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STANDARDIZATION OF AYURVEDIC POLYHERBAL FORMULATION (CHURNA) FOR ANTIOXIDANT ACTIVITY

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ABSTRACT

As per Ayurvedic Pharmacopeia, many types of preparations are presents, one of them Antioxidant churna is very essential, known to be effective in all types of allied disorders related to oxidation in cellular-molecular level in the living system has been standardized by following modern scientific quality control procedures both for the raw material and finished product. The obtained values of physical and chemical parameters can be adopted to lay down new pharmacopoeial standards to be followed for traditional preparations of Antioxidant churna with batch-to-batch consistency. The Physicochemical constituents found to be present in the raw material used the preparation of Antioxidant churna possible facilitate the desirable therapeutic efficacy of the medicinal formulation, and also could help in knowing the underlying mechanisms of Pharmacological Action.

INTRODUCTION

Now-a-day's worldwide believe on our ancient system of medication which is Ayurveda, As per the Ayurvedic concept of Preparations, Churna is a fine powder of a drug or drugs which is prepared by mixing clean, finely powdered and sieved drugs. Churna shows its effects mainly on gastrointestinal tract. It increases peristaltic movements of GI tract. It is used as antiflatulent (admana), antirheumatic (amavata) all disease are allied disorders of Antioxidant Activity. It is also used in the treatment of abdominal disorders (udara roga), pain (sula) and in treatment of piles (arsa) etc Practitioners usually do the identification of different herbs used in Pancasama churna according to Ayurvedic parameters. The preparation of Churna is based on traditional methods in accordance with the procedures given in classical texts [1]. Due to lack of modern pharmacopoeial standards laid down and followed for processing of Churna, the medicine prepared using traditional methods may not have the desired quality and batch to batch consistency. Hence this formulation required standardization of following scientific parameters including organoleptic characters, chemical analysis, chromatographic pattern and microbial screening. The work was undertaken in trust as part of a program of testing and validation of traditional practice of using the Ayurvedic medicine formulation like churna in management of admana. Some standards already exist for Ayurvedic churna in Ayurvedic Pharmacopoeia. However, the work deals with the details of following latest standardization guidlines involving Good Manufacturing Practices (GMP) for preparation of Ayurvedic medicines. Standardization guidelines to be followed for herbal products provided by international bodies like World Health Organization (WHO), European Agency for the evaluation of Medicinal Products (EMEA) and United States Pharmacopeia (USP) have also been considered.

The concept of developing drugs from plants used in indigenous medical system is much older, while in some cases direct links between a local and biomedical use exists, in other cases the relationship is much more complex [2]. Traditionally, 'Rasayana' drugs are used against a plethora of seemingly diverse disorders with no pathophysiological connections according to modern medicine. Looking at these diverse applications adaptogenic agents from this group of 'Rasayanas' were identified [3]. It has been reported that the

'Rasayanas' are rejuvenators, nutritional supplements and possess strong antioxidant activity. They also have antagonistic actions on the oxidative stressors which giving rise to the formation of different free radicals. Therefore, the therapeutic indication of these drugs can include the diseases relating to all the above systems. Their antistress/adaptogenic actions have made them therapeutically far more important [4] . The strong antioxidant activity of any 'Rasayana' was found to be 1000 times more potent than ascorbic acid, α-tocopherol, and probucol [5]. For example, oral administration of 'Brahma rasayana' (50 mg/animal for 10 and 30 days) significantly increased the liver antioxidant enzymes such as SOD, CAT along with tissue and serum levels of GSH. Thus, indicating that 'Brahma rasayana' could ameliorate the oxidative damage produced in the body by radiation [6]. 'Rasayana' preparations also increased stem cell proliferation and also prevented free radical-induced injury produced by radiation [7][8] also reported the prevention of oxidant stress by 'Student Rasayana'.

#Since free radicals are implicated in a number of physiological disorders as described above and with the 'Rasayana'drugs ofAyurveda used in the treatment of diverse physiological disorders, there is a strong case to believe that 'Rasayana' drugs exert their therapeutic actions by their ability to scavenge free radicals or by their antioxidant potential. There has been a review on some plants of Indian traditional medicine with antioxidant activity [9] and a reviewof immunemodulators from 'Ayurveda' especially of the 'Rasayana' drugs [10]. But there is not a single reviewof the 'Rasayana' drugs of the 'Ayurveda', as antioxidants. Some important plants like *Allium sativum*, *Centella asiatica*, *Ocimum sanctum*, *Vitis vinifera* and *Zingiber officinale* have been extensively reviewed in the recent past. Important 'Rasayana' drugs are reviewed for their antioxidant activity here.

