed the presence of carbohydrates, sugar, and proteins whileglucose, volatile oil, tannins, fats and polysaccharides were absent. The results of the phytochemical test are summarized in table1. The organoleptic properties of the gum were observed and were found to be acceptable. The color of the powdered gum was White. The gum was odor and taste was found to be characteristic and agreeable. The fracture was rough. The solubility profile of the gum is shown in table 2. Solubility analysis showed that mardi gum formed viscous colloidal solution with hot water, swells and forms a gel with cold water and was insoluble in most of the organic solvents.

The different parameters of the gum were evaluated and are shown in table 3. The pH of the mardi gum (1% w/v solution) was found to be 5.6 ± 0.01, which was slightly acidic. The pH of gum indicated that adjustment of pH might be required in the formulation of oral and buccal drug delivery systems. The swelling index of the gum was found to be 44 ± 0.58, which suggests that the gum has optimum swelling property; gum can be used for control delivery system.

Total ash, acid insoluble as and water-soluble ash were calculated 3.20 ± 0.01, 0.82 ± 12, and 1.62 ± 0.13 respectively. Bulk density and tapped density were

calculated as 0.73 ± 0.01 g/cm3 and 0.91 ± 0.01 g/cm3, respectively. Bulkiness was found to be 1.37 ± 0.19 cm3/g. Hausner's ratio and carr's index calculated were 1.25 ± 0.01 and 19.78 ± 0.12%. The angle of repose of 27.62° ± 0.40 suggested that the powdered gum possesses good flow property. Viscosity was 40 ± 0.58, the result for loss on drying was found to be 8.2 ± 0.10 %.this indicated that gum was hygroscopic in nature and need to be stored in air-tight container, and Figure 1 shows the IR spectra of the purified gum and is illustrated in table 4. the IR spectra of mardi gum shows the wavenumbers (cm–1) 3558.67, 2887.44, 2509.39, 2117.84, 1604.77, 1313.52, 1055.06 etc., which confirms the presence of alcohol, alkanes, carboxylic acid, amines, alkynes, and esters.

The results showed that the extracted gum possesses optimum organoleptic as well as micromeritic properties and gum can be further used as excipient in pharmaceutical dosage form.

Table 1. Chemical characterization of isolated Mardi gum.

Tests	Present/Absent
Carbohydrates	+
Sugar	+
Glucose	-
Tannins	-
Proteins	+
Polysaccharides (starch)	-
Fats	-
Volatile oils	-
Gum	+

2. Solubility profile of Mardi gum

Solvents	Solubility
Cold water	Swell to form a gel
Hot water	Form viscous colloidal solution
Methanol	Insoluble
Ethanol	Insoluble
Diethyl ether	Insoluble
Petroleum ether	Insoluble
Acetone	Insoluble

3. Parameters of Mardi gum

Parameters	Observations
pH (1% w/v solution)	5.6 ± 0.01
Swelling Index (%)	44 ± 0.58
Bulk density (g/cm^3)	0.73 ± 0.01
Tapped density (g/cm^3)	0.91 ± 0.01
Bulkiness	1.37 ± 0.02
Hausner's ratio	1.25 ± 0.01
Carr's index	19.78 ± 0.12
Angle of repose	27.62 ± 0.40
Viscosity (1% w/v)	40 ± 0.58
LOD (% w/w)	8.2 ± 0.10
Total Ash (%)	3.20 ± 0.10
Acid Insoluble ash	0.82 ± 0.12
Water Soluble ash	1.62 ± 0.13

4. IR study

S.N.	Wave numbers (cm^{-1})	Group present
1	3558.67	O-H Stretch (Alcohol)
2	2887.44	C-H Stretch (Alkanes)
3	2509.39	O-H of $–CO_2H$ Stretch (Carboxylic Acid)
4	2117.84	CΞC Stretch (Alkynes)
5	1604.77	C-N Stretch (Amines)
6	1313.52	C-N Stretch (Amines)
7	1055.06	C-O Stretch (Ester)

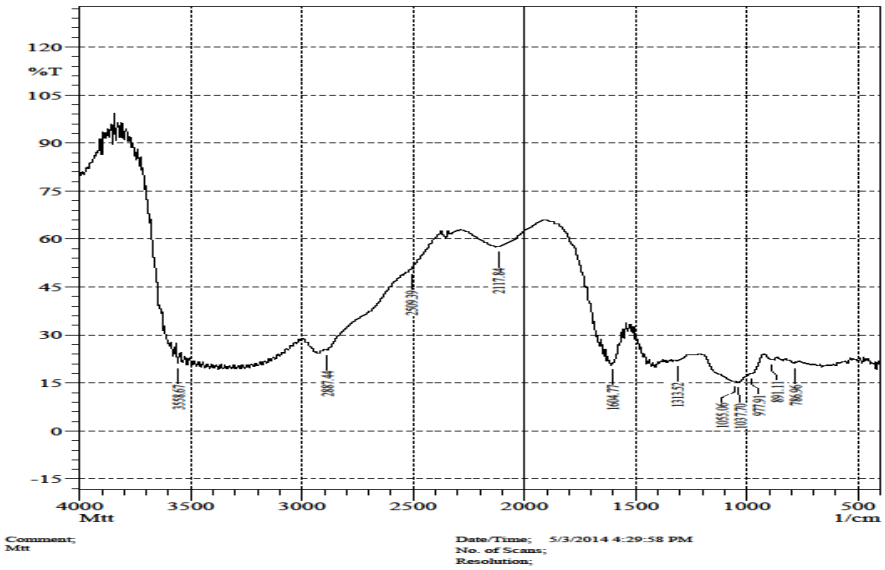

Fig1. IR spectra of Mardi (terminaliatomentosa) gum

CONCLUSIONS:

It is concluded from the research work that the gum extracted from mardi (terminalia tomentosa) shows the presence of carbohydrates after chemical tests. All the organoleptic properties evaluated were found to be acceptable. The pH was found to be slightly acidic. Swelling Index reveals that the gum swells well in water. Total ash value was within the limits. The values of angle of repose and Carr's Index of powdered gum powder showed that the flow property was good. IR spectra confirmed the presence of presence of alcohol, alkanes, carboxylic acid, amines, alkynes, and esters.

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