

# EVIDENCE BASED PHYTOPHARMACOLOGICAL VALIDATION OF AN AYURVEDIC FORMULATION OF PUSHYANUGA CHURNA.

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**ABSTRACT:** Polycystic Ovary Syndrome (PCOS) is a complex endocrine disorder affecting women of reproductive age, characterized by hormonal imbalance, metabolic disturbances, and reproductive dysfunction. The increasing prevalence of PCOS and the limitations associated with conventional therapies have led to growing interest in herbal and polyherbal formulations as alternative treatment approaches. The present study focuses on the evidence-based phytopharmacological validation and standardization of Pushyanuga Churna, a classical Ayurvedic polyherbal formulation widely used in gynecological disorders. Comprehensive evaluation of marketed samples of Pushyanuga Churna was carried out using physicochemical, phytochemical, and chromatographic (HPTLC) analyses to establish quality control parameters. Organoleptic properties, pH, ash values, extractive values, moisture content, and flow properties were assessed. Preliminary phytochemical screening confirmed the presence of bioactive constituents such as flavonoids, phenolics, alkaloids, saponins, and terpenoids, contributing to its therapeutic potential. Chromatographic fingerprinting demonstrated variations among different marketed formulations, indicating differences in composition and processing methods. The study highlights the importance of standardization and quality assessment of polyherbal formulations to ensure safety, efficacy, and reproducibility. The findings provide scientific validation for the traditional use of Pushyanuga Churna in PCOS management and establish baseline data for its inclusion in modern therapeutic systems.

**KEYWORDS:** PCOS, Pushyanuga Churna, Polyherbal formulation, Phytopharmacology, Standardization, HPTLC, Phytochemical analysis, Ayurvedic medicine, Quality control, Antioxidant activity

## 1. INTRODUCTION:

Women undergo continuous physiological and hormonal changes from menarche to menopause, during which they are susceptible to various gynecological disorders, including dysmenorrhea, amenorrhea, polycystic ovary syndrome (PCOS), uterine fibroids, and endometriosis. These conditions are influenced by genetic, lifestyle, and environmental factors and significantly impact physical and psychological well-being, highlighting the importance of maintaining reproductive health throughout life.

PCOS is a complex and heterogeneous endocrine disorder characterized by menstrual irregularities, hyperandrogenism, insulin resistance, and metabolic disturbances such as type 2 diabetes and dyslipidemia. It is also associated with increased risks of cardiovascular diseases and chronic inflammation, contributing to long-term health and economic burdens.

Globally, PCOS affects approximately 4–20% of women, with a prevalence of 3.7–22.5% reported in India, depending on diagnostic criteria. Projections indicate a rising global burden, potentially reaching up to 199.1 million cases by 2026. Current management strategies focus on symptom control and targeting hormonal and metabolic pathways, including androgen and estrogen receptors and insulin signaling mechanisms. However, due to its multifactorial nature, PCOS requires comprehensive and individualized treatment approaches.[1]

## 2. HERBAL MANAGEMENT OF PCOS AS AN ALTERNATE THERAPY:

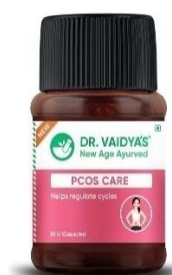
The adverse effects associated with synthetic drugs and hormonal therapies have led to increased interest in plant-based medicines as potential therapeutic alternatives for the management of polycystic ovary syndrome (PCOS). Herbal medicines offer a multi-targeted therapeutic approach due to the presence of diverse bioactive phytoconstituents, in contrast to conventional single-target pharmaceutical agents. Several phytochemicals, including curcumin, berberine, and resveratrol, have demonstrated promising therapeutic potential in the management of PCOS and may serve as alternatives to standard treatments such as clomiphene citrate. Furthermore, numerous medicinal plants have been reported to exert beneficial effects in PCOS by modulating hormonal balance, reducing oxidative stress, regulating the estrous cycle, and improving insulin resistance and lipid metabolism.[2]



