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"Evaluation of Quality Standards of Poly-Herbal Ayurvedic Compound (*Kalingadi Churna*) and Its Anti-Oxidant Activity"

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KEYWORDS

Kalingadi Churna, IBS and ayurveda ,anti-oxidant and ayurveda ,*Grahani roga* .

ABSTRACT:

Introduction: The science of *Ayurveda* withholds innumerable formulations that are single drug, poly herbal and Herbo-mineral compounds. Only a few of these have been researched upon and are explored fully to their potential. One such formulation is *Kalingadi churna* which is said to have its effect in the management of *Grahani roga*, which can nearly be understood in terms of IBS. It is composed of the drugs *Kalinga* (Holarrhena antydysentrica), *Ativisha* (Aconitum heterophyllum), *Hingu* (Ferula narthex), *Vacha* (Acorus calamus), *Haritaki* (Terminalia chebula) and Saindhava lavana (Unaqua sodium chloride).

Material and Methods: The current study involved the preparation of a polyherbal *Churna* and the assessment of its constituents for macroscopic characters, physicochemical parameters, thin layer chromatography (TLC), and also conducting in vitro antioxidant activity. The antioxidant activity was examined using in vitro antioxidant models through DPPH free radical scavenging, Iron Chelating activity, and nitric oxide scavenging activity.

Result and Discussion: Based on the findings of this study, the formulation demonstrated a notable antioxidant capacity, which may be attributed to the presence of certain phytochemicals in the formulation. It also indicated that the polyherbal *churna* can serve as a readily available source of natural antioxidants and thereby helping in understanding its probable mode of action and thus making it a potential candidate for usage as a drug in various ailments of the gastrointestinal tract.

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1. Introduction

Ayurveda a traditional system of medicine which has its roots in India, provides numerous herbal compositions that may play a pivotal role in contemporary healthcare for the treatment of various illnesses that are challenging to address. *Ayurveda* has gained worldwide recognition in recent times because of its comprehensive approach to disease treatment and the limited occurrence of adverse medication reactions. One of the dosage forms practiced in Ayurveda is *churna* (powder). *Churna*[1], the dried, finely powdered form of a medication is obtained by pounding it in a stone mortar known as *Khalwa Yantra* and then sieving it through linen.

Grahani which can nearly be understood as duodenum which is said to be the seat of the digestive fire. Disease that manifests in this particular part or alters the physiology of it is known *grahani* roga. The symptoms of grahani roga have some similarity with that of irritable bowel syndrome. Irritable bowel syndrome (IBS) is a chronic and relapsing functional gastrointestinal disorder that affects 9-23% of the population across the world[2] . Abnormal gut-brain interaction, an imbalance of the gut microbiome, and prolonged stress are the causative factors of irritable bowel syndrome.

IBS commonly presents with stomach discomfort or pain, as well as changes in bowel movements such as constipation, diarrhoea, or a combination of both[3]. Additional problems expressed by persons with IBS comprise bloating and distention. *Kalingadi churna*[4] is one of the formulation which is indicated in the treatment of *Grahani* (IBS). It contains six drugs ' *Kalinga* (Holarrhena antydysentrica), *Ativisha* (Aconitum heterophyllum), *Hingu* (Ferula narthex), *Vacha* (Acorus calamus), *Haritaki* (Terminalia chebula) and *Saindhava lavana* (Unaqua sodium chloride). These drugs possess the properties of antispasmodic, antidepressant, increase intestinal motility and carminative activity.

Oxidative stress plays a significant role as a potential component in the development of various clinical diseases, the process is said to be initiated by extremely reactive free radicals that form stable pairs involving biomolecules. The human body possesses an inherent antioxidant enzyme defence system to counteract free radicals, antioxidants prevent or minimize damage caused by free radicals through their ability to reduce or scavenge these harmful molecules, antioxidants that are natural serve a crucial role in avoiding oxidative damage in disorders where oxidative stress has a key factor [5]. The current polyherbal formulation contains components that possess antioxidant action [6].

There is no available literature or studies that have assessed all the quality control parameters and antioxidant effect of the poly-herbal compound (*kalingadi churna*) as a whole. Hence, the present study was taken up to document its physicochemical characters, phytoconstituents and antioxidant activity.

