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PHYTOCHEMICAL EVALUATION OF KARISALAI KARPA CHOORNAM- A POLY HERBAL SIDDHA FORMULATION

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ABSTRACT

Karisalai Karpa Choornam (KKC) is a Polyherbal formulation mentioned in Siddha to treat aging, liver disorders, immunodeficiency and general weakness. In this present study, preparation and extraction of polyherbal formulation using various solvents based on polarity was carried out. Fluorescence analysis of powder and different extracts under UV light and ordinary light were noted. Preliminary qualitative phytochemical analysis for different extracts shows the presence of carbohydrates, phytosterols, triterpenoids, saponins, tannins, phenolics and flavanoids. Physiochemical analysis such as Ash content, water soluble ash, acid insoluble ash, sulphated ash, pH, moisture content were studied to evaluate the purity and quality of the formulation. Physical characteristics like bulk density, tapped density, angle of repose and carr's index also performed to find out flow property of the formulation. The results of this study could be used as a diagnostic tool for the standardization of the polyherbal formulation.

INTRODUCTION

Herbal medicines play an important role in traditional system of medicine. They have been used for thousands of years and have made a great contribution to maintaining human health. A majority of the world's population in developing countries still relies on herbal medicines to meet its health care needs [1]. The World health organization (WHO) suggested the importance of herbal drugs for human beings in developing nations and has developed guidelines to support the member states in their attempt to formulate national policies on herbal medicine and to study their quality, safety and efficacy [2]. Phytochemical analysis of herbal formulation is necessary in order to assess quality of drugs. Both quantitative and qualitative assessment of poly herbal formulation is of vital importance in order to justify their acceptability in modern system of medicine [3]. In the traditional Indian system of medicine plant formulation and several cases, combined plant extracts used as drug choice rather than individual. Many of these have shown promising effect [4]. Karisalai Karpa Chooranam (KKC) is a polyherbal formulation which consists dried whole plant powders of *Eclipta prostrata* (*Asteraceae*) ,*Wedelia chinensis* (*Asteraceae*), *Centella asiatica* (*Apiaceae*), *Acalypha indica* (*Euphorbeaceae*), *Indigofera tinctoria* (*Fabaceae*) and *Sphaeranthus indicus* (*Asteraceae*). In traditional Siddha system of medicine it has been used as an Antioxidant, immunomodulator and hepatoprotective. The results of previous GC-MS analysis of Karisalai Karpa Choornam indicated the presence of bioactive compounds such as 2,2' Bioxirane, Butanoic acid, Methyl 2-oxopropanoate, Phenol, 2,4 dihydroxyacetophenone -, D-Erythro-Pentose, 2-Deoxy-,Cyclopentasiloxane, decamethyl-, 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethyl siloxy) tetrasiloxane, Dodecanoic acid, 2 (3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4a-methyl-,Myristic acid, 9-

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Octadecanoic acid (Z), 2,6,10-Trimethyl,14-Ethylene-14-Pentadecen-,2-Pentadecanone,6,10,14-Trimethyl-, Penta decanoic acid , 3,7,11,15-Tetramethyl-2-Hexa decen -1-ol ,Hexadecanoic acid, methyl ester ,cis-10-Nonadecenoic acid,1-(+)-Ascorbic acid 2,6-dihexadecanoate, Andro grapholide ,Methyl 9-cis,11-trans-octade cadienoate ,11-Octadecanoic acid, methyl ester ,Methyl stearate ,Cyclopentadecanone,2-hydroxy- , Octadecanoic acid [5]. Further study is designed to evaluate the preliminary phytochemical, physiochemical and physical characteristics of Karisalai Karpa Choornam using standard laboratory procedures.

MATERIALS AND METHODS

Plant material

The Plant species of *Eclipta prostrata* (*Asteraceae*), *Wedelia chinensis* (*Asteraceae*), *Centella asiatica* (*Apiaceae*), *Acalypha indica* (*Euphorbeaceae*), *Indigofera tinctoria* (*Fabaceae*) and *Sphaeranthus indicus* (*Asteraceae*) were collected from rural areas and Herbal garden, PRIST University, Thanjavur, TamilNadu, India and authenticated by scientist Dr.G.V.S.Murthy, Botanical Survey of India (BSI), Ministry of Environment and Forests, Coimbatore, Tamilnadu, India. Voucher specimens (BSI/SRC/5/23/2011-12/Tech-1046, 47, 48, 49, 50, 51) of the same have been deposited in the Department of Pharmacy, PRIST University for future reference.

Preparation of the sample

KKC was prepared by the method described in the Siddha literature *'Bogar 7000'*. As mentioned in the text, leaf, stem, root, flower and seeds of each plant were collected. After collection, the raw medicinal plant materials were subjected to proper preliminary processing, including elimination of unwanted materials and contaminants, washing to remove excess soil, sorting and cutting. As per the reference, shade drying was preferred to avoid the loss of active chemical constituents of the herbals. All medicinal plant materials individually spread out in thin layers on drying frames and turned repeatedly. In order to maintain adequate air circulation, the drying frames were placed at a sufficient height above the ground. Efforts were made to achieve uniform drying of medicinal plant materials. After 15 days, each plant was powdered separately and passed through 40# sieve. Finally each plant powder was weighed accurately and mixed together in specific proportions to get moderately coarse powder. Finally it was stored in airtight container and used for further analysis. The composition of KKC is mentioned in Table 1.

Organoleptic evaluation

The organoleptic characters such as appearance, colour, odour, taste and texture of the formulation were carried out [6]. The results are shown in Table 2.

Solvent Extraction

The formulation was subjected to solvent extraction taking from non-polar to polar solvents like Petroleum ether, Benzene, Chloroform, Ethyl acetate, Methanol, Ethanol and Water. The sample was subjected to Soxhlet extraction with various solvents. Then the excessive solvents were removed by using Rotary vacuum evaporator (MAC Buchi). These extracts were stored in desiccators for further analysis [7, 8]. The characteristics of different extracts such as colour, consistency and percentage yield were observed. The results are shown in table 3.

Qualitative phytochemical analysