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Pharmacognostic and phytochemical investigations on *Pyrus pashia* Buch.-Ham. ex D. Don stem bark

Vikrant Arya, Richa Gupta and *Vivek Kumar Gupta

Department of Pharmacognosy, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, Bela, Ropar, Punjab, India

ABSTRACT

Pyrus pashia Buch.-Ham. ex D. Don is a small to medium sized tree, one of the important member of Rose family (Rosaceae), commonly known as Indian Wild Pear and has been employed as herbal medicine especially in the treatment of digestive ailments. In pharmacognostic studies, various parameters viz. ash values, extractive values, foreign organic matter content, swelling index, foaming index, moisture content, fluorescence analysis, analysis of powdered drug reaction with different chemical reagents etc. were being determined. The powdered crude drug was successively extracted using soxhlet apparatus with different solvents (in terms of increasing polarity), n-hexane, chloroform, acetone, n-butanol and water. Qualitative phytochemical analysis of these extracts revealed the presence of flavonoids, phenolics, steroids, saponins, carbohydrates and tannins in some extracts, which later on were also being confirmed by TLC technique. The morphological, microscopic, physicochemical and chromatographic studies would serve as a standard reference for identification, authentication and distinguishing the plants from its adulterants. This is the first such study on standardization on Pyrus pashia Buch.-Ham. ex D. Don stem bark.

Keywords: Rosaceae, pharmacognostic standardization, extracts, Thin Layer Chromatography.

INTRODUCTION

The genus *Pyrus*, is a tree member of subfamily Maloideae, family Rosaceae and comprising of approximately 38 species in temperate regions of the Northern hemisphere (except North America) and exceptionally enters the Northwestern tip of Africa [1]. *Pyrus* species (Pear) have

been known to have various physiological activities and have various useful polyphenol pytoconstituents *viz.* chlorogenic acids, flavan-3-ols, arbutin *etc.* [2]. These polyphenols have already been reported for their antioxidant potential [3-6], antimutagenic and anti-carcinogenic effects [3].

Pyrus pashia Buch.-Ham. ex D. Don is a small to medium sized tree with ovate-oblong leaves rounded at base, glabrous; flowers appear as white in clusters; fruits are globose shaped, dark yellow brown in colour, covered with raised white round spots. The plant is distributed in Himalayan region, Meghalaya, Manipur, Myanmar (India) and internationally found in many places including Hazara to Bhutan, Afghanistan and Pakistan. Dried ripe fruits are edible; Leaves and twigs when tender lopped as fodder; wood is used for walking sticks, combs, tobacco pipes and as fuel. *Pyrus pashia* Buch.-Ham. ex D. Don has been employed as herbal medicine and therapeutically used in the treatment of digestive ailments [7-12].

The morphological, microscopic, physicochemical and chromatographic studies would serve as a standard reference for identification, authentication and distinguishing the plants from its adulterants. This is the first such study on standardization on *Pyrus pashia* Buch.-Ham. ex D. Don stem bark**.**

EXPERIMENTAL SECTION

Collection and identification of plant

The stem bark was collected in the month of January from Disstt. Kangra, Himachal Pradesh, India. The bark was identified and authenticated (NISCAIR/RHMD 1691/289) by Dr. H. B. Singh, Scientist, Head, Raw Materials Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources, New Delhi, India. The voucher specimen of the plant (VPP/10/03) has been retained in Pharmacognosy Research Laboratory – 02, Department of Pharmacognosy, in our institution for the future reference. The collected bark was shade dried for 40 days and finally pulverized by electronic grinder machine into coarse powder. It was stored in a well closed container free from environmental climatic changes or any other contamination till usage for the further studies.

Pharmacognostic standardisation

The specimen was processed to the pharmacognostic standardisation [13-18].

Macroscopic investigations: The stem bark of plant was subjected to macroscopic studies which comprised of organoleptic characteristics *viz*. color, odour, appearance, taste, smell, shape, touch, texture, fracture, *etc*. of the drug. These parameters are considered as quite useful in quality control of the crude drug.

