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PHARMACOGNOSTIC STUDIES OF "*PTEROLOBIUM HEXAPETALUM* (ROTH.) SANT. AND WAGH."

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ABSTRACT

Pterolobium hexapetalum "Yerracheeki" is an herbal medicine used by the Chenchu tribes of Nallamalai region against diarrhoea, skin and veneral diseases, cold and cough, jaundice, bone fractures and ulcers. Phytochemical screening of all parts resulted important secondary metabolites like flavonoids, phenols, alkaloids, glycosides, tannins and steroids. Also showed antibacterial activity against four pathogenic bacteria with aqueous and methanol extracts effectively at 0.312 to 1.25 mg of MIC values to that of *Gentamycin* and antifungal activity against two strains with 0.312 and 0.625 mg of MIC values compared to *Nystatin*. Hence various plant parts have been tested for their morphological, physicochemical, histochemical and qualitative analysis of phenols, flavonoids and anthocyanidins to standardize the crude drug for future formulations and also as a check for adulteration. Qualitative analysis resulted 38 phenols, 12 flavonoids and 10 anthocyanidins components in all parts. Histochemistry proved the distribution of various phytoconstituents in all parts supported various herbal formulations of each part. Especially Phenols like protocatechuic acid acts as antioxidant and anti-inflammatory; Chlorogenic acid as antimicrobial; p-hydroxy benzoic acid antifungal also acts as anti rheumatic antipyretic; Ferulic acids as antioxidants and antifungal; Scopoletin and vanilic acid as antimicrobial; Melilotic acid and syringic acid against hepatoprotective and antimicrobial; Coumarins against inflammations, hypertension, osteoporosis and analgesic; Salicylic acid anti-inflammatory; Cinnamic acid as antifungal, flavonoids like myricetin reduces prostate cancer and jaundice. Quercetin and kaempferol anti-inflammatory; Luteolin, orientin and vitexin reduces respiratory disorders and chronic bronchitis; Anthocyanidins like delphinidin is major component used in ophthalmic disorders. Cyanidin and peonidin are responsible to

reduce cardiovascular diseases and cancer. Hence the herbal drugs are proved as the best anti microbial against pathogenic bacteria which causes skin diseases, liver damage, bronchial disorders, haemorrhages, inflammations, arthritis, ulcers as used by the herbalists. The herbal formulations are further recommended for testing in the respective bioactivity to prove scientifically for future studies in the isolation of bioactive compounds and in drug designing.

KEYWORDS: Phenols, flavonoids, anthocyanidins, ash values, extractive values, qualitative analysis.

INTRODUCTION

Pterolobium hexapetalum (Caesalpiniaceae) is one of the important medicinal plant with significant herbal uses against diarrhea, skin disorders, constipation, piles and venereal diseases with leaf, stem, bark, fruit and flower parts. Herbal drug also possess high quantities of significant phytoconstituents in leaf and stem bark extracts like alkaloids, flavonoids, phenols, glycosides, tannins, quinones and steroids showed effective antibacterial activity with aqueous and methanol extracts at 10mg/well with 0.312- 0.625mg of MIC values.[1] Antifungal activity of fruit extracts on *A. niger* and *C. candida* supports its herbal uses against diarrhea, constipation, piles and ulcer with fruit aqueous, methanol, benzene and alcohol extracts at 10mg/well proved effective with 26-36mm zone of inhibition on both organisms with 0.156-0.625mg of Minimum Inhibitory Concentrations.[2] Hence pharmacognostic studies of the leaf, stem, stem bark, flower and fruit fresh materials and dry powders morphological, physico chemical, histochemical and qualitative studies of phenols, flavonoids and anthocyanidins was carried out to standardize the herbal drug quality, purity and authenticity for further studies in testing the biological activities, isolating the bioactive components and in designing the drugs against respective traditional uses of the *P. hexapetalum* crude drugs.

MATERIALS AND METHODS

Collection and identification of plant material

The plant material *P. hexapetalum* was collected during July-December 2011 from Nallamallai forests of Mahanandi area of Kurnool district, Andhra Pradesh, India. The taxonomic identification of the species is confirmed by Prof. N. Yasodamma and voucher specimens B.K:7, B.K:12 has been deposited in the department herbarium (SVUTY), Department of Botany, Sri Venkateswara University, Tirupati for future reference as per standard methods.[3]

Macroscopic/ Morphological and Organoleptic characters

Habit, morphology; color, odour, taste, texture, fracture of stem, leaf, flower and fruit were observed.[4]

Physico-Chemical Analysis

For the determination of ash values of leaf, stem bark, flower and fruit powders were tested the following tests as per the methods.[5]

Total ash

About 1g of each powder is accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air-dried powder.

Acid insoluble ash

The ash obtained as described above was boiled with 25ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Water soluble ash

The ash obtained as described for the total ash, was boiled for 5 minutes with 25ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference of weight was considered as water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried parts respectively.

Sulfated ash

A silica crucible was heated to red for 10min and was allowed to cool in desiccators and weighed. A gram of substance was accurately weighed and transferred to the crucible. It was ignited gently at first, until the substance was thoroughly charred. Then the residue was cooled and moistened with 1ml of concentrated sulfuric acid, heated gently until white fumes are no longer evolved and ignited at $800^{o}C \pm 25^{o}C$ until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool. A few drops of concentrated sulfuric acid were added and heated ignited as before and was allowed to cool and weighed.

Moisture content

Air dried material of 1g was dried in an oven at 105°C. The loss of weight was calculated and values were tabulated.

Foreign matter

100g of the powdered drug is taken and spread out in a thin layer on a slide and observed free from foreign matters like soil, insect parts or animal excreta. They are separated and weighed and the percentage was calculated.

Fluorescence analysis

A small quantity of dried and finely powdered leaf, stem bark, flower and fruit were placed on a porcelain tile and 1-2 drops of freshly prepared organic reagents were added and mixed gently and waited for 1-2min to observe the colors under Ultra Violet viewer chamber and viewed in Day light, Short (245nm) and long (360nm) Ultra Violet radiation. The colors observed by application of different concentration of acids in different radiations were recorded. Inorganic and organic reagents like 50% sulphuric acid, Conc. HCl, 50% HCl, 50% Nitric acid, Ethanol, Sodium hydroxide + Water, 10% Sodium hydroxide, Conc. Sulphuric acid, Conc. Nitric acid, 5% Ferric chloride, 5% Potassium hydroxide, with water, Acetic acid, 1N HCl and 1 N NaOH were used.[6]

Extractive value determination

Fifty grams of coarsely powdered air dried material of leaf, stem bark, flower and fruit were macerated with 250ml of each solvents, placed in a glass stoppered conical flask (cold water, hot water, methanol, ethanol, ethyl acetate, benzene, chloroform, acetone and petroleum ether) shaking frequently, and then allowing it to stand for 18 hours. Filter it rapidly through what man No.1 filter paper, taking care not to lose any solvent. Transfer 25ml of filtrate to

flat bottom dish and evaporate the solvent on a water bath. Dry at 105^0 C for 6 hours, cool in a desiccator for 30 minutes and weigh it immediately. Calculate the content of extractable matter in % of air-dried material.[7]