2. Materials & methods

All the drugs were procured from KLE Ayurveda Pharmacy in Khasbhag, Belagavi, Karnataka, India. These drugs were identified and authenticated at The Central Research Facility of KAHER's Shri BMK Ayurveda Mahavidyalaya, Shahapur, Belagavi, Karnataka, India. The collected drugs are dried and then mixed in equal proportion, following the instructions mentioned in classical texts. Then it is reduced to powder in a mechanical grinder, sieved through 80 number mesh, and sealed in airtight packets at KLE Ayurveda Pharmacy, Khasbhag, Belagavi, Karnataka, India. Thus, obtained *churna*(powder) was analysed for various analytical parameters such as organoleptic, physicochemical, phytochemical & microbial limits in AYUSH approved ASU drug testing Laboratory, Central Research Facility of KAHER's Shri BMK Ayurveda

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Mahavidyalaya, Shahapur, Belagavi, Karnataka, India. CRF No: CRF/FG/95/2023-24).

Organoleptic Character

Methods:

Colour and form were determined by examining the untreated sample under day light. Odour was determined by slowly keeping sample on the hand then slowly smelling it. Taste was determined by chewing a pinch of polyherbal *churna.*

Physio-chemical Parameters [7]

Alcohol soluble extract

5 gm of polyherbal powder was taken with 100 ml of ethanol and closed in flask for 24 hours. Solution is shaken for first 6 hours in flask shaker and was kept stable for next 18 hours, 25 ml of filtrate was evaporated to dryness in tared flattened dish by boiling in a water bath and percentage of alcohol extractive was calculated.

Water soluble extract

3gm of polyherbal powder and 100 ml of chloroform water was taken in conical flask. It was continuously shaken for 6 hours and allowed to stand for 18 hours. After 24 hours extract was filtered by filter paper, 25 ml of filtrate was taken and transferred to dried and weighed evaporating dish. Solution was evaporated till we get constant weight. Later extractive value was calculated.

Acid Insoluble Ash

Ash was prepared of polyherbal powder by placing it in the muffle furnace, 25 ml of 2ml dilute HCL was added to ash mixture. Mixture was heated in water bath and was filtered through ashless filter paper, followed by washing of filter paper by hot boiling water. Ashless filter paper was collected in a crucible and weighted. Later it was incinerated in muffle furnace till ash was obtained. Then it was cooled in desiccator and weight on machine and percentage was calculated.

Total Ash

Polyherbal powder and crucible weight was obtained on Digital weighing machine. Powder was incinerated at 450 c in muffle furnace for 30 minutes, after 30 minutes carbon free ash was obtained. Later it was transferred to desiccator at cooled at room temperature and then percentage of total ash was calculated.

Loss of Drying

Polyherbal powder was taken in watch glass and weighed. It was kept in hot air oven for 45 minutes at 110 c temperature, after 45 minutes again weight was taken and checked until a constant weight is obtained after intervals. It is marked as W2 and then percentage of loss on drying was calculated.

Thin layer chromatography

Alcoholic extract of polyherbal powder was taken; by using capillary tube sample was touched gently on TLC plate, TLC Plate was dried and fresh TLC solvent of Toluene: Ethyl Acetate was prepared in the flask. T LC plate was placed in the flask and was closed for some time, after developing of spots the plates were taken and dried. Then the spots were observed for under UV light [8].

Phytochemical tests [9]

Tests for carbohydrates (Molisch test)

5 gm of PHP was taken in 100 ml of water mixed and filtered.

2-3 ml of aqueous extract extract and few drop of alpha-napthtol was added in alcohol, to it concentrated H2SO4 was added from side of tube. Appearance of violet ring at the bottom indicates the presence of carbohydrates.

Test for monosaccharides (Barfoed test)

Barfoed solution and test solution were taken in equal amount. They were heated for 1-2 minutes and

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appearance of red precipitation indicates presence of monosaccharide.

Test for protein (millons test)

3 ml of test solution and 5 ml of millons reagent were mixed. White precipitation indicates a positive test

Test for tannin and phenolic compounds

In 2-3 ml of alcholic extract few drops of $FeCl_2$ were added, deep blue - black color if seen indicates a positive test.

Test for hexose sugar (selwinoffs test)

3 ml of selwinoffs solution and 1 ml of test solution were heated in water bath for 1-2 minutes. Red color if seen indicates a positive test.

