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"Pharmacognostical Evaluation and Quantification of Phytoconstituents in Herbs of *Asthimajja Pachak Kwath* Formulation"

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ABSTRACT:

Introduction: The growth of herbal medicines in the recent era is a significant trend in healthcare and wellness. Ayurveda, being one of the oldest traditional systems of medicine, carries immense value in terms of holistic health and natural remedies. O**bjectives**: The need for standardization in herbal drug formulations is of paramount importance to ensure their safety, efficacy, and consistency. **Methods:** Study involving standardization and evaluation of a traditional formulation. The goal of this study seems to be to ensure the quality, safety, and effectiveness of the formulation. Standardization formulation was performed by checking its extractive value, physicochemical evaluation, heavy metal analysis, qualitative and quantitative evaluation and phytoconstituents quantification by HPTLC to assess the quality and safety of formulation. **Results:** The parameters of standardization confirms that all the raw material which was purchased from local market and athleticized by NISCAIR were good quality material as quality of crude drugs directly affects the pharmacological activity of formulation. Preliminary phytochemical evaluation of formulation and ingredients revealed that presence of bioactive phytoconstituents for activity. **Conclusion:** Standardization of ayurvedic formulation is an essential factor to assess the quality of the drugs which are used for the formulation. Various types of evaluations were carried out on all individual sample and mixture itself like organoleptic, microscopically, physical and chemical evaluation. The parameters of standardization confirms that all the raw material which was purchased from local market and authentified by NISCAIR are good quality material as quality of crude drugs directly affects the pharmacological activity of formulation.

KEYWORDS: *Asthimajja Pachak Kwath*, Evaluation, Preparation, Standardization, Quantification.

INTRODUCTION:

The ancient Indian medical system *Ayurveda,* is based on ancient writings that depend on a "natural" and holistic approach for treatment of disease.[1] It has been passed down through generations and continues to be practiced as a primary form of healthcare in many parts of the country. A substantial portion of India's rural population, approximately 70%, depend on the traditional Ayurvedic system of medicine for their healthcare needs.[2] The World Health Organization (WHO) recognizes the importance of medicinal plants for public health in developing countries. It provides guidelines to support efforts to evaluate quality, safety, and efficacy of traditional herbal medicines.[3] Prosses of evaluating the quality and purity of crude drug by evaluating different parameters like morphological, microscopical, physical, chemical and biological observation is called as standardization.[4] Physicochemical parameter for checking quality of raw material of herbal formulation which include morphological and microscopical evaluation, moisture content, ash value, extractive value, powder flow property, heavy metal analysis and phytochemical evaluation.

MATERIALS AND METHODS

Asthimajja Pachak Kwath Composition and Preparation

Asthimajja Pachak Kwath formulation which was mentioned in *Charak Samhita*. *Asthimajja Pachak Kwath* was prepared by three ingredients Amla, Giloy and Musta.[5] Drug powder was prepared from the shade dried raw drug material and mixed in equal proportion. It was transferred into the earthen pot and 16th part of water was added into it. The mixture and water were left to soak for overnight, approximately 12 hours. After soaking, the mixture is boiled traditionally until it reduces to 1/4th of its original volume. it was allowed to cool down and the cooled mixture is then filtered using a cloth to remove any solid particles, resulting in the preparation of *Asthimajja Pachak Kwath*. [6-8]

Collection and Authentication

The ingredients used in this formulation were *Amla (Emblica officinalis)*[9-11] were purchases from local market and *Giloy (Tinospora cordifolia)*[12-14,] *Musta (Cyprus Rotundus)*[15-16] were purchased from *Sanjivani Aushadhalaya*, Saurastra, Gujarat, India. These drugs were Authenticated by NISCAIR, New Delhi. The ingredients used in the formulation of *Asthimajja Pachak Kwath* have

been carefully sourced and authenticated. The authentication process ensures that the herbal ingredients were of high quality and meet the required standards.

Chemical and Requirements

Water bath, Compound microscope STC-1000, Camag HPTLC system (Switzerland) with Linomat 5 automatic sample applicator and Camag TLC Scanner 3, UV Spectrophotometer (1800 Shimadzu Corporation, Kyoto, Japan). Gallic acid (> 98% Purity), Ferulic acid (98.1% Purity), Rutin (> 95% Purity) and Berberine (97.4 % Purity) biomarkers were purchased from Yucca Enterprise Ltd., Mumbai. Analytical grade chemical Reagents of Finar Chemicals were used.

Pharmacognostical Evaluation of Powdered Crude Drug[17-20]

Morphological study: Study was conducted on the size, shape, color, odor, and taste of the individual ingredients (*Amla, Giloy, and Musta*) present in the *Asthimajja Pachak Kwath* formulation.

Microscopical study: The powder microscopy and transverse section study of shade dried drug *Emblica officinalis, Tinospora cordifolia*, and *Cyprus rotundus* was carried out by using various reagents for the identification. Stained and unstained slide was prepared, Powder characteristics of the drug were studied under the microscope for examination of different powder characters.

Procedure: Powder drug was boiled with chloral hydrate for 1-2 minutes, staining reagents phloroglucinol and concentrated HCl was added. Slide was prepared and mounted with 50% glycerin, slide was covered by cover slip and observed under microscope. For starch grain identification-stained slide was prepared using iodine solution.

Physicochemical Evaluation Parameters[21-23]

Loss on Drying (LOD) A known amount of the powdered herbal drug (10 grams) is accurately weighed and placed in a Petri dish. and The Petri dish containing the herbal drug is placed in an oven at a specific temperature of 110 °C. At fixed intervals of 10 minutes, the Petri dish was taken out from oven it was cooled in desiccator and observed loss of moisture at each time interval. The procedure was repeated for at least five times or more.

Extractive Value

The extractive value of the crude drug determines the quality as well as purity of the drug. Extractive value plays an important role in evaluation of crude drugs. Water, alcohol and petroleum ether solvent were used. It was performed by two techniques: i) Cold Extraction technique (Maceration) ii) Hot Extraction technique (by Successive Soxhlet extraction)

Cold Extraction technique (Maceration): 2 gm powdered drug was taken in stoppered flask and 100 ml absolute alcohol; Petroleum ether and water contain 1% chloroform was added. The flask was shaken frequently for 6 hrs. using magnetic stirrer and stand it for 18 hrs. after that it was filtered using Whatman filter paper and loss of solvent is adjusted. A measured portion of the filtrate was taken and evaporated to dryness in a China dish. This is typically done on a hot plate with a controlled temperature range of 60-70°C. Extract weight was calculated.

Hot Extraction technique (Successive Soxhlet extraction): Extraction procedure was done by using the Soxhlet apparatus for *Amla, Galo, Musta* and *Asthimajja Pachak Kwath Churna* by giving the different fraction of solvent Petroleum ether, chloroform, alcohol and water and % extractive value was calculated. [29-30]

Procedure: 25 grams of the drug material were taken into a thimble. Petroleum ether, chloroform, Absolute alcohol and water solvent were used. During the extraction procedure 60-70°C temperature and continues water flow was maintained. After each solvent fraction the extract was collected in a China dish and evaporated on hot plate till dryness and extract was collected & % extractive value was calculated.

Ash Value

Total ash value: 2 gm drug was taken in crucible. It was incinerated into the muffle furnace till drug converted into carbon free ash with increasing temperature up to 600°C. The percentage total ash was calculated.

Acid insoluble ash value: 0.5 gm of ash was boiled with 25 ml 10% HCl in a reflux condition for 1 hr. the acid insoluble ash was filtered using ash less filter paper and it is washed using water till filter paper free from acid. Acid insoluble ash containing filter paper was incinerated into the muffle furnace till it converted into ash. Acid insoluble ash value was calculated.

Water soluble ash value: 0.5 gm ash was boiled with 25ml water in a reflux condition for 1 hr. the water-soluble ash was filtered using ash less filter paper. Filtrate was taken in China dish and it was evaporated on hot plate and weight of water-soluble ash was determined.

Powder Flow Property Determination[26-27]

Powder flow properties of *Amla, Galo, Musta* and powdered Formulation mixture was determined by following describe parameter as below:

a) Bulk and Tapped density: 10 gm of drug was transferred into 25 ml measuring cylinder and bulk density was determined and after 100 tapping from fixed height, tapped density was determined as per USP. It was calculated using following equations:
   Bulk density = weight of powder/ bulk vol. of powder
   Tapped density = weight of powder/ tapped vol. of powder
b) Carr's index (compressibility index): It was determined by compressibility index by following equation:
   $$\text{Carr's index (\%)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Bulk density}} \times 100$$
c) Housners ratio: it was calculated by using following equation:
   Housners ratio = tapped density / bulk density
d) Angle of repose: It was performed by funnel method. Funnel was fixed on a stand at 5 cm from the surface and powder was passed through funnel till powder touch to the tip of funnel. Cone Diameter was measured and it was calculated using following equation:
   $\tan^{-1}(2h/d)$

Heavy Metal Identification in Formulation[28-30] Heavy metal analysis was done using Inductive couple plasma mass spectrometry (ICP-MS) at Aum research Lab Pvt. Ltd., Ahmedabad, Gujarat. Solid sample was directly introduced using electrothermal vaporization technique and amount of Mercury (Hg), Cadmium (Cd), Arsenic (Ar) and Lead (Pb) were measures in *Amla, Galo, Musta* and Formulation Water extract.

Qualitative Preliminary Phytochemical Studies[31]

Preliminary plants metabolites such as carbohydrates, lipids, proteins and secondary plants metabolites such as alkaloid, tannin, steroids, flavonoid, amino acid, etc. was determined by performing the qualitative test.

Test for alkaloids: A small portion of the solvent free extract was stirred separately with a few drops of dilute HCL and filtered. The filtrate was tested with various reagents.

i) Dragendrof's Test: To the 1 ml of extract add 1 ml of reagent (potassium bismuth iodide). An orange red precipitate indicates the presence of alkaloids.

ii) Mayer's Test: To the 1 ml of extract add 1 ml of reagent (potassium mercuric iodide). Whitish yellow or cream color precipitate indicates the presence of alkaloids.

iii) Hager's Test: To the 1 ml of extract add 1 ml of reagent (saturated aqueous solution of picric acid). Yellow color precipitate indicates the presence of alkaloids.

iv) Wagner's Test: To the 1 ml of extract add 1 ml of reagent (iodine in potassium iodide). Reddish brown precipitate indicates the presence of alkaloids.

Test for flavonoids:

i) Shinoda's test: the alcoholic extract was treated with magnesium foil and conc. HCL gives intense cherry red color, indicates the presence of flavones.

ii) Alkaline Ammonium Test: The Ethanolic extract is treated with 10% sodium hydroxide solution and ammonium was added. Dark yellow color indicates the presence of flavonoids.

Test for proteins and amino acids:

i) Biuret's test: Add 1 ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color is produced and then add to the 1ml of the extract. Formation of pink or purple violet color indicates the presence of proteins.

ii) Ninhydrin test: Add two drops of freshly prepared 0.2% Ninhydrin reagent (0.1%solution in n-Butanol) to the small quantity of extract and heat. Development of blue color reveals the presence of proteins and amino acids.

iii) Xanthoproteic test: To 1 ml extract, add 1ml of concentrated nitric acid, a white precipitate is formed, it is boiled and cooled. Then 20% sodium hydroxide or ammonia is added. Orange color indicates the absence of aromatic amino acids.

Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration.

Test for steroids: Two ml of acetic anhydride was added to 0.5 g Ethanolic extract of each sample with 2 ml H_2S0_4. The color changed from violet to blue or green in some samples indicated the presence of steroids.

Estimation of Total Tannin Content[32]

Total tannin content in water extract of *Amla, Galo, Musta* and *Asthimajja Pachak Kwath Churna* were measured by redox titration method as per reported method.

Reagents:

Preparation of *Asthimajja Pachak Kwath Churna* and its ingredients extract: 1 gm powder of *Amla, Galo, Musta* and *Asthimajja Pachak Kwath Churna* were extracted with 100 ml distilled water by heating at 70-80˚C for 1 hr. separately. Extract was filtered and volume adjusted to 100 ml in volumetric flask.

Preparation and Standardization of 0.1 N $KMnO_4$.: It was prepared by 3.16 gm $KMnO_4$ was dissolved in 1000 ml distilled water. For standardization 0.1 gm Oxalic acids was transfer in 100 ml conical flask. 25 ml of water, 10 ml of dilute H_2SO_4 was added in to conical flask and heated at 70˚C. it was titrated with 0.1 N $KMnO_4$. End point was characterized by color change of solution from colorless to pink.

Procedure: 10 ml of extract from stock solution was transferred into 250 ml conical flask. 180 ml water was added into it and 10 ml indigo carmine was added in flask as an indicator. Solution was heated at 70˚C for 20min. Solution was titrated with 0.1 N $KMnO_4$. End point was characterized by change color of solution from blue to green to golden yellow.

Factor: 1 ml of 0.1 N $KMnO_4$ = 0.004157 gm. of total tannin calculated as tannic acid.

Estimation of Total Phenolic Content[33]

The phenolic content in water extract of *Amla, Galo, Musta* and *Asthimajja Pachak Kwath* formulation was measured according to Folin ciocalteu reagent method.

Preparation of standard stock solution: 1000 µg/ml of gallic acid stock solution was prepared by using methanol. From stock solution 30-80 µg/ml working standard solution was prepared for calibration curve. 1 ml of each concentration solution was taken into Eppendorf tube and 1 ml of folin ciocalteu reagent and 2ml of 20% Na_2CO_3 solution was added. Place it into dark place for 1 hr. & Blank determination was also done. Absorbance was taken by using UV spectrophotometer at wavelength of 760 nm. And calibration curve was plotted.

Procedure for Phenol estimation: 1 ml of extract *Asthimajja Pachak Kwath Churna* and its ingredients, 1 ml of folin ciocalteu reagent and 2ml of 20% Na_2CO_3 solution was taken into Eppendorf tube. Place it into dark place for 1 hr. Absorbance was taken by using UV spectrophotometer at wavelength of 760 nm. Amount of phenol was calculated by using regression equation (Gallic acid calibration curve was prepared).

TLC Fingerprinting of *Amla, Galo and Musta*.[34-37]

TLC fingerprinting of Gallic acid in *Amla*:

Precoated Silica Gel G60 F_{254} aluminum sheets (10 x 10 cm, pre-washed with methanol and dried in Hot air oven) was used as stationary phase. Standard stock solution of Gallic acid 1000 µg/ml concentration and Amla extract test solution (10mg/ml) was prepared and 100-600 ng/band and 2 µl test solution was spotted on TLC plate using HPTLC automatic sample applicator. TLC plate was run into mobile phase having Toluene: Ethyl acetate: Formic acid (5:3.5:0.5, v/v/v) in TLC chamber and it was scanned using TLC scanner at 254 nm. And area was measured. Calibration curve of gallic acid was plotted.

TLC fingerprinting of Ferulic acid and Rutin in *Musta*:

Precoated Silica Gel G60 F_{254} aluminum sheets (10 x 10 cm, pre-washed with methanol and dried in Hot air oven) was used as stationary phase. Standard stock solution of Ferulic acid and Rutin 1000 µg/ml and Musta extract test solution (10mg/ml) was prepared and 10-60 ng/band for ferulic acid and 10-35 ng/band for rutin and 2 µl test solution was spotted on TLC plate

using HPTLC automatic sample applicator. TLC plate was run into mobile phase having Toluene: Ethyl acetate: Formic acid (6:4:1, v/v/v) for Ferulic acid and Ethyl acetate: Methanol: Formic acid (10:2:1, v/v/v) for rutin in TLC chamber and it was scanned using TLC scanner at 315 nm for ferulic acid and 366nm for rutin. And area was measured. Calibration curve of ferulic acid and rutin was plotted.

TLC fingerprinting of Berberine in *Galo*:

Precoated Silica Gel G60 F_{254} aluminum sheets (10 x 10 cm, pre-washed with methanol and dried in Hot air oven) was used as stationary phase. Standard stock solution of Berberine 1000 µg/ml concentration and Galo extract test solution (10mg/ml) was prepared and 20-120 ng/band and 20 µl test solution was spotted on TLC plate using HPTLC automatic sample applicator. TLC plate was run into mobile phase having Toluene: Ethyl acetate: Methanol: Formic acid (9:9:3:2, v/v/v/v) in TLC chamber and it was scanned using TLC scanner at 266nm. And area was measured. Calibration curve of berberine was plotted.

RESULTS AND DISCUSSIONS

Microscopical Evaluation: Drug microscopical evaluation was confirming the presence of diagnostic powder characters in the powdered drug and transverse section of raw material which were used for the *Asthimajja Pachak Kwath* formulation shown similarity. Transverse section of *Amla, Galo* and *Musta* were shown in Figure 1, Figure 2 and Figure 3. Powder microscopical characters of *Amla, Galo* and *Musta* were shown in Figure 4, Figure 5 and Figure 6.

Physicochemical Evaluation Parameters: Evaluation of loss on drying in Formulation and its ingredients which were within a limit result were shown in Table 1. The observation that the extractive value of *Amla, Galo, Musta,* and the Formulation is higher when using water as the solvent compared to alcohol, chloroform, and petroleum ether suggests that the water-soluble compounds in these herbal materials were more abundant or readily extracted than the compounds soluble in the other solvents. As per the pharmacopoeia the results were found satisfactory and results was shown in Table 2 and Table 3. Ash value of formulation and its ingredients as per the pharmacopoeia monograph it was found within a limit and results was mentioned in Table 4.

Powder Flow Property:

For checking powder flow properties of *Asthimajja Pachak Kwath* ingredients and formulation. As per the results *Amla, Galo, Musta* and *Churna* found poor flow properties in all the tested materials. Its results show in Table 5.

Heavy Metal Analysis: Heavy metal analysis of aqueous extract of *Amla, Galo, Musta* and formulation was tested by Inductive couple plasma mass spectrometer (ICP-MS). This technique is used for accurate and sensitive detection of heavy metals in samples. Analysis revealed the absence of mercury and acceptable levels of cadmium, lead and arsenic ensuring the safety of the product. The results were shown in table 6.

Chemical Evaluation:

Phytochemical Evaluation: Phytochemical present in *Amla, Galo, Musta* and Formulation aqueous extract were shown in Table 7. Data suggested that carbohydrates, proteins, steroids, tannins and phenolic and glycosides components were presents.

Estimation of Total Tannin Content: The data suggested that tannin content in *Amla* is high as compare to *Galo* and *Musta*. Prepared *Asthimajja Pachak Kwath* formulation contain good amount of tannin. Results were shown in table 8.

Estimation of Total Phenol Content: The data suggested that phenolic content in *Amla, Galo* and *Musta* found satisfactory results. Prepared *Asthimajja Pachak Kwath* formulation also contain good amount of phenol. Calibration curve were shown in figure 7. Results were shown in table 9.

TLC Fingerprinting of *Amla, Galo, Musta* and *Asthimajja Pachak Kwath*

TLC fingerprinting for gallic acid in Amla, Ferulic acid in Musta and Berberine in Galo were shown in figure 8, Figure 9 and Figure 10 and Figure 11. With the corresponding R_f value for gallic acid was found at 0.3, for Ferulic acid was found at 0.58, rutin at 0.42 and for Berberine was found at 0.52. Calibration curve for Gallic acid, Ferulic acid and Berberine were plotted**.** Results were shown in Figure 12, Figure 13, Figure 14 and Figure 15. The found R^2 value for Gallic acid was 0.999, Ferulic acid was 0.9973, Rutin was 0.9991 and Berberine was 0.998.

As per the quantification 4.26 %w/w of Gallic acid was present in *Amla*, 0.03 %w/w Ferulic acid was present in *Musta* and 0.35%w/w Berberine was present in *Galo*.

CONCLUSION:

Standardization of *Asthimajja Pachak Kwath* and its ingredients was done by checking its physicochemical parameters. Moisture content, Ash value, Extractive value measures the solubility of active constituents in a particular solvent this can give insights into the potential bioactive compounds present of *Asthimajja Pachak Kwath* and its ingredients were in limit. Heavy metal analysis was done by Inductive couple plasma mass spectrometer (ICP-MS), Mercury was not detected in the formulation and its ingredients. Cadmium, Lead, Arsenic Levels within acceptable limits, indicating the product's safety. Successive Soxhlet extraction is a method to extract compounds from plant material using various solvents. Water and alcohol yielding high extractive values for the formulation and its ingredients as compare to petroleum ether and chloroform. Phytochemical like tannins, flavonoids, saponin, carbohydrates, steroids, glycosides, amino acids were presents in formulation.

Quantification of phytoconstituents was done by HPTLC fingerprinting. 4.26 %w/w of Gallic acid was present in *Amla*, 0.03 %w/w Ferulic acid and 1.23%w/w Rutin was present in *Musta* and 0.35%w/w Berberine was present in *Galo*.

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38. **TABLES:**

Table 1: Results of Loss on Drying.

Sr. No.	Sample	% Loss on drying
1.	*Amla*	3.1 ± 0.3
2.	*Galo*	3.36 ±0.26
3.	*Musta*	2.8±0.14
4.	Formulation	4.1±0.1

Table 2: Extractive value by cold extraction technique of Ingredients and *Asthimajja Pachak Kwath*

Sr. No.	**Sample**	**Alcohol extractive value** (%±S.D.)	**Water extractive value** (%±S.D.)	**Pet. ether extractive value** (%±S.D.)
1.	*Amla*	21.66±0.57	18.66±0.6	0.01±0.002
2.	*Galo*	14±2.0	13.33±4.16	2.66±1.15
3.	*Musta*	14.66±1.15	4.0±0.0	0.0±0.0
4.	Churna	10.66±2.3	21.33±1.15	1.33±1.2

Table 3: Extractive value by hot extraction technique for *Asthimajja Pachak Kwath* formulation and ingredients.

Sr. No.	**Sample**	**Pet. ether extractive value** (% W/W)	**Chloroform extractive value** (% W/W)	**Alcohol extractive value** (% W/W)	**Limit NLT**	**Water extractive value** (% W/W)	**Limit NLT**
1.	*Amla*	0.295±0.45	5.1± 0.57	39.4±0.12	31.0	50.02±0.37	46.0
2.	*Galo*	1.48± 0.72	1.5± 0.39	9.38±0.56	6.0	17.23±0.64	13.0
3.	*Musta*	0.25± 0.36	1.05± 0.87	16.08±0.75	14.0	23.75±0.28	20.0
4.	Churna	1.12± 0.81	1.075± 0.33	20.83±0.08	-	29.2±0.721	-

Table 4: Ash value of Ingredients and *Asthimajja Pachak Kwath* Formulation.

Sr. No.	**Sample**	**Total ash value** (%±S.D.)		**Acid insoluble ash** (%±S.D.)		**Water soluble ash** (%±S.D.)	
		%	STD Limit	%	STD Limit	%	STD Limit
1.	*Amla*	2.3±0.01	NMT 12.0	0.86±0.04	NMT 2.0	74±0.3	NLT 46.0
2.	*Galo*	5.6±0.32	NMT 7.0	0.51±0.08	NMT 0.8	32±0.004	NLT 13.0
3.	*Musta*	1.7±0.07	NMT 6.5	1.83±0.05	NMT 2.5	47.19±0.02	NLT 20.0
4.	Formulation	3.25±0.41	-	1.27±0.12	-	39.7±0.067	-

Table 5: Powder Flow Property of Ingredients and Asthimajja Pachak Kwath Formulation.

Powder flow property	***Amla***	***Galo***	***Musta***	**Formulation**
Bulk density (gm/ml)	0.64±0.01	0.34±0.01	0.32±0.2	0.4±0.01
Tapped density (gm/ml)	0.84±0.02	0.52±0.01	0.45±0.06	0.633±0.01
Carr's index %	23.6±0.76	35.5±0.78	27.94±0.9	37.3±0.03
Housners ratio	1.3±0.02	1.55±0.02	1.17±0.39	1.6±0.03
Angle of repose	41.36±0.13	45±0.24	42.75±0.59	41.63±0.32

Table 6: Heavy metal analysis of *Asthimajja Pachak Kwath* formulation and ingredients.

Sr. No.	**Sample**	**Cadmium (ppm)**	**Arsenic (ppm)**	**Lead (ppm)**	**Mercury (ppm)**
1.	*Amla*	0.072	0.572	1.652	Not detected
2.	*Galo*	0.067	0.635	1.407	Not detected
3.	*Musta*	0.044	2.209	0.853	Not detected
4.	Formulation	0.073	1.177	1.121	Not detected

Table 7: Results for Phytochemical Evaluation of *Asthimajja Pachak Kwath formulation* and ingredients.

Test	***Amla***	***Galo***	***Musta***	**Formulation**
Carbohydrates	+	+	+	+
Proteins	+	+	+	+
Steroids	+	+	+	+
Alkaloids	-	+	+	+
Tannins & phenols	+	+	+	+
Glycosides	+	+	+	+
Cardiac glycosides	+	-	-	+
Anthraquinone glycosides	-	-	+	+
Flavonoids	+	+	+	+
Saponin glycosides	-	+	-	+
Coumarin glycosides	-	-	+	+

Table 8: Total tannin content in drug and formulation

Sr. No.	Drug	Tannin Content (%w/w)
1.	Amla	76.08±1.02
2.	Galo	33.75±0.84
3.	Musta	11.81±1.51
4.	Formulation	42.18±1.11
		Mean ± S.D. (n=3)

Table 9: Total Phenol content in drug and formulation

Sr. No.	Drug	Phenol Content (%w/w)
1.	Amla	9.05±0.114
2.	Galo	6.95±0.048
3.	Musta	7.79±0.278
4.	Formulation	8.28±0.119
		Mean ± S.D. (n=3)

Table 10: Quantification of Gallic acid, Ferulic acid and Berberine

Drug	Phytoconstituent	%w/w
Amla	Gallic acid	4.29±0.26
Musta	Ferulic acid	0.03±0.71
	Rutin	1.23±0.62
Galo	Berberine	0.35±1.07
		Mean ± S.D. (n=3)

FIGURES:

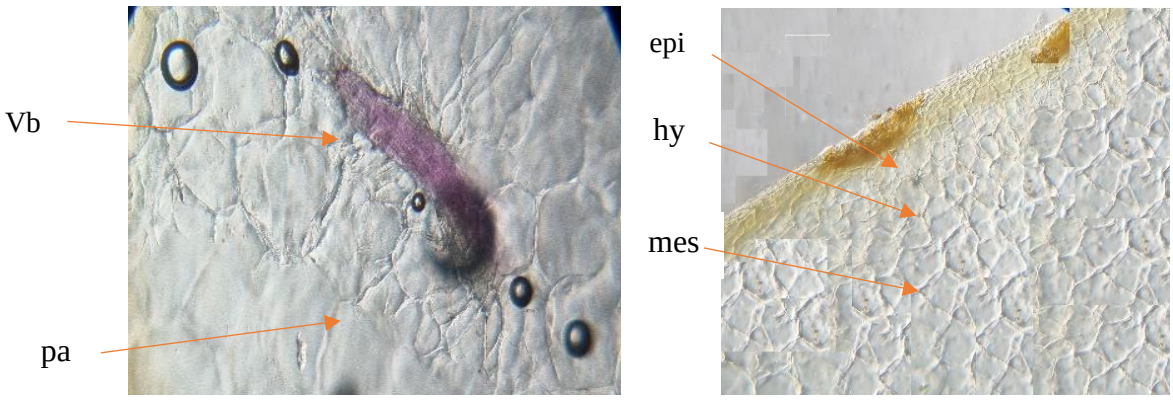


Figure 1: Transverse section of *Emblica Officinalis* Fruit epi, epicarp; hy, hypodermis; mes, mesocarp; vb, vascular bundle; pa, parenchyma.

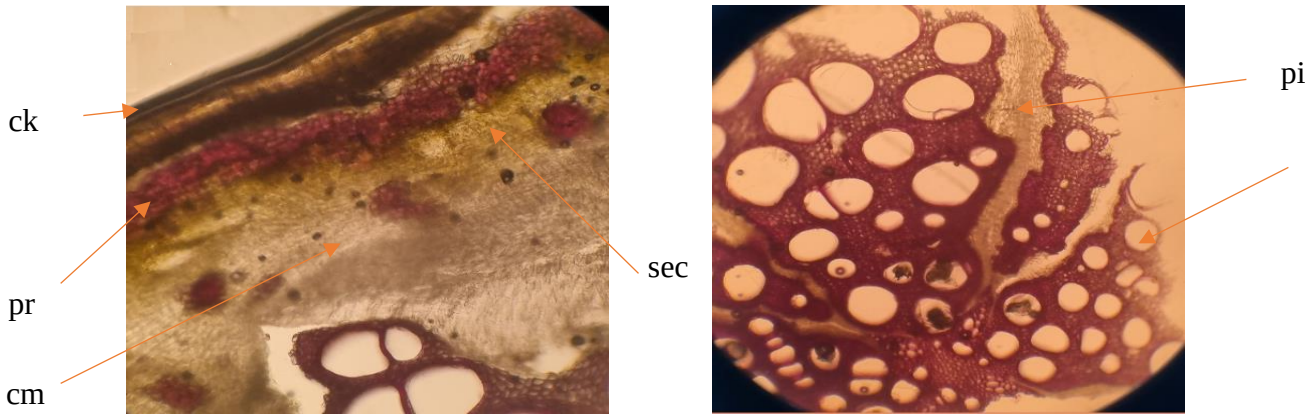


Figure 2: Transverse section of *Tinospora Cordifolia* Stem ck, cork; pr, pericycle; cm, cambium, sec, secretory cell; pi, pith; xy, xylem.

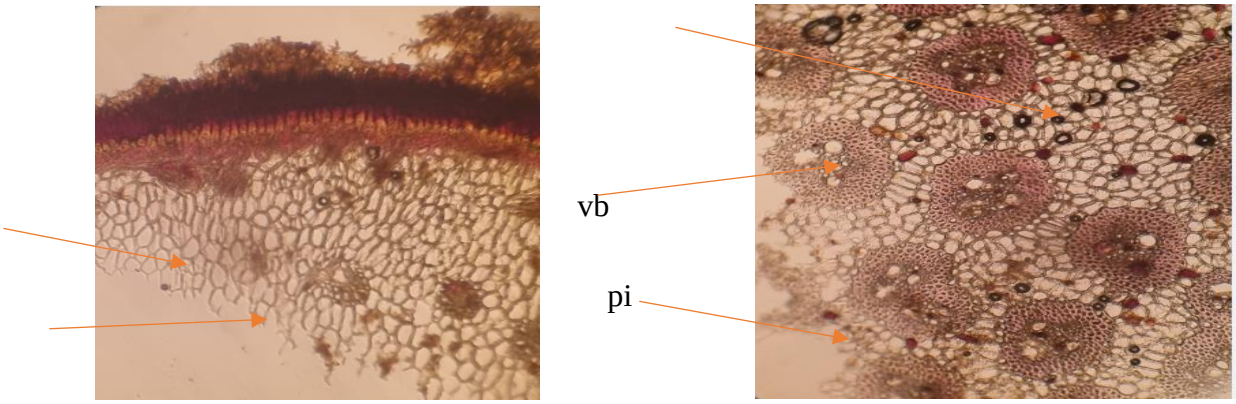
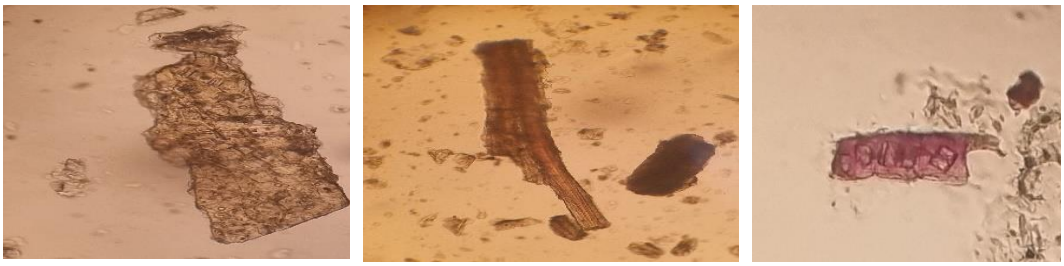


Figure 3: Transverse section of *Cyperus Rotundus* Rhizome e, epidermis; ct, cortex; pr, pericycle; end, endodermis; vb, vascular bundle; pi, pith



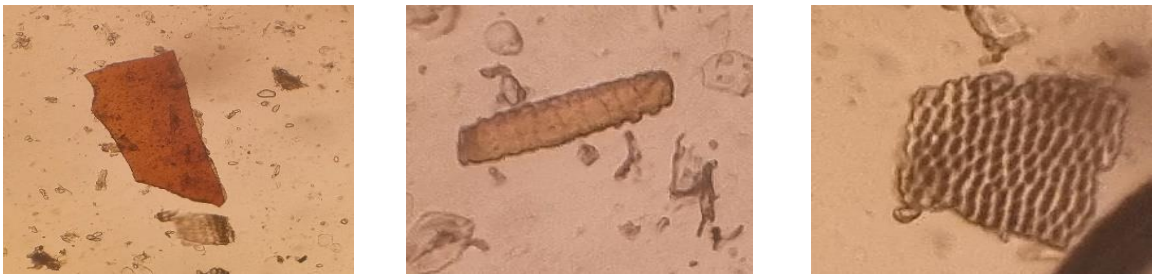
Epidermal cell

Pitted vessel attached with parenchyma

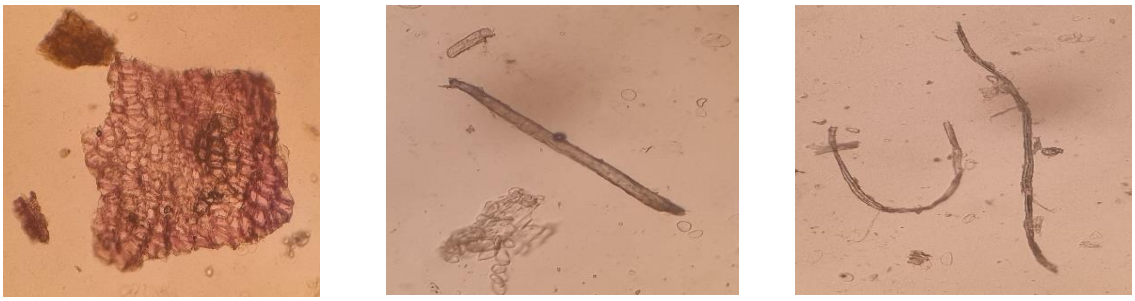
Bundle of Stone cell

Sclereids Lignified Fibers Starch grain

Figure 4: Microscopical character of *Emblica Officinalis* (Fruit powder).



Vessel elements Vessel Vessels with reticulate secondary wall thickening

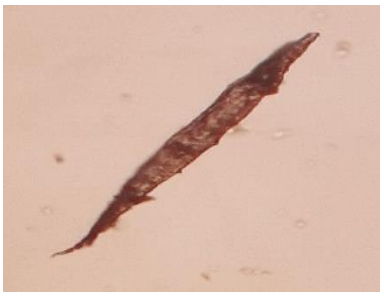


Bundle of stone cell Tracheid Fibers

Figure 5: Microscopical characteristic of *Tinospora Cordifolia* (Stem powder).

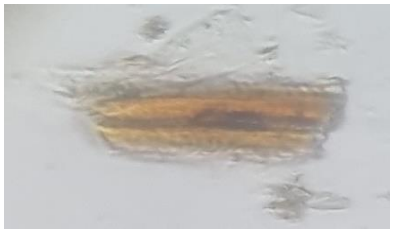
Vessel elements with spiral thickenings

Tissue fragments with thin-walled cells

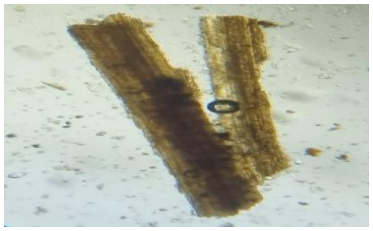


Fibers

Oil gland



Scalariform xylem vessels



Scalariform xylem vessels

Figure 6: Microscopical character of *Cyperus Rotundus* Rhizome Powder

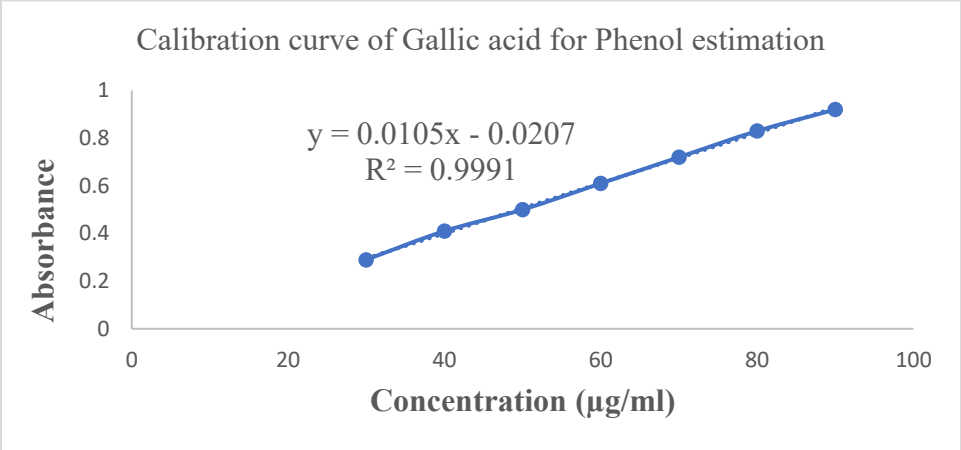


Figure 7: Calibration curve of Gallic acid for phenol estimation

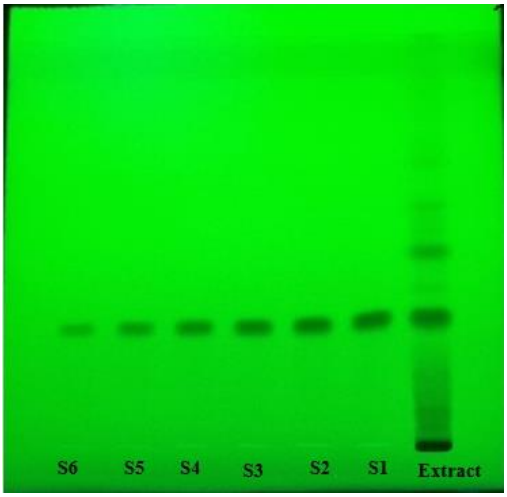