**Gynoveda**



**Herbalance for PCOS**



**PCOS Care**



**Bodywise: PCOSBalance Capsules**

**PCOS Balance with Herbs**

**Namaya Aarthava Kahaya PCOD And PCOS tablets**

*Figure 1: Proprietary medicine for PCOS management*

### 3.PUSHYANUGA CHURNA:

Pushyanuga churna (PC) is an Ayurvedic polyherbal formulation composed of twentyfive plant ingredients and one mineral as described in Ayurvedic Formulary of India. Ayurvedic texts prescribe it for various female reproductive disorders such as Ashwagandha (menorrhagia), Shweta pradara (leukorrhea) Rajodosa (menstrual disorders), Arsa (piles), and Yonidosa (disorders of female genital tract). Several clinical studies on Pushyanugachurna and Lodhrasava reported its clinical efficacy in controlling Shweta pradara (leukorrhea) with the treatment period of fifteen days. Clinical evaluation of Pushyanuga churna and Kukkutabdatwak Bhasma on 121 patients showed significant positive effect on Rakta Pradara (uterine hemorrhage) after treatment for twenty-one days.

Plants like Cyperus and Glycyrrhiza present in PC are also utilized in Chinese traditional medicines falling in the list of top five most prescribed single herbs for the treatment of PCOS while Cyperus is most commonly used in Taiwan.

‘Evidence-based standardization of an ayurvedic formulation: Pushyanugachurna’ reported Pushyanugachurna as a single unifying therapy in PCOS management by providing concrete and valid baseline data to support its traditional claims. The generated results have provided significant data, justification and much needed rationale for its inclusion in mainstream medicine and highlighted the importance of proper collection procedures and legitimate preparation methods for any polyherbal formulation.[3]





### 4.THERAPEUTIC EFFICACY OF PUSHYANUGA CHURNA:

The therapeutic activity of polyherbal formulation does not depend upon the single phytoconstituent but it is believed that the unique blend of herbs with their varying biopotency gives synergistic therapeutic effect to polyherbal formulation through the phenomenon of ‘positive herb-herb interaction. Being a polyherbal formulation, Pushyanugachurna provides a multitarget treatment approach for PCOS management, particularly suited to the multifactorial symptoms associated with PCOS. Each plant ingredients in Pushyanuga churna was selected by the Acharya based on their individual clinical efficacy, well described





in the classical text ‘Bhaisajyaratnawali, Strirogadhikara; 46-49’ . On the basis of this on ancient text Bhaisajyaratnawali, the ayurvedic formulary has also given a detailed procedure for preparation of Pushyanuga churna and some preliminary quality control parameters

Pushyanuga churna includes ingredients that are reported for their antidiabetic activity, anti-androgenic activity, estrogenic activity, anti-inflammatory activity and anti-oxidative stress potential . Therefore, amalgamation of therapeutically potent individual ingredients makes Pushyanuga churna as one of the precious formulation of Ayurveda, efficacious in management of PCOS.[4]

**Table 1: Therapeutic efficacy of Pushyanuga churna**

<b>Cissampelos Pareira</b>	<b>Syzygiumcumini</b>	<b>Mangifera indica</b>	<b>Bergenia ligulata</b>
			
Anti-inflammatory activity, Cardioprotective effect, Anti-hyperglycemic activity, Antioxidant activity, Immuno-modulatory activity.	Anti-Diabetic Activity, Cardioprotective activity, Anti-hyperlipidemic activity, Anti-oxidant activity, Enhancement of ovarian Health.	Anti-Diabetic activity, Anti-inflammatory activity, Antioxidant activity.	Anti-Diabetic activity, Free radical scavenging activity, Cardioprotective activity, Excessive uterine haemorrhage, menorrhagia Anti-inflammatory activity.

**Table 2: Therapeutic efficacy of Pushyanuga churna**

Berberis aristata	Hibiscus sabdariffa	Salmalia malabarica	Nelumbo nucifera
			
Anti-Diabetic Activity, Antihyperglycemic activity, Antioxidant activity	Anti-diabetic activity, Anti-anaemic activity, Anti-obesity activity, Antioxidant activity,	Anti-diabetic activity, Anti-hyperlipidemic activity, Anti-obesity activity, Anti-Inflammatory activity, Immunomodulatory activity, Antioxidant activity.	Antioxidant activity, Anti-Inflammatory activity, Anti-diabetic activity, Anti-obesity activity.

