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Study on elemental analysis, pesticides, antioxidant, marker compound and validation of conversion of Ajmodadi Churna, an Ayurvedic formulation into suitable dosage form

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With the change in the present scenario of globalization, the importance of standardization of traditional formulation has been more stressed out than before. The major objective of this study was the development of an analytical method for the estimation of some important constituent, pesticides, elemental study of the formulation anti-oxidant activity and to study the effect of conversion of churna to granules of in-house and marketed samples. So, a suitable plan was designed to get comprehensive standardization of this formulation Ajmodadi churna. The paper demonstrates the feasibility of the conversion of Ajmodadi churna in granules form and estimation of piperine: cucalyptol, caryophyllene, and eugenol were done using HPTLC, these constituents have an important role in the activity. Eugenol and Eucalyptol were found more in sample A. Our results demonstrate that Piperine was found more in sample B. A study on pesticide residue was done by the TLC method. None of the samples showed the presence of pesticide in the solvent system. Elements are toxic for the body so its determination is also important for the formulation. The micromeritic properties of prepared granules were also studied. The granules showed better flow property than the powders. Antioxidant activity in different samples was observed. Sample A (in-house formulation) showed better antioxidant activity than sample B (marketed formulation). Superior results are seen from the conducted experiments. Importantly, our results provide evidence for a new approach that could also be useful for the standardization of ayurvedic formulation using modern techniques.

Keywords: Antioxidant, Ayurvedic formulation, Eugenol, HPTLC, Piperine, Pesticides

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Now the scenario has changed, new technology is used for the manufacturing of herbal medicines on a large scale in the pharmaceutical industry[1]. In past several decades Ayurveda played an important role and trusted worldwide in healthcare system which consists of various formulations such as asava, arishta, ghruta, taila, churna, and much more. A variety of medicinal plants has been used in ayurvedic system of medicine since ancient time. A model for comprehensive clinical management has been developed based on the scientific approach. Ayurvedic medicine is as old as the Vedic age[2-3]. With the changing scenario in view of globalization, the importance of standardization has been more emphasized than before. India is rich in traditional medicinal heritage such as Ayurveda, Siddha, Unani and other. Therefore, in this direction, we have chosen an ayurvedic formulation Ajmodadi churna. Ajmodadi churna is an ayurvedic medicine, which has many properties such as a carminative, antispasmodic, vermifuge, and also in many treatments of painful conditions like sciatica and stiffness. It is also used in the proper functioning of the digestive system[4]. It is widely considered to be a good way to the conversion of Ayurvedic formulation into the modern formulation using advanced technology.

Methodology

The raw materials used in the study were obtained from the local market in Ranchi and underwent authentication by the Department of Pharmaceutical Sciences & Technology, B.I.T. Mesra, Ranchi. The authentication process followed the guidelines of the ayurvedic pharmacopeia, which involved microscopic examination of the raw materials. A commercially available formulation of Ajmodadi Churna (referred to as Sample B) was purchased from the local market, while an in-house formulation (referred to as Sample

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A) was also prepared. Ajmodadi churna is composed of twelve ingredients: *Apium graveolens* L-12 g, *Embelia ribes* Burm-12 g, Rock salt-12 g, *Cedrus deodar* (Roxb) Loud-12 g, *Plumbaigo zeylanica* Linn-12 g, *Piper longum* Linn-12 g, *Foeniculum vulgare* Gaertn-12 g, *Piper nigrum* Linn-12 g, *Terminalia chebula* Retz-60 g, *Argyreia speciosa* (L.f.) sweet-120 g, and *Zingiber officinale* Roscoe-120 g. Each ingredient was finely powdered using a sieve with a fineness of 44/85. The wet granulation method was employed to prepare ajmodadi churna granules. Analysis of piperine, eucalyptol, caryophyllene, and eugenol in the ajmodadi churna formulations was conducted using standards procured from Sigma Aldrich, India. Silica gel TLC plates (60 F254, dimensions 20 cm×20 cm, with a layer thickness of 0.2 mm) from E. Merck, Darmstadt, Germany, and supplied by Anchrom Technologies, Mumbai, India, were used for the analysis.

Material and Methods

The Rankem India chemicals were procured for analysis. Ajmodadi Churna formulations as well as all the ingredients of the formulations were used for analysis.

