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International Journal of Pharmaceutical Chemistry and Analysis

Journal homepage: https://www.ijpca.org/

Original Research Article

Standardization & evaluation of anti-oxidant, anti-inflammatory and antimicrobial potential of *Panchnimba Churna*

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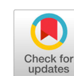

A R T I C L E I N F O

Article history:
Received 20-11-2022
Accepted 13-12-2022
Available online 06-02-2023

Keywords:
Standardization
Panchanimba Churna
DPPH
Anti-Microbial
Anti-Inflammatory
Anti-Oxidant

A B S T R A C T

Background: Ayurvedic medication pancha nimba choorna is a herbal powder. The panchanimba is a well-known medication stated in the old Ayurvedic texts used to cure kshudra kushtas (minor skin disorders), such as eczema, acne, and Maha kushtas (major skin ailments). It is used to treat a variety of skin conditions, including dermatitis, pruritis, psoriasis, sinus, non-healing wounds, headaches, uti, diabetes, toxins, and arthritis. It is employed to treat infections of the skin.

The traditional use of the neem tree's leaves, blossoms, seeds, fruits, roots, and bark for the treatment of inflammation, infections, fever, skin conditions, and dental problems includes all of these parts. Neem leaf's therapeutic benefits have been specifically discussed. The immunomodulatory, anti-inflammatory, anti-hyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, and anticarcinogenic activities of neem leaf and its components have been proven. Protein, carbohydrates, flavonoids, phenol, saponin, glycosides, tannin, and alkaloids are all present according to phytochemical analysis, whereas terpenoids, sterols, and other substances are not present.

Results: To guarantee the quality of the product, standardization employing various parameters was used which was performed as per the Indian Pharmacopoeia, WHO guidelines and various literature reports. The antioxidant activity may be attributes to flavonoids and phenolics present in extracts of *Panchanimb Churna*. In terms of gallic acid equivalent, Panchanimb Churna contained 105 mg/g of total phenols and the total flavonoid concentration was 43.8214 mg/g, which is the quercetin equivalent. Antioxidant property was evaluated using DPPH activity.

Conclusion: According to a forementioned quality criterion, Panchanimb Churna was determined to be competent under every examined aspect. The current study strengthens belief in herbal remedies of natural origin to combat infectious diseases and other illnesses that may be included in medications in the future. Overall the product is very beneficial in day to day life for humans as it possess various qualities and activities.

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1. Background

The ability of the skin to shield internal organs from harmful external impacts makes it one of the body's most vital organs. Without unbroken skin, no person can survive. Physical, chemical, biological, and other environmental factors that might affect the skin can all be classed. Biological agents are any of the various infectious or infesting organisms that can flourish on the skin and cause diseases. The skin contains a number of built-in defences

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https://doi.org/10.18231/j.ijpca.2022.032
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against environmental irritants, and most of the time the skin is able to defend itself.[1] The concept of skin problems is significantly different in Ayurvedic medicine. All skin conditions are regarded as Kushtha roga. Kushtha rogas is mostly brought on by poor eating habits, specifically consuming heavy, inappropriate, unsuited, and healthy foods as well as using medications for indigestion. Taking dip water after having been heated by sunlight exposure, fire etc. Due to all of these factors, inflamed pitta and kapha dosha reach the obliquely moving channels, where they further aggravate and disperse themselves all over the place in the direction of the exterior flow. When dispersed doshas move, patches develop as a result. If untreated, doshas that started in the skin grow and go within, vitiating dhatus.[2] The simultaneous vitiation of all three doshas is linked to skin infections. However, the symptoms are indicative of the dominant doshas.[3]

Eczema can be related to "Vicharchika" in Ayurveda. There is a lot of overlap between eczema and the symptoms of extreme itching, boil or pustule formation, discoloration/ hyperpigmentation, profuse oozing and later noticeable lichenification, discomfort, and excessive dryness.[4] Atopic dermatitis, another name for eczema, is characterised by skin irritation. Patches of skin that are scaly or crusty, frequently accompanied by redness, blistering, and itching, are the main symptoms of the disorder. Children and adults can both be affected by atopic dermatitis (AD).[5] The primary sign of this illness is pruritus, which lowers quality of life in terms of health (HRQOL).[6] In addition to cutaneous immunological and epidermal failure, eczema has a pathogenesis that includes systemic dysfunction.

