

EFFECT OF PYRUS COMMUNIS FRUIT EXTRACT IN DEXAMETHASONE INDUCED DELAYED WOUND HEALING MODEL IN RATS

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ABSTRACT

Nature has blessed us with super flora and fauna, which had made our life lovely. One of the wonders is pear fruit. Pear is gently sweet and juicy fruit. Pears (*Pyrus Communis* Linn) are grown in the temperate regions of the world including India. Nutritionally, pears are considered to be a fairly good source of fiber, which malic acid is the major organic acid. The genus of *pyrus* has probably originated in central Asia, the mountainous region or western and southern China. Arbutus (hydroxyquinone- β -D-glucopyranoside) is a natural phenolic glucoside found in various species of *Pyrus Communis* Linn. Flavanoids also has been isolated and identified. The chlorogenic acid is isolated and identified from the flowers of the *Pyrus Communis* Linn. The triterpsnoids were isolated from the stem bark of *Pyrus Communis* Linn. Pears fruits are excellent source of carbohydrates,

optimum quantity of sugars and dietary fiber. Chemically they are mainly composed of carbohydrates. Different sugars, starch and cellulose are the main carbohydrates forms found in the pear fruits. In ripe fruit, sugars constitute about 70 percent of the total carbohydrates percentage. A protein content of 0.6 percent has been recorded in pear fruit. *Pyrus communis* Linn also known as Amritphala has astringent, sedative activity and act as febrifuge. Its leaves and bark can be used in wound healing and thus also act as anti-inflammatory.

KEYWORDS: *Pyrus Communis*, Pears, Wound healing, Astringent.

INTRODUCTION

HERBAL PLANTS

Since time immemorial man has used various parts of plants in the treatment and prevention of many ailments historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts, mixtures, etc. Today a substantial number of drugs are developed from plants which are active against a number of diseases. The majority of these involve the isolation of the active ingredient found in a particular medicinal plant and its subsequent modification. In the developed countries 25 percent of the medical drugs are based on plants and their derivatives and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries. In the past our ancestors made new discoveries of the healing power of plants through trial and error. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and thousands of years.

PLANT PROFILE



Taxonomy of *Pyrus communis*

Botanical name: *Pyrus communis* Linn

Common name Common Pear

Hindi name: Babbu-goshaa

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order Rosales

Family: Rosaceae

Genus: *Pyrus* L

Species: *Communis* Linn.

Vernacular Names

Common Name: common pear

Hindi: Babbu-goshaa

Sanskrit: Amritphala

Telugu: Berikaya

Tamil: Berikkay

ORIGIN AND DISTRIBUTION

The genus *Pyrus* has probably originated in Central Asia, the mountainous regions or western and southern China, from Asia Minor to India and further diversified and moved both in eastern and western directions from primary centre of origin. Speciation has occurred mainly in eastern and central Asia in the Himalayas, Caucasus, Asia Minor and Eastern Europe. Distribution of wild species of *Pyrus* extends from the Balkans in Europe, through Caucasus, Turkmenistan, Altai Mountains, Siberia to China and Japan. Some species of this genus although naturalized in America but America is not a native home of this genus proposed three centres of origin (centre of biodiversity) for cultivated pears *i.e.* Chinese centre, Central Asiatic centre and Near Eastern centre.

Fruit

The morphological characteristics of fruit in *Pyrus* genotypes are variable with respect to genetics and environments. Generally, all species have pome fruits along with variable fruit shape like oblong, bulbous or pyriform, round and pear shaped with persistent or deciduous calyx. In fruit shapes as round or apple shaped and ovate to oblong in various pear genotypes. Fruit size is generally 0.5-20 cm long with or without grit cell. Ground colour changes from green to yellow or red during maturation. Browning russet is mainly due to increase in humidity. Ripening season is generally from June to November. Precisely. *P.communis* fruits vary in shape mostly oblate/bulbous and pyriform, calyx persistent, fleshy pedicels and pulp with gritty The pulp is melting and buttery in texture. *P. pyrifolia* fruits are round and *P. pashia* with small, dark brown fruits, which become soft and sweet to some extent when

ripened but gritty and astringent with poor quality. This species is commonly used as a rootstock for commercial varieties in India and Pakistan.