- *Acorus calamus : - Acorus calamus* Linn. (family: Acoraceae, Ayurvedic name: 'Vacha')
- *Aloe vera :- Aloe vera* Linn. (family: Aloaceae, Ayurvedic name: 'Kumari').
- *Andrographis paniculata :- Andrographis paniculata* (Burm. f.) Wall ex. Nees (family: Acanthaceae, Ayurvedic name: 'Kalmegh').
- *Asparagus racemosus :-Asparagus racemosus* Willd. (family: Asparagaceae, Ayurvedic name: 'Shatavari').

- *Azadirachta indica :-Azadirachta indica* A. Juss. (family: Meliaceae,Ayurvedic name: 'Nimba') is commonly known as neem.
- *Bacopa monnieri :- Bacopa monnieri* (Linn.) Penn. (family: Scrophulariaceae, Ayurvedic name: Brahmi).
- *Desmodium gangeticum :- Desmodium gangeticum* (L.) DC. (family: Fabaceae, Ayurvedic name: 'Shalparni').
- *Phyllanthus emblica :- Phyllanthus emblica* L. (family: Euphorbiaceae, Ayurvedic name: 'Amalaki') is considered best among 'Rasayana' so called 'Acharasayana' [7].
- *Glycyrrhiza glabra : Glycyrrhiza glabra* Linn. (family: Fabaceae, Ayurvedic name: 'Yashtimadhu') is commonly known as licorice.
- *Picrorhiza kurroa:- Picrorhiza kurroa* Royle ex Benth (family: Scrophulariaceae, Ayurvedic name: 'Kutki').
- *Psoralea corylifolia:-Psoralea corylifolia* Linn. (family: Leguminoceae, Ayurvedic name: 'Vakuchi').
- *Semecarpus anacardium :-Semecarpus anacardium* Linn. f. (family: Anacardiaceae, Ayurvedic name: Bhalatak).
- *Terminalia chebula :-Terminalia chebula* Retz. (family: Combretaceae, Ayurvedic name: 'Haritaki').
- *Tinospora cordifolia :-Tinospora cordifolia* (Willd.) Miers. (family: Menispermaceae, Ayurvedic name: 'Guduchi').
- *Withania somnifera :-Withania somnifera* Dunal (family: Solanaceae, Ayurvedic name: 'Ashwagandha') has been in use for more than 2500 years.

Miscellaneous plants :- *Curculigo orchioides* Gaertn. had high antioxidant activity in 2,2_-azinobis[3-ethylbenzothiazoline-6-sulfonate] (ABTS) assay [11]. *Hygrophila auriculata* (Schum.) Hiene. extract showed good radical scavenging activity against DPPH with moderate scavenging activity against Nitric oxide, hydroxyl radical, ferryl bipyridyl Complex and LPO [12]. Alcoholic extract of the seeds of *Mucuna pruriens* (Linn.) DC. has an antilipid peroxidation property, which is mediated through the removal of superoxides and hydroxyl radicals [13]. The antioxidant components of Piper species, viz., *Piper cubeba*, green pepper, *Piper brachystachyum*, *Piper longum*

and *Piper nigrum* constitute a very efficient system in scavenging a wide variety of reactive oxygen species. Antioxidant potential of Piper species was further confirmed by their ability to curtail in vitro LPO by around 30–50% with concomitant increase in GSH content [14]. In ferric reducing/antioxidant power (FRAP)/DPPH assays, boiled ethanolic extracts of *Plumbago zeylanica* L. were the most effective, while in the ABTS assay boiled aqueous extracts were the most efficient. These extracts also significantly inhibited LPO induced by cumene hydroperoxide, ascorbate-$Fe^{(2+)}$ and peroxynitrite. Thus, Extracts of *Plumbago zeylanica* and its active ingredient plumbagin have significant antioxidant abilities that may possibly explain some of the reported therapeutic effects [15]. ABTS assay showed that the ethanolic extract of *Sida cordifolia* L. was found to be most potent along with relative antioxidant capacity for the water infusions and potent inhibition of LPO. *Evolvulus alsinoides* and *Cynodon dactylon* were also found to be moderately active [16].

