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PREPARATION AND STANDARDISATION OF AYURVEDIC POLYHERBAL FORMULATION: AN ANTIDIABETIC CHURNA

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ABSTRACT

Traditional medicines refer to the knowledge, approaches, beliefs and health practices that incorporate plants, animals based medicines, manual techniques, exercises, spiritual therapies etc., which are applied individually or in combination to maintain well being or prevent, treat and diagnose illnesses. Diabetes mellitus becomes a severe threat to human life and several approaches are adopted to control the ill effects of diabetes and its complications; still herbal remedies are considered as the preferred choice due to low cost and lesser side effects. The variability in the efficacy of herbal medicines, unavailability of rigid quality control parameters and lack of authentic scientific based documentation procedures for herbal materials and their formulations are considered as a major problem in herbal medicine industry. The present study focuses on standardization of polyherbal churna of seven well-known traditional medicinal plants used in the treatment of diabetes. The polyherbal churna is prepared by using the different parts of *Phyllanthus emblica* (fruit), *Phyllanthus amarus* (whole plant), *Tinospora cordifolia* (whole plant), *Curcuma longa* (rhizome), *Syzygium aromaticum* (flower)**,** *Piper longum* (fruit) and *Moringa oleifera* (leaf). The outcome of the present research may be applied as a reference standard for setting quality control limits for the polyherbal medicine and can be further utilized in the formulation development of different dosage forms.

KEYWORDS: Antidiabetic, Polyherbal churna, Physicochemical screening, Standardization, GC-MS.

INTRODUCTION

An estimated worldwide population of 108 million people had diabetes in 1980 that was increased to 387million people in 2014 and further estimated to reach 592 million people by 2035.[1,2] Herbal medical formulations extended widespread suitability as remedial agents for diabetes mellitus, cough remedies, liver diseases, arthritis and memory enhancers. For the first time, the risks associated with herbal products were identified on the plants of Asteraceae family (Genus: Hypericin and Aristolochia) and kava-kava.[3] Over 50% of the populations used complementary or alternative medicine at least once in North America, Europe and other industrialized nations. The global market for herbal medicines is over 60 billion US dollars annually and is increasing steadily. The increase in the use of herbal medicines worldwide as a result of rapid expansion of the global market and hence the safety, efficacy and quality of herbal products became a serious concern for public, pharmaceutical industries and health authorities. The strong scientific evidence from randomized clinical trials are available only for few herbal remedies, many acupuncture medicines and for few manual therapies. Further research studies can be conducted to establish the safety and efficacy of herbal medicines.[4] High quality standardization methods and regulations shared at global levels are mandatory for safe use of herbal products apart from the use of phototherapy as efficacy and safety criteria. The legislative and regulatory framework should establish the basic parameters to guarantee high quality standards and safety for the use of herbal medicinal products.[5]

Methods of standardization shall consider all the important parameters that assure quality, efficacy, safety and reproducibility of herbal medicines.[6] There are many factors that may influence the quality and phytochemical profile such as genetic variants, geographical variations, seasonal variations, unavailability of selective analytical methods, differences in method of agriculture etc.[7] Hence, standardization of herbal formulations is essential to assess the quality of drugs based on the active principle concentration, standardization tools, in-vitro/in-vivo parameters etc.[8] The assessment of various semi-volatile and volatile phytochemical components are mostly accomplished by combining optimum separation method with definitive identification method such as gas and liquid chromatography (GC-MS).[9,10] In the present study, standardization parameters are assessed for polyherbal churna of seven traditional medicinal plants; *Phyllanthus emblica* (fruit), *Phyllanthus amarus* (whole

plant)**,** *Tinospora cordifolia* (whole plant), *Curcuma longa* (rhizome)**,** *Syzygium aromaticum* (flower**)**, *Piper longum* (fruit) and *Moringa oleifera* (leaf).

MATERIALS AND METHODS

Collection and Authentication of Plant Materials

The Plants were collected from different areas of Thrissur district (Kerala state) in the month of October. Dr. M Kesavan M.S.A.M (Chief Physician, Amala Ayurvedic Hospital and Research Centre, Amala Nagar, Thrissur, Kerala) authenticated the plants.

Preparation of polyherbal churna[11]

The selected part of plants were washed and dried under shade for 20 days; pulverized for further study. The individual drugs were separately weighed for the preparation of polyherbal churna as mentioned in the Ayurvedic Formulary of India. The polyherbal drugs were mixed using a double cone blender. The mixed formulations are unloaded, weighed and packed in labeled glass bottles.