Estimation of constituents by using HPTLC

HPTLC estimation was conducted using precoated plates specifically designed for HPTLC analysis. The setup consisted of a TLC scanner connected to a computer, equipped with software, and a Linomat IV sample applicator connected to a nitrogen tank, utilizing 100 µL syringes. Two plates, namely plate 1 and plate 2, each measuring 10 cm × 20 cm, were utilized in the analysis. Plate 1 contained tracks of six ingredients, two formulations, and four standards. The following settings were employed: A bandwidth of 6 mm, a sample distance of 8 mm, and a gas flow rate of 100 µL/s. Development of plate1 took place in a glass chamber using a solvent system comprising Toluene: Ethyl acetate: Diethylamine (in a ratio of 4.8:4.2:0.1). Plate 2 accommodated tracks of five other ingredients and two formulations, utilizing the same settings as plate 1. Development of plate 2 occurred in a glass chamber using a pre-prepared solvent system consisting of Toluene: Ethyl acetate: Formic acid (in a ratio of 5:4:1). Scanning of the plates was performed using a Camag TLC scanner with CATS software (IV 1.1.4.0. Camag). The scanner was optimized for maximum light utilization, employing a slit dimension of 4×0.30 mm, a micro scanning speed of 20 mm/s, a data resolution of 100 µm/step, and multiple wavelengths scanning in absorbance reflectance mode. The remaining measurement parameters were kept at their default settings. Regression analysis and statistical data were generated using Win CATS Planar chromatography version 1.1.4.0 software[5,6].

Method of preparation of standard and sample solution

A standard solution of piperine (0.1 µg/µL), eucalyptol (46.2 µg/µL), caryophyllene (45.1 µg/µL), and eugenol (53.3 µg/µL) were prepared in methanol. Sample solutions of Ajmodadi churna (0.5 g each) were macerated in 25 mL of methanol. It was kept for maceration for 7 days. After 7 days, it was filtered through Whatman filter paper size no. 41 and used for analysis. After the method validation, the standard and sample solutions were prepared and spotted on a 10×20 silica plate, and the content of standards was estimated in the formulations[7].

Pesticide residues were identified using Thin Layer Chromatography (TLC) in ajmodadi churna formulation. A known amount powder sample was taken and dissolved in 5 mL distilled water. In the solution, 3.75 g of NaCl, ethyl acetate (1:1), 20 mL acetone, and 12.50 mL cyclohexane were added. The mixture was homogenized in a beaker. 7.5 g of anhydrous sodium sulfate was added to the mixture, shaken vigorously for 30 min 5 mL aliquot was filtered and evaporated to dryness. Then the extract was diluted with 1 mL of ethyl acetate and used for TLC analysis. A pre-coated aluminum TLC plate (0.25 mm) was activated at 105°C for few seconds before use; detection was done under a UV lamp (254 and 365 nm). Ethyl acetate was used as a solvent system for analysis.

Pesticide solutions were spotted in the TLC plate at 1.4 cm keeping the minimum distance between the spots. The plate was eluted with ethyl acetate in the developing tank. The plate was placed in UV chamber at 254 nm for a few minutes. Black colored spots appeared on a colorless or light-grey background[8].

Evaluation of micromeritic properties of ajmodadi churna granules

Bulk Density

The sample was surpassed *via.,* No.18 sieve to interrupt up agglomerates that had been fashioned throughout storage. It turned into introduced in a dry stopper 100 mL measuring cylinder and mass of the powder was measured. The bulk density in g/cc was measured as follows[9,10].

$$Bulk\ density = \frac{Mass}{Unsettled\ Apparent\ Volume(Vo)}$$

Tapped density

The measuring cylinder was mechanically tapped by using a suitable mechanical tapped density test at a nominal rate of 300 drops each moment. The chamber was tapped multiple times at first and tapped volume Va was estimated. Tapping was rehashed 750 extra times and tapped volume Vb was estimated. The tapped thickness in g/cc was estimated as depicted in

Powder flow property

A good flow of powder or granules to be packed is important to guarantee productive blending and satisfactory weight consistency for the compacted tablets.

The angle of repose was determined by free flowing of powder from an orifice which form a conical heap on the horizontal surface. The angle the heap obtained on the horizontal surface and determined by the formula[9]:

$$\tan \emptyset = h/r$$

Where,
Ø is the angle of repose.
h is the height of the of the powder.
r is the radius of powder.
Carr's Index: Carr's index gives information about the compressibility factor during the manufacturing of the tablets[9].
Hausner's Ratio: This was calculated as the ratio of tapped density to bulk density of the samples[11,12].

Elemental analysis of ajmodadi churna

Elements were determined by utilizing ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) (Optical 2100 dv, Perkin Elmer, USA). 0.2 g of dried samples of formulations was digested with a mixture of Nitric acid and Hydrogen peroxide in a microwave absorption unit. The reasonable arrangements acquired were sifted into a 100 mL volumetric and made up the volume with Millipore water and elements present were determined using a thermal conductivity detector[13,14].