- As per literature *Emblica officinalis* [Syn: *Phyllanthus Emblica*] or Amla, *Ocimum sanctum* (Tulsi), *Azadirachta indica* (Neem), Ginger (*Zingiber officinale* Roscoe, Zingiberacae) Amla (*Embilica Officinalis*) all the herbs having Anti-oxidants activity,so preparing an Ayurvedic formulation with two of then herbal ingredients and standardized that particular formulation.
- ✓ **About the selection of Herbs :-**Ginger (*Zingiber officinale* Roscoe, Zingiberacae) is easily available in market, Amla (*Embilica Officinalis*) is a seasonal friut and also easily available in markaet,both are common ingredients, cheap in compare to other herbal ingredients and already established about both are potent Anti-oxidant ingredient.[17][18].

The process for preparation of Churna :-Churna is a fine powder of a drug or drugs which is prepared by mixing clean, finely powdered and sieved drugs. The term churna may be applied to the powder prepared by a single drug or a combination of more drugs [19]. Ayurvedic formulary of India has given the specification for the composition of churnas [20][21].

MATERIAL AND METHODS

Plant material was collected from Nadaun,Himachal Pradesh, The authenticity of the species of herbs was checked and confirmed. For microscopically study, properly washed plant material was cut in to desirable size. Free hand sections were taken and stained with phoroglucinol and HCl.[22]. Physico-chemical studies like total ash, water soluble ash, acid insoluble ash, water extract at different temparature, loss on drying at 105°C, heavy metals and successive extractive values by Decoction process were carried out as per the WHO guide lines [23].

An Antioxidant churna was prepared ,This is a polyherbal formulation, consisting of 2 ingredients in all, with specific morphological parts of the plants (herbs) used and each ingredient being of equal quantity. For standardization of Antioxidant churna, some modifications were made, Ginger, *Zingiber officinale* Roscoe, Family:-Zingiberacae, Rhizomes; Amla (*Embilica Officinalis*) Family:- Phyllanthaceae., Fruit ; known to have antioxidant properties. The Antioxidant herbs was procured from the local market and prepared a ayurvedic churna , after a preliminary identification was made based on the ayurvedic parameters such as Varna(colour),Gandha (odour),Ruchi (taste),aakruti varna (Shape) and parimana (size).The material was examined for probable adulterants and foreign matter adhering to the surface was removed. Organoleptic evaluation was used for identification of sensory characteristics like colour, odour, taste, shape, size, texture and fracture. Microscopic evaluation and cytomorphological evaluation were not done as detailed then considered for quality analysis. Active phytochemical constituents like Alkaloids, glycosides, flavonoids, acids, gums, tannins, carbohydrates, proteins, fixed oils etc were identified through qualitative chemical analysis in each of the ingredients.

The formulation like Vaiśvānara Cūr´a is a powder preparation made with the ingredients in the Formulation composition given below,

FORMULATION COMPOSITION

Table 1: **Ingredients of An Antioxidant Churna:**

Serial No.	Sanskrit name	English name	Scientific name	Part Used	Quantity
1.	Ardraka	Ginger	*Zingiber officinale* Roscoe, Family:- Zingiberacae	Rhizomes	1 Part
2.	Amla	Indian Gooseberry	*Embilica Officinalis*. Family:- Phyllanthaceae.	Fruit	1 Part

Table 2:- Analytical Specification of Individual churna Preparation:-

Standardisation : *Emblica officinalis*,Family:-Phyllanthaceae dry extract is standardized to 30% Tannins.

S.No	Parameters	Specification
1.	Description	Light brown powder
2.	Organoleptic Tests	
	Odour	Astringent
	Taste	Sour
3.	Solubility	
	In Water	Soluble
	In Alcohol	Insoluble
	Heavey Metals	Not more than 20PPM
	Assay (on dried basis)	Not less Than 30% W/W
	Taninns by Tritrimetry	

Table.3.Antioxidant Churna Individual Formulation Specifications for *Emblica officinalis*, Family:-Phyllanthaceae dry extract

S.No	Parameters	Specification
1.	Description	Light brown powder
2.	Organoleptic Tests	
	Odour	Astringent
	Taste	Sour
3.	Identification Tests for Tannins :-	
	By Ferric Chloride solution	+
	By Lead Acetate Solution	+
4.	Loss on Drying (at 105^0C)	Not More Than 6% W/W
5.	Ash content	Not More Than 10% W/W
6.	Solubility	
	in water	Not Less Than 80% W/V
	in alcohol (50%)	Not Less Than 70% W/V
7.	Heavy Metals	Not More Than 20 PPM
8.	Assay :- (on dried basis)Tannins by Titrimetry	Not Less Than 20% W/W
	Vitamic C (as per IP method)	Not Less Than 20% W/W

Table.4:- Standardisation : Ginger *Zingiber officinale* Roscoe, Family:-Zingiberacae dry extract is standardized.