Tests for Reducing Sugars

Equal amount of test solution and benedict solution are heated in water bath for 5 minutes, red color if seen indicates a positive test.

Tests for Glycosides (Saponin)

Foam test

Drug extract was shaken vigorously with water and a persistent foam if observed indicates a positive test.

Cardiac Glycosides (Keller-Killiani test)

2 ml of extract was taken in which glacial acetic acid, 1 drop of 5% of FeCl3 and conc. H2SO4 were added if a reddish brown color appears test is said to be positive.

Antimicrobial Activity

Microbial Analysis

Microbial analysis was carried out as per standard procedure mentioned in Ayurvedic Pharmacopoeia of India [10-11] by conducting minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC). It included total bacterial count, total fungal count, and presence of pathogens like *Escherichia coli*, *Salmonella ebony*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

Antioxidant activities of the *Kalingadi churna*

DPPH free radical scavenging assay

The radical-scavenging processes in each sample were examined utilising a stable DPPH radical, as outlined by Blois (1958) with some modifications (Sudarshan et al., 2019). Various concentrations (100 to 300 µg/mL) of each sample were combined with 2 mL of DPPH (100 µM), adjusted to a total volume of 3 mL with methanol, and the reaction solution was allowed to stand in dark for 45 minutes at ambient temperature. At the end of of the time of incubation, the absorbance was measured using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 517 nm compared to the blank (without sample/standard). The free radical scavenging effectiveness of each sample was quantified and represented as IC50 values relative to BHT, which served as the standard antioxidant [12] .

Ferrous-Ion Chelating Assay

The ferrous-ion chelating capacity of the specimens was assessed following the methodology of Danis et al. (1994), with modifications by Lakshmegowda et al. (2020). In summary, varying concentrations (0 to 300 µg) of suitably diluted samples were individually combined with 0.05 mL of 2 mM FeCl2. The reaction that followed was initiated by the addition of 0.1 mL of 5 mM Ferro-zine and subsequently incubated for 10 minutes at ambient temperature. The absorbance of the generated colour was determined using a spectrophotometer at 562 nm compared to the blank (without sample/standard). The percentage suppression of Ferro zineFe2+ compound formation was determined, and the data were shown as IC50 values

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relative to EDTA, which served as a standard antioxidant [13] .

Nitric oxide scavenging activity

In this experiment, the material sodium nitro (10 mM) in phosphate buffered saline (PBS, pH 7.4) was incorporated with varied quantities of samples and standards, and the mixture was then incubated at room temperature for a period of one hundred fifty minutes. The control substance was a similar reaction combination as the sample and the standard, but it did not include either of those components. Subsequent to the period of incubation, 0.5 mL of Griess reagent [1% sulfanilamide, 2% H3PO4, and 0.1% N-(1-naphthyl)ethylenediamine HCl] was included, and the absorbance of the produced chromophore was evaluated at 546 nm (Sreejayan and Rao, 1997). The scavenging activity percentage of the produced nitric oxide was assessed by comparing the absorbance values of control and test preparations, with findings represented as IC50 values relative to ascorbic acid, used as a reference antioxidant [14-15] .

3. RESULTS

Analytical Observations / Organoleptic Characters

On macroscopic description, polyherbal *Churna* was found to be brown in colour, bitter in taste and has a characteristic odour. The results are depicted in table 1 below.

Table 1. Organoleptic Character of *Kalingadi churna*

SI.No.	Organoleptic Characters	Kalingadi churna
1	Form	Churna
2	Color	Light Brown
3	Odour	Characteristic
4	Taste	Bitter

Physio-chemical Parameters

As polyherbal *churna* is not in a liquid form, the 5% solution was prepared by adding 5gms of *churna* in 100ml of distilled water, after shaking the mixture well, the solution was filtered through filter paper. Thus, obtained filtrate was used for calculation of pH. The pH of the sample was 4.3 in 5% solution. (Table 2)

Loss on drying indicated the moisture content in the sample to be 7.167% which is less moisture content and within the permissible limits this would also not facilitate growth and microbial contamination. (Table 2)

Ash value indicates presence of inorganic residues in the plant sample, which valuated to be 18.921% and is in accepted limits. (Table 2)

Acid insoluble ash also known as silica test was done to detect the amount of inorganic residue in the sample. The given sample has shown the presence of 2.365% acid insoluble ash which is within the permissible limits. (Table 2)