Determination of the antioxidant activity of ajmodadi churna

About 5 g of powder was taken and macerated with 100 mL of methanol for 7 days. After 7days the macerated sample was filtered. About 25 mL of the filtrate was evaporated on a water bath to get the extract with a concentration of 500 mg/mL. Then 50 mg extract was taken and dissolved in 100 mL of methanol. From this stock solution 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, and 0.5 mL of the solution were pipetted out to get the solutions with a concentration range of 50 µg/mL to 250 µg/mL[15,16].

DPPH radical scavenging method

The ability of the concentrates and a few pure substances to donate hydrogen molecules or electrons is assessed by observing the fading of a purple-colored methanol solution containing DPPH. 4 mL of different groupings of the concentrates in methanol was added to a 1 mL arrangement of DPPH methanol (last convergence of DPPH was 0.2 mM). The arrangement was shaken and afterward it was permitted to represent 30 min. After 30 min, the absorbance of was estimated at 517 nm. Restraint of free extremist DPPH in percent (%) was determined in the accompanying way:

$$\%\ Inhibition = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control} \times 100$$

Where, a clear absorbance serves as a reference for the reagents, and an example includes the EC50 value (in mg/mL) of the test compound, which represents the effective concentration at which DPPH radicals were scavenged by 50%, determined through linear regression analysis. Ascorbic acid was used as a control.

Results

Ajmodadi churna granules were prepared through the developed formula and all properties were studied. Nowadays powders are being dispensed in the newer packages like sachets.

The quantification of important constituents in both the samples A and B were also determine Such constituents are piperine, eucalyptol, caryophyllene, and eugenol in both the ajmodadi churna samples. The HPTLC procedure was optimized with a view to quantifying the abovementioned constituents in the formulations. The mobile phase, Toluene: Ethyl acetate: Diethylamine (4.8:4.2:0.1) was used that gave the Rf value 0.64 for piperine, Rf 0.59 for eucalyptol, Rf 0.81 for caryophyllene and Rf 0.30 for eugenol. Piperine was found to be more in sample B. Eugenol was found more in sample A. Caryophyllene was absent in sample A. Eucalyptol was more in sample A. The Rf value results showed the presence of phytoconstituents in the sample. The observations are shown in the Table 1 and Figure 1 & 2.

Identification of pesticide residue in Ajmodadi churna samples

Pesticides are substances employed to prevent or eradicate various forms of pests. Their primary application is in safeguarding plants against damage caused by weeds, diseases, or insects. The primary objective of the study was to assess the reproducibility of Rf values for selected pesticides using laboratory-based techniques such as elution and discovery, with a focus on ensuring and evaluating residue levels in samples. Specifically, two pesticides, namely Dichlorvos and Quinalphos, were selected to determine the presence of pesticide residues in powder tests. Ethyl acetate was employed as the solvent for elution. For meticulous analysis, the two chosen pesticides, Dichlorvos (ps1) and Quinalphos (ps2), were subjected to TLC investigation. Elution of the TLC plates was carried out using ethyl acetate. However, the fluorescence patterns observed from the pesticides in the solvent systems did not correspond with those of the Churna samples (Sample A & B). The Rf values obtained for Dichlorvos (0.458) and Quinalphos (0.631) were determined using the ethyl acetate solvent system. None of the samples showed the presence of pesticides in the solvent system. The result is shown in Figure 3.

Table 1 — Estimation of standard in formulations

DRUG	Rf	Amount found in mg	Present in 100 mg
A	0.60	0.007062	0.7062
B	0.60	0.008679	0.8671
Piperine	0.59	0.00100	0.1
A	0.30	0.0055375	0.55375
B	0.31	0.004000	0.4
Eugenol	0.30	0.002	0.2
A	-	-	-
B	0.75	0.0345	3.45
Caryophyllene	0.74	0.002	0.2
A	0.83	0.011795	1.1795
B	0.83	0.0065	0.65
Eucalyptol	0.81	0.002	0.2