There are many different treatments available in ayurveda. Several indigenous plants are discovered to be highly beneficial in the treatment of vicharchika, according to eczema treatment methods. Even today, outstanding progress is being made in the study of these remedial approaches. The appropriate treatment for vicharchika mentioned in Bhaishjya Ratnavali and Bharat Bhaishajya Ratnakar is panchnimba churna (abhyantar prayoga).

In order to evaluate the quality of medications based on the concentration of their active principles, physical, chemical, phytochemical, and standardisation, in-vitro and in-vivo characteristics, it is crucial to standardise herbal formulation.[7] To demonstrate why herbal formulations are acceptable in the current medical system, it is crucial to evaluate their quality. The authenticity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity are a few of the factors that affect the quality of herbal drugs. The phytochemical profile is crucial in these standardisation procedures.[8] The quantification of the marker compound would be an extra criterion in evaluating the quality of the sample, while the fingerprint acts as a guideline to the phytochemical profile of the medicine in ensuring the quality. Chromatographic techniques are also utilised to assess the herbal formulation's quality. The use of techniques like HPTLC, HPLC and LC-MS, and GC-MS to standardise plant material in relation to marker compounds is crucial. WHO establishes recommendations for herbal formulation in order to standardise it.[9]

2. Materials and Methods

2.1. Material

Panchanimb Churna was collected from pharmacy department of RARI, Gwalior, Madhya Pradesh. To make it easier for the study, the materials were kept at ambient temperature (25 ± 2) ° C.

2.2. Reagents and standards

All of the chemicals and solvents used were analytical quality or HPLC grade and were from the scientific businesses E-Merck, Ranchem, and Fisher. Ellagic Acid, Epicatechin, Gallic Acid, Quercetine reference standards were procured from Tokyo chemical industry with 98% purity.

2.3. Methods

2.3.1. Organoleptic evaluation

Organoleptic evaluation of churna was performed for color; odor and taste were performed according to Indian Pharmacopoeia methods and WHO guidelines.[10,11]

2.3.2. Phytochemical and physicochemical analysis

Qualitative analysis of the phytochemicals such as Alkaloids, Coumarins, Flavonoids, Glycosides, Phenols, Proteins, Saponins, Steroids, Sugar, and Tannins was carried out as per standard methods. The standardization and quality measures are followed as mentioned in Indian Pharmacopoeia methods and WHO guidelines.[10,11] Physico-chemical parameters such as pH, total ash, acid-insoluble ash, loss upon drying at 105° C, and others are evaluated. According to the Indian Ayurvedic Pharmacopeia, extractives that are soluble in alcohol and water were conducted.[12]

3. Antioxidant Assay

3.1. Total phenolic content

Total phenolic content (TPC) was quantified using the Folin-Ciocalteu technique[13,14] with a few minor adjustments. The extract's dark blue colour (k = 765 nm) changes as a result of the FCR reagent's oxidation of the phenols, and the UV-visible spectrophotometer tracks this change.

Table 1: Results of organoleptic evaluation

Organoleptic Evaluation S. No.	Parameter Name	Result
1	Color	Greenish Brown
2	Odor	Pleasant
3	Taste	Bitter, Pungent

Table 2: Phytochemical analysis in ethanol and water extracts of Panchanimb Churna

S. No .	Phytochemical test	Name of the test	present (+) absent (-)	
			Ethanol extractive	Water extractive
1	Test for carbohydrate	Molish test	+ve	+ve
		Benedict test	+ve	+ve
2	Test for glycoside	Sodium hydroxide reagent	+ve	+ve
3	Test for protein and amino acid	Biuret test	+ve	+ve
		Ninhydrin test	+ve	+ve
4	Test for alkaloids	Mayer's reagent	-ve	-ve
		Hager's test	-ve	-ve
		Wagner test	-ve	-ve
5	Test for terpenoids	Salkowaski reagent test	-ve	-ve
6	Test for flavonoids	Ferric chloride reagent test	+ve	+ve
7	Test for saponin	Froth test	+ve	+ve
8	Test for tannins	Alcoholic ferric chloride test	+ve	+ve
9	Test for phenolic compound	Aqueous $fecl_3$ test	+ve	+ve
10	Test for steroids	H_2S0_4 and chloroform test	-ve	-ve

Table 3: Results of physicochemical parameters

Physicochemical Experiment S. No.	Parameter Name	Result
1	Water extractive	22.031
2	Ethanol extractive	21.485
3	LOD	3.470
4	Total ASH	10.135
5	Acid insoluble	5.201
6	pH	4.68 @ 22.9 ^{o}C

Table 4: Results of calibration curve of gallic acid for TPC

S. No.	Standard Solution for calibration curve	Concentration (μl)	Absorbance @ 765 nm
1	20 μg/μl GA solution (B) + 3 ml water+ 1 ml FRC A + 3 ml Na2CO3 (7 %)+ water=10 ml.	200	0.23
2	40 μg/μl GA solution (B) + 3 ml water+ 1 ml FRC A + 3 ml Na2CO3 (7 %)+ water=10 ml.	400	0.44
3	60 μg/μl GA solution (B) + 3 ml water+ 1 ml FRC A + 3 ml Na2CO3 (7 %)+ water=10 ml.	600	0.64
4	80 μg/μl GA solution (B) + 3 ml water+ 1 ml FRC A + 3 ml Na2CO3 (7 %)+ water=10 ml.	800	0.78
5	100 μg/μl GA solution (B) + 3 ml water+ 1 ml FRC A + 3 ml Na2CO3 (7 %)+ water=10 ml.	1000	0.94

Table 5: Results of TPC in terms of gallic acid equivalents of panchanimb churna

S. No.	Name of formulation	Test Solution for Estimation of phenolics	Abs. @ 765 nm	X = Y- 0.078 /0.0009	GAE (mg/g)
1	Panchanimb Churna	1 ml of MEF B solution + 3 ml water+ 1 ml FRC A + 3 ml Na2CO3 (7 %) + water=10 ml.	0.678	840	105

Table 6: Results of calibration curve of quercetin for TFC

S. No.	Standard Solution for calibration curve	Concentration (μl)	Absorbance @ 510 nm
1	20 μg/μl of quercetin solution (B) + 4 ml water+ 0.3 ml 5% NaNO3 +wait for 5 minutes+ 0 .3 ml of 10% AlCl3 + wait for 5 minutes + 2 ml of 1M NaOH + water=10 ml.	200	0.4
2	40 μg/μl of quercetin solution (B) + 4 ml water+ 0.3 ml 5% NaNO3 +wait for 5 minutes+ 0 .3 ml of 10% AlCl3 + wait for 5 minutes + 2 ml of 1M NaOH + water=10 ml.	400	0.55
3	60 μg/μl of quercetin solution (B) + 4 ml water+ 0.3 ml 5% NaNO3 +wait for 5 minutes+ 0 .3 ml of 10% AlCl3 + wait for 5 minutes + 2 ml of 1M NaOH + water=10 ml.	600	0.69
4	80 μg/μl of quercetin solution (B) + 4 ml water+ 0.3 ml 5% NaNO3 +wait for 5 minutes+ 0 .3 ml of 10% AlCl3 + wait for 5 minutes + 2 ml of 1M NaOH + water=10 ml.	800	0.83
5	100 μg/μl of quercetin solution (B) + 4 ml water+ 0.3 ml 5% NaNO3 +wait for 5 minutes+ 0 .3 ml of 10% AlCl3 + wait for 5 minutes + 2 ml of 1M NaOH + water=10 ml.	1000	0.99

