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PHYTOCHEMICAL ANALYSIS AND PHARMOLOGICAL STUDIES OF ALMOND GUM OBTAINED FROM PRUNES AMYGDALES VAR. DULCIS

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ABSTRACT

Objective and background: Gums are tree exudates usually formed following injury or by unfavorable conditions such as drought. Gums are considered as the pathological products or abnormal products of metabolism. Gums are natural plant hydrocolloids and are heterogeneous in composition. Prunes amygdales var dulcis (Sweet almond) is a medium sized tree with purplish-brown bark and its leaves are bright green, Lanceolate, serrate. Flowers are white in colour and tinged with red. The gum exudates from the injured wounds on almond trees. The injured bark secretes the gum and it gets dried up, which is later collected and used for various medicinal purposes. It is commonly used as emulsifier, thickener, adhesive and stabilizer. In Unani System of Medicine Almond Gum (AG) is a drug of choice for chronic cough, Tuberculosis, Nephrolithiasis and haemoptysis. **Methods:** Preliminary phytochemical studies were carried out on extracted samples. The extracts were subjected to various qualitative phytochemical tests such as alkaloids, glycosides, carbohydrates, phenolic compounds, tannins, phytosterols, fixed oils, flavonoids, proteins, amino acids and presence or absence of gum was done by Ruthenium red test. Pharmacological studies of Almond Gum have also been thoroughly analyzed. **Results:** In the current Study the preliminary phytochemical analysis of aqueous extract of AG has been done which showed presence of carbohydrates and The Ash of Almond Gum showed the presence of sulphates, iron, and chloride. **Conclusion:** The present study has provided evidence based scientifically validated data for standardization of AG (Almond Gum) and will serve as a useful tool to minimize adulteration and substitution of AG.

KEYWORDS: Almond Gum; Phytochemical analysis; scientific studies.

INTRODUCTION

Gums are easily available plant ingredients having a wide range of applications both in pharmaceuticals and cosmetic industries. Gums have been extensively explored as pharmaceutical excepient. Natural materials are safe as compared to synthetic ones because they are chemically inert, nontoxic, less expansive, biodegradable and are widely available.[1] Almond Gum is commonly found in Kashmir, Punjab, Baluchistan, Afghanistan, Persia and the Meditarrian region.[2] The best almond gum is that which is white in color and is obtained from young tree. Usually there are two verities of almond trees from which gum is obtained, the gum obtained from sweet almond tree is of cold temperament while gum obtained from bitter almond tree is of hot temperament and has same action as that of Babool gum (Gum acacia).[3,4] Almond gum is used commonly as emulsifier, thickener, Binder, adhesive and stabilizer in pharmaceutical industries.[5]

MATERIALS AND METHODS

Preliminary phytochemical studies were carried out on extracted samples. The extracts were subjected to various qualitative phytochemical tests such as alkaloids, glycosides, carbohydrates, phenolic compounds, tannins, phytosterols, fixed oils, flavonoids, proteins, amino acids and presence or absence of gum was done by Ruthenium red test.[6,7]

Test for Carbohydrates

Fehling's solution test: To 2 ml test extract of the drug, 1 ml of a mixture of equal parts of Fehling's solutions A and B was added, and then the contents of the test tube was boiled for few mints. Formation of red or brick red colour of precipitate was observed. A red or brick red precipitate of cuprous oxide indicates the presence of reducing sugars.[8,9]

Molish,s Test: In a test tube containing 2 ml of aq. extract of the drug 2 drops of freshly prepared 20% alcoholic solution of α-naphthol was added and shaken, and then 2 ml of concentrated Sulphuric acid was gently

poured. Formation of red-violet colour ring at the junction of the two solutions, which disappears on the addition of an excess of alkali solution indicates the presence of reducing sugars.[9]

Benedict's test: To 0.5 ml of test solution, 0.5 ml of Benedict's reagent was added in a test tube and heated in the boiling water bath for five minutes. The colour of the solution was observed. The solution colour appears green, yellow, or red depending on amount of the reducing sugars present in the test solution.[9]

Test for gum

Ruthenium red test: powdered samples were treated with ruthenium red solution and observed for the appearance of pink colour.[10,11]

Test for Cardiac Glycosides[12]

Test for deoxysugars (Keller-killaini test): 5ml of each extract was added with 2 ml of glacial acetic acid which was followed by the Addition of few drops of ferric chloride solution and 1ml of concentrated Sulphuric acid. Formation of brown ring at interface confirms the presence of glycosides.[13]

Test for bufadenoloids (Liebermann's test): 2 ml of the test extract of each was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added and the solution was cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a Steroidal nucleus that is, a glycone portion of glycoside.[14]