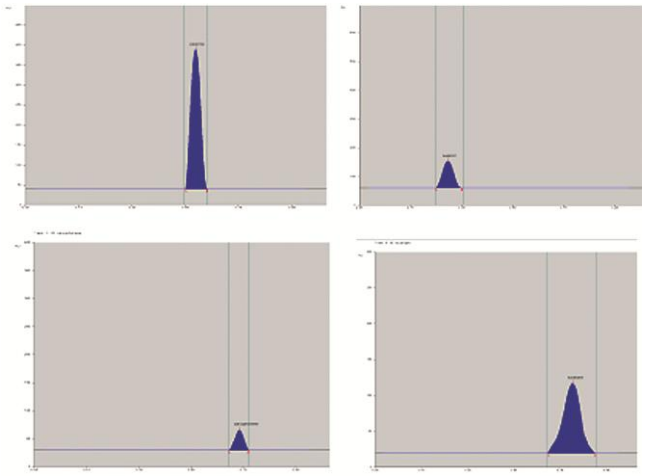

Fig. 1 — HPTLC chromatogram of standard Piperine, Eugenol, Caryophyllene and Eucalyptol

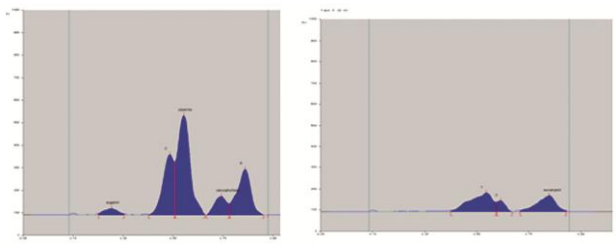

Fig. 2 — HPTLC fingerprint of marketed formulation & In-house formulation (300 & 400 nm)

As in this study, apart from finding out the bulk density which determines the bulk of the powder which will help in deciding the size of the sachets, the angle of repose is also quantified to study the flow property of powder while flowing through the hopper at the time of packing. Bulk density of the churna samples lies in the range of 0.34-0.42 g/cc while the granules values were found to be in the range of 0.0.45-0.57g/cc. The granules showed better flow property than the powders as the values in the range of 34.3-39 angle of repose, 20.83-26.7 as Carr´s index, 1.26-1.36 Hausner's ratio, respectively while for the powders the angle of repose was 37.6-40.4, Hausner's ratio was in the range of 1.38 to 1.5 and Carr´s index ranged from 27.6 to 33.3 as shown in Table 2.

Elemental analysis

Elements with toxic property, on the other hand, are injurious to the human body, even at relatively

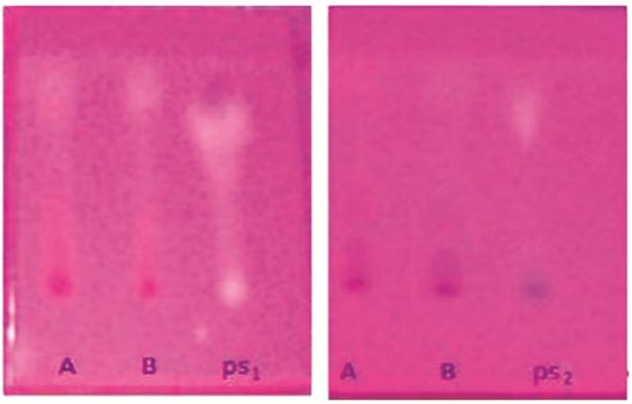

Fig. 3 — Finger print TLC profile of sample A (In house formulation of Ajmodadi chuma extract), sample B (Marketed formulation) and pesticides samples (Dichlorvos – psl and Quinalphos –ps2) in Ethylacetate

Table 2 — Micromeritic properties of Ajmodadi Churna samples

S.No.	Parameters	Ajmodadi Churna Sample A Mean± SEM	Ajmodadi Churna Sample B Mean±SEM	Ajmodadi Granules Sample A Mean±SEM	Ajmodadi Granules sample B Mean±SEM
1	Bulk density (g/mL)	0.42±0.06	0.34±0.04	0.45±0.03	0.57±0.02
2	Tapped density (g/mL)	0.63±0.16	0.47±0.05	0.74±0.04	0.72±0.03
3	Angle of repose	40.4°±1.17	37.6°±1.70	39°±1.2	34.3°±1.6
4	Hausner's ratio	1.5±0.06	1.38±0.06	1.36±0.05	1.26±0.06
5	Carr's index	33.3±0.71	27.6±0.71	26.7±0.06	20.83±0.07

Table 3 — Elemental Analysis of Ajmodadi churna samples

S.No.	Name of Elements	Concentration of elements (mg/L)	
		Sample A	Sample B
1	Al	1.462	2.622
2	Bi	0.011	0.012
3	Ca	15.9	32.32
4	Cd	0.001	0.001
5	Co	0.002	0.001
6	Cr	0.056	0.059
7	Cu	0.130	0.134
8	Fe	1.008	2.759
9	K	29.94	32.41
10	Mg	5.765	5.882
11	Mn	1.628	0.404
12	Na	35.99	33.38
13	Pb	0.035	0.037
14	Zn	0.373	0.324

low concentrations. Therefore, the detection of elements and their equivalent concentrations in food products as well as in herbal formulations, in addition to finding any possible genotoxic effect represents crucial steps before releasing such products into the markets. ICP-OES is used for elemental analysis in the field of quality control measures and research concerned with plant samples[17]. In the present investigation, Samples A and B were studied for their element constituents. The elements were detected within the permissible limits. The result is given in Table 3.