**Pushyanug Churna**  
Ingredients: Shloka

पाठा जम्बवाग्रयोर्मध्यं शिलोद्भेदं रसाञ्जनम्॥९०॥  
अम्बष्ठा शाल्मलीश्लेषं समङ्गां वत्सक त्वचम्  
बाह्लीकातिविषे बिल्वं मुस्तं लोध्रं स गैरिकम्॥९१॥  
कट्वङ्गं मरिचं शुण्ठीं मृद्धीकां रक्त चन्दनम्  
कटफलं वत्सकानन्ता धातकी मधुकार्जुनम्॥९२॥  
पुष्येणीद्धृत्य तुल्यानि सूक्ष्म चूर्णानि कारयेत्  
तानि क्षौद्रेण संयोज्य पिबेत्तण्डुल वारिणा॥९३॥  
अर्शःसु चातिसारेषु रक्तं यच्चोपवेश्यते।  
दोषागन्तुकृता ये च बालानां तांश्च नाशयेत्॥९४॥  
योनिदोषं रजोदोषं श्वेतं नीलं सपीतकम्  
स्त्रीणां श्यावारुणं यच्च प्रसह्य विनिवर्तयेत्॥९५॥  
चूर्णं पुष्यानुगं नाम हितमात्रेयपूजितम्॥९६॥  
इति पुष्यानुग चूर्णम्।

च० वि० 30/90-95  
www.ayurmedia.in

**Figure 2: Formula of Pushyanuga churna in Ayurvedic text****Figure 3: Pushyanuga churna Dabur**

**Table 3: Details on container – Dabur**

<b>Name of formulation as on bottle</b>	Pushyanuga churna-- Ayurvedic medicine
<b>Manufacture details (Mfd by/ Mktd by)</b>	Dabur India Ltd, Rajasthan.
<b>Reference for preparation</b>	Bhaishajya Rantavali/Pradar Roga Chikitsa, without Rakta chandana as per API Part-II, Vol-III
<b>Indications</b>	Useful in excessive discharge & disorders of genital tract in females.
<b>Monograph if available</b>	--
<b>No. of ingredients (Label claim)</b>	26
<b>Changes if any (plant part)</b>	--
<b>Additions/ substitutions</b>	--
<b>Absent ingredients</b>	Pterocarpus santalinus
<b>Shelf life on package</b>	2 years
<b>Recommend storage condition</b>	--
<b>Dosage</b>	1/4to 1/2 teaspoonful (1 to 3g) twice a day with honey or as directed by the physician
<b>MRP (Maximum retail price)/100gm</b>	₹72

## 5. MATERIAL AND METHOD :

The polyherbal formulation which encompasses all possible information and assures the reproducibility of phytoconstituents. To have good coordination between the quality of raw materials and final products, it has become essential to develop reliable and specific quality control methods using a combination of classical and modern instrumental methods of analysis. Standardization of polyherbal formulation will provide passport data for raw materials authentication and selection as well as macroscopic, microscopic and chromatographic evaluation . The data can be used to decide the identity, purity, potency, and quality standards for formulations. Among the many analytical tools, HPTLC is widely used to construct reference fingerprints for polyherbal formulations and their raw materials due to its simplicity, versatility and simple sample preparation. In the present research work, various physicochemical parameters, rheological parameters, phytochemical analysis and analytical quality control tools were developed for Pushyanuga churna.

### 5.1 Equipments:

Analytical weighing balance, horizontal shaker, hot plate, Karl Fischer titrator, muffle furnace, oven, reflux apparatus, sonicator, Soxhlet apparatus, vortex mixer and water bath were used in this research. The HPTLC system consisted of TLC Scanner 4 supported by winCATS software version 1.4.7 equipped with Linomat 5 sample spotter and Reprostar 3 system.[5]

### 5.2 Chemicals and reagents:

Whatman filter paper (no. 1 and 41), nylon micro filter paper (0.45 µm), kieselguhr, Karl Fischer reagent, Dragendorff's reagent, Folin-Ciocalteu phenol reagent, chemicals for Mayer's reagent (HgCl<sub>2</sub> and KI), (CH<sub>3</sub>COO)<sub>2</sub>Pb, NaOH, FeCl<sub>3</sub>, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, KMnO<sub>4</sub> Wagner's reagent, DPPH [2,2-Diphenyl-1-picrylhydrazyl (98% purity)], galvinoxyl (98% purity), phosphate buffer reagents (NaCl, KCl, Na<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>), K<sub>3</sub>Fe(CN)<sub>6</sub> were obtained from Sigma-Aldrich Chemicals (St. Louis, USA). Ethanol, methanol, petroleum ether, toluene, chloroform, ethyl acetate, vanillin sulphuric acid, glacial acetic acid, HCl, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub> were obtained from Merck Specialities (Mumbai, India). Ultra-pure water was obtained using a Milli-Q purification system (Millipore, USA). All other chemicals used were of analytical grade. Derivatizing reagent (10% methanolic sulphuric acid) was prepared as per the method described. Glassware from Borosil was used throughout the study.