CHEMICAL CONSTITUENTS

Arbutin (hydroxyquinone- β -D-glucopyranoside) is a natural phenolic glucoside found in various plant species of diverse families such as Rosaceae (*Pyrus communis* Linn.). The leaves contain arbutin, isoquercitrin, sorbitol, ursolic acid, astragalins and tannin. The bark contains friedelin, epifriedelanol and β -sitosterol. Phloridzin is present in the root bark. Flavonoid glycosides have been isolated and identified: quercetin 3-O- β -D-glucopyranoside, kaempferol 3-O- β -D-(6''-O- α -L-rhamnopyranosyl)-glucopyranoside and quercetin 3-O- β -D-(6''-O- α -L-rhamnopyranosyl)-glucopyranoside. Sterols and triterpenes (β -sitosterol and α -amyrin), phenolics and coumarins are present in *Pyrus communis* Linn. Flowers Chlorogenic acid is also isolated and identified from *Pyrus communis* Linn. flowers. The triterpenoids were isolated from the stem bark of *Pyrus communis* Linn.

NUTRITIONAL IMPORTANCE

Composition Pear fruits available in the world market belong either to *P. communis* or *P. prafolia* or hybrid group of these two species. They are excellent source of carbohydrates, optimum quantity of sugars and dietary fiber. Chemically they are mainly composed of carbohydrates. Different sugars, starch and cellulose are the main carbohydrate forms found in the pear fruits. In ripe fruits, sugars constitute about 70 percent of the total carbohydrates percentage. Fructose is the predominant sugar (6.5% to 11.2%) followed by glucose (0.5% to 3.5%) and sucrose (0.1% to 2.4%) in the fruit juice. Round starch granules of pear resemble very much in shape and amylose content to that of apple starch. Variability in taste and colour of fruits is mostly due to changes in contents and ratios of sugar contents.

A protein content of 0.6 percent has been recorded in pear fruits. However all the essential amino acids except tryptophan have been identified in fruits. Organic acids detected in pear fruits include malic, citric, quinic, α -ketoglutaric, succinic, lactic, glycolic, shikimic, glyceric and mucic. Citric and malic acid ratios in pear fruit juice correlate the organoleptic evaluation of taste and quality criterion. Different minerals found as mg per 100 g of fruit pulp are calcium 8, phosphorus 15, copper 0.4, sulphur 14, and chlorine 1. Pectin is present abundantly in pear fruits. The exact amount, however, depends on the cultivar, stage of ripening and storage conditions. At harvest, in unripe Bartlett' fruits, 0.07 percent soluble pectin was recorded which rose to 0.7-0.8 percent on the ripening of fruits, 103).

USES

Pyrus communis Linn. Also known as Amritphala has astringent, sedative activity and act as febrifuge. Its leaves and bark can be used in wound healing and thus also acts as anti-inflammatory. Leaves, buds, and bark of the tree are domestic remedies among the Arabs on account of their astringent action (0) Pear is a rich source of Vitamin C, ascorbic acid and it is an antioxidant. It acts against reactive gen species 110710, Arbutin is commonly used in urinary therapeutics and as a human skin whitening agent, It decreases melanin in the skin. In the past, the presence of arbutin in Pear has been correlated with biochemical processes that operate as defence mechanism against bacterial invasion. Therefore acts as antibacterial too the flowers of common pear are used in folk medicine as components of analgesic and spasmolytic drugs.