Determination of ash values: The ash values are useful to determine the purity and quality of the crude drug. Ash contains inorganic radicals like phosphate, carbonates and silicates of sodium, potassium, magnesium, calcium *etc*., sometime inorganically variables like calcium oxalates, silica, carbonate content of the crude drug affects ‘total ash value’. Hence, determination of ash values is also considered as important parameters in pharmacognostic evaluation of the drug.

Determination of extractive values: Extractive values give an idea about the nature of the chemical phyto-constituents present in the crude drug. The extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. The composition of these phyto-constituents in that particular solvent depends upon the nature of the drug and solvent used. The use of a specific solvent can be the means of providing information on the quality of a particular drug sample.

Foreign matter: Drug containing appreciable quantities of potent foreign matter, animal excreta, insects or mould may produce critical impact on the health. Therefore, the parameter must not be neglected. In case of whole drug a weighing quantity sample (100-500g) is spread in a thin layer on paper. It is examined at x6 magnification and foreign matter is picked out and weighed and its percentage to be recorded.

Swelling index: It is defined as the volume in milliliters occupied by 1g of a drug. The drug is treated with 1.0 ml ethanol (96%) and 25 ml water in a graduated cylinder, shaken every 10 minutes for 1 h and allowed to stand. The drugs have mucilage (has a property to swell after absorbing plenty of water) as a phyto-constituent may have different swelling index and therefore, provide the useful information.

Foaming index: The drug containing saponins has the capability to form froth which depends upon the nature of drug and/or quantity of saponins present. This parameter also provides useful information and help in quality control of the drug.

Moisture content: The drug containing excessive water beyond the normal limit, in conjunction with a suitable temperature will lead to the activation of enzymes and may lead to growth of various microorganisms *viz.* moulds, insects and mites. In the present paper, *loss on drying* method was taken in determining the moisture content.

Determination of fluorescence character: The powder material was treated separately with different reagents and exposed to short and long-wave ultraviolet light (254nm, 366nm) in studying their fluorescence behaviour.

Powdered drug reaction with different reagents: The powdered drug was treated separately with different reagents and acids like picric acid, hydrochloric acid, nitric acid, glacial acetic acid, potassium hydroxide *etc.* the colour shown by that treatment is noted as such and under the microscope

Microscopic studies (Powdered drug): Powder (#60) of the bark was used for observation of various microscopic features. The powdered drug was separately treated with phloroglucinol, hydrochloric acid, glycerine and iodine solution in determining the presence of lignified cells, calcium oxalate crystals and starch grains.

Extraction of the plant material: The powdered material (400 g) was successively extracted using soxhlet apparatus with different solvents in order to increase in their polarity *n*-Hexane, chloroform, acetone, *n*-butanol and water. The resultant extracts were concentrated by rota-

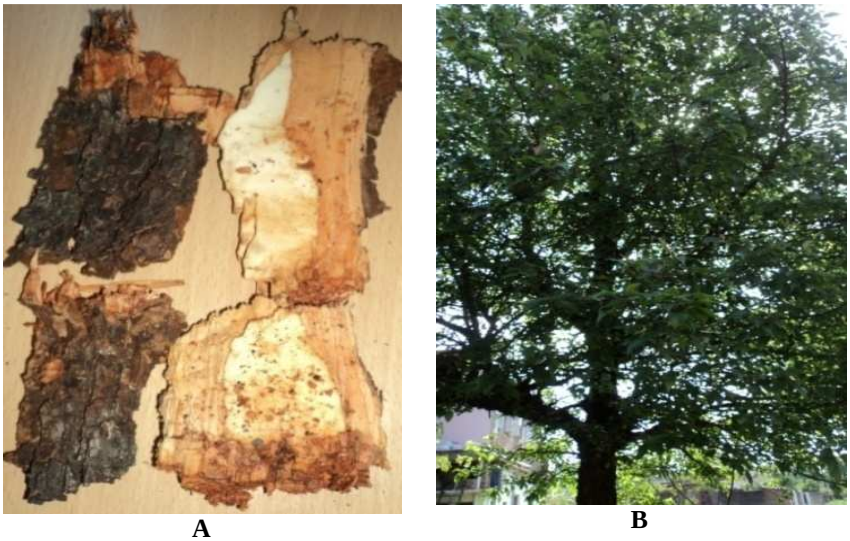
evaporator (Heidolph-Germany). The % yield *mg/g* with respect to air-dried drug has also been calculated.

Qualitative phytochemical analysis: Various types of phytoconstituents *viz.* Alkaloids, glycosides, saponins, steroids, flavonoids, tannins, proteins and amino acids *etc.* may be present in the plant. Therefore, Qualitative phytochemical analyses of all the extracts were being carried out by employing standard methods [13-18].

Thin layer chromatography: After concentration and drying of each extract in vacuum desiccator, identification of phytoconstituents was carried out by thin layer chromatography using different detecting reagents. The test extract was dissolved by using appropriate solvent in a concentration of 1 mg/mL and subjected for spotting. Silica gel G was used as a stationary phase and four solvent systems were used as mobile phase: *n*-Hexane-EtOAc (Ethyl acetate) (8:2), $CHCl_3$-MeOH (methanol)-H_2O (8:2:0.2), EtOAc-$CHCl_3$-MeOH-H_2O (12:8:8:2) and $CHCl_3$-MeOH-H_2O (65:35:10). Spots have been detected by using both non-destructive and destructive visualisation techniques. Non-Destructive technique involved use of UV light, iodine chamber and destructive technique involved use of spraying reagents (Anisaldehyde sulphuric acid and Vanillin-sulphuric acid). Various extract were applied with the help of micro capillary, just 2 cm above from the bottom. The spots were equally sized, dried, and developed and finally the R_f values have also been observed.

RESULTS AND DISCUSSION

Results of all above mentioned physio-chemical parameters for pharmacognostic studies have been provided and discussed.



A **B**

Fig.1: Photograph of *Pyrus pashia* Buch.-Ham. ex D. Don plant; A: Stem bark; B: Tree

Morphological evaluation: The results of various organoleptic characteristics *viz*. color, odour, taste, fracture, touch *etc*. of the stem bark of Pyrus *pashia* Buch.-Ham. ex D. Don (Fig. 1) have been provided in Table 1.

Table 1: **Morphological evaluation of *Pyrus pashia* Buch.-Ham. ex D. Don Stem bark**

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Features	Observations
Colour	Greyish brown (outer surface), pale yellow (inner surface)
Odour	Disagreeable
Taste	Bitter
Fracture	Irregular
Touch	Rough
Extra feature	The bark shows minute longitudinal wrinkles and fibrous fracture, internal surface smooth to touch

Pharmacognostic standardization: Water soluble extractive were found 4.4 % w/w as compared to alcohol soluble extractives 2.8% w/w, which showed bark had more water soluble phytoconstituents. Total ash was found to be 5.21% w/w so bark was definitely having inorganic content. Foaming index was less than 100 which indicated the presence of saponins in bark which was finally proves by phytochemical screening. Swelling index was 0.0 mL indicated the absence of mucilage in bark as represented in Table 2.

Table 2: Standardization parameters

Parameter	Determined value
Moisture content	34 %w/w
Total ash	5.21 % w/w
Acid insoluble ash	0.9 % w/w
Water soluble ash	1.5% w/w
Water soluble extractive	4.4 % w/w
Ethanol soluble extractive	2.8 % w/w
Foreign matter	0.0
Swelling index	0.0 mL
Foaming index	Less than 100

Fluorescence analysis: Fluorescence study is an essential parameter for first line standardization of crude drug. A scientist Stokes stated that 'in fluorescence the fluorescent light is always of greater wavelength than the exciting light'. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which do not visibly fluoresce in daylight (Table 3).