### 5.3 Procurement of Formulation:

Marketed samples of Pushyanuga churna of six brands Dhootpapeshwar, Patanjali, Baidhyanath, Arkashala, Dabur and Kottakkal were purchased from a registered Ayurvedic pharmacy at Matunga, Mumbai. The Marketed samples of Pushyanuga churna coded as PC I, PC II, PC III, PC IV, PC V and PC VI for further studies.[6]

**Table 4: Sample code for marketed samples of Pushyanugachurna**

Sample name	Sample code
Pushyanuga churna-Dhootpapeshwar	PC I
Pushyanuga churna-Patanjali	PC II
Pushyanuga churna-Baidhyanath	PC III
Pushyanuga churna-Arkashala	PC IV
Pushyanuga churna-Dabur	PC V
Pushyanuga churna-Kottkkal	PC VI

### 5.4 Organoleptic evaluation:

Each PC-formulation was macroscopically characterised in terms of colour, odour, taste, and texture to validate the authenticity and quality of formulations. Comparative evaluation of *Pushyanuga churna* formulations showed variation in color, odor and texture. [7,8]

**Table 5: Organoleptic properties of Pushyanugachurna**

Samples	Appearance	Color	Odor	Taste	Texture
Reference PC	Powder	Light brown	Musty	Bitter	Moderately fine
PCI	Powder	Light brown	Musty	Bitter	Fine
PCII	Powder	Brown	Musty	Bitter	Moderately fine
PCIII	Powder	Dark brown	Characteristic	Slightly bitter	Fine
PCIV	Powder	Dark brown	Musty	Bitter	Moderately fine
PCV	Powder	Reddish brown	Musty	Bitter	Fine
PCVI	Powder	Light brown	Aromatic	Bitter	Fine

## 5.5 Physicochemical evaluation:

### 5.5.1 PH value (1% w/v solution):

The pH of solution provides a useful practical means for the indication of the acidity or alkalinity of a solution. pH value of reference PC, PC-I, PC-II and PC-III was found to be within prescribed limit given by Ayurvedic formulary of India. pH value of PC-IV, PC-V and PC-VI was found to be below the prescribed limit.

**Table 6 : PH of Pushyanuga churna**

Samples	pH(1%w/v solution)		Prescribed limits
	Mean $\pm$ SD (n = 3)	% CV	
ReferencePC	5.80 $\pm$ 0.015	0.264	5 to 6 (AFI,2007)
PCI	5.31 $\pm$ 0.012	0.218	
PCII	5.32 $\pm$ 0.015	0.282	
PCIII	5.12 $\pm$ 0.020	0.391	
PCIV	4.72 $\pm$ 0.015	0.324	
PCV	4.82 $\pm$ 0.015	0.317	
PCVI	4.82 $\pm$ 0.025	0.522	

**5.5.2 Ash value:** Ash values are important quantitative standards that can be used to determine drug authenticity and purity. The formulation's total ash value measures the amount of material that remains after ignition (physiological ash), whereas acid-insoluble and water-soluble ash indicate the amount of silica present, particularly as sand and siliceous earth (non physiological ash). The content of total ash, acid insoluble ash and water-soluble ash in *Pushyanuga churna* is summarized. The content of total ash and acid-insoluble ash was found to be higher in PC III and PC VI, whereas the content of water-soluble ash was found to be more in PC III, PC IV and PC V. The results indicated the presence of more foreign inorganic matter as carbonates, phosphates, silicates, silica in these formulations as compared to other tested formulations. These inorganic contents can be considered 'impurities', which may be due to improper collection, contamination, substitution, or adulteration. The content of total ash, acid-insoluble ash and water-soluble ash for the remaining formulations was within the prescribed limit as reported in the Ayurvedic Formulary of India, indicating the quality and purity of the formulations. [9,10]

**Table 7: Ash value of *Pushyanuga churna***

Samples	Total ash (%)		Acid in soluble ash (%)		Water-soluble ash (%)	
	Mean $\pm$ SD (n = 3)	% CV	Mean $\pm$ SD (n = 3)	% CV	Mean $\pm$ SD (n = 3)	% CV
ReferencePC	11.70 $\pm$ 0.053	0.450	3.12 $\pm$ 0.025	0.806	3.37 $\pm$ 0.044	1.293
PCI	13.70 $\pm$ 0.156	1.140	1.25 $\pm$ 0.010	0.800	4.35 $\pm$ 0.031	0.702
PCII	12.76 $\pm$ 0.053	0.415	2.59 $\pm$ 0.040	1.544	3.20 $\pm$ 0.050	1.563
PCIII	16.13 $\pm$ 0.125	0.775	4.73 $\pm$ 0.042	1.115	6.26 $\pm$ 0.031	0.488
PCIV	11.65 $\pm$ 0.136	1.165	1.24 $\pm$ 0.021	1.355	8.26 $\pm$ 0.049	0.597
PCV	12.79 $\pm$ 0.087	0.683	2.71 $\pm$ 0.026	0.976	8.82 $\pm$ 0.035	0.398
PCVI	16.58 $\pm$ 0.065	0.392	6.22 $\pm$ 0.047	0.760	4.62 $\pm$ 0.015	0.331
<b>Prescribed/Suggested limits</b>						
<b>Total ash (%)</b>		NMT 15%		AFI,2007		
<b>Acid in soluble ash (%)</b>		NMT 4%				
<b>Water-soluble ash(%)</b>		NMT 6%				

**5.5.3 Loss on drying and moisture content:** Loss on drying and moisture content are especially critical factors for *Pushyanuga churna* as the smaller particle size of *churna* may absorb moisture easily and deteriorates expeditiously in the presence of water. A high water content in herbal formulations promotes microbial growth, eventually causing degradation of the formulation. The total amount of moisture and any volatile components in the sample are taken into account when calculating the loss on drying. Moisture content of any sample is determined using Karl Fischer titration method, which is

based on the reaction of water with iodine and sulphur dioxide in pyridine solution (Bajwa et al, 2013). The percent loss on drying and moisture content of all PC formulations were found to be within the prescribed limits. Results indicate that Good Manufacturing Practices (GMP) have been implemented during the drying and processing of raw materials. Slightly higher values of moisture content in PC-III and PC-IV samples showed susceptibility to bacterial, fungal, or yeast growth as compared to other formulations analysed in the study (table 8).[11,12]

**Table 8: Loss on drying and moisture content of Pushyanuga churna**

Samples	Loss on drying (%)		Moisture content (%)	
	Mean $\pm$ SD (n = 3)	% CV	Mean $\pm$ SD (n = 3)	% CV
Reference PC	6.10 $\pm$ 0.055	0.903	8.95 $\pm$ 0.118	1.323
PCI	6.62 $\pm$ 0.035	0.231	8.93 $\pm$ 0.087	0.978
PCII	6.41 $\pm$ 0.067	1.039	7.20 $\pm$ 0.070	0.972
PCIII	6.39 $\pm$ 0.059	0.917	9.28 $\pm$ 0.075	0.666
PCIV	7.82 $\pm$ 0.040	0.512	9.58 $\pm$ 0.079	0.829
PCV	8.51 $\pm$ 0.159	1.873	7.65 $\pm$ 0.130	1.699
PCVI	3.06 $\pm$ 0.021	0.681	8.84 $\pm$ 0.171	1.233
<b>Prescribed/Suggested limits</b>				
<b>Loss on drying(%)</b>		NMT 11%	AFI,2007	
<b>Moisture content (%)</b>		NMT 10%	Suggested limit	

**5.5.4 Solvent extractive value:** Extractive value is indicative of the extent of polar, medium polar and non-polar components present in polyherbal formulation. The results are represented in Table 3.7. It was observed that the content of the water-soluble extractives was in the following order: reference PC > PC-I > PC-II > PC- IV > PC-V > PC-III > PC-VI, which were found within the prescribed limits as per the ayurvedic pharmacopeia of India. The higher water-soluble extractive value of all formulation reveals that formulations consist of higher content of polar constituents such as sugar, acids and inorganic compounds . It was observed that the content of the ethanol-soluble extractives was in the following order: PC-I > PC-II > referencePC>PC-V>PC-IV>PC-VI>PC-III.LessalcoholextractivevaluesforPC-III and PC-VI indicates improper post harvesting conditions and addition of exhausted material.[13]