S.No	Parameters	Specification
1.	Description	Brown powder
2.	Organoleptic Tests	
	Odour	Astringent
	Taste	Pungent
3.	Solubility	
	In Water	Soluble
	In Alcohol	Sparingly soluble
	Heavey Metals	Not more than 20PPM
	Assay (on dried basis)	Not less Than 30% W/W

Table .5:-Antioxidant Churna Individual Formulation Specifications for Ginger *Zingiber officinale* Roscoe, Family:-Zingiberacae.

S.No	Parameters	Specification
1.	Description	Brown powder
2.	Organoleptic Tests	
	Odour	Astringent
	Taste	Pungent
3.	Loss on Drying (at 105^0C)	Not More Than 10% W/W
4.	Ash content	Not More Than 15% W/W
5.	Solubility	
	in water	Not Less Than 85% W/V
	in alcohol (50%)	Not Less Than 60% W/V
6.	Heavy Metals	Not More Than 20 PPM
7.	**Active Ingredients :-** **Phenolic compounds**: shogaols and gingerols. **Sesquiterpenes:** bisapolene, zingiberene, zingiberol, sesquiphellandrene, curcurmene.	
	Other: 6-dehydrogingerdione, galanolactone, gingesulfonic acid, zingerone, geraniol, neral, monoacyldigalactosylglycerols, gingerglycolipids.	

Then the Both Churna Mixing in 1:1 Ratio.

Method of preparation:

Take both the ingredients of pharmacopoeia quality, in a stainless steel pan at a low temperature till it becomes free from moisture. Powder the ingredients 1 to 2 individually in a pulverizer and pass through sieve number 85. Weigh separately each ingredient, mix together in specified ratio (1:1) and pass through sieve number 44 to obtain a homogeneous blend. Pack it in tightly closed containers to protect from light and moisture. Store in a Cool and Dry place.

Fig.1:- After Mixing of both Churna in (1:1) ratio.

Description:

Dark Brownish in colour, smooth powder with the characteristic smell, taste salty, astringent, sour taking after some time sweet in taste due to the present of Amla (*Embilica Officinalis*) with a tingling sensation. The powder completely passes on through sieve number 44 and not less than 50 per cent pass on through sieve number 85.

Analysis process for Standardization:-For the purpose of standardization of Ayurvedic Churna by the very known analytical procedure, Thin Layer chromatography (TLC) was performed and R_f values were calculated for standardization parameters. Quantitative analysis of the raw material was done organic matter, water soluble extractive, Alcohol soluble extractive, total ash and acid insoluble ash .Their values were calculated and found to be well within the available standard ranges. Other parameters, moisture content, P^H, were also evaluated. The approved raw material was packed in sterilized airtight container with proper labeling and stored in a cool place. Hygienic conditions were maintained by regular disinfecting of the work areas. The finished product obtained, was subjected to chemical treatment similar to that give to the raw material to inhibit microbial growth. It was then dried at 60ºC.The above detailed procedure were adopted for 5 batches of Antioxidant churna prepared. Statistical analysis was done "Mean", "Standard Error of the Mean" values are given also "Range" and "Median" values wherever applicable.

RESULT AND DISCUSSION

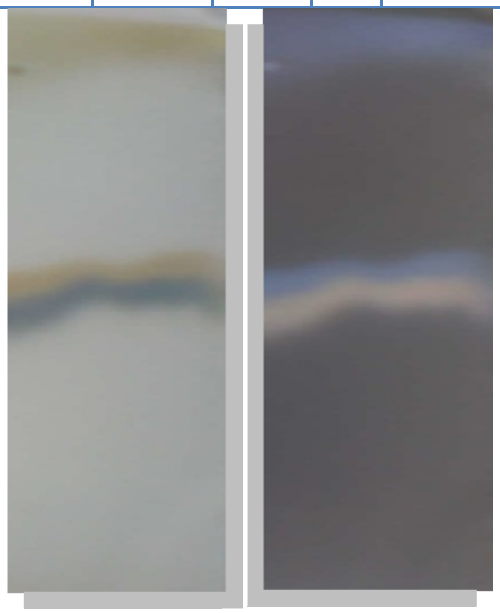
As part of standardization procedure, all 5 batches of the finished product of Antioxidant churna were tested for relevant physical and chemical parameters Quality tests (*Table.7*)

for Antioxidant churna were performed for moisture content, Water soluble extractive, Alcohol soluble extractive, Ash content and acid insoluble ash and were found to be within standard ranges. Values of P^H and alcohol soluble extractive were assessed. In addition, TLC was done (Fig.2(a) and 2(b)) with water extract of Antioxidant churna. 4gms of sample (Water extract form prepared churna) in *alcohol* (25 ml x 3) under reflux on a water-bath for 30 min. Filter, concentrate to