Table 3: Fluorescence analysis

Treatment	Short UV (254 nm)	Long UV (366 nm)
Powder as such	Light brown	Dark brown
1N NaOH	Black	Dark black
Acetic acid	Brown	Black
5% Iodine	Brown	Black
Methanol	Dark brown	Black
HNO_3+ NH_3	Black	Dark black
5% $FeCl_3$	Dark brown	Dark black
25% NH_3	Light brown	Dark brown
10%potassium dichromate	Black	Dark black
1M HCl	Dark brown	Dark black

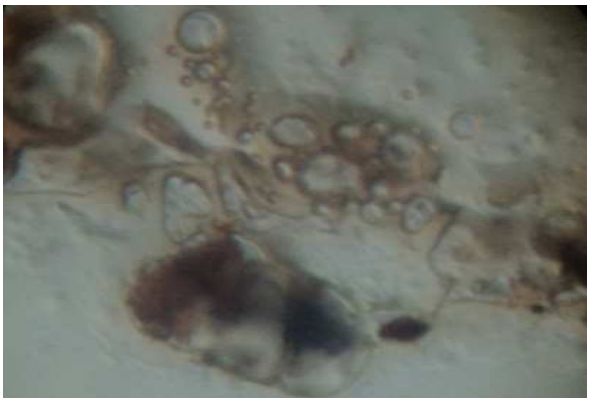
Powdered drug reaction with different chemical reagents: *Pyrus pashia* Buch.-Ham. ex D. Don stem bark powdered drug was treated with several acids and bases and other reagents. These all indicated the presence of some particular type of constituents in the plants confirmed by the phytochemical screening have been provided in Table 4.

Table 4: Colour analysis of stem bark powder after treated with different chemical reagents

Treatment	Bark
Powder as such	Light brown
Conc. HCl	Dark brown
Conc. H_2SO_4	Dark black
Conc. HNO_3	Yellowish brown
Glacial acetic acid	Brown
5%$FeCl_3$	Dark green
5%KOH	Reddish brown
Picric acid	Yellow

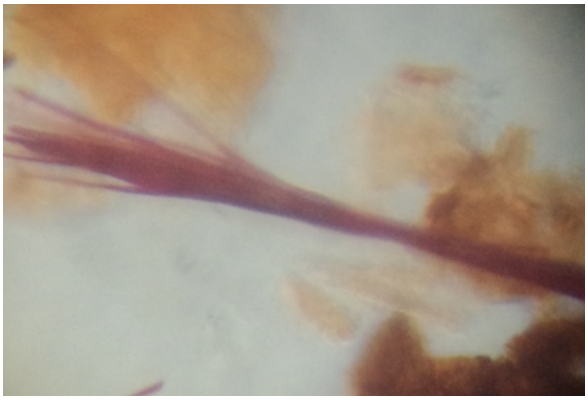
Microscopic studies: Powder was light brown in colour with bitter and astringent taste having characteristic odour. Histochemical colour reactions were applied with Hydrochloric acid-Phloroglucinol to reveal the lignified elements, weak iodine solution for starch grains, 40% H_2SO_4 for the calcium oxalate crystals. Microscopically powdered *Pyrus pashia* Buch.-Ham. ex D. Don bark showed the presence of oval shaped cork cells (Fig. a). Pericyclic fibers were lignified long slender tapering towards both the end (Fig. c). Phloem fibers arranged radially, in vertical columns, intermingled with phloem parenchyma (Fig. d) and xylem fibres were non-lignified (b). Medullary rays multiseriate run in parallel rows separating phloem (Fig. h). Thick walled stone cells were observed (Fig. e). Starch grains were very few (Fig. f) along with monocyclic (Fig. i, j) and tetragonal type of prismatic crystals calcium oxalate crystals (Fig. k, l).

Extraction: Successively soxhlet extraction with different polarity solvents revealed that maximum percentage of extract were obtained in case of acetone (3.11%w/w) and minimum with *n*-hexane (0.27%w/w) as represented in table 5.



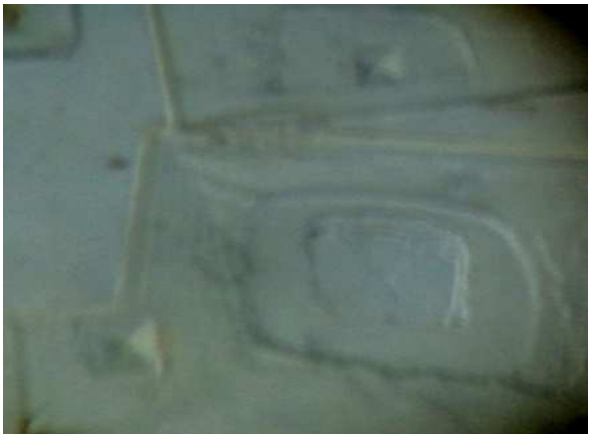
a: Cork cell

b: Xylem fibre

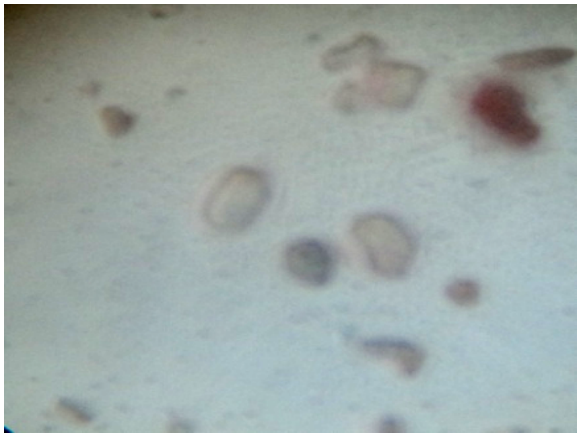


c: Lignified pericyclic fibres

d: Pholem fibre



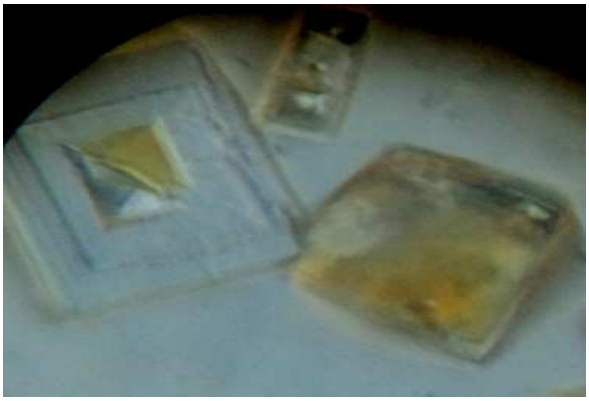
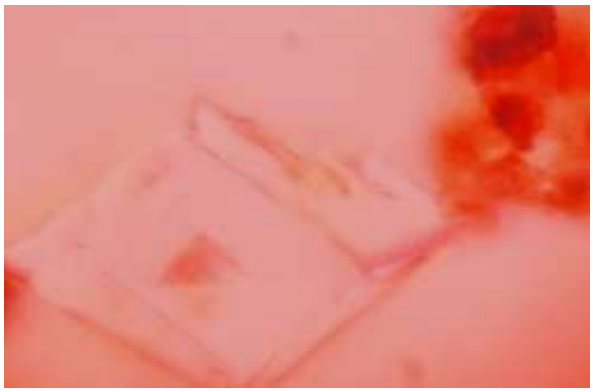
e: Stone cell with calcium oxalate prisms



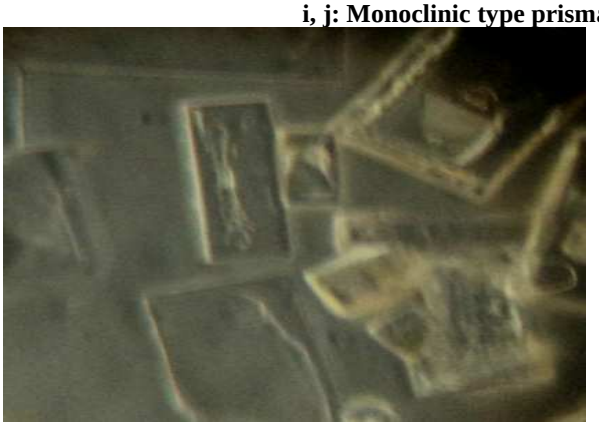
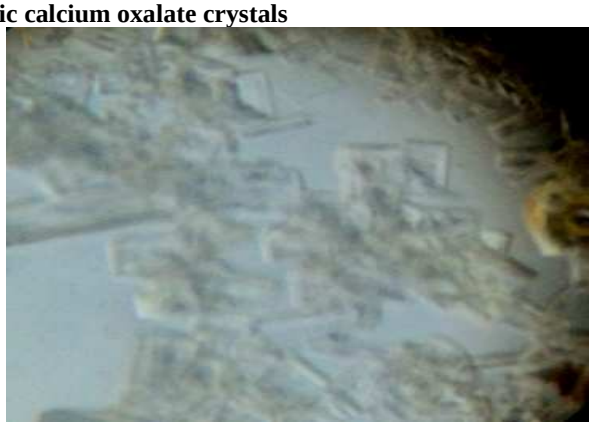
f: Starch grains