**Table 9: Ethanol and water soluble extractive of *Pushyanuga churna***

Samples	Ethanol soluble extractive (%)		Water-soluble extractive(%)	
	Mean±SD (n = 3)	%CV	Mean±SD (n = 3)	%CV
ReferencePC	12.92±0.025	0.195	70.13±0.042	0.059
PCI	13.76±0.035	0.252	68.27±0.072	0.106
PCII	13.34±0.126	0.943	67.96±0.017	0.028
PCIII	9.08±0.068	0.750	59.74±0.047	0.079
PCIV	11.44±0.036	0.315	66.53±0.036	0.054
PCV	12.17±0.055	0.452	65.19±0.040	0.062
PCVI	9.74±0.040	0.415	48.19±0.015	0.032
<b>Prescribed limits</b>				
Ethanol soluble extractive (%)		NLT12%		AFI,2007
Water-soluble extractive(%)		NLT13%		

**5.5.5.Flow properties:** The rheological properties of formulations, such as bulk density, tapped density, hausner's ratio, and Carr's index, were measured and compared with the acceptance limit given in USP Pharmacopeia. The results showed a wide range of variations, possibly due to improper preparation, grinding, storage and packaging methods of the finished product. The variation in particle characteristics of PC formulations suggested that there was a difference in raw material processing during manufacturing process of *Pushyanuga churna*. Comparative evaluation of physical characters showed that PC-II and PC-III have better flow properties as compared to other tested PC-formulations. Therefore, the ease of administration and absorption of *Churna* by the oral route is superior in these marketed formulations. Furthermore, the poor flowability of other PC formulations suggests that more efficient raw material handling methods should be used to improve flowability. The results are represented in Table 8.

**Table 10: Physical characteristics USP limits**

Flow property	Compressibility Index (%)	Angle of repose (degree)	Hausner ratio
Excellent	<10	25-30	1.00-1.11
Good	11-15	31-35	1.12-1.18
Fair aid not added	16-20	36-40	1.19-1.25
Passable may hang up	21-25	41-45	1.26-1.34
Poor must agitate, vibrate	26-31	46-55	1.35-1.45
Very poor	32-37	56-65	1.46-1.59
Very,very poor	>38	>66	>1.60

**Table 11:Physical characteristics of Pushyanuga churna**

Parameters		Refere nce-PC	PCI	PCII	PCIII	PCIV	PCV	PCVI
Bulkdensity (g/ml)	Mean ± SD(n=3 )	0.38±0. 006	0.37±0. 003	0.45±0. .008	0.45±0.0 05	0.40±0.0 05	0.40±0.0 05	3.35± 0.02
	%CV	1.579	0.811	1.778	1.111	1.250	1.500	0.597
Tapdensity (g/ml)	Mean ± SD(n=3 )	0.52±0. 009	0.56±0. 006	0.56±0. .003	0.55±0.0 03	0.55±0.0 07	0.54±0.0 03	5.00± 0.003
	%CV	1.731	1.071	0.536	0.545	1.273	0.556	0.060
Hausner ratio	Mean ± SD(n=3 )	1.38±0. 015	1.56±0. 0181	1.22±0. .0171	1.22±0.0 24	1.38±0.0 11	1.32±0.0 25	1.50± 0.002
	%CV	1.087	1.154	1.393	1.967	0.797	1.894	0.133

<b>Carr's index</b>	<b>Mean</b> ± <b>SD(n=3)</b> )	26.85±0.053	35.71±0.049	18.18±0.016	18.18±0.012	28.00±0.027	24.00±0.028	33.33±0.185
	<b>%CV</b>	0.197	0.137	0.088	0.066	0.096	0.117	0.555
<b>Angleof repose</b>	<b>Mean</b> ± <b>SD(n=3)</b> )	38.20±0.02	41.47±0.021	34.95±0.024	41.60±0.042	32.37±0.034	41.12±0.035	41.12±0.021
	<b>%CV</b>	0.052	0.051	0.069	0.101	0.105	0.085	0.051

## 6. PRELIMINARY PHYTOCHEMICAL PROFILE:

The presence of a broad range of secondary metabolites such as flavonoids, phenolics, alkaloids, saponins, terpenoids, etc. in polyherbal formulations gives a multidimensional therapeutic effect to polyherbal formulations. The intensity of the reaction in different tests was graded qualitatively as low, average and high for showing the presence of phytochemical constituents in the formulations. Preliminary phytochemical screening of various PC-formulations revealed the presence of different primary and secondary metabolites.