Histochemistry

Hand sections of fresh stem, stem bark and fruit were stained with a series of histochemical reagents as a) Safranine (1% Safranine in 50% alcohol) for lignins b) Iodine solution for cellulose c) Ruthenium red for mucilage d) Iodine for starch e) Wagner"s reagent for alkaloids f) Dilute $FeCl_3$ solution for tannins g) Conc. H_2SO_4 for saponins h) 20% aq. NaOH for sugars i) heating with strong KOH along with sulphuric acid for Suberins.[6,8,9]

Qualitative Analysis of Phenols

Extraction of Phenols

Fresh leaves, stem bark, flowers and fruits were collected and extracted.[10-11] About 30 gm of the healthy and fresh, washed plant material was macerated in approximate 100ml of 2N HCl. The homogenate was digested on a boiling water bath for about 30mts. The contents were cooled and filtered through whatman no:1 filter paper. The filtrate was extracted with peroxide free diethyl ether (solvent ether) repeatedly. The cooled extract was concentrated to a small volume and was treated thrice with 25ml of 5% anhydrous Na_2CO_3 solution. The cooled Na_2CO_3 solution was adjusted to pH 2.0 with concentrated HCl. The acidified fraction at pH 2.O was then extracted with equal volumes of fresh diethyl ether for three times and the combined ether extracts were washed with 2ml of distilled water for 3mts to remove the traces of HCl. The ether soluble water was removed by freezing the extract and then the ether was evaporated to dryness on water bath at 98°C. The resulting residue was dissolved in 1ml of 95% ethanol and was preserved at low temperature in a dark container until used.

Separation of Phenols

1gm of the extract was spotted on 23 × 29cm whatman No: 1 chromatographic paper with the help of a micropipette. The origin of the spot was dried immediately with the help of hair drier. The dried sheets were run in bidimensional ascending chromatography by using rectangular chromatographic glass tanks which can accommodate 4 sheets at a time. The chambers are saturated with the chromatographic solvents one day before. The development of the chromatograms has to be carried out at 22°-24°C. Solvent I Benzene: Acetic acid: water (60: 70: 30) v/v/v (upper layer of this mixture used at first direction). Solvent II Sodium formate: Formic acid: water (10: 1: 200) v/v/v (used for second direction). The

papers after development were removed from the tanks and dried at room temperature. The dried sheets were examined under UV light and the fluorescent regions were marked. Then the papers exposed to ammonia vapours were also observed under UV light and the new fluorescent spots were also marked.

Identification of Phenolic Compounds using Chromatogenic Spray Reagents

To identify the phenolic compounds separated on the chromatograms they were sprayed with Diazotised Sulphanilic acid, Paranitranilins and ferric chloride reagent with the help of an automizer. The phenolic compounds were identified by calculating their Rf values of individual spot colors with chromogenic sprays and finally confirmed with authentic samples by chromatography. Diazotised Sulphanilic acid: 25 ml of freshly prepared 5% Sodium Nitrite solution was slowly added to 5 ml of Sulphanilic acid solution at 0°C to intensify the colors of the developed spots. 20% Sodium Carbonate solution was also sprayed at 0°C immediately after spraying the above solution. The solutions can be prepared as below. Sulphanilic acid: 900mg of Sulphanilic acid was dissolved in 9ml of concentrated HCl until entire Sulphanilic acid was dissolved in 9ml of HCl then it was diluted to 100ml with distilled water and cooled to 0°C. 5% Sodium Nitrite: 5gms of Sodium Nitrite was dissolved in glass distilled water and made up to 100ml and cooled to 0°C before use. 20% Anhydrous Sodium Carbonate: 20gms of Anhydrous Sodium Carbonate was dissolved in distilled water and made up to 100ml and cooled at 0°C. Diazotised Para Nitraniline Reagent: This reagent was prepared by mixing 10ml of Para Nitraniline reagent with 0.2 ml of 5% Sodium Nitrite and 10ml of 10% Sodium Carbonate solutions respectively. The solutions can be prepared as below (Smith, 1960). Para Nitriniline: 0.5 gms of Para Nitriniline were dissolved completely in 15ml of concentrated HCl and it was made up to 317ml glass distilled water. 5% Anhydrous Sodium Nitrite: 5gms of Sodium Nitrite was dissolved in 100ml of distilled water. 10% Sodium Carbonate: 10gms of Sodium Carbonate was dissolved in distilled water and made up to 100ml. Ferric Chloride reagent: This solution was prepared by dissolving 1gm of Ferric Chloride in distilled water diluting it to100ml.

Qualitative Analysis of Flavonoids

Extraction of Flavonoids: The flavonoid compounds were extracted.[12] About 2gm of plant part dried powder at 40°C was taken in a boiling test tube and then 18ml of methanol and 2ml of water (9:1) was added. Shaken well and was kept for about 24 hours at room temperature. After that the upper clear solution of the extract was transferred to another test

tube. To the remaining residue in the test tube, again 5ml of methanol and 5ml of water (1:1) was added. Stirred well and the contents were kept for 24 hours and the clear extract thus obtained was cooled up with the earlier sample. The combined extract was mixed well and filtered through the cotton. The filtrate was evaporated to about 1/3 of the original volume and the resultant aqueous extract was taken into a separatory funnel and then extracted with 10ml of chloroform. This process was repeated 2or 3 times. All the chloroform extracts were combined and evaporated to dryness under vaccum in a rotatory evaporator and the dried residue was dissolved in 1ml of 95% alcohol which was stored at low temperature in the dark until used.

Separation of Flavonoids

1 gm of the extract was spotted on 23x29cm Whatmann No: 1 chromatographic filter papers with help of a micropipette. The origin of the spot areas was dried immediately with the help of hair drier. The dried sheets were run in unidimensional ascending chromatography by using rectangular chromatographic glass tanks which can accommodate 4 sheets at a time. The chromatographic chambers were saturated with any of the following solvents one day before the development of the chromatograms at 22-24°C.

1. Iso-Propyl alcohol: Ammonia (25%): Water (8: 1: 1) v/v/v
2. n-Butanol: Acetic acid: Water (4: 1: 5) v/v/v (top layer was used)
3. Conc. HCl: Acetic acid: water (3: 30: 10) v/v/v
4. Phenol: Water (3: 1) v/v/v

The chromatograms after unidimensional development were removed from the tanks and dried at room temperature. The dried sheets were observed under UV light and the fluorescent regions were marked. The papers while exposed to ammonia were also observed under UV light and the new fluorescent spots were also marked.

Identification of Flavonoids Using Chromogenic Spray Reagents

To identify the flavonoids compounds separated on the chromatograms they were sprayed with chromogenic spray reagents with the help of an automizer. The flavonoid compounds were identified by calculating their Rf values of individual spot colors with authentic samples by co-chromatographic studies.