Figure 8: TLC fingerprinting of Gallic Acid in *Amla* water extract

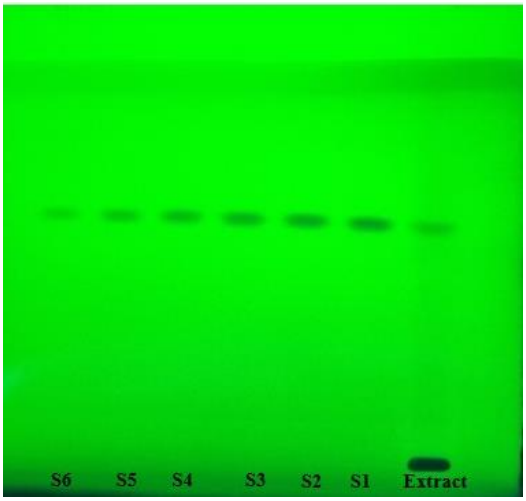


Figure 9: TLC fingerprinting of Ferulic Acid in *Musta* water extract

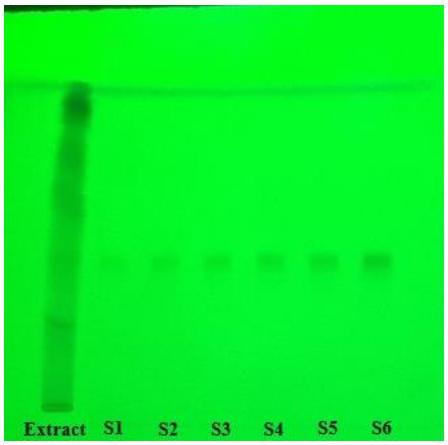


Figure 10: TLC fingerprinting of Rutin in *Musta* water extract

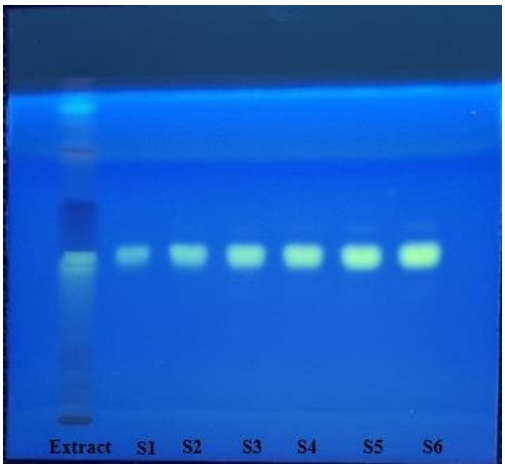


Figure 11: TLC fingerprinting of Berberine in *Galo* water extract

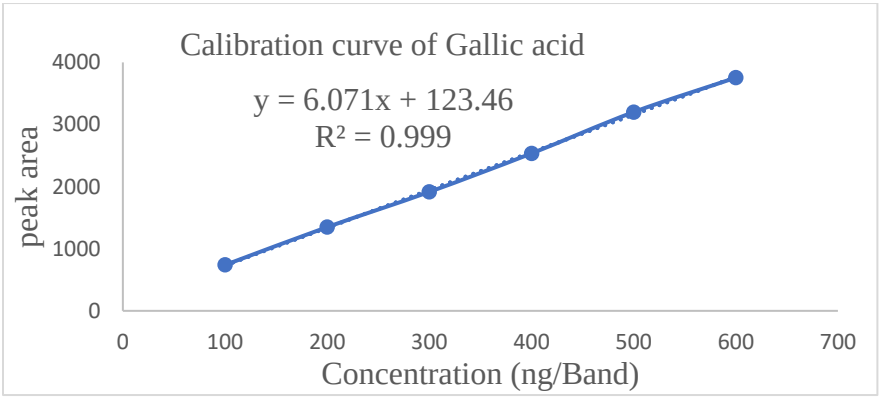


Figure 12: Calibration curve of Gallic acid

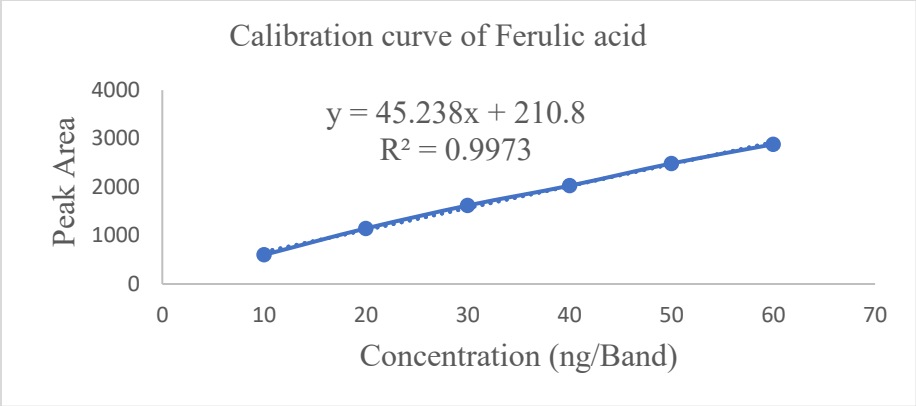


Figure 13: Calibration curve of Ferulic acid

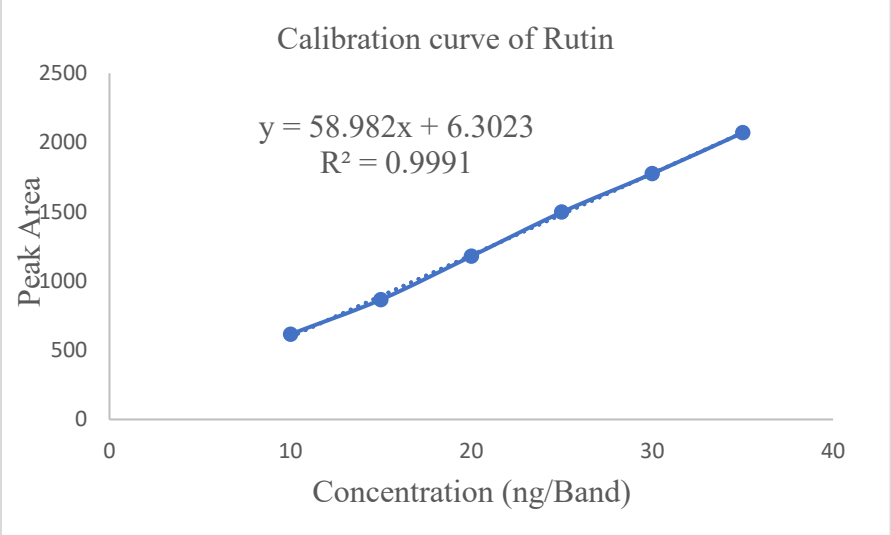


Figure 14: Calibration curve of Rutin

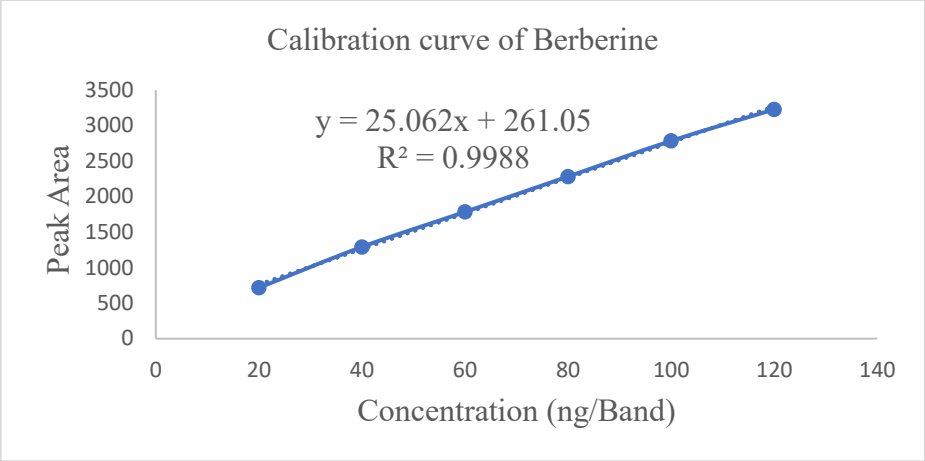


Figure 15: Calibration curve of Berberine