### 6.1 Major phytochemical screening by Soxhlet extraction and fractionation:

Soxhlet extraction of the formulation with organic solvents (methanol, distilled water, and ethyl acetate) followed by fractionation with chloroform and chloroform: methanol separated crude fibre by producing four distinct extracts (neutral, polar, moderately polar, and basic). Comparative analysis of the major phytochemical fractions of the PC formulations revealed a variation in the percent content of fats and waxes, terpenoids and phenolics, quaternary alkaloids, and N-oxides and alkaloids, which could be attributed to variations in the plant composition of the Pushyanugachurna formulations.

The data provides a comparative account of the phytochemical fractions of the formulations rich in various phytochemical constituents such as fats and waxes (neutral extract), terpenoids and phenolics (moderately polar extract), quaternary alkaloids and N-oxides (polar extract) and alkaloids (basic extract)[14]

**Table 12: Qualitative data on the presence of phytochemical constituents in the Pushyanuga churna.**

Testperformedwith	Inference						
	Reference-PC	PCI	PCII	PCIII	PCIV	PCV	PCVI
<b>Flavonoids</b>							
(CH <sub>3</sub> COO) <sub>2</sub> Pbsolution	+	+	+	+	+	+	+
Increasing amount of NaOH solution	-	-	-	-	-	-	-
<b>Phenolics</b>							
5% FeCl <sub>3</sub>	+	+	+	+	+	+	+
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> solution	+	+	+	+	+	+	+
Dilute KMnO <sub>4</sub> solution	+	+	+	+	+	+	+
Acetic acid solution	+	+	+	+	+	+	+
Dilute HNO <sub>3</sub> solution	+	+	+	+	+	+	+
<b>Alkaloids</b>							
Dragendorff's reagent	+	+		-	+	+	+
Mayer's reagent	+	+		-	+	+	+
Wagner's reagent	+	+		-	+	+	+
<b>Terpenoids</b>							
Chloroform+carefully addition of concentrated H <sub>2</sub> SO <sub>4</sub>	+	+	+	+	+	+	+
<b>Saponins</b>							
Water with vigorous shaking	+	+	+	+	+	+	+
<b>Glycosides</b>							
Water+NaOH solution	+	+	+	+	+	+	+
<b>Essential oils</b>							
Vanillin sulphuric acid	-	-	-	-	-	-	-
<b>Resins</b>							
Concentrated H <sub>2</sub> SO <sub>4</sub>	+	+	+	+	+	+	+
<b>Reducing sugar</b>							
DNS Areagent+boil	+	+	+	+	+	+	+

**Table 13: Content of major phytochemical constituents by Soxhlet extraction present in*****Pushyanuga churna***

Parameters		Referen ce-PC	PC I	PC II	PC III	PC IV	PC V	PC VI
Crude fibre (%)	Mean ±SD (n = 3)	66.48±0 .546	77.53± 0.020	66.52± 0.015	72.56± 0.029	80.16± 0.036	79.91± 0.015	82.22± 0.026
	% CV	0.821	0.026	0.023	0.040	0.045	0.019	0.032
Neutral extract (fats and waxes) (%)	Mean ±SD (n = 3)	0.73±0. 013	0.85±0. 004	0.93±0. 004	1.79±0. 005	1.87±0. 003	1.58±0. 004	0.74±0. 008
	% CV	1.720	0.423	0.433	0.280	0.134	0.228	1.107
Moderately polar extract (terpenoid s and phenolics) (%)	Mean± SD (n=3)	2.95±0. 015	3.76±0. 015	3.97±0. 006	2.14±0. 036	1.97±0. 015	2.87±0. 015	1.95±0. 025
	% CV	0.517	0.406	0.145	1.685	0.777	0.533	1.293
Polar extract (quaternary alkaloid sand- oxides) (%)	Mean ±SD (n = 3)	24.24±0 .031	25.37± 0.173	20.09± 0.075	19.93± 0.114	22.22± 0.040	14.16± 0.136	17.26± 0.021
	% CV	0.126	0.684	0.376	0.570	0.182	0.961	0.121
Basic extract (most alkaloids) (%)	Mean± SD (n=3)	1.17±0. 006	1.07±0. 015	1.28±0. 010	0.37±0. 006	0.66±0. 012	0.71±0. 010	0.73±0. 012
	% CV	0.492	1.432	0.781	1.546	1.855	1.408	1.648

## 7. GROUP DETERMINATION USING UV-VISIBLE

**SPECTROPHOTOMETER:** The phytochemical constituents like flavonoids, terpenoids, saponins, phenolics, etc. are known to have numerous pharmacological effects that are responsible for the biological activities of polyherbal formulations. UV-Visible spectroscopy was successfully used to distinguish the content of phytoconstituents between different formulations of Pushyanuga churna thus defining their chemical quality. From the results, the content of phytochemicals in the PC-formulations was found to be in the following order: flavonoids > phenolics > saponins > terpenoids. The results obtained for group determination assays are discussed in the succeeding subsections.