Sulphanilic acid

200mg of Sulphanilic acid was dissolved first in 2ml of Conc. HCl and to this 18ml of distilled water was added.

Sodium Nitrite

1gm of Sodium Nitrite was dissolved in 20ml of glass distilled water.

Anhydrous sodium carbonate

4gms of anhydrous Sodium Carbonate was dissolved in 40ml of distilled water. The above 1 and 2 solutions were mixed and allowed to stand for about 5minutes at room temperature and cooled in ice until it was used. The reagent was kept in air for some time to melt in cooled reagent and sprayed on sheets, to intensify the colors of the developed spots the 3rd reagent was a lso sprayed, while the sheets were in wet condition.

Qualitative Analysis of Anthocyanidins

Extraction for Anthocyanidins

Anthocyanidins were extracted.[11] About 5 gms of leaf, stem bark and fruit powders were heated in 20ml of 2N HCl in a boiling test tube for 40 minutes at 100ºC. The colored extract was cooled and decanted from the plant tissue. The cooled extract was twice washed with 20ml of ethyl acetate to remove flavonoid compounds. The ethyl acetate layers discarded and the remaining aqueous extract heated at 80º C for 30mts to remove the last traces of ethyl acetate. The pigment was then extracted into a small volume of Iso-amyl alcohol in a separatory funnel. The Iso-amyl alcohol extract was evaporated to dryness on a boiling water bath. The anthocyanidins in the residue was eluted with 1ml of 1% methanolic HCl and was preserved at low temperature in a dark container until used.

Separation of Anthocyanidins

1 gm of anthocyanidin extract was spotted on 23x29 cm whatman No: 1 chromatographic filter paper with the help of micropipette. Origin of the spot areas was dried immediately with the help of hair drier. The dried sheet was ran in uni dimensional ascending chromatography by using rectangular chromatographic glass tanks. The chromatographic chambers were saturated with an appropriate quantity of suitable solvent one day before the development of the chromatogram at 22-24ºC.

1. Conc HCl: Formic acid: Water (2:5:3) v/v/v
2. n-Butanol: Acetic acid: Water (4:1:5) v/v/v
3. n-Butanol: 2N HCl (1:1) v/v/v

Identification of Anthocyanidins

The chromatograms after unidimensional development were taken from the glass chambers and dried at room temperature. The dried papers were observed under UV light and the fluorescent regions were marked. The sheets were also observed under UV Light while exposing to ammonia vapours and fluorescent intensified spots were recorded. The Rf values and colors of the spots were determined.

RESULTS

Table -1: Macroscopic / Organoleptic characters

Characters	Plant parts			
	Leaf	**Stem bark**	**Flower**	**Fruit**
Colour	Green	Brown	Yellowish red	Reddish brown
Odour	Characteristic	Slight	Pleasant aroma	Slight characteristic
Taste	Bitter	Astringent	Light sweet	Astringent
Texture	Fine	Coarse	Coarse	Coarse
Fracture	Smooth	Fibrous	Smooth	Rough

Macroscopic / Organoleptic characters: (Table -1) (Plate: 1)

Leaf powders green, characteristic odour, bitter taste and fine texture with smooth fractures. **Stem bark** brown with ruff in nature, slight odour, astringent and coarse texture with fibrous fractures. **Flower** powders yellowish red, with pleasant aroma, light sweet taste and coarse texture with smooth fractures. **Fruit** powders reddish brown, slight characteristic odour, astringent and coarse texture with rough fractures.

LEAF **STEM BARK**

FLOWER **FRUIT**

Plate-1: Powder drugs – Organoleptic characters

Physico chemical study

Table 2: Ash values: Powdered drug: (%)

Plant parts	Total ash	Water soluble ash	Acid insoluble ash	Sulphated ash	Moisture content
Leaf	46.0	25.0	3.62	1.18	2.80
Stem bark	50.0	26.9	4.68	2.34	2.12
Flower	41.2	20.9	0.98	1.12	3.12
Fruit	45.6	22.6	3.26	2.50	2.92

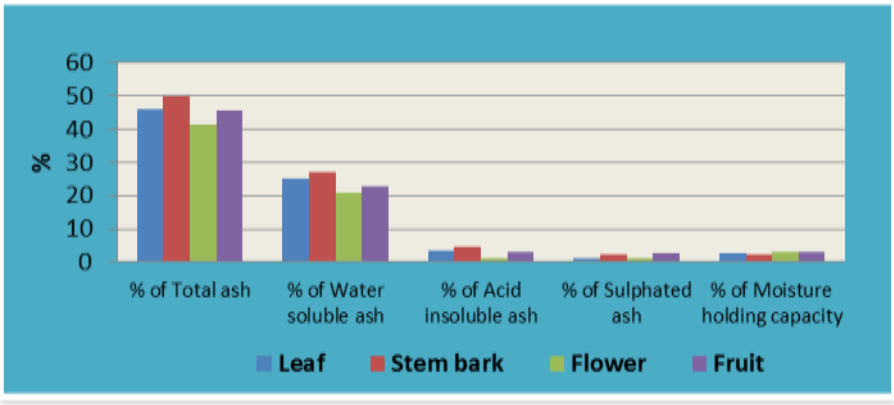


Figure 1: Ash values: Powdered drug: (%)

Ash Values: (Table: 2; Figure: 1)

Total ash

Total ash of leaf, stem bark, flower and fruit was found to be 46.0%, 50.0%, 41.2%, 45.6%, respectively. It is highest in stem bark followed by leaf, fruit and flower. Water soluble ash was found to be 25.0%, 26.9%, 20.9%, and 22.6%, respectively. It was least in flower and highest in stem bark. Acid in soluble ash in soluble, ash valves are 3.62%, 4.68%, 0.98%, 3.26% respectively, it was highest in stem bark and lowest in flower. Sulphated ash is 1.18%, 2.34%, 1.12%, and 2.50% of sulphated as was obtained respectively. It was highest in fruit and lowest in flower. Moisture content / Loss on drying were 2.80%, 2.12%, 3.12%, 2.92% respectively. It was highest in flower followed by fruit, leaf and stem bark.

Table 3: Extractive values (%)

Treatment	Plant parts	Extraction	Filtrate color	Extract nature and color
Cold water	Leaf	4.40	Light brown	Sticky brown
	Stem bark	2.54	Light brown	Semisolid brown
	Flower	1.54	Light brown	Semisolid brown
	Fruit	5.25	Brown	Semisolid brown
Hot water	Leaf	15.35	Dark brown	Sticky dark blackish brown
	Stem bark	10.32	Dark brown	Semisolid dark brown
	Flower	5.25	Reddish brown	Sticky dark reddish brown
	Fruit	14.27	Golden brown	Crystalline dark blackish golden

Methanol	Leaf	14.5	Dark green	Sticky dark blackish green
	Stem bark	10.38	Dark brown	Semisolid reddish brown
	Flower	5.24	Dark green	Sticky dark green
	Fruit	15.25	Dark green	Sticky dark blackish green
Ethanol	Leaf	8.54	Green	Sticky dark green
	Stem bark	7.28	Yellowish brown	Semisolid dark yellowish brown
	Flower	2.25	Green	Semisolid green
	Fruit	7.98	Brownish green	Sticky dark brownish green
Chloroform	Leaf	3.56	Dark green	Semisolid dark blackish green
	Stem bark	3.76	Yellowish green	Semisolid green
	Flower	1.19	Light green	Semisolid green
	Fruit	4.0	Green	Semisolid green
Benzene	Leaf	4.25	Green	Semisolid dark green
	Stem bark	3.22	Light green	Semisolid green
	Flower	1.25	Light green	Semisolid green
	Fruit	4.35	Yellowish green	Semisolid dark green
Ethyl acetate	Leaf	2.25	Green	Semisolid dark green
	Stem bark	1.55	Light yellowish	Semisolid green
	Flower	1.17	Light green	Semisolid green
	Fruit	3.0	Green	Semisolid dark green
Acetone	Leaf	12.50	Green	Semisolid dark green
	Stem bark	8.38	Light green	Semisolid green
	Flower	4.24	Yellowish brown	Semisolid light yellowish brown
	Fruit	12.25	Yellowish brown	Semisolid dark yellowish brown
Petroleum ether	Leaf	2.80	Green	Semisolid green
	Stem bark	2.26	Yellowish green	Semisolid green
	Flower	1.50	Light green	Semisolid green
	Fruit	2.58	Light green	Semisolid green

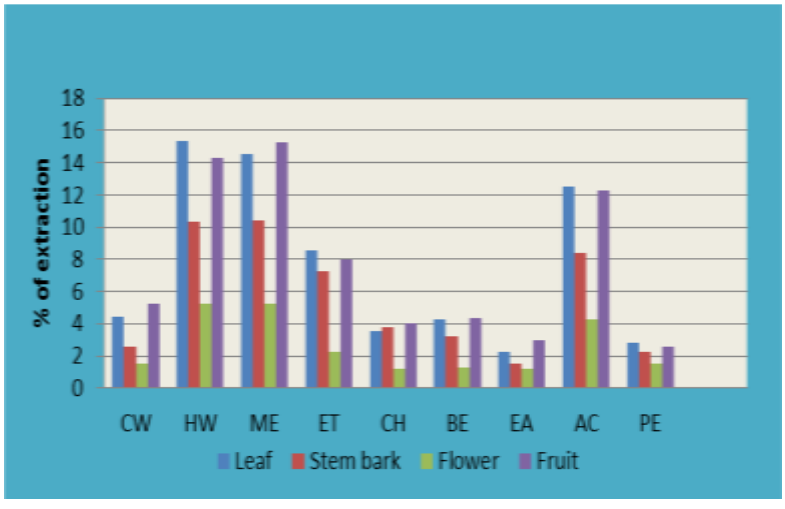


Figure- 2: Extractive values (%)

CW: Cold water**, HW:** Hot water, **ME:** Methanol, **ET:** Ethanol **CH:** Chloroform**; BE:** Benzene. **EA:** Ethyl acetate**, AC:** Acetone, **PE:** Petroleum ether.

Extractive values: (Table -3; Figure -2)

Extractive values of leaf stem bark, flower, and fruit with different solvents hot water, coldwater, methanol, alcohol, benzene, chloroform, petroleum ether and acetone were observed. Hot water leaf and methanol fruit yielded highest amounts of extractive 15.35%, 15.25% followed by ethanol and acetone extracts and chloroform, ethyl acetate flower yielded very low quantities 1.19%, 1.17% of extract values than other extracts. **Extractive color and nature of residues** of leaf, stem bark, flower, and fruit shows specific colors and nature in all solvents. Fruit hot water extracts yielded crystalline dark blackish golden yellow color residue nature it is very differing than other extracts.

Table- 4: Fluorescence analysis

Treatment	Leaf			Stem bark			Flower			Fruit		
	Day light	Short UV (250-270)	Long UV (365-390)	Day light	Short UV (250-270)	Long UV (365-390)	Day light	Short UV (250-270)	Long UV (365-390)	Day light	Short UV (250-270)	Long UV (365-390)
Powder as such	G	DG	B	B	B	BR	LG	LG	B	LG	LG	B
Conc. H_2SO_4	BLG	DBL	BL	BG	BG	B	DG	BLG	BL	BLG	BLG	BL
50% H_2SO_4	DG	DG	BL	DG	DG	B	DG	DG	BL	DG	DG	BL
Conc. HCl	DG	DG	BL	G	DG	B	G	DG	BL	G	DG	BL
50% HCl	G	DG	BL	G	DG	B	G	G	B	G	DG	BL
1N HCl	G	G	DG	LG	LG	B	LG	G	B	LG	LG	B
Conc. HNO_3	BR	Y	BR	BR	Y	BR	BR	Y	BR	BR	Y	BR
50% HNO_3	BR	GY	BL	BR	B	BR	B	B	BR	B	B	BR
Acetic acid	DG	DG	BL	LB	G	B	G	G	B	G	G	B
NaOH + H_2O	DG	DG	BL	DG	DG	BL	G	G	B	DG	DG	BL
10% NaOH	DG	DG	DB	DG	DG	DB	G	G	B	DG	DG	BL
Powder with 1 N NaOH	DG	DG	BL	DG	DG	B	G	G	B	G	DG	BL
5% KOH	G	DG	DBL	G	DG	DB	G	DG	DB	G	DG	DB
5% $FeCl_3$	BLG	DG	DBL	BLG	DG	DBL	G	DG	BL	G	DG	DBL
Ethanol	G	G	BL	G	DG	BL	G	DG	B	G	DG	B
With Water	G	G	BL	LB	B	B	G	G	B	G	G	B

B: Brown, **G:** Green, **Y:** Yellow**, BL:** Black, **DG:** Dark green, **LB:** Light brown, **LY:** Light yellow, **LG:** Light green, **GY:** Greenish yellow, **BR:** Bright red, **DB:** Dark brown, **BG:** Brownish green, **BLG:** Blackish green, **DBL:** Dark black.

Fluorescence analysis: (Table -4)

The color of the leaf, stem bark, flower and fruit extracts was observed both under ordinary and UV light at 250 to 270 and 365 to 390 nm showed various color radiations which help in identifying the drug in powder forms. **Leaf** powder exhibited different colors green, dark green, brown, black, brick red yellow, greenish yellow and red; **Stem bark** exhibited brown, dark green, black, brownish green light green light brown; **Flower** with light green, brown, dark green, yellow, brick red, brownish red; **Fruit** with light green, brown, dark green, brick red, black, blackish green when treated with different chemicals and observed under UV and day light.

Table-5: Histochemical studies

Test	Reagent used	Plant part	Nature / % of change		Histological zone
Lignins	Safranine (1%)	Stem	Red	++	Epidermis, stele
		Stem bark	Red	++	Secondary phloem
		Fruit	Red	++	Exocarp, mesocarp, endocarp
Cellulose	Iodine solution	Stem	Pale yellow	+	Epidermis, cortex, protoxylem
		Stem bark	-	-	-
		Fruit	Pale yellow	+	Exocarp
Mucilage	Ruthenium red	Stem	Pink	++	Trichomes, cuticle, epidermis
		Stem bark	-	-	-
		Fruit	Pink	+	Exocarp
Starch	Iodine	Stem	Blue	+	Xylem
		Stem bark	Blue	+	Secondary phloem
		Fruit	Blue	+	Mesocarp
Sugars	20% Aq. NaOH	Stem	Yellow	++	Cortex, pith
		Stem bark	Yellow	+	Secondary cortex
		Fruit	Yellow	+	Mesocarp
Alkaloids	Wagner's reagent	Stem	Brown	++	Cortex, phloem
		Stem bark	Brown	++	Secondary cortex
		Fruit	Brown	+	Mesocarp
Tannins	Dil. $FeCl_3$ solution	Stem	Blackish blue	++	Epidermis, hypodermis, phloem, pith
		Stem bark	Blackish blue	++	Cork cambium, secondary phloem
		Fruit	Blackish blue	++	Mesocarp

Saponins	Conc. H_2So_4	Stem	Light yellow	+	Epidermis, hypodermis
		Stem bark	Light yellow	+	Secondary cortex
		Fruit	Light yellow	+	Exocarp, mesocarp
Suberins	KOH+ H_2So_4	Stem	Light brown	+	Epidermis, hypodermis
		Stem bark	Light brown	++	Cork cambium
		Fruit	Light brown	+	Exocarp

Histochemical study: (Plate- 2, 3; Table -5)

Lignins are localized in epidermis, cortex, stele of stem; secondary phloem of stem bark; exocarp, mesocarp, endocarp of fruit, pink in colour. **Cellulose** in epidermis, cortex, protoxylem of stem; exocarp of fruit, pale yellow. **Mucilage** in trichomes cuticle epidermis of stem; exocarp of fruit, pink. **Starch** in xylem of stem; secondary phloem of stem bark; mesocarp of fruit, blue. **Sugars** in cortex, pith of stem; secondary cortex of stem bark; mesocarp of fruit, yellow in colour. **Alkaloids** in cortex, phloem of stem; secondary cortex of stem bark; mesocarp of fruit brown. **Tannins** in epidermis, hypodermis, phloem and pith of stem; cork cambium, secondary phloem of stem bark; mesocarp of fruit, blackish blue. **Saponins** in epidermis, hypodermis of stem; secondary cortex of stem bark; exocarp, mesocarp of fruit, light yellow. **Suberins** in epidermis, hypodermis of stem; cork cambium of stem bark; exocarp of fruit, light brown.

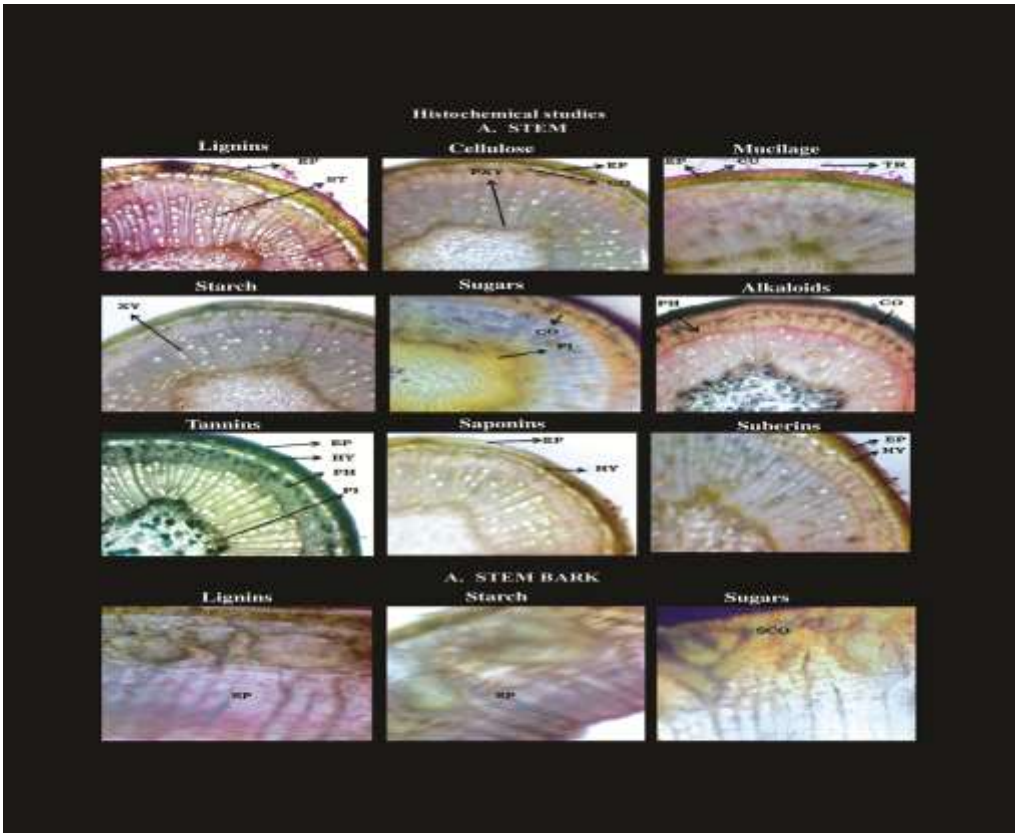


Plate-2: Histochemical Studies of Stem and Stem bark

Plate-3: Histochemical Studies of Stem bark and Fruit

EP: Epidermis; **CU:** Cuticle; **TR:** Trichomes; **HY:** Hypodermis; **CO:** Cortex; **SCO:** Secondary Cortex; **CC:** Cork Cambium; **ST:** Stele; **PH:** Phloem; **PXY:** Primary Xylem; **XY:** Xylem; **SPH:** Secondary phloem; **PI:** Pith; **EX:** Exocarp; **ME:** Mesocarp; **EN:** Endocarp.

Qualitative Analysis

Phenolic acids: (Plate-4); (Tables- 6)

A total of 37 phenolic compounds identified and 1 unidentified represented in leaf, stem bark, flower and fruit among them 9 in leaf, 8 in stem bark, 10 in flower and 11 in fruit respectively. Highest compounds present in fruit. **Leaf** consists Protocatechuic acid, homo-Protocatechuic acid, p-hydroxy benzoic acid, m-hydroxy benzoic acid, trans-p-coumaric acid, vanillic acid, salicylic acid, syringic acid and phloretic acid. **Stem bark** phloroglucinol,

chlorogenic acid, p-hydroxy benzoic acid, cis-p-coumaric acid, m-hydroxy benzoic acid, melilotic acid, cis-ferulic acid and salicylic acid. **Flowers** iso-chlorogenic acid, neo-chlorogenic acid, cis p-coumaric acid, trans-p-coumaric acid, o-coumaric acid, vanillic acid, trans-ferulic acid, salicylic acid, coumarin, phloretic acid. **Fruit** neo-chlorogenic acid, trans-p-coumaric acid, chlorogenic acid, m-hydroxy benzoic acid, o-coumaric acid, ferulic acid, phloretic acid, scopoletin, o-pyro catechuic acid, cis-ferulic acid and one unidentified compound. Fruit and flower and exhibit highest number of phenolic constituents.

The Common compounds present in flower and fruit are neo -chlorogenic acid, trans -p-coumaric acid, o-coumaric acid, phloretic acid; leaf and stem bark with, p-hydroxy benzoic acid, m-hydroxy benzoic acid; stem bark and flower with cis- p-coumaric acid; stem bark and fruit chlorogenic acid, m-hydroxy benzoic acid; leaf and fruit trans-p-coumaric acid, m-hydroxy benzoic acid, phloretic acid specific compounds present in leaf protocatechuic acid, homo protocatechuic acid, syringic acid; in stem bark phloroglucinol, melilotic acid, cis-ferulic acid; in flower iso-chlorogenic acid, trans ferulic acid, coumarin; in fruit scopoletin, ferulic acid and o-pyro catechuic acid.

Flavonoids: (Plate- 4) (Table-7)

A total of 12 flavonoid compounds were represented in leaf, stem bark, flower and fruit. **Leaf** consists 3 compounds Myricetin, Quercetin, Vitexin; **Stem bark** with 2 Luteolin, Vitexin; **Flower** with 3 Myricetin, Kaempferol, Orientin and **Fruit** consists 4 Myricetin, Quercetin, Luteolin, Vitexin. **Myricetin** is the common compounds present in leaf, flower and fruit; **Quercetin** in leaf and fruit; **Vitexin** in leaf, stem bark and fruit; **Luteolin** in stem bark and fruit. Significant compounds present in flowers are **Kaempferol, Orientin**. Flavonoid components also highest in fruit.

Anthocyanidins: (Plate-4); (Table-8)

A total of 10 anthocyanidins were represented in leaf, stem bark, flower and fruit. **Leaf** with Cyanidin, Peonidin, Rosinidin; **Stem bark** Cyanidin, Luteolinidin; **Flower** Delphidin, Petunidin, Cyanidin, Malvidin and only one in **Fruit** Luteolinidin. The common compound Cyanidin present in leaf, stem bark and flower; **Luteolinidin** is the specific component in stem bark and fruit. Flower represents highest number of constituents.

Table- 6: Qualitative analysis of Phenols

S. No	Rf values in solvent		UV Florescence	Sulphanilic acid reagent	Compounds	L	Sb	Fl	Fr
	1	2	With NH_3						
1	0.01	0.76	Duck egg green	Orange	chlorogenic acid	-	+	-	+
2	0.01	0.31	Duck egg green	Light orange	iso-chlorogenic acid	-	-	+	-
3	0.02	0.58	None	Yellow	phloroglucinol	-	+	-	-
4	0.05	0.58	None	Buff	protocatechuic acid	+	-	-	-
5	0.05	0.78	Bright egg green	Brown	Neo-chlorogenic acid	-	-	+	+
6	0.18	0.80	None	Buff	Homo Protocatechuic acid	+	-	-	-
7	0.30	0.51	Very bright blue	Yellow	Scopoletin	-	-	-	+
8	0.37	0.58	Deep blue	Yellow	o-Pyro catechuic acid	-	-	-	+
9	0.38	0.68	None	Bright yellow	p-hydroxy benzoic acid	+	+	-	-
10	0.42	0.77	Deep blue	Dark brown	cis - p-coumaric acid	-	+	+	-
11	0.44	0.73	None	Yellow	m-hydroxy benzoic acid	+	+	-	+
12	0.49	0.68,	Very light blue	Blue green	Unknown	-	-	-	+
13	0.50	0.45	Deep blue	Light brown	trans- p-coumaric acid	+	-	+	+
14	0.51	0.76	None	Orange	melilotic acid	-	+	-	-
15	0.64	0.60	Yellow green	Light brown	o-coumaric acid	-	-	+	+
16	0.66	0.77	None	Yellow	Phloretic acid	+	-	+	+
17	0.66	0.20	Blue	Purple	Ferulic acid	-	-	-	+
18	0.78	0.57	None	Orange	Vanillic acid	+	-	+	-
19	0.80	0.31	Blue	Purple	trans-Ferulic acid	-	-	+	-
20	0.89	0.65	Blue	Purple	cis-Ferulic acid	-	+	-	-
21	0.92	0.48	None	Red	Syringic acid	+	-	-	-
22	0.93	0.31	Dark violet	Yellow	Salicylic acid	-	-	+	+
23	0.96	0.04	None	Yellow	cinnamic acid	+	+	-	-
24	0.99	0.66	None	Yellow	coumarin	-	-	+	-
Total						9	8	10	11

Solvent I: Benzene: Acetic Acid: Water (60:70:30) v/v/v

Solvent II: Sodium Formate: Formic Acid: Water (10:1:200) v/v/v

L: Leaf; **Sb:** Stem Bark**; Fl:** Flower**; Fr:** Fruit.

Table-7: Qualitative analysis of Flavonoids

S No.	R_f Values in solvent	UV Fluorescence With NH_3	Sulphanilic reagent	Compound	L	Sb	Fl	Fr
1	0.07	Bright yellow	Light green	Myricetin	+	-	+	+
2	0.26	Light yellow	Bright yellow	Quercetin	+	-	-	+
3	0.37	Bright yellow	Orange	Kaempferol	-	-	+	-
4	0.44	Yellow	Light red	Luteolin	-	+	-	+
5	0.78	Yellow green	Grey	Orientin	-	-	+	-
6	0.91	Yellow	Bright red	Vitexin	+	+	-	+
Total					**3**	**2**	**3**	**4**

Solvents: Isopropyl Alcohol: Ammonia (25%): Water (8: 1: 1) v/v/v

L: Leaf; **Sb:** Stem Bark**; Fl:** Flower**; Fr:** Fruit.

Table-8: Qualitative analysis of Anthocyanidins

S. No.	Rf values in Solvent	Visible Colour	U V Fluorescence With NH_3	Compound	L	Sb	Fl	Fr
1	0.15	Purple	Blue green	Delphidin	-	-	+	-
2	0.26	Purple	Mauve	Petunidin	-	-	+	-
3	0.38	Megenta	Blue green	Cyanidin	+	+	+	-
4	0.45	Purple	Blue green	Malvidin	-	-	+	-
5	0.60	Megenta	Blue	Peonidin	+	-	-	-
6	0.61	Orange	Reddish brown	Luteolinidin	-	+	-	+
7	0.77	Bluish red	Blue	Rosinidin	+	-	-	-
Total					3	2	4	1

Solvent key: Conc. HCl: Formic Acid: Water (2: 5: 3) v/v/v.

L: Leaf; **Sb:** Stem bark; **Fl:** Flower; **Fr:** Fruit.