Water soluble extractive and alcohol soluble extractive represents the loss of biomass proportion as a result of extraction and also indicates the potency of the drug. The values for polyherbal powder (*kalingad*i *churna)* were 58.932% and 5.837% respectively. (Table 2)

Table 2. Physio-chemical Parameters of *Kalingadi churna*

SI. No	Parameters	Results
1	pH	4.3
2	Loss of Drying	7.17%
3	Total Ash	18.92%

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4	Acid insoluble Ash	2.37%
5	Water soluble Extractive	58.93%
6	Alcohol Soluble Extractive	5.83%

Phytochemical Screening

The sample was screened for phytochemicals in water & alcohol media. Carbohydrates, monosaccharaides, reducing sugar , flavonoids & tannis are present in both water & alcohol ,

whereas cardiac glycosides and saponin glycosides were present in water media. Penstose ,hexose,proteins,amino acids, alkaloids were present in alcohol media. Results are depicted in table 3 and table 4.

Table 3. Preliminary Phytochemical Screening

SI. No	Tests	Water	Alcohol
1	Tests for carbohydrates	Positive	Positive
2	Tests for Reducing Sugars	Positive	Positive
3	Tests for monosaccharides	Positive	Positive
4	Tests for pentose sugar	Negative	Positive
6	Tests for hexose sugars	Negative	Positive
7	Tests for proteins	Negative	Positive
8	Tests for amino acids	Negative	Positive
9	Tests for flavonoids	Positive	Positive
10	Tests for alkaloids	Negative	Positive
11	Tests for tannins	Positive	Positive

Table 4. Tests for Glycosides

SI .No	Tests	Water	Alcohol
1	Cardiac Glycosides	Positive	Negative
2	Saponin glycosides	Positive	Negative

Tests Thin Layer Chromatography

The sample was also subjected for chromatography in which TLC was performed through alcohol extract. The Rf value was calculated based on mobile phase.

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The Rf value in Toluene : Ethyl acetate at 7:3 ratio was found to be 0.16,0.25,0.35,0.48,0.64,0.78 for short wave and 0.22,0.32,0.44,0.55,0.64,0.77,0.91 for long wave. Results in depicted table 5 and figure 1.

Table 5. Tests for Thin Layer Chromatography

TLC (Alcohol Extract)	Rf Value
Short Wave	0.16,0.25,0.35,0.48,0.64,0.78
Long wave	0.22,0.32,0.44,0.55,0.64,0.77,0.91
Mobile Phase - Toluene: Ethyl acetate (7:3)	

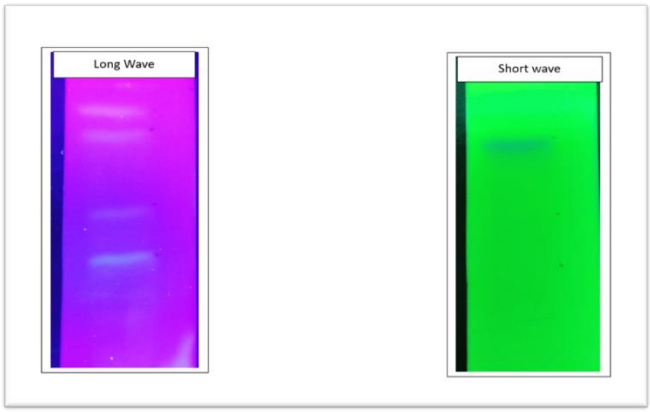

Figure 1. TLC of *Kalingadi Churna*

Anti-microbial activity

The microbiological character of the *Kalingadi Churna* appeared to be adequate, with total microbial plate count, yeast, and mould counts revealing no growth and below the API limit, and total yeast and mould also showing no development below the API limit. Furthermore, the pathogenic bacteria, namely *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. ebony*, were discovered to be missing. Results depicted in table 6 and table 7.

Table 6. Tests for Micro-organisms (Qualitative)

SI. No	Bacteria	Limits	Results
1	*E. Coli*	Absent/100	Absent
2	*S. aureus*	Absent/100	Absent
3	*P. aeruginosa*	Absent/100	Absent
4	*S. ebony*	Absent/100	Absent

Table 7. Microbial Limit Tests (Quantitative)

SI. No	Tests	Limits	Results
1	Total Bacterial count	30-300cfu/ml	No growth
2	Total Fungal count	10-100cfu/ml	No growth

Antioxidant activity

Table 8. Antioxidant properties of *Kalingadi churna*

Samples	DPPH /mL)	Ferrous (µg/mL)	NO (µg/mL)
Churna	31.304	50.045	71.194
Std.	22.835 (Vit-C)	23.431 (EDTA)	47.658 (Vit-C)

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Table 9. DPPH Assay

Con (µg/mL)	OD	% Inhibition	IC50 (µg/mL)
Blank	2.717		
Sample			
0	2.514	0	31.30462
10	2.137	14.99602	
20	1.731	31.14558	
30	1.295	48.48846	
40	0.924	63.24582	
50	0.547	78.24185	
Std. Vit-C			
10	1.737	30.90692	22.82552
20	1.331	47.05648	
30	0.995	60.42164	
40	0.624	75.179	
50	0.347	86.1973	

Figure 2: DPPH Assay

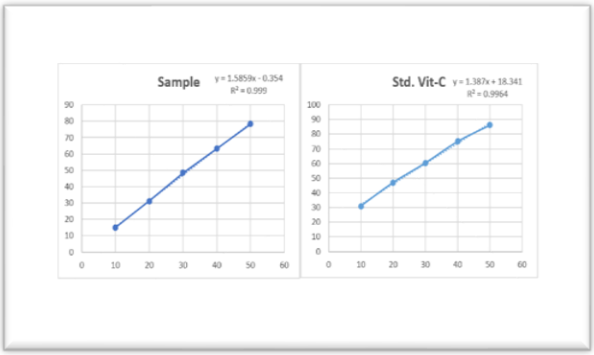

Table 10: - Iron Chelating activity

Con (µg/mL)	OD	% Inhibition	IC 50 (µg/mL)
Blank	2.624		
Std. Vit-C			
0	2.624	0	47.65821
20	1.625	38.07165	
40	1.378	47.48476	
60	1.178	55.10671	
80	0.978	62.72866	
100	0.718	72.6372	
Sample			
20	1.916	21.1847	71.19434
40	1.647	32.2501	
60	1.358	44.13821	
80	1.095	54.95681	
100	0.826	66.02221	

Figure3: Iron Chelating activity

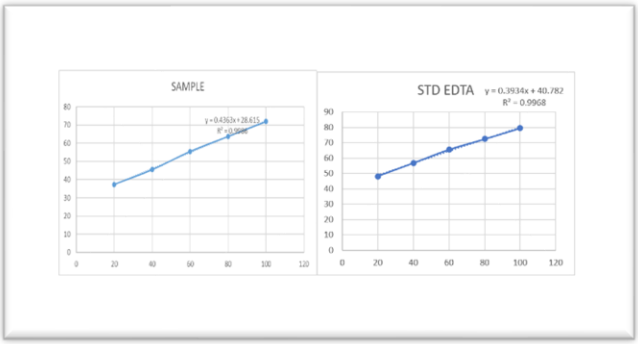

Table 11: - NO Scavenging properties

Con (µg/mL)	OD	% Inhibition	IC50 (µg/mL)
Std. EDTA			
Blank	2.354	0	23.43162
20	1.224	48.0034	
40	1.021	56.62702	
60	0.813	65.46304	
80	0.651	72.34494	
100	0.483	79.48173	
Sample			
20	1.474	37.38318	50.04584
40	1.284	45.45455	
60	1.047	55.52251	
80	0.854	63.72133	
100	0.662	71.87766	

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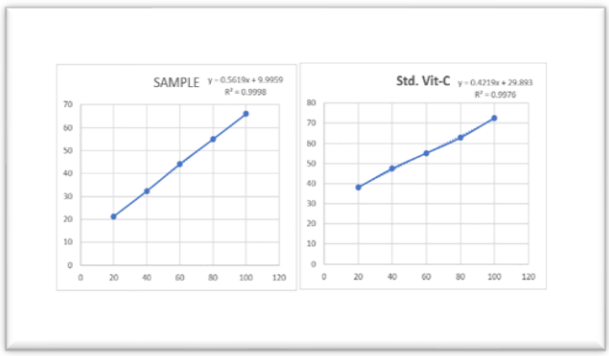
Figure 4: NO Scavenging properties