Table 7: Results of total flavonoid contents in terms of quercetin equivalents

S. No.	Name of formulation	Test Solution for Estimation of Flavonoids	Abs. @ 510 nm	X = Y+ 0.254/0.0007	QE (mg/g)
1	Panchanimb Churna	1 ml of MEF B solution + 3 ml water+ 1 ml FRC A + 3 ml Na2CO3 (7 %)+ water=10 ml.	0.22	350.5714	43.8214

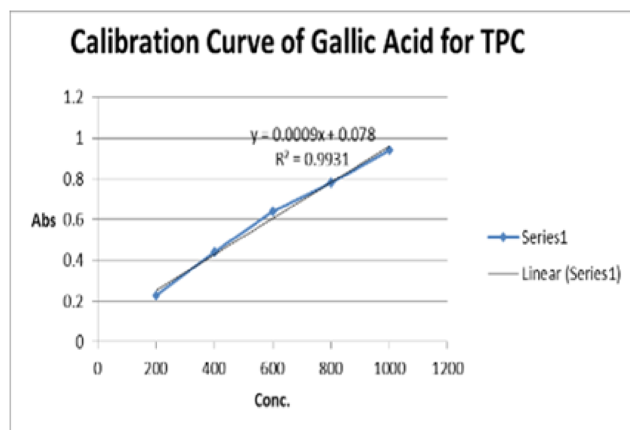

Fig. 1: Calibration curve of gallic acid for TPC

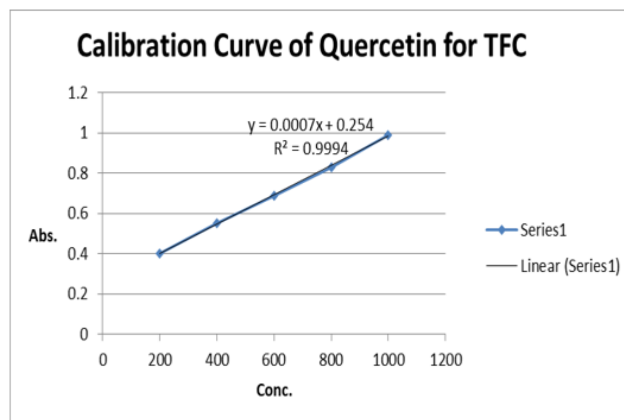

Fig. 2: Calibration curve of gallic acid for TFC

In a nutshell, 1 ml of aliquot (1 mg/ml) was combined with 5 ml of 10% (FC reagent in distilled water) Folin Ciocalteu reagent. After properly combining the solutions in the test tube, 4 ml of Na2CO3 was added (1M). Once more combined, the solutions were kept at room temperature for 15 minutes. Using the calibration curve, the phenolic content was calculated as Gallic acid equivalents (g/ml). Gallic acid solutions ranging from 20 g/ml to 100 g/ml (standard phenolic) were used to create the standard curve.

4. Total Flavonoids Content

By using the aluminium chloride colorimetric method as reported by Hossain et al., the total flavonoids contents of Panchanimb Churna were ascertained using the methanolic

Table 8: Rf values of extracts of Panchanimb Churna

Wave length	Track 1		Track 2		Track 3		Track 4		Track 5		Track 6	
	Rf	Color	Rf	Color	R_f	Color	Rf	Color	Rf	Color	R_f	Color
	0.23	Green										
	0.34	Green							0.34	Dark Green		
UV 254 nm												
	0.48	Green					0.48	Dark green				
	0.6	Green										
					0.7	Green					0.7	Green
	0.75	Green										
Cont.. Rf values of extracts of panchanimb churna												
Wave length	**Track 1**		**Track 2**		**Track 3**		**Track 4**		**Track 5**		**Track 6**	
UV 366 nm	Rf	Color	Rf	Color	R_f	Color	Rf	Color	Rf	Color	R_f	Color
	0.48	Blue					0.48	Blue				
	0.51	Red										
	0.58	White										
	0.71	Blue										
	0.77	Red										
	0.81	Red										
Cont.. Rf values of Extracts of Panchanimb Churna												
Wave length	**Track 1**		**Track 2**		**Track 3**		**Track 4**		**Track 5**		**Track 6**	
Derivatized with 5% Anisaldehyde Sulphuric acid At R White	Rf	Color	Rf	Color	R_f	Color	Rf	Color	Rf	Color	R_f	Color
	0.3	Dark green									0.3	Dark green
	0.34	Pale yellow							0.34	Pale yellow		
							0.48	yellow				
	0.59	Blue	0.59	blue								
	0.7	Light blue			0.7	Light blue						
	0.75	Dark blue									0.7	Green
	0.88	Dark blue										

extracts[15]. [To create a solution of 5% sodium nitrate using this approach, sodium nitrate (2.5 g) was taken in a volumetric flash (50 mL) and water was added. Another volumetric flash (50 mL) of sodium hydroxide (2.5 g) was collected, and water was added until the target concentration of 4% sodium hydroxide. Then, using the same method, 10% aluminium chloride solution was created. The extracts (0.25 mg) were then added to a test tube together with 1.25 mL of water and 0.75 mL of sodium nitrate. The test tubes were then left in the dark for 6 minutes. The reaction was then completed by adding 0.150 mL of 10% aluminium chlorides and allowing it to run for 5 minutes in total darkness. The test tube was then filled with the 5% sodium hydroxide solution (0.5 mL) and water (0.275 mL). The samples' absorbance was determined at a constant wavelength of 510 nm. For the calibration curve in this method, quercetin solutions at concentrations of 20, 40, 60, 80, and 100 g/ml were prepared as the quercetin standard. Using a linear equation based on the calibration curve, the estimation of the total flavonoid content in the extracts was

Table 9: Effect of *Panchanimb Churna* on DPPH radical scavenging

Sample Concentration (μg/ml)		% DPPH Inhibition	IC50 Value
Panchnimb Churna (PNC)	100	2.41±0.772	
	200	13.34±0.686	
	400	25.73±0.915	662.5 μg/ml
	600	47.32±0.858	
	800	65.17±1.101	
	1000	75.07±0.386	
BHT	10	13.34±0.686	
	20	25.73±0.915	
	40	47.32±0.858	50.173 μg/ml
	60	65.17±1.101	
	80	75.07±0.386	
	100	80.27±0.444	

Table 10: Effect of sample (Panchnimb churn) on protein denaturation inhibition

Sample Concentration (μg/ml)		% protein denaturation inhibition	IC50 Value
PNC	50 μg/ml	42.86±0.989	95.49 μg/ml
	100 μg/ml	58.78±1.832	
	200 μg/ml	69.76±3.595	
	400 μg/ml	77.26±3.189	
	800 μg/ml	88.53±4.682	
	1000 μg/ml	97.66±0.427	
Diclofenac sodium	50 μg/ml	36.09±2.850	237.104 μg/ml
	100 μg/ml	40.79±2.805	
	200 μg/ml	47.98±1.424	
	400 μg/ml	61.46±2.768	
	800 μg/ml	91.96±1.574	
	1000 μg/ml	95.18±0.346	

Table 11: Effect of sample (SAMPLE) on anti-proteinase activity

Sample Name Concentration ((μg/ml))		% anti-proteinase activity	IC50 Value
PNC	100	29.61±0.462	
	200	31.40±0.346	
	400	38.75±0.541	593.6 μg/ml
	600	46.86±0.139	
	800	58.52±0.749	
	1000	71.47±1.930	
Aspirin	100	20.99±0.282	
	200	23.40±0.380	
	300	47.25±0.454	330.206 μg/ml
	400	60.72±0.118	
	500	75.53±0.216	

done in triplicate. The average result of the total flavonoid content was represented as quercetin equivalent per gramme weight of sample.

5. Chromatography Procedure

TLC was performed using an aluminium pre-coated plate with silica gel 60 GF254 (10x10 cm2; 0.2 mm thick) with a mobile phase of toluene, ethyl acetate, and formic acid (5:3.5:1) V/V. Using a Linomat 5 applicator, alcohol extracts of the samples and markers, epicatechin, gallic acid, quercetin, and a ellagic acid standard solution were applied to the plate. For the development of the TLC plate, a Camag Twin Trough Glass Chamber (10x10 cm2) with an SS lid was utilised. For 20 minutes, mobile phase was saturated in the Twin Trough Glass Chamber. Up to 8 cm above the location of the sample application, a TLC plate was created. After being taken out of the chamber, the plates were left to air dry at room temperature. The HPTLC finger print profile was captured by the Camag visualizer

UV 254 nm	UV 366 nm	Derivatized with Anisaldehyde Sulphuric acid
1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6
Track 1: Panchanimb Churna; **Track 2:** Nimbine; **Track 3**: B-Sitosterol; **Track 4:** Quercetin; **Track 5**: Gallic Acid & **Track 6:** Azadirachtin.		

Fig. 3: HPTLC fingerprint profiling of panchanimb churna

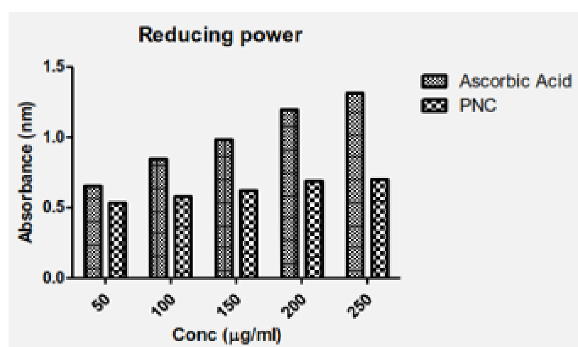

Fig. 4: Effect of sample on reducing potential

II before derivatization under UV 254 nm, 366 nm, and after derivatization at white light. This plate was sprayed (derivatized) with Anisaldehyde Sulphuric acid reagent, followed by heating at 105 ^{o}C for 5 minutes.

6. Preparation of Soxhlet extract

Utilizing a methanolic extract of the mixture that was made following a conventional methodology, antioxidant, anti-inflammatory, and anti-microbial activity was tested. Briefly 10 gm of dry formulation (sample) was kept in Soxhlet assembly with 150 ml methanol and after 2 to 3 days all the nutrients in the extract or sample pocket become colorless. The extract was collected in beaker and the moisture content was dried on water bath at 60 to 70 ^{o}C. After being weighed, the collected extract was sealed in a container and chilled to 4 °C for analysis.

7. In Vitro Antioxidant Activity

7.1. DPPH radical scavenging assay

Using a slightly modified version of the approach previously described (Brand-Williams et al., 1995)[16]. The DPPH radical scavenging activity of Sample Drug was assessed. In a nutshell, 0.5 ml of the sample drug (100-500 g/ml, distilled water as solvent) or reference antioxidant (BHT, 10-100 g/ml) was added to 3.5 ml of DPPH solution (0.1 mM DPPH in methanol), carefully mixed, and allowed to stand at room temperature for 30 min. In a spectrophotometer, the absorbance of the resulting mixture was measured at 517 nm (UV 1800, shimadzu, japan). The experiment was carried out three times.

The percent DPPH radical scavenging was calculated as - % scavenged DPPH= Ac-At/Ac*100

Where Ac = is the absorbance of the control and At = is the absorbance, when the sample drug or BHT is present.