g: Fibres with stone cells

h: Medullary rays

i, j: Monoclinic type prismatic calcium oxalate crystals

k, l: Tetragonal type prismatic calcium oxalate crystals

Table 5: Percentage extractives and physical characteristics of the various extracts

Extract	%Dry wt. (w/w)	Colour	Odour	Consistency
n-Hexane	0.27	Light greenish	Characteristic	Sticky
Chloroform	0.47	Dark brown	Pungent	Waxy
Acetone	3.11	Orange brown	No odour	Powdered
n –Butanol	0.85	Red	Characteristic	Sticky
Water	1.52	Reddish brown	No odour	Powdered

Table 6: Qualitative Phytochemical analysis of various extracts of *Pyrus pashia* Buch.-Ham. ex D. Don stem bark

Extract Constituents	Hexane	Chloroform	Acetone	Butanol	Aqueous
Alkaloids	-	-	-	-	-
Carbohydrates	-	-	-	-	+
Glycosides	-	-	-	-	-
Flavonoids	-	-	-	-	+
Proteins and amino -acids	-	-	-	-	-
Saponins	-	-	-	-	+
Tannins	-	+	+	+	-
Lipids/fats	+	-	-	-	-
Steroids	-	+	+	-	-

(+) Positive test, (-) Negative test

Preliminary phytochemical screening: Preliminary phytochemical screening of various extracts of *Pyrus pashia* Buch.-Ham. ex D. Don stem bark showed the presence of carbohydrates, tannins, flavanoids, saponins. Main attraction of phytochemical screening was presence of tannins and sterols in maximum of extracts (chloroform, acetone, *n*-butanol). Flavonoids were present only in aqueous extract. These phytoconstituents were known to show medicinal activity as well as exhibiting physiological activity [3-10]. The presence of phenolics in the present study was supported the opinion of *Challice and Williams 1968* [19]; *Challice and Westwood 1972* [11-12] who noted the presence of phenolics in *Pyrus* genus (Table 6).

Thin layer chromatography (TLC): Thin layer chromatography of different extracts using various solvents in different ratios as a mobile phase showed presence of various compounds as represented in fig. 3.