A. Organoleptic properties

Organoleptic properties of polyherbal formulation was evaluated for appearance, colour, odour, taste and texture.

B. Preliminary phytochemical screening[12,13,14]

The preliminary phytochemical analysis of polyherbal extract was performed by the following standard procedures for estimation of glycosides, terpenoids, flavonoids, phenols, steroids, tannins, acids, glycosides, saponins and alkaloids.

C. Phytochemical screening using GC-MS[9,10]

The phytochemical screening and identification for bioactive chemical constituents of hydroalcoholic polyherbal extracts was carried out using GC-MS analysis.

D. Physicochemical parameters[12,13,14,15]

The physicochemical analysis of polyherbal churna was performed on 3 different lots as mentioned below:

Determination of loss on drying

Weight loss was noted after drying at 105°C. The difference in the weight gave the loss on drying of powdered drug.

Determination of ash values

The percentage of ash was calculated with the reference to the air-dried powder as per the procedure mentioned in Indian pharmacopoeia.

Determination of extractive values

The extractive value was calculated with the reference to the air-dried drug as per the procedure mentioned in Indian pharmacopoeia.

Determination of pH values

The pH (1% and 10 % solution) of drug was prepared in distilled water and pH of filtrate was checked using pH apparatus.

Bulk Density and Tapped Density

The bulk density indicates to the measure used to explain the packing of particles or granules. The initial volume gave the bulk density value and reduced volume after tapping is the tapped density.

Carr's Index.

Carr's index (% Compressibility) = 100 X (1 - Bulk density / Tapped density).

Hausner's Ratio.

Hausner's ratio = Bulk density / Tapped density.

Angle of repose.

Angle of repose (Tan θ) = Height of the conical pile / Radius of the conical pile.

E. Fluorescence analysis

The powdered drug was sieved through 60# and treated separately with different reagents. The fluorescence was observed under short UV (254 nm & 365 nm) and visible light.

F. Microbiological Quality[16,17]

Microbial analysis was carried out as per procedure mentioned in British Pharmacopeia 2014 and ICH Guidelines.

RESULTS

The standardization parameters for polyherbal churna of seven traditional medicinal plants are assessed and the results. are mentioned below:

A. Organoleptic properties

The polyherbal churna was studied for organoleptic characteristics and the results are tabulated below:

Table- I Organoleptic properties of the polyherbal churna.

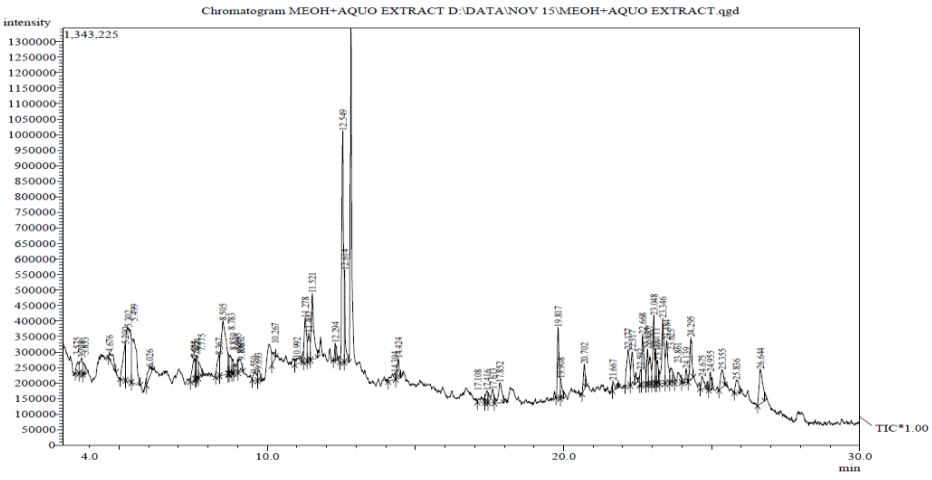
Organoleptic properties				
Appearance	***Colour***	***Odour***	***Taste***	***Texture***
Powder	Brownish yellow	Characteristic	Slightly bitter	Moderately fine

B. Preliminary phytochemical screening[12,13,14]

Preliminary phytochemical analysis revealed the presence of glycosides, terpenoids, flavonoids, phenols, steroids, tannins, glycosides, carbohydrates, saponins and alkaloids.