8. Reducing Power Assay

The procedure previously described was used to determine the sample drug's reducing power (Oyaizu, 1986)[17] Equal volumes of varied sample drug/ascorbic acid concentrations (50–250 ug/ml in distilled water) were combined with 1.5 ml of phosphate buffer (0.2 M, pH 6.6) and 1.5 ml of potassium ferricyanide (1%), resulting in a final mixture of 0.5 ML. For 20 minutes, the mixture was incubated at 50 OC. After adding 1.5ml of trichloroacetic acid (10% in distilled water), the mixture was centrifuged at 3000 rpm for 10 minutes. 1.5ml of the solution's top layer were diluted with distilled water (1.5ml). Finally, 300 l of fecl3 (0.1%) was added. The mixture was centrifuged again for 5 minutes at 3000 rpm, and the absorbance of the resulting solution was determined at 700 nm. The increase in absorbance indicates increase in reducing potential. The experiment was performed in triplicate.

9. In-Vitro Anti-Inflammatory Activity

9.1. Protein denaturation inhibitory activity

The procedure outlined by Chandra et al. (2012)[18] was followed in order to measure the protein denaturation inhibitory activity. In a test tube, 2.0 ml of SAMPLE DRUG (20-100 g/ml) or diclofenac sodium (31.25, 62.5, 125, 250, 500, 1000 g/ml) in PBS were added, along with 0.2 ml of egg albumin (diluted with twice as much purified water). The control had PBS. The mixture was heated for 5 minutes at 70° C after 15 minutes of incubation at 37+2 ° C. After cooling, the mixture underwent a 10-minute centrifugation at 3000 rpm. In a spectrophotometer (UV 1800, Shimadzu, Japan), the absorbance of supernatant was measured at 660 nm against a blank. The following formula was used to estimate the % inhibition. By graphing the percentage of inhibition with respect to the control versus

the treatment concentration, the concentration that inhibits albumin denaturation by 50% (IC50) was discovered.

9.2. Anti-proteinase activity

The anti-proteinase activity was carried out using a modified version of Oyedepo et al(1995).'s[19] approach. 1.0 ml of 20 mM Tris HCl buffer (pH 7.4), 0.06 mg of trypsin, and 1 ml of the sample drug/standard solution produced in distilled water make up the reaction mixture (2.0 ml). After 5 minutes of incubation at 37 ° C, 1 ml of 1.0% (w/v) albumin was added to the mixture. An extra 20 minutes were spent incubating the combination. To stop the reaction, 0.1 ml of 70% perchloric acid was added. The absorbance of the supernatant was measured at 210 nm against a buffer as a blank after centrifuging the cloudy suspension at 3000 rpm for 10 min. Aspirin was the go-to medication, with concentrations ranging from 100 to 500 g/ml. The experiment was carried out three times. The following formula was used to determine the percent inhibition of proteinase inhibitory activity.

% Inhibition= Ac-At/ Ac x100

Where, Ac = absorbance of the control, At = absorbance in the presence of Sample Drug/ standard.

10. Results

10.1. Organoleptic evaluation

The results of organoleptic evaluation are tabulated in Table 1.

10.2. Phytochemical screening test

The results of qualitative phytochemicals screening revealed that all phytochemicals are present except terpenoids and steroids, which is tabulated in Table 2.

11. Physicochemical Analysis

The physicochemical analysis was done to ascertain the quality of the raw material used in the study. The physicochemical Parameters are pH value, water-soluble extractive, and alcohol soluble extractive values, Total ash content, Acid-insoluble ash and Loss on drying at 105° C. Obtained results are highlighted in Table 3.

12. Antioxidant Assay

12.1. Total phenolic content (TPC)

Total phenolics content of Panchanimb Churna in terms of gallic acid equivalent was 39.0277 mg/g. which was calculated from the equation of linearity X = Y-0.078/0.0009, drawn by calibration curve by taking gallic acid as standard at different concentrations. Results of TPC are highlighted in Table 4 and Table 5. While calibration of gallic acid is given in Figure 1.

12.2. Total flavonoid content (TFC)

Total Flavonoids content of Panchanimb Churna in terms of quercetine equivalent was 44.714 mg/g. which was calculated from the equation of linearity X = Y-0.078/0.0009, drawn by calibration curve by taking quercetine as standard at different concentrations. Results of TFC are highlighted in Tables 6 and 7. While calibration of gallic acid is given in Figure 2.