Tests for Alkaloids

Dragendroff's test: A few mg of alcoholic extract of *Almond gum* was dissolved in 5 ml of distilled water, 2 M hydrochloric acid was added until an acid reaction occurs, then 1 ml of Dragendroff's reagent (solution of potassium bismuth iodide) was added and colour of precipitate was observed. Formation of orange or orange red precipitate indicates the presence of alkaloids.[9]

Mayer's test: A few drops of Mayer's reagent (potassium mercuric iodide) was added to 1 ml of Hydroalcholic extract of the drug, formation of white or pale yellow precipitate indicates the presence of alkaloids.[9]

Hager's test: To 1 ml of alcoholic extract of the drug taken in a test tube, added a few drops of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate indicates the presence of alkaloids.[9]

Wagner's test: 1 ml of alcoholic extract of the drug was acidified with 1.5 % v/v of hydrochloric acid and added a few drops of Wagner's reagent (Iodine in potassium iodide). Formation of Yellow or brown precipitate indicates the presence of alkaloids.[9]

Test for flavonoids[15]

1. Alkaline reagent Test: 2 ml of extract was treated with few drops of 20 % sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of few drops of dilute acid, indicates the presence of flavonoids.[16]

2. Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour Precipitate indicates the presence of flavonoids.[17]

Test for Tannins

Ferric chloride test: To 1-2 ml of extract of the drug a few drops of 5% ferric chloride solution was added. A bluish black colour, which disappears on the addition of a few ml of dilute Sulphuric acid solution followed by the formation of a yellowish brown precipitate, indicates the presence of tannins.[9]

Lead acetate Test: 2ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicates the presence of tannins.[9]

Test for Phytosterols/Terpenes[18,19]

1. Salkowski's Test: 5ml of extract was taken in a test tube and 2ml of chloroform was added to it followed by the addition of 3ml of concentrated sulphuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpanoids.[9]

2. Hosse's reaction test: The extract was mixed with chloroform and 2ml of concentrated Sulphuric acid was poured from the side of the test tube. The colour of the ring, at the junction of the two layers was noted. A red colour ring indicates the presence of sterols/ Terpenes.[20]

3. Liebermann Burchard's test: 2 ml of extract was mixed with chloroform. 1-2 ml acetic anhydride and 2 drops of Conc. H_2SO4 was added from the sides of the test tube. Formation of brown ring at the Junction indicates the presence of phytosterols.[21]

Test for Fixed oils: Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of test extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.[22]

Filter paper test / Spot test: Small amount of extract was separately pressed between the two filter papers. Appearance of oil stains on the paper indicates the presence of fixed oil.[22]

Tinc. Alkane test: To the extract, 2-3 drops of tincture Alkane was added. The colour of the extract was observed. The red colour of the extract indicates the presence of fixed oil.[23]

Test for saponins

Froth test: The presence of saponins was determined by Frothing test. The extract was vigorously shaken with distilled water and was allowed to stand for 10 minutes and classified for saponins content as follows: no froth indicates absence of Saponins and stable froth of more than 1.5 cm indicates the presence of saponins.[24]

Foam test: 2ml of extract was taken in a test tube and 6ml of distilled water was added to it. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.[20]

Test for Phenols

Lead acetate test: To 1ml of the extract 3ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.[9]

Tests for Proteins and Amino acids: Hydro alcoholic extract of the test drug was subjected to following tests.[23]

1. Ninhydrin test: Test solution was mixed with few drops of Ninhydrin solution and heated. After heating gently on water bath for few minutes, formation of blue to red- violet colour indicates presence of Amino acids.[17]

2. Biuret's test: To 1 ml of hot aq. extract of the drug added 5-8 drops of 10 % w/v concentrated sodium hydroxide solution, followed by 1 or 2 drops of 3 % w/v copper sulphate solution. A violet or red colour indicates the presence of proteins.[9]

3. Million's reaction: A small quantity of the extract of the drug was dissolved in 1 ml of distilled water and 5-6 drops of Million's reagent was added and the colour of precipitate was observed. Formation of white precipitate which turns to red upon heating indicates the presence of proteins.[9]

4. Xanthroprotenic test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.[17]

Test for inorganic constituents of Almond gum: Ash of Almond gum was prepared separately**.** To the ash 50% v/v hydrochloric acid and 50% v/v Nitric acid was added and was kept for an hour then filtered. The followings tests were performed with the filtrate.[23,25]

Test for sulphate

Few drops of 5% Barium chloride were added to 5 ml of filtrate. White crystalline precipitate formed which was insoluble in hydrochloric acid indicates the presence of sulphate.