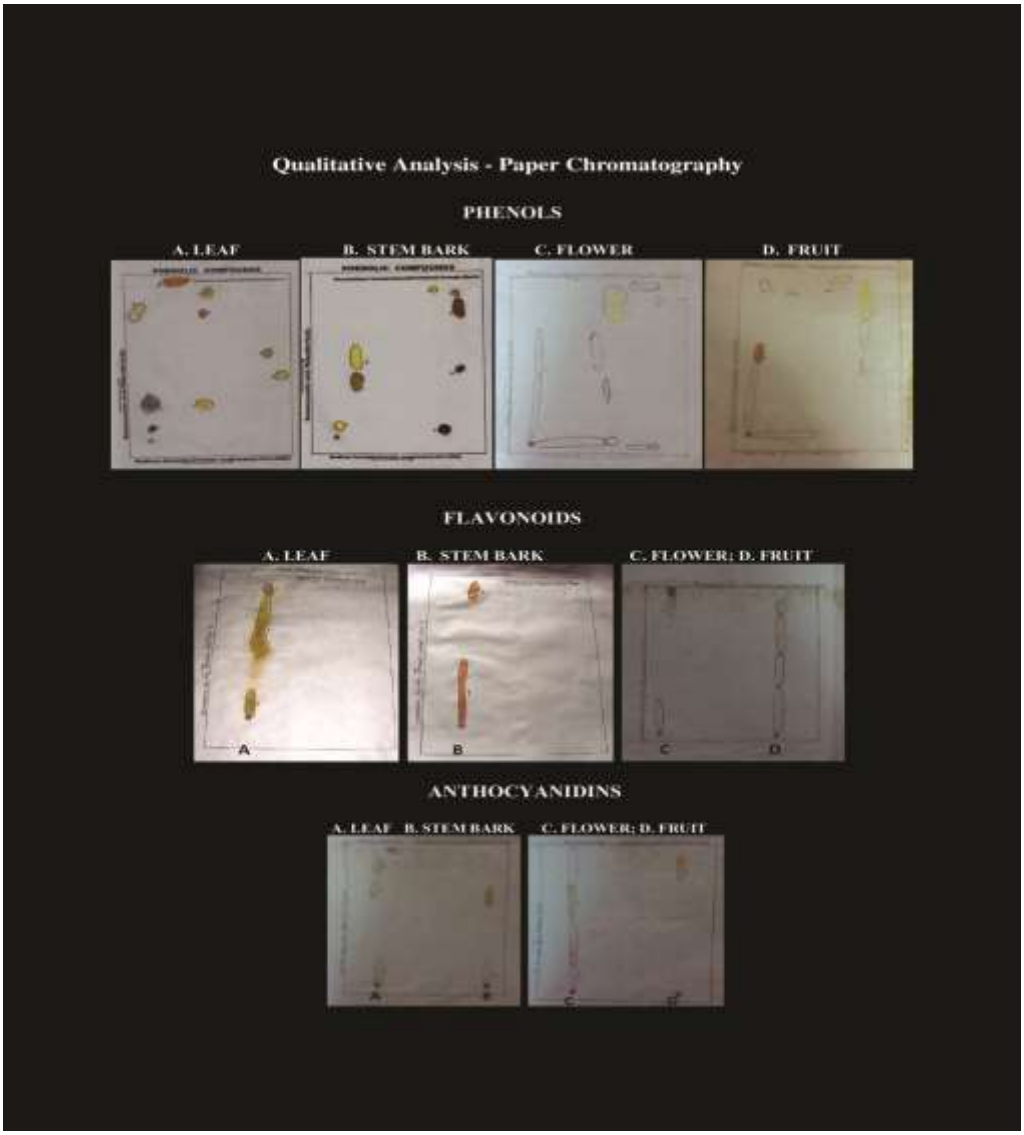
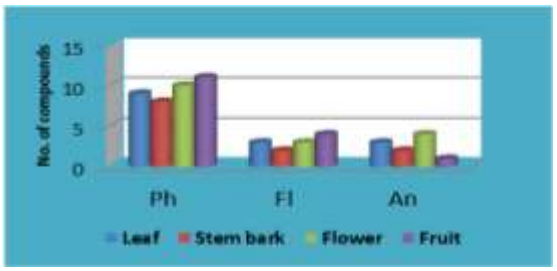


Plate-4: Qualitative Analysis- Paper Chromatography

Comparative Account of Phenols, Flavonoid and Anthocyanidins (Table-9; Figure- 3)

Plant parts	Ph	Fl	An	Total
Leaf	9	3	3	15
Stem bark	8	2	2	12
Flower	10	3	4	17
Fruit	11	4	1	16
Total	**38**	**12**	**10**	**60**



Ph: Phenols; **Fl:** Flavonoids; **An:** Anthocyanidins

Comparative account of Phenols, Flavonoids and Anthocyanidins: (Table -9; Figure -3)

Comparative analysis of phenols, flavonoids and anthocyanidins reveals a total of 60 compounds present in all parts where as phenolics are 38, flavonoids 12, and anthocyanidins 10. Highest number of compounds 17 found in the flower followed by fruit 16, leaf 15 and stem bark 12. Phenolic compound are highest in all parts.

DISCUSSION

Ash values are useful in determining authenticity and purity of drug and also important in qualitative and quantitative standards.[6,10] Ash values of any crude drug gives an idea about the presence of earthy matter and /or inorganic composition and /or other impurities present along with the crude drug. In the present study total ash and water soluble ash are more in stem bark. Similar studies were reported in leaves of *B. vareigata and Caesalpinia bonduc*.[13,14] Moisture content of the *P. hexapetalum* leaf, stem bark, flower and fruit powder drugs is very less ranging from 2.12 to 3.12%, by which life span of the drug increases. Fluorescence analysis of plant powders treated with different acids and bases showed different colors in daylight as well as in short and long UV lights as in dark green to blackish green helps to check in various formulations. Extractive values represented the presence of compounds in polar and non-polar solvents. It is useful for the diversity of chemical nature and property of drug contents. The highest extractive values were noticed in leaf hot water and fruit methanol extracts than other solvents. Extract color and nature of residues of all parts showed very specific in the selected crude drug.

Histochemistry showed the Presence of phytoconstituents in different parts indicates the usage of respective part in the formulation is very much important. Major phytoconstituents like lignins, cellulose, mucilage, starch, sugars, alkaloids, tannins, saponins, suberins are localized in the secondary cortex, secondary phloem and in cork cambium of Stem bark.

Epidermis, hypodermis, cortex and phloem of Stem and Exocarp, mesocarp, endocarp of Fruit.