4. DISCUSSION

Polyherbal powder (*kalingadi churna*) is one of the medicines extensively mentioned in Ayurveda classics in the context of *Grahani* (irritable bowel syndrome). *Ayurveda* herbs must be devoid of extraneous substances, including other plant parts, foreign plants, Molds, insects, excrement, visible impurities such as dust and crystals, as well as toxic and hazardous foreign materials and chemical residues. Thus, analysis of the drug becomes a need of the hour to assess quality control measures in turn to prove its safety and potency for further therapeutic use and evidence-based practice. There are no standard analytical parameters mentioned for the *kalingadi churna*, here an attempt was made to analyse & create the data of macroscopic, physicochemical, phytochemical, Thin Layer chromatography & microbial limit parameters for further references. Our investigation revealed that the foreign matter quantity in the constituents of the *churna* was below the permissible limits established by the API.

Organoleptic characteristics are the characteristics of compounds as encountered by the sensations, particularly smell, vision, taste, and sensation. Variance in those characteristics provides a major indicator regarding manufacturing difference.

Developed preliminary and physicochemical criteria provide vital details for subsequent investigations and aid in recognizing of formulations. Extractive values are essential for assessing the consistency of the kind and quantity of chemical components in a medicinal product. Given the significance of these physicochemical parameters, *churna* was characterized by assessing water-soluble extractive, Alcohol-soluble extractive, total ash content, acid-insoluble ash, ph, and loss on drying. The test for the percentage of loss on drying measures both moisture and volatile substances. Total ash analyses the residual elements post-ignition. Acid insoluble ash estimates the silica content, particularly sand and siliceous materials

Phytochemicals constitute naturally occurring compounds found in plants. Regional location, period of harvesting, plant component utilized, and separation process influence the chemical content that results from crude material extracted from the plant. A comparative phytochemical study of *churna* and its constituent components in liquids of various polarity is conducted.

Presence of functional carbohydrates [16,17] helps in glucose homeostasis, oxidative metabolism, gastrointestinal function, prevention of diabetes, obesity, cardiovascular disease, metabolic syndrome, and also are proven to have anti-cancerous activity.

Presence of proteins[18,19] in the sample maintain cholesterol levels and exhibit antitumor activity, antithrombotic effects, antihypertensive effects, antimicrobial properties, antioxidant and neuro modulatory activities.

The presence of tannins [20,21] suggests anticancer, virucides, antioxidant, antimicrobial and anti-inflammatory, antidiabetic, wound healing, cardiovascular protection and antidiarrheics activity.

Presence of saponins[22] in the sample suggests that they have many medical uses including Anti-hypercholesterolemic, antifungal, antiparasitic and

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antibacterial anti-tumor, hepatoprotective, haemolytic and anti-inflammatory activities.

The sample showed the presence of flavonoids [23,24] which possess anticancer, antioxidant, anti-inflammatory, antiviral properties, neuro-protective and cardio-protective activity.

TLC helps to determine the number of components, identification of compound and the purity of a compound.

Due to their natural source, herbal medications are susceptible to invasion by microbes from soil, air, and water, which may introduce potentially dangerous organisms to humans. The existence of microbial contamination in herbal products might diminish or completely negate their therapeutic efficacy, potentially harming patients who consume these medications.
The microbiological load for *kalingadi Churna* was determined to be within the permissible limits set by API, specifically for the pathogen *E. coli. S. aureus, P. aeruginosa, and S. Abony* were shown to be not present. This confirmed its microbiological safety for human administration.

Antioxidants [25] present on the polyherbal *churna* inhibit the creation of free radicals by preventing their effects, there by safeguarding the human body against oxidative stress.

Based on the study conducted, the data is in line with the standards set by the API. However, there is a little difference in the acid insoluble ash and water-soluble extracts, which might be attributed to changes in the dosage form and environmental conditions.

5. CONCLUSION

The study analysed polyherbal powder (*kalingadi churna*) as there is limited or no data on standardized limits for the same in API. The validation and quality control criteria of polyherbal powder (*kalingadi* churna) given above adhere to established standards, indicating the purity and potency of this sample. In -vitro Antioxidant activity showed it had an anti-oxidant activity which lays a further scope to explore its biomolecules in depth and understand it's mechanism.

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