C. Phytochemical Analysis by GC-MS

The GC-MS chromatogram for hydroalcoholic polyherbal extracts of antidiabetic churna indicates the presence of fifty-nine compounds. The biochemical constituents identified in the polyherbal extracts are presented in fig.1 along with their retention time, percentage area etc.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.575	3.517	3.650	203683	0.66	29712	0.53	6.86		2-AMINO-4-HYDROXY-6-OXO-1,6-I
2	3.728	3.650	3.775	314588	1.03	51090	0.92	6.16	V	PROPANEDIOIC ACID, DIMETHYL
3	3.833	3.775	3.858	162398	0.53	36085	0.65	4.50	V	2-[2-(2-AMINOETHOXY)ETHOXY]E
4	4.676	4.642	4.892	295400	0.96	21859	0.39	13.51		3-Ethyl-3-pentyl methylphosphonofluor
5	5.200	5.033	5.217	705612	2.30	115323	2.07	6.12		Acetamide, 2-(2-hydroxyethoxy)-
6	5.302	5.217	5.417	1916466	6.25	172956	3.10	11.08	V	3-Dimethylsilyloxytetradecane
7	5.499	5.417	5.708	1555220	5.08	143237	2.57	10.86	V	Dimethyl(1-cyclopentylethoxy)silane
8	6.026	5.925	6.117	285401	0.93	33461	0.60	8.53	V	Triethylenediamine
9	7.533	7.408	7.558	415911	1.36	71437	1.28	5.82	V	OXIRANECARBOXAMIDE, 2-ETHY
10	7.575	7.558	7.600	159619	0.52	66119	1.19	2.41	V	5-Methyl-1-hexyn-3-ol
11	7.621	7.600	7.675	285600	0.93	75656	1.36	3.77	V	2-Hepten-1-ol, (E)-
12	7.775	7.675	7.800	258326	0.84	25582	0.46	10.10	V	4'-Pentylbicyclohexyl-4-carboxamide
13	8.367	8.258	8.392	371373	1.21	67078	1.20	5.54	V	4-Hydroxypiperidine
14	8.505	8.392	8.675	2025382	6.61	174875	3.13	11.58	V	4H-PYRAN-4-ONE, 2,3-DIHYDRO-3,

Fig. I GC-MS Chromatogram and report of polyherbal extract (Continued on next page)