The pear fruit is highly delectable which helps in maintaining a desirable acid basebalance in human body. The European and West Asian pears are relished for their buttery juicy, fine texture and flavour, whereas East and North Asian pears for their crisp and sweet taste. Juice of Bartlett' fruits can be utilized in jellies and syrups and after acidification as a beverage. Pear fruit is appreciated for its delicious taste and aroma. Consumers prefer yellow, elongated and concave pear fruits or round shaped with golden colour. Pear fruits have pharmacological properties like anti-inflammatory, anti-tumour, anti-allergenic and also help in reducing body weight. Dietary fibre of pear and its phenolic compounds help to reduce the risk of cardiovascular diseases. Moreover, it is preferred by diabetic persons due to its low sugar content. The processed pears, almost all are canned: small amounts are pureed for baby food, dried, or juiced for use in pure or blended fruit juices or in perry (i.e. pear cider) and wine products. Bartlett' is the cultivar used for canned halves, puree and most pear juice and nectar. Both sun and oven-drying are employed for preserving the pear fruits. Oven-dried product is better than sun-dried one which is tender, translucent and light in colour. Pears can also be candied and sweet pickled.

According to literature and plant profile of the plant *Pyrus communis* possess the wound healing property and antidiabetic activity, but it was not scientifically proved yet. So the present study was designed for evaluation of diabetic induced delayed wound healing with ethanol extracts of *pyrus communis* using experimental animals.

MATERIAL AND METHODS

COLLECTION AND AUTHENTICATION OF PYRUS COMMUNIS

The fruit of *pyrus communis* had been collected from central market, Tirupati, Chittoor District, and Andhra Pradesh, India. The fruit was identified and authenticated by the Botanist Dr. K. Madhava Chetty, Assistant Professor, Department of botany Sri Venkateswara University, Tirupathi.

PREPARATION OF EXTRACTS OF FRUITS OF PYRUS COMMUNIS

Extract preparation

The collected fruit were shade dried completely and ground into powder with mechanical grinder. The powder was passed through sieve no. 40 to get uniform powdered.

Extraction Procedure

The 500gm powder of *Pyrus* fruits also defatted and marc was successively extracted with chloroform, ethyl acetate, ethanol & water by maceration.

Maceration process

Maceration process involves the separation of medicinally active portions of the crude drugs. The drug material is taken in a stoppered container and immersed in the bulk of the solvents in the ratio of 1:2 (Drug & Solvent) and allowed to stand for 7 days in a room temperature with frequent shaking of every 30 min up to 6 hours on each day. The solvent was separated by filtration and concentrated under reduced pressure. The resulting semisolid mass was vacuum dried and percentage yield was calculated.

Data showing the % yield values of fruit of *Pyrus communis* Linn

Plant Name	Part Used	Method of Extraction	solvent	Colour of extract	Nature of extract	% yield of extract
<i>Pyrus communis</i>	Fruit	Maceration	Chloroform	green	semisolid	14.38
			Ethyl acetate	Dark brown	semisolid	18.24
			Ethanol	Dark brown	semisolid	15.00
			Aqueous	Brown	semisolid	19.62

FRELIMINARY PHTOCHEMICAL SCHEENING

The different extracts fruits of *pyrus commiunls* was sereened for the presence of various phytoconstituents like alkolides, flavonoids, saponin, tannin and glycosides.

TEST FOR CARBOHYDRATES

A small quantity of extracts was dissolved separately in distilled water and filtered. The filtrate was subjected to molisch test for detecting carbohydrates.

Fehling's Test

The extracts when treated with 5ml Fehling's A & B solutions give an orange red precipitate showing the presence of reducing sugar.

Benedict's test

The extracts on heating with 3ml Benedict's reagent gives brown precipitate showing the presence of sugar.

Barfoed's Test

3ml of Barfoed's reagent was added to the given extracts and boiled on a water bath for 5 minutes reddish precipitate was observed for the presence of carbohydrates.

Molisch test

Filtrate was treated with 2-3 drops of alcoholic alpha-naphthol solution and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of the two liquids shows the presence of carbohydrates.

TEST FOR GLYCOSIDES**Borntrager's test**

A few ml of dilute sulphuric acid were added to the extract solution, boiled, filtered and treated the filtrate with chloroform and shaken well. Then separated the chloroform layer and tested with a few ml of ammonia solution. The ammoniacal layer gets pink or red colour.

Modified Borntrager's test

Add a few ml of 5% Fee, solution and dilute hydrochloric acid to the extract. Then boiled for 5 min, cooled and shaken well with organic solvent. Then added equal quantity of dilute ammonia. The ammoniacal layer acquires pinkish red colour.

Legal's test

Extract and Dissolved with pyridine and made alkaline with sodium nitroprusside solution. The solution becomes pink or red.

Keller-killani test

To the powdered drug extract, add 10ml of 70% ethanol for 2 min, then filtered and added 10 ml of water and 0.5 ml of strong solution of lead acetate and filtered. The filtrates were shaken with 5 ml of chloroform and separate the chloroform layer and evaporated. Added 3 ml of glacial acetic acid and cooled. Then added 2 drops of 1% ferric chloride solution. Transferred the content into a test tube containing 2 ml of concentrated sulphuric acid. Reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green on standing.

TEST FOR FLAVONOIDS**Shinoda test**

Add extract plus magnesium turnings or foil and add conc. HCl. Intense cherry red colour or orange red colour is formed.

General tests

To small quantity of residue, add lead acetate solution. Yellow color precipitate is formed. Addition of increasing amount of sodium hydroxide to the residue shows yellow coloration, which decolorizes after addition of acid.

TEST FOR SAPONINS**Foam test**

Add small quantity of extract add 20 ml of distilled water, shaken in a graduated cylinder for 15 minutes, 1 cm layer of foam develops.

Hemolytic test

Add drug extract or dry powder to one drop of blood, Placed on the glass side. Hemolytic zone appears.

TEST FOR STEROIDS**Libermann-Buchard test**

add extract and dissolved in few ml of chloroform, add 3 ml of acetic anhydride and add conc H₂SO₄ of slides of the test tube, bluish green colour is formed.

Salkowski test

Extract and dissolved in few ml of chloroform add equal volume of conc. H₂SO₄ red colour is formed.

TEST FOR ALKALOIDS**Dragendrof's test**

Add Dragendroffs reagent (potassium bismuth iodide solution) to the extract. Crag redprecipitate is formed.

Mayer's test

To the 1 ml of extract add 1ml of Mayer's reagent (potassium mercuric edide. Cream coloured precipitate is formed.

Hager's test

To the 1 ml of extract add 3 ml of Hager's reagent (saturated aqueous solution of picric acid). A yellow coloured precipitate indicates the prevence of alkaloids.

Wager's test

To the 1 ml of the extract add 2 ml of Wager's reagent (iodine in potassium iodide) formation of reddish brown precipitate indicates the presence of alkaloids.

TEST FOR GUMS**Mucilage test**

To the 10 ml of aqueous extract and add slowly to 25 ml of absolute alcohol with constant stirring. Filtered the precipitate and dried in air. Examine the precipitate for its swelling properties and for the presence of carbohydrates.

Hydrolytic test

Hydrolyse the test solution using dilute Hcl. Perform Benedict's and Fehling's test Red colour is formed.

TEST FOR FIXED OILS AND FATS**Spot test**

Press a small quantity of extract between the filter paper. Oil stains on the paper indicates fixed oils.

Saponification test

To the 1 ml of the extract add few drops of 0.5N alcoholic KOH and drop of phenolphthalein. Heat the mixture on water bath for 1-2 hours. Formation of soap or partial neutralization of alkali.

TEST FOR PROTEINS AND AMINO ACIDS**Ninhydrin test**

Take small quantity or extract add 2 drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n - butanol). Heat for few minutes, blue colour is formed.

Biuret test

To the 1 ml of 40% NaOH solution and 2 drops or 1% copper sulphate solution, add 1 ml of extract. Blue colour is formed.

Xanthoproteic test

To the test solution and add 1 ml of conc nitric acid and boil. Yellow precipitate is formed. After cooling add 40% sodium hydroxide solution orange colour is formed.

Tryptophan test

To the 3 ml of test solution, a few drops of glyoxalic acid and conc. H₂SO₄. Reddish violet appears at the junction of two layers.

Cysteine test

To the 5 ml of test solution add few drops of 40% NaOH and 10% lead acetate solution. Boil precipitate of lead sulphate is formed.