10 ml and carry out the thin layer chromatographer Apply 10 µl of the extract on TLC plate, develop the plate to a distance of 8 cm using Benzene: Ethanol : Methanol (3:5: 2) as mobile phase. After development of the plate, allow it to dry in air and examine under ultraviolet light (254 nm) and the spots were visualized after using Spraying reagent Vanilline-H_2SO_4 mixture. The R_f Values (Table-7) were calculated.Standardization of Antioxidant churna parameters concerning the quality of the raw material was possible by considering various scientific used,keeping intact procedure in accordance with Ayurvedic System. Values obtained after conducting tests for moisture content,Water soluble extractive,alcohol soluble extractive,ash content,acid standards laid down for hebal meterial.The "Mean"insoluble ash for Antioxidant churna were in conformance with the value obtained for each parameter was found to be consistent across 5 batches with minimun 'SEM'. For p^H,alcoholic soluble extractive. The 'Mean' value obtained for each of these parameters was also found to be consistent across 5 batches with minimum'SEM'.The occurrence of spot at the same respective location in TLC (*Fig.2(a) & 2(b)*) obtained for all 5 consecutive batches confirms the batch-to-batch consistency of the finished product. The phytochemical constituents in Antioxidant churna like Alkaloids, Glycosides, Flavonoids, Acids, Gums, Tannins, could have Pharmacological action on their own or in cojunction with body fluids in terms of efficacy.

Table 6: Photochemical Constituents presents in Anti-oxidant churna

Name of Herb	Part used	Scientific name	Alkaloids	Acids	Carbohydrats	Fixed Oils	Flavonoids	Glycosides	Gums	Resins	Saponins	Sterols	Tanins	Terpens
Ginger	Rhizomes	*Zingiber officinale* Roscoe, Family: - Zingiberacae	+	+	+	+	+				+	+	+	+
Amla (*Embilica Officinalis*)	Fruit		+	+	+		+	+	+	+			+	



2.(a) *2.(b)*

Fig.2.TLC profile of Antioxidant Churna

2.(a). TLC Profile of Antioxidant Churna after using Spraying reagent.

2.(b). TLC Profile of Antioxidant Churna in U.V Chamber.

***Fig.3.*Ginger *: Zingiber officinale* Roscoe, Family:-Zingiberacae (Rhizomes)**

***Fig.4.* Amla (*Embilica Officinalis*) Fruits Family:- Phyllanthaceae.**

***Table 7:* Result of Evaluation test:-**

Parameter	Standard values	(Obtained Value)					Mean values + SEM
		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	
Loss of Drying at 105°C	≤ 6%	5.50	5.60	4.90	5.00	5.40	5.28+0.139
Water soluble extractive (%)	Not less than 42	66.70	72.80	70.20	67.50	68.90	69.22+1.076
Alcoholic soluble extract(%)	Not less than 34	44.20	50.30	46.80	43.50	53.60	47.68+ 1.900
Ash contain (%)	15	10.30	12.40	12.30	12.90	13.00	12.18+0.489
Acid insoluble ash(%)	≤ 1.8	0.80	0.70	0.80	0.90	0.60	0.76+0.050
p^H	-	5.4	4.8	5.3	5.0	5.0	5.1+0.109
TLC (No of spots)	-	1.00	1.00	1.00	1.00	1.00	1.00+ 0.00
R_f Value	-	0.67	0.65	0.68	0.66	0.62	0.65+0.010

* No of Spot 01 but in that churna two different ingredient present form that give two different colour in the prepared churna..

* The evaluation after mixing of two Churna in (1:1) ratio.

*** Storage**: The Ayurvedic formulation ,Store in a cool place in tightly closed containers, protected from light and moisture.

CONCLUSION

That type of Ayurvedic formulations are present in market, in a huge numbers but their standardization process as per the scientific view and to the compare with Ayurvedic pharmacopeia, doubtful the process of standardization, but by follow of this process may be standardized all Ayurvedic herbal formulations.

The result obtained would be used to lay down a set of new pharmacopoeia Standards for the preparation of Anti-oxidant churna to obtained optimal efficacy of the medicine. The phytochemical screening churna shows effect like positive tests of Alkaloids, Glycosided, Tannins, Saponnins, Acids etc as per specifications chemical constituents present on the prepared Ayurvedic polyherbal churna.

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