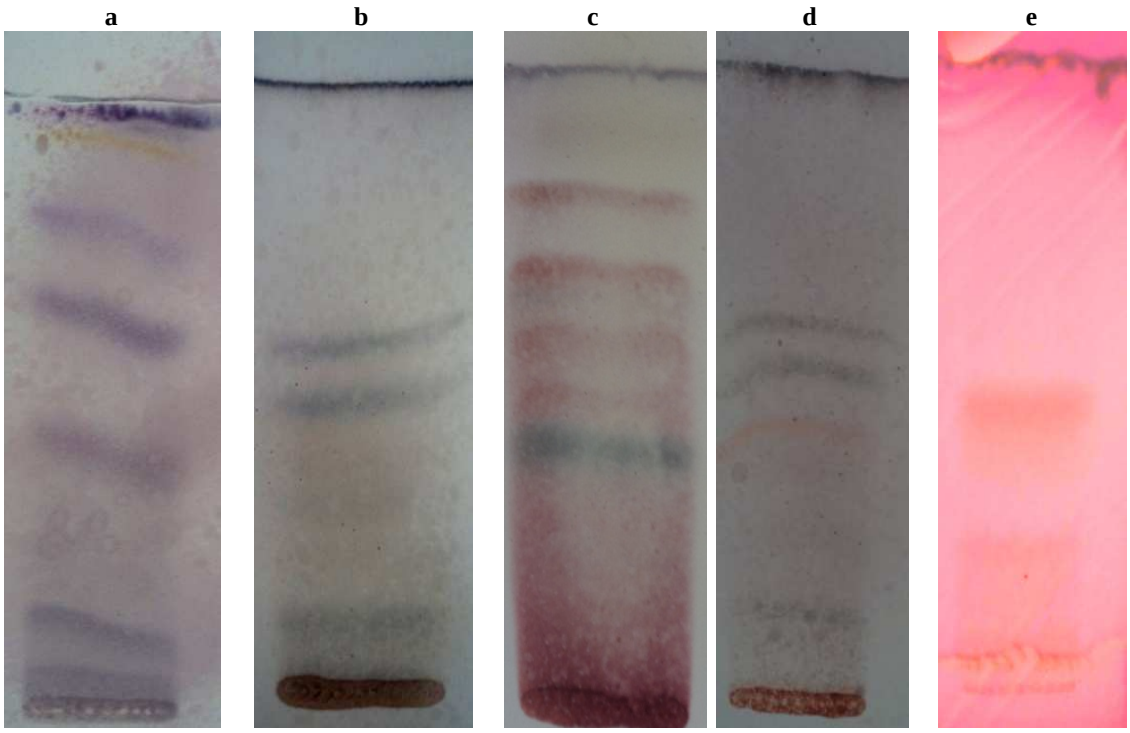


Fig.3: TLC of various extracts (a) TLC of *n*-hexane extract, solvent system *n*-Hexane-EtOAc (8:2), (b) TLC of Chloroform extract, solvent system $CHCl_3$-MeOH-H_2O (8:2:0.2), (c) TLC of acetone extract, Solvent system EtOAc-$CHCl_3$-MeOH-H_2O (12:8:8:2), (d) TLC of *n*-butanol extract $CHCl_3$-MeOH-H_2O (8:2:0.2), (e) TLC of aqueous extract $CHCl_3$-MeOH-H_2O (65:35:10)

TLC of the *n*-Hexane extract on Silica gel 'G' plate using mobile phase as *n*-Hexane-$CHCl_3$ (8:2) showed six different colour spots at R_f 0.19, R_f 0.20, R_f 0.34, R_f 0.55, R_f 0.77 and R_f 0.92. TLC of the Chloroform extract on Silica gel 'G' plate using mobile phase as $CHCl_3$-MeOH-H_2O (8:2:0.2) showed four different colour spots at R_f 0.17, R_f 0.37, R_f 0.49, R_f 0.52. TLC of the Acetone extract on Silica gel 'G' plate using mobile phase as EtOAc-$CHCl_3$-MeOH-H_2O (12:8:8:2) showed five different colour spots at R_f 0.32, R_f 0.34, R_f 0.36, R_f 0.42, R_f 0.49. TLC of the *n*-Butanol extract on Silica gel 'G' plate using mobile phase as $CHCl_3$-MeOH-H_2O (8:2:0.2) showed four different colour spots at R_f 0.12, R_f 0.22, R_f 0.35, R_f 0.41. TLC of the

aqueous extract on Silica gel 'G' plate using mobile phase as $CHCl_3$-MeOH-H_2O (65:35:10) showed three different colour spots at R_f 0.23, R_f 0.42, R_f 0.50

CONCLUSION

Pyrus pashia Buch.-Ham. ex D. Don is traditionally important medicinal plant. The pharmacognostic parameters which are being reported for the first time could be useful in the identification and standardization of crude drug. The data produced in the present investigation is also helpful in the preparation of the *Pyrus pashia* Buch.-Ham. ex D. Don monograph and inclusion in various pharmacopoeias. The bark of *Pyrus pashia* Buch.-Ham. ex D. Don contains phytoconstituets like steroids, saponins, fats, flavonoids, tannins, carbohydrates. The TLC results of the *n*-hexane, chloroform, acetone, *n*-butanol and aqueous extract showed that four or five different phytoconstituents were present in each extract of *Pyrus pashia* Buch.-Ham. ex D. Don stem bark. More detailed study must be done for further isolation leading to pure compounds.

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