**7.1 Total flavonoid content:** The results were derived from a calibration curve ( $y = 0.00025x + 0.02912$ ;  $R^2=0.998$ ) of quercetin (23.40-1500  $\mu\text{g/mL}$ ) and expressed as mg QEa/g present in Pushyanuga churna. The content of flavonoid compounds in ethanolic extract of Pushyanuga churna ranged from 46.99 mg QEa/g to 92.25 mg QEa/g.

## 8. CHROMATOGRAPHIC FINGERPRINT OF THE PUSHYANUGA CHURNA:

Chromatographic fingerprints can reliably depict the "chemical integrity" of traditional formulations even when the concentration and/or a number of their chemically distinctive ingredients vary across different samples of the traditional formulation. Thus, chromatography-based fingerprints are useful for quality control since they may be used to authenticate and identify traditional formulations and their raw materials (Kelly, 2001). In HPTLC analysis, the fingerprint of Pushyanuga churna was developed using toluene: ethyl acetate: formic acid (8: 2: 1, v/v/v) as a mobile phase. The plates were derivatized with 10% methanolic sulphuric acid and 1% anisaldehyde sulphuric acid reagent. Figure 3.2 depicts the HPTLC plate photo and chromatograms in the form of 3-D overlay of fingerprint of Pushyanuga churna formulations under 254nm, 366 nm (before derivatization), 366nm (after derivatization with 10% methanolic sulphuric acid) and 540 nm (after derivatization with 1% anisaldehyde sulphuric acid). The detailed information on the  $R_f$  values and response obtained under different wavelengths has been summarized in Table 3.13 A, B, C & D.

**9.EVALUATION OF ANTIOXIDANT ACTIVITY:** A polyherbal formulation contains multiple antioxidant phytochemicals, including steroids, alkaloids, tannins, flavonoids, and phenolic compounds. Among all phytochemicals, flavonoids and phenolic acids are considered decisive factors in the antioxidant activity of any plant or plant-based drug. Compared to synthetic antioxidants, natural antioxidants are quite safe and can be used for a longer duration chemicals are desired since they not only counteract reactive oxygen species but also have little or no side effects.[15]

**10.RESULT AND DISCUSSION:** The scenario for manufacturing and dispensing herbal medicines has changed tremendously over the last few decades. Lack of a rigid quality control profile and reproducible therapeutic activity are the main barriers to the acceptance of traditional medicines. Quality evaluation of polyherbal formulations is a vital requirement to ensure potency, safety, batch-to-batch consistency and product acceptance. Therefore, the marketed formulations of Pushyanuga churna and the reference

Pushyanuga churna were subjected to a series of relevant physical and chemical quality parameters and chromatographic fingerprints using HPTLC. The results obtained are discussed in the succeeding subsections.

**11.CONCLUSION:** In the present study, *Pushyanuga churna* was subjected to comprehensive quality evaluation, including physicochemical, phytochemical, and chromatographic (fingerprint) analyses. The findings revealed significant variations in quality control parameters among the evaluated marketed formulations. These discrepancies may be attributed to differences in plant composition, processing methods, or the possible presence of substitutes and adulterants. The standardization parameters established in this study provide a scientific basis for assessing the quality, purity, and authenticity of *Pushyanuga churna*. Furthermore, these findings may contribute to the development of pharmacopeial standards and serve as a reference monograph for quality assurance. Comparative evaluation indicated that the marketed formulations Pushyanuga churna I (Dhootpapeshwar) and Pushyanuga churna II (Patanjali) demonstrated superior performance in quality control assessments. Both formulations also exhibited the highest similarity in HPTLC fingerprint profiles when compared to the reference standard. Based on these results, these two formulations were selected for further marker-based chromatographic analysis, as discussed in the subsequent chapter

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