Lead acetate reagent was added to the test solution. White precipitate formed which was soluble in sodium hydroxide indicates the presence of sulphate.[23]

Test for Iron

Potassium ferrocyanide test: Few drops of 2% potassium ferrocyanide were added to 5 ml of test solution. Dark blue coloration was observed which indicates the presence of iron.

Potassium thiocyanate test: Few drops of 5% potassium thiocyanate or ammonium thiocyanate were added to 5ml of the test solution. The colour of the solution turned to blood red colour indicates the presence of Iron.[23]

Test for phosphate: To 5 mL of test solution prepared in Nitric acid, added few drops of ammonium molybdate solution. Then heated for ten minutes, after cooling yellow crystalline precipitate of ammonium phosphomolybdate was observed which indicates the presence of phosphate.[23]

Test for chloride: Few drops of 10% silver nitrate solution were added to 3 ml of test solution prepared in nitric acid. White precipitate of silver chloride was observed; precipitate was soluble in dilute ammonia solution which shows the presence of chloride. To 5 to 7ml of filtrate, 3 to 5 ml of lead acetate solution was added, white precipitate formed, which was soluble in hot water indicates the presence of chloride.[23]

Test for Carbonate: To a test solution mercuric chloride was added. A brownish red precipitate was formed which indicates the presence of carbonate. To the test solution magnesium sulphate solution was added. White precipitate was formed which indicate the presence of carbonates.[23]

Test for Nitrates: Solution of ferrous sulphate was added to the test solution which yields no brown colour, if sulphuric acid was added slowly from the side's of test tube, a brown colour was produced at the junction of two liquids which indicates the presence of nitrates.[23]

RESULTS

Preliminary Phytochemical analysis

The results of Aqueous extract of *Almond Gum* showed presence of carbohydrates. The Ash of AG showed the presence of sulphates, iron, chloride and nitrates. Results are given in (Table 6).

Test for carbohydrate (Molish,s and Benedict's test)	**+ve**
Test for Gums (Ruthenium Red)	**+ve**
Test for Glycosides (Keller-killaini test)	-ve
Test for Glycosides (Keller-killaini test)	-ve
Test for Alkaloids (Dragendroff's, Wagner's, Hager's, Mayer's test)	-ve
Test for flavonoids (Alkaline reagent & Lead acetate tests)	-ve
Test for Tannins (Ferric chloride and Lead acetate test)	-ve
Test for phytosterols /Terpenes (Salkowski's test, Hosse's reaction test, Liebermann Burchard's reaction)	-ve
Test for Fixed oils (Filter paper test and Tinc. Alkane test)	-ve
Test for saponins (Froth test, Foam test)	-ve
Test for Phenols (Lead acetate test)	-ve
Test for proteins and Amino acids (Ninhydrin test, Burette's reaction, Millions reaction, Xanthroprotenic reaction)	-ve
Test for sulphate	**+ve**
Test for Iron (potassium ferrocyanide test, Potassium thiocyanate test)	**+ve**
Test for Phosphate	-ve
Test for Chloride	**+ve**
Test for Carbonate	-ve
Test for Nitrates	-ve

Pharmacological studies
The following Pharmacological studies have been done on *Almond Gum.*

Wound Healing activity of almond gum was evaluated by Bouaziz F *et al.* on dermal wounds of adult rats.

Antioxidant and Antibiotic Activities
Antioxidant and antibiotic activities of almond gum were found in a study conducted by Bouaziz F *et al.*

Multifactorial excepient
Almond Gum has been studied for its Multifactorial excepient activity by Kulkarni GT *et al.*

Best Preservative and Non toxic
It has been found that the fruits coated with Almond gum have increased the shelf life of fruits, e.g. Shelf life of apple increases upto 46 days. Toxicity study were performed which shows 0% mortality rate.

DISCUSSION

Gums and resins have been used for a long time in Unani System of medicine to treat different ailments in a single dosage form or in combination with other herbal drugs as a Demulcent, Astringent, Aphrodisiac, Anti- obesity, Diuretic, Emmenagogue, Stimulant, Carminative, and Hypolipidemic, etc.[26,27] The preliminary phytochemical analysis of aqueous extract of AG showed positive test to ruthenium red which indicates the purity of gum sample.[28] it revealed the presence of carbohydrates and shows negative tests to glycosides, alkaloids, flavonoids, tannins, fixed oil, saponins, phenols, proteins and Aminoacids. For inorganic constituents, the test of AG Ash shows presence of sulphate, iron and chloride, and negative results to phosphate.

Carbonate and nitrate. A pharmacological study revealed that the Almond Gum posses wound healing activity, Antibiotic activity, Antioxidant and is multifactorial excepient.

CONFLICT OF INTEREST: Nil

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