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
15	8.692	8.675	8.717	152449	0.50	66414	1.19	2.30	V	6-FLUOROBICYCLO[3.3.0]OCTAN-
16	8.783	8.717	8.808	296423	0.97	54901	0.98	5.40	V	Propanal, 2,3-dichloro-2-methyl-
17	8.850	8.808	8.867	138656	0.45	45082	0.81	3.08	V	CYCLOHEXANOL, 3,5-DIMETHOXY
18	8.995	8.867	9.017	247153	0.81	42131	0.76	5.87	V	2-[2-(DODECYLOXY)ETHOXY]ETH
19	9.108	9.017	9.175	284844	0.93	36806	0.66	7.74	V	2-PENTADECYN-1-OL
20	9.550	9.492	9.675	212502	0.69	21676	0.39	9.80		3-Deoxy-d-mannoic lactone
21	9.693	9.675	9.808	178714	0.58	36007	0.65	4.96	V	GERMACYCLOPENTANE
22	10.267	10.150	10.283	210554	0.69	17859	0.32	11.79	V	1,1,1,3-TETRACHLOROHEPTANE
23	10.992	10.925	11.167	216928	0.71	18001	0.32	12.05	V	3,4,5,6-TETRAHYDROXY-2-OXO-HI
24	11.278	11.225	11.350	747868	2.44	140438	2.52	5.33	V	1,3,7-TRIMETHYL-2,6-OCTADIENY
25	11.402	11.350	11.458	456622	1.49	89600	1.61	5.10	V	1-HEXEN-4-YNE, 3-ETHYLIDENE-2
26	11.521	11.458	11.700	1290158	4.21	212816	3.81	6.06	V	2,6-Octadienoic acid, 3,7-dimethyl-, m
27	12.294	12.233	12.308	140931	0.46	57426	1.03	2.45		2-Myristynoyl pantetheine
28	12.549	12.458	12.600	3107468	10.14	745137	13.36	4.17	V	Benzene, 1-chloro-2-methoxy-
29	12.614	12.600	12.758	618301	2.02	296813	5.32	2.08	SV	(1R,2R,3S,4S)-1-METHYL-1,2,3,4-TE
30	14.304	14.083	14.333	206398	0.67	21879	0.39	9.43		2-(1-METHYL-2-OXOHYDRAZINO)I
31	14.424	14.333	14.500	294579	0.96	64611	1.16	4.56	V	4,7-Methanobenzofuran, 2,2'-oxybis[oc
32	17.108	17.092	17.342	147291	0.48	7721	0.14	19.08		BENZENAMINE, 2-METHYL-
33	17.416	17.342	17.450	189394	0.62	37495	0.67	5.05	V	3-Methoxy-2-nitrobenzaldehyde
34	17.613	17.450	17.667	331453	1.08	43908	0.79	7.55	V	Cyclotetradecane P835
35	17.852	17.667	17.975	553361	1.81	65596	1.18	8.44	V	Phthalic acid, di-(1-hexen-5-yl) ester
36	19.817	19.750	19.892	966026	3.15	226338	4.06	4.27		Ar-tumerone
37	19.908	19.892	20.000	232830	0.76	63255	1.13	3.68	V	4-[3-(4-Methylbenzyloxy)propyl]-1H-ir
38	20.702	20.625	20.742	336886	1.10	80848	1.45	4.17	V	Spiro[4.4]nona-1,6-diene, (S)-
39	21.667	21.650	21.850	150230	0.49	31781	0.57	4.73	V	Ethinamate P681
40	22.177	22.075	22.258	799887	2.61	115833	2.08	6.91		Tumerone
41	22.317	22.258	22.408	574376	1.87	107595	1.93	5.34	V	BICYCLO[3.1.1]HEPTANE, 2,6,6-TRI
42	22.592	22.558	22.625	168621	0.55	48507	0.87	3.48	V	3-OXO-20-METHYL-11-.ALPHA.-HY
43	22.668	22.625	22.783	853836	2.79	163421	2.93	5.22	V	Spiro[3-phenyl-1,4,2dioxazolin-5,2'-bor
44	22.835	22.783	22.867	399050	1.30	113861	2.04	3.50	V	Caryophyllene oxide
45	22.917	22.867	22.975	444860	1.45	90833	1.63	4.90	V	(-)-THUJOPSEN
46	23.048	22.975	23.092	878716	2.87	221891	3.98	3.96	V	3-ALLYL-6,6-DIMETHYL-2-METHY
47	23.111	23.092	23.142	262414	0.86	112033	2.01	2.34	V	BENZENE, 1-(1,1-DIMETHYLETHYI
48	23.169	23.142	23.208	253635	0.83	75270	1.35	3.37	V	Ethinamate P681
49	23.346	23.250	23.433	1174262	3.83	210302	3.77	5.58	V	2-CYCLOHEXENE-1,4-DIOL, 5-(1-H
50	23.484	23.433	23.583	733118	2.39	137682	2.47	5.32	V	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trim
51	23.625	23.583	23.683	256209	0.84	48714	0.87	5.26	V	1-METHYL-4-(2-METHYL-2-OXIRA
52	23.861	23.750	23.983	259486	0.85	33443	0.60	7.76	V	6-Amino-1,2,3,4-tetrahydroindan-5,7-di
53	24.139	23.983	24.208	164145	0.54	44196	0.79	3.71	V	(1S-(1ALPHA,2BETA,5ALPHA))-2-M
54	24.295	24.208	24.383	652074	2.13	137111	2.46	4.76		Butylphosphonic acid, 1-adamantylmetl
55	24.675	24.608	24.842	279401	0.91	39670	0.71	7.04	V	2,5-Norbornanediol
56	24.955	24.900	24.992	203427	0.66	58114	1.04	3.50		NONANOIC ACID, METHYL ESTER
57	25.355	25.250	25.450	399481	1.30	57046	1.02	7.00		11-Hexadecyn-1-ol
58	25.836	25.767	25.950	321382	1.05	42369	0.76	7.59		9-OCTADECENOIC ACID (Z)-
59	26.644	26.550	26.783	901641	2.94	112338	2.01	8.03		Tumerone
				30643019	100.00	5579238	100.00			

Fig. I GC-MS Chromatogram and report of polyherbal extract

D. Physicochemical Analysis

The physicochemical analysis of polyherbal churna was performed on three different lots (F1, F2 and F3) and the results are tabulated below:

Table– II Physicochemical parameters of polyherbal churna

Physicochemical parameter	Results of 3 different lots			
	F1	F2	F3	Mean
Loss on drying (% w/w)	3.24	3.16	3.21	3.20
Total ash value (% w/w)	6.01	5.86	5.66	5.84
Acid insoluble ash (% w/w)	0.98	0.90	1.02	0.97
Water soluble ash (% w/w)	3.31	3.47	3.22	3.33
Alcohol soluble extractive value (% w/w)	18.1	20.2	19.3	19.2
Water soluble extractive value (% w/w)	28.7	24.3	26.7	25.6
pH (10% aqueous solution)	4.20	4.14	4.22	4.18
pH (1% aqueous solution)	4.29	4.26	4.31	4.28
Bulk density	0.65	0.58	0.59	0.61
Tapped density	0.77	0.71	0.68	0.72
Hausner ratio	1.18	1.22	1.15	1.18
Compressibility index (Carr's index)	15.6	16.67	13.2	15.15
Angle of repose	29.7	31.3	28.4	29.8

E. Fluorescence analysis

The polyherbal churna was studied for Fluorescence activity with different reagents and observed under ultra violet light and daylight. The results are tabulated below:

Table- III Fluorescence analysis

Treatments	Observations	
	Day Light	UV Light
Powder	Brownish yellow	No Fluorescence
Powder+ Methanol	Greenish brown	Fluorescent green
Powder+ petroleum ether	Light green	Fluorescent green
Powder+ Conc. HNO3	Orange yellow	Fluorescent yellow
Powder+ FeCl3	Greyish green	Brownish yellow
Powder+ Conc. HNO3	Orange yellow	Fluorescent Green
Powder+ Conc. H2SO4	Orange yellow	Fluorescent Green
Powder+ 5% H2O2	Brownish yellow	Fluorescent green
Powder+ 1M aqueous NaOH	Brownish yellow	Greenish yellow

F. Microbial Quality

Table- IV Microbial Quality powdered polyherbs

Test	*Specification*	*Results*
Total Aerobic Microbial Count	NMT 10^5cfu/g	Absent
Total Combined Yeasts/Moulds Count	NMT 10^4cfu/g	Less than 10cfu/g
Bile-tolerant gram negative bacteria	NMT 10^4cfu/g	Absent
Escherichia coli	Absent	Absent
Salmonella	Absent	Absent

DISCUSSION

Standardization of drugs is the vital part for establishing the quality, purity, safety and correct identity of herbal medicines.[18] Several occurrences of substandard drugs and adulterated herbs come into existence due to lack of standardized quality parameters for herbal preparations and thus standardization is the principal responsibility of herbal drug industry.[19] This can be attained only if the herbal medicinal products are evaluated using sophisticated modern analytical techniques such as GC-MS, HPLC, HPTLC etc. and stringent quality standards are established. In the present study, polyherbal churna is tested for relevant phyto-constituents, physical parameters and chemical parameters as per standardization procedure. The preliminary phytochemical studies reveal that the polyherbal anti-diabetic churna contains almost all types of secondary metabolites that are accountable for their desired therapeutic activity. The phytochemical screening of the polyherbal extract using GC-MS reveals the presence of many phyto-constituents that aids as a guideline for the phytochemical profile of the polyherb.

The organoleptic properties of the polyherbal churna was evaluated and reported during the study. The quality tests were performed for LOD, ash content and extractive values and results were found to be within standard specification ranges. Acid- insoluble ash value of less than 1% indicates quality, purity and less content of silicious matter in the polyherbal churna. The water-soluble ash value of 3.3% also indicates the quality and purity of the churna. The low value noted for loss on drying further indicates water and volatile matter of the formulation. The high values of alcohol soluble extractive test reveals the presence of polar chemical constituents in the ingredients and high water soluble extractive value occurs due to the presence of many phyto-constituents in the polyherbal churnam. The pH of aqueous solutions reveals that the formulation is acidic in nature. The result of solvent treated samples under ultraviolet light and day light shows the presence of fluorescence. The values noticed for Hausner ratio, compressibility index and angle of repose and physical characteristics indicate good flowability of polyherbal churna.

CONCLUSION

The polyherbal churna is standardized using modern scientific quality control measures. The results of phytochemical studies reveal the presence of almost all the phytoconstituents in the polyherbal churna that will have a synergetic effect on the antidiabetic activity. Pharmacognostic characters establish for the polyherbal churna shall be employed as quality control standards for routine analysis. Also isolation, identification and characterization of the active compounds can give deeper insight for further developments in the field of diabetes and can be further used during formulation development of different dosage forms.

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