Phenolic compounds are the major bioactive compounds in leaf, stem bark, flowers and fruits than flavonoids and anthocyanidins. Protocatechuic acid (PCA) is present in leaf is capable of modulating certain cellular enzymes as well as having anti-oxidative and anti-mutagenic activities.[15] It is extracted from *Hibiscus sabdariffa* and showed hepatoprotective, antioxidant and anti-inflammatory activities.[16] Homo-Protocatechuic acid present in leaf is an antioxidant. Chlorogenic acid in stem bark and fruit is a naturally occurring compound very important antioxidant, present in green coffee, beans extract helps to reduce high blood pressure and as an antibacterial[17], anti-fugal.[18] Neo-chlorogenic acid in both flower and fruit, is a natural polyphenolic compound in fruits.[19] It may have potential chemo preventive dietary compound, involved as laxative.[20,21]

Phloroglucinol only in the stem bark was originally isolated from the bark and fruits is useful for the industrial synthesis of pharmaceuticals and explosives. It is also used against gastro intestinal disorders.[22] p-Coumaric acid is a hydroxyl cinnamic acid exists in two forms as Trans-p-Coumaric acid present in leaf, flower and fruit Cis-p-Coumaric acid in stem bark, flower. It is a major component of lignin and has antioxidant properties and is believed to reduce the risk of stomach cancer[23] by reducing the formation of carcinogenic nitrosamines.[24] Trans-p-Coumaric acid is antibacterial.[25] p-hydroxy benzoic acid in leaf and stem bark is antifungal against *Candida albicans*.[26] m-hydroxy benzoic acid in leaf, stem bark and fruit may used as intermediate for pharmaceuticals especially as antipyretic, analgesic, antirheumatic. Ferulic acid in fruit is a hydroxyl cinnamic acid, an antioxidant, antitumor against breast cancer[27] and liver cancer.[28] It is also having antifungal properties on onion epidermis.[29] Trans-Ferulic acid in flower is very common in multi vitamins and anti aging supplements. It is an important part of gamma-oryzanol, a plant sterol complex used by athletes, due to its antioxidant and adaptogenic properties. Cis-Ferulic acid only in stem bark is an antioxidant used against skin problems.

Scopoletin in fruit is a coumarin acts as anti-microbial on potato leaf and tubers.[30,31] Antifungal on sweet potato.[32] o-coumaric acid in flower and fruit, is a hydroxyl cinnamic acid can be found in vinegar. Vanillic acid in leaf and flower is a dihydroxy benzoic acid derivative used as a flavoring agent. The highest amount of vanillic acid is found in the roots of *Angelica sinensis*, which is used in traditional chinese medicine.[33] It is also used as

diuretic, purgative and antimicrobial[34] Melilotic acid which is present only in the stem bark is known to function at the level of liver metabolism. The presence of melilotic acid might be suggestive with broad spectrum antimicrobial and antispasmodic drugs.[35] *Capparis zeylanica* having antimicrobial activity against skin diseases might be due to the presence melilotic acid.[36] Syringic acid only in fruit may have hepatoprotective activity.[37] Coumarin in flower is a fragrant organic compound used against anti HIV, antitumor, antihypertension, antiarrhythmic, anti-inflammatory, antiosteoporosis, antiseptic, analgesic, asthma[38] and also used in lymphedema.[39] Salicylic acid present in flower and fruit is a monohydroxyl benzoic acid, is a phenolic phytohormone and used as anti inflammotary drug[40], used antirheumatic, analgesic and anti-inflammatory.[41] Cinnamic acid in leaf and stem bark is a kind of self - inhibitor of fungal spore germination.

Flavonoids are readily ingested by humans and they seem to display important anti-inflammatory, anti cancer activities and also found to be powerful anti oxidants. Myricetin in leaf, flower and fruit is a naturally occurring flavonal has antioxidant properties. A finish study correlated high myricetin consumption with lowered rates of prostate cancer[42] also used against jaundice and hepatitis.[43] Quercetin present both in leaf and fruit may have anti inflammatory properties[44, 45], antihaemorrhagic activity from *Thuja orientalis*.[46] Kaempferol in flowers has been identified in many species commonly used in traditional medicine also found in most of the Caesalpinaceae members like *B. forficate, B. microstachya*, and *C. alata* showed anti oxidant, anti inflammatory properties[47], acts as anticancerous.[48] Luteolin in stem bark, fruit displays strong antileishmanial activity due to its anti-inflammatory and smooth muscle relaxing properties, luteolin has potential in the prevention and treatment of many respiratory disorders, including asthmatic conditions, chronic bronchitis.[49,50] Orientin present only in flower may be used for respiratory troubles by the herbalists.[51] Vitexin in leaf, stem bark and fruit may be used for treating cough and cold the symptoms caused only by bacterial infections.[52]

Anthocyanidins are members of the flavonoid group having antioxidant potential.[53] Delphidin in flower may act as antioxidant and also used in the treatment of ophthalmic diseases.[54] Petunidin in flower is used for food coloring and responsible for the petal colors. Cyanidin in leaf, stem bark and flower has putative antioxidant and radical scavenging effects may protect cells from oxidative damage and reduce risk of cardiovascular disease and cancer, also inhibits the development of obesity and diabetes, and also acts as anti

inflammatory.[55] Malvidin present in flower is responsible for the color of red wine *Vitis vinifera*. Peonidin present in leaf has potent inhibitory and apoptoxic effects on cancer, notably metastatic human breast cancer cells.[56]

CONCLUSION

Morphological studies of the herbal drug *P. hexapetalum* gives the standard values for future studies. Physicochemical analysis resulted in highest water soluble ash content which helps in the drug designing. Moisture content is very less which proved its long viability and storage of crude drug compared to that of *Bauhinia variegata* and *Caesalpinia bonducella*. Fluorescence analysis exhibits dark blackish green nature under different lights helps to check its purity and quality in different formulations. Crude drug extracts yielded high quantities in leaf aqueous and fruit methanol extracts for the standardizations. Histochemistry proved the distribution of various phytoconstituents in all parts supported the usage of various herbal formulations. Qualitative analysis of phenols along with flavonoids and anthocyanidins which supports various medicinal uses of the plant. Especially Phenols like protocatechuic acid acts as antioxidant and anti-inflammatory; Chlorogenic acid as antimicrobial; Coumaric acids against the risk of cancers; p-hydroxy benzoic acid as antifungal also acts as anti rheumatic, antipyretic; Ferulic acids as antioxidant and antifungal; Scopoletin and vanilic acid as antimicrobial; Melilotic acid and syringic acid against hepatoprotective and antimicrobial; Coumarins against inflammations, hypertension, osteoporosis and analgesic; Salicylic acid as anti-inflammatory; Cinnamic acid as antifungal, flavonoids like myricetin reduces prostate cancer and jaundice. Quercetin and kaempferol acts as anti-inflammatory; Luteolin, orientin and vitexin reduces respiratory disorders and chronic bronchitis; Anthocyanidins like delphinidin is major component used in ophthalmic disorders. Cyanidin and peonidin are responsible to reduce cardiovascular diseases and cancer. Hence the herbal drug *P. hexapetalum* is proved as the best anti microbial against pathogenic bacteria which causes skin diseases, liver damage, bronchial disorders, haemorrhages, inflammations, arthritis, ulcers as used by the herbalists. The herbal formulations are further recommended for testing the respective bioactivity to prove scientifically and also in the isolation of bioactive compounds and the drug designing.

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