Antioxidant studies

From the literature survey, it has been revealed that many of the diseases are treated by drugs having antioxidant properties. Oxidative stress being one of the common causes of abdominal pain can be counteracted with a formulation having considerable antioxidant activity. Hence, the determination of *in vitro* antioxidant activity was carried out using DPPH radical scavenging activity. Antioxidant activity in different samples was compared with ascorbic acid. Sample-A exhibited better antioxidant activity than the sample-B (Table 4 and Fig. 4.)

One of the principle purposes of stomach treatment these days is the oxidative pressure caused because of

Table 4 — DPPH free radical scavenging activity of samples

S.No.	Conc.(µg/mL)	% inhibition		
		Ascorbic acid	Sample A	Sample B
1	50	96.95	85.11	79.93
2	100	97.25	88.48	82.61
3	150	97.45	94.31	93.15
4	200	97.66	94.36	94.41

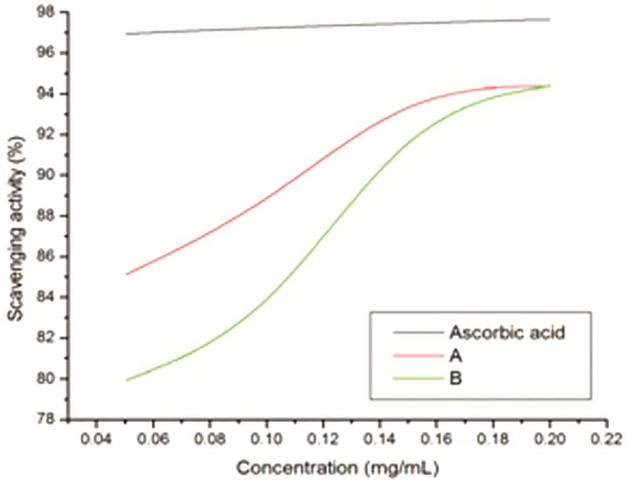

Fig. 4 — Antioxidant activity of Ajmodadi churma formulation, sample A (In house formulation), sample B (Marketed formulation) and Standard Ascorbic acid using DPPH method

the age of responsive oxygen species or the free extremist. These free extreme harm parts of cell which can cause cell demise, slow irritation, and annihilation prompting torment in the midsection. Since Ajmodadi churna is very valuable in stomach treatment, along these lines cancer prevention should also be thought about.

Discussion

The findings of our research are highly compelling, leading to the following conclusion that Piperine was observed to be present in excess in sample B. Eugenol was tracked down additional in sample A (in- house plan). Caryophyllene was not present in sample A Eucalyptol was more in sample A. Due to various advantages of granules; the approach was made to convert traditional formulation ajmodadi churna into

granules form. The granules showed better flow property than the powders as the values of angle of repose, carr´s index and Hausner's ratio were in the range of 34.3°-39°, 20.83-26.7, 1.26-1.36, respectively while for the powders the angle of repose was 37.6-40.4, Hausner's ratio was in the range of 1.38 to 1.5 and Carr´s index ranged from 27.6 to 33.3. Other parameters such as antioxidants and pesticides were also determined. Some elements are having toxic properties, so it is important to identify the presence of these elements in the formulation. Antioxidant activity was compared with ascorbic acid and it was observed that sample-A exhibited better activity than sample B. Pesticides determination was done using TLC showed that no pesticides were present in samples.

Conclusion

Ajmodadi churna is a polyherbal ayurvedic medication utilized as a carminative and an antispasmodic, is a solid vermifuge, and helps in lessening numerous difficult circumstances like sciatica and firmness in back and it additionally ordinary help in restoring the digestive functions.

The present findings confirm the standardization of formulation and evaluation of conversion of formulation in the different dosage forms. These important findings help in the standardization of ayurvedic formulation using modern techniques.

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Conflict of Interests

The authors declare that they do not have any conflict of interest.

Authors' Contributions

AP design the experiments. NT performed all experiments. AP drafted the manuscript with the help of NT.

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