Millon's test

To the 3 ml of test solution, mix with 5 ml of Millon's reagent. White ppt warm ppt turns brick red or the ppt. dissolves giving red colour solution, indicates the presence of tyrosine.

TEST FOR POLYPHENOL

1. Ferric chloride test-To extracts few drops of neutral ferric chloride solution, blackish red colour.

TEST FOR TANNINS

Diluted small quantities of extracts with distilled water and subjected to

1. Ferric chloride Test-Extracts treated with ferric chloride solution. blue colour
2. Gelatin test -Extracts treated with gelatin solution, white precipitate
3. Lead acetate test -Extracts treated with lead acetate solution, yellow precipitate

SOLUBILITY PARAMETERS

Very soluble : One part of soluble in less than one part of the solvent

Free soluble :1:10

Soluble. :1:30

Sparingly Soluble. : 1:100

Slightly Soluble : 1:1000

Very slightly Soluble : 1:10:000

Practically insoluble : More than 10,000

Solubility of various extracts of *pyrus communis* in varios solvents

Extracts	Water	Hot water	1% v/v Tween 80	0.9% CMC	1% DMSO
CEPC	Insoluble	Sparingly Soluble	Freely soluble	Soluble	Sparingly Soluble
AEPC	Freely soluble	Freely soluble	Freely soluble	Sparingly Soluble	Sparingly Soluble
EAEPc	Slightly soluble	Slightly Soluble	Freely soluble	Soluble	Sparingly Soluble
EEPC	Soluble	soluble	Freely soluble	Sparingly soluble	Sparingly Soluble

EXPERIMENTAL ANIMALS

All the experiments were carried out using Swiss Albino mice (25-30 g) and Wister a (150-200 g). The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of $24 \pm 2^\circ\text{C}$ and relative humidity of 30-70% A 12 hrs day : 12 hrs night cycle was followed. All animals were allowed free access to water and fed. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) of Sri Krishna Chaithanya College of pharmacy, Madanapalle, Andhra Pradesh.

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) followed by Dunnett's meth of multiple comparisons was employed using Graph pad InStat 5.0 software. $p < 0.05$, $p < 0.01$ & $p < 0.001$ was considered to be statistically significant.

RESULTS**PRELIMINARY PHYTOCHEMICAL SCREENING TEST OF DIFFERENT EXTRACTS OF PYRUS COMMUNIS FRUIT**

The preliminary phytochemical analysis of ethyl acetate and ethanol extracts of *Pyrus communis* revealed the presence of Alkaloids, Carbohydrates, glycosides, phenolic compound, flavonoids, tannin and proteins. The chloroform extracts show alkaloid, sterols, protein and carbohydrate. In case of aqueous extracts showed alkaloid, carbohydrate, glycoside, proteins, amino acid, tannin and flavanoid etc.

PRELIMINARY PHYTOCHEMICAL SCREENING TEST OF DIFFERENT EXTRACTS OF PYRUS COMMUNIS L

S.NO	Constituents	Tests	Chloroform	Ethyl acetate	Ethanol	Aqueous
1	Alkaloids	Mayers test	+	+	+	+
		Dragendoffs test	+	-	-	+
		Hagers test	+	-	-	+
		Wagners test	+	+	-	+
2	Sterols	Burchard test	+	-	-	-
		Salkowski test	+	-	-	-
3	Carbohydrates	Molich test	-	+	+	+
		Fehlings test	+	+	+	+
		Benedicts test	+	+	+	+
		Barfodes test	+	-	+	+
4	Glycosides	Legal test	-	+	+	+
		Kellerkillani test	-	+	+	+
		Borntragers test	-	+	+	+
5	Fixed oil & fats	Spot test	-	-	-	-
		Saponification test	-	-	-	-
6	Phenolic compound	Ferric chloride	-	+	+	+
7	Proteins & amino acid	Biuret test	+	+	+	+
		Ninhydrin test	-	+	+	-
		Millons test	-	+	+	-
		Xanthoproteic test	-	+	+	+
		Cystine test	-	+	-	+
		Tryptophan test	-	-	-	-
8	Saponins	Foam test	-	-	-	-
		Haemolysis test	-	-	-	-
9	Tannins	Gelatin test	-	+	+	+
		FeCl ₃ test	+	+	+	-
		Lead acetate test	-	+	+	-
10	Gum & mucilage	Mucilage test	-	+	-	+
		Hydrolytic test	-	-	-	-
11	Flavonoids	Shinoda test	-	+	+	+
		Conc. H ₂ SO ₄	-	+	+	+
		Lead acetate	+	+	+	+

Where += Present, -= Absent

DISCUSSION

Wound healing involves various phases Initially involves acute inflammatory phase followed by the synthesis of collagen and other extra cellular macromolecules which are later removed to form a scar. Drugs, which influence one phase, may not necessarily influence another.

Dexamethasone is a potent and highly selective glucocorticoid used in the treatment of inflammation. Side effects of glucocorticoid treatment include steroid diabetes.

High exposure to glucocorticoids impairs insulin sensitivity, contributing to the generation of metabolic syndrome including insulin resistance and hypertension The mechanism by which dexamethasone induces peripheral insulin resistance is by inhibiting GLUT-4 translocation. The underlying mechanism involves increased α adrenoceptor signaling, increased Potassium channel activity and impaired glucose metabolism reduction of β cell mass in long-standing glucocorticoid therapy may contribute to the consecutive development of steroid diabetes. The increased blood glucose level leads to delayed wound healing Patients with diabetes often have difficulty to heal wounds. The initial barrier to healing is an increased blood glucose level, which causes cell walls to become rigid, impairing blood flow through the critical small vessels at the wound surface and impeding red blood cell permeability and flow and impaired hemoglobin release of oxygen results in oxygen and nutrient deficits in the wound. A less than optimal immune function also contributes to poor wound healing in the patient with diabetes When blood glucose levels are persistently elevated, Chemotaxis and phagocytosis are compromised.

Within a few hours after injury, inflammatory cells invade the wound tissue. Neutrophils arrive first within a few minutes, followed by monocytes and lymphocytes. They produce a wide variety of proteinases and reactive oxygen species as a defense against contaminating microorganisms, and they are involved in the removal of cell debris. In addition to these defense functions, inflammatory cells are also an important source of growth factors and cytokines, which initiate the proliferative phase of wound repair.

Healing is a physiological process and does not normally require much help but still wounds cause discomfort and are prone to infection and other complications.

CONCLUSION

The present study indicates the ethyl acetate, ethanol and aqueous extracts fruits of *Prunus communis* consist of tannin, flavonoid and phenolic compound it may interact with various phases of wound healing like inflammation, angiogenesis, proliferative repair and remodeling. So the plant could serve as potent natural drug for wound healing in normal rats and Diabetes has implications for acute and chronic wound healing.

Type 2 (non-insulin dependent) diabetes continues to increase in incidence and is more prevalent in older patients in whom age-related skin changes already negatively impact on the healing process. Maintaining normoglycaemia (normal blood sugar levels) is important as hyperglycemia has been correlated with impaired wound healing. The present study on *Prunus communis* proved it could be useful in management of diabetes associated with poor wound healing study conducted on diabetic model rats. Moreover the plant increase the collagen synthesis which will involve in proliferative phase of wound healing and reverse the dexamethasone delayed wound healing. So we concluded plants *Prunus communis* speed up the inflammatory and proliferative phases of wound healing and its more valuable plants to treat chronic wound or wounds in diabetic and immunosuppressed patient. The effect of extracts are ordered according to the results of dexamethasone delayed wound model as EAEPC - 10% EAEPC > EEPC - 10% EEPC > AEPC - 10% AEPC. Except chloroform, all extracts possesses a definite wound healing activity but oral as well as topical administration of ethyl acetate extract of *Prunus communis* exhibited significant wound healing activity in delayed wound healing model in rats, which is comparable to marketed standard drug hydrogel. These interesting activities require further research to identify and isolate the compounds involved followed by mechanism of action.

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