12.3. HPTLC chromatography

The sample application of 10 ul was done while 5 ul of reference standards were taken. The TLC plate was developed in toluene: Ethyl Acetate: formic acid (5:3.5:1) V/V mobile phase for Panchanimb Churna and markers (Panchanimb Churna, Nimbin B-sitosterol, quercetin, gallic acid and Azadirachtin). The TLC plate was visualized in UV at 254 and 366 nm for different Rf values and at R white in white light after derivatizing the plate with Anisaldehyde Sulphuric acid reagent at 105 ºC for 5 minutes. HPTLC fingerprint profiling of Panchanimb Churna with reference standards is given in Figure 3 and Rf values are given in Table 8.

13. In-vitro Antioxidant activity

13.1. DPPH scavenging assay

The results are explained in Table 9.

Values are mean± SEM; n=3; IC_{50}= 50% Inhibitory concentration

13.2. Reducing power assay

By increasing the green colour absorbance measured at 700 nm, Panchanimb Churna in the concentration range of 50-250 g/ml demonstrated concentration-related reduction of ferricyanide to ferrocyanide. Ascorbic acid, a common antioxidant, was also found to have a similar impact when used at concentrations between 50 and 250 g/ml. Figure 4 shows a concentration versus absorbance graph comparing ascorbic acid with Panchanimb Churna. [Results are expressed as Mean±SEM; n=3]

14. In-vitro Anti-Inflammatory Activity

14.1. Protein denaturation inhibitory activity

Egg albumin denaturation was inhibited by Panchanimb Churna at various doses (50–1000 g/ml) and diclofenac sodium (standard) at concentrations between (50–1000 g/ml), as shown by a concentration-dependent drop in solution absorbance. For Panchanimb Churna and diclofenac sodium, the linear regression study of

concentration versus percent egg albumin denaturation showed r2= 0.9162 and 0.9787, respectively. From a linear regression analysis, the IC50 values for Panchanimb Churna and diclofenac sodium were 95.49 g/ml and 237.104 g/ml, respectively. Table 10 provides explanations of the findings. (Values are mean± SEM; n=3; IC_{50}= 50% Inhibitory concentration).

14.2. Anti-proteinase activity

Aspirin (100-500 g/ml) and panchanimb churna (100-1000 g/ml) significantly inhibited proteinase activity against albumin. The IC50 values for aspirin and Panchanimb Churna were 593.6 g/ml and 330.206 g/ml, respectively, as determined by linear regression analysis. Table 11 presents the findings.

15. Conclusions

In the current study, standardization, HPTLC in accordance with API, and other literature reports were used to evaluate the quality of the herbal product Panchanimb Churna. Additional in-vitro research was done to examine the biological activities, including the total phenolic content, total flavonoid content, antioxidant, anti-inflammatory, and antimicrobial potential. Panchanimb Churna was found to meet the criteria for the investigated parameters under specific experimental conditions and it also showed promising therapeutic qualities. Recent research is strengthening belief in the ability of herbal remedies with natural origins to combat infectious diseases and other illnesses. Since there are several potential presented by this investigation—quantification of plant phytochemicals, ensuring the legitimacy of herbal products. It is obvious that the effort is justified if herbal items are allowed to undergo standard quality control examination so there is an increasing demand with passing time.

16. Declarations Ethics Approval and Consent to Participate

No any animal or human were used during this experimental study.

17. Source of Funding

None.

18. Conflict of Interest

The author declares no potential conflict of interest.

Acknowledgments

All authors have read and approved the final manuscript.

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Cite this article: Meena AK, Rajput S, Chaturvedi S, Parashar D, Sharma L. Standardization & evaluation of anti-oxidant, anti-inflammatory and antimicrobial potential of *Panchnimba Churna*. *Int J Pharm Chem Anal* 2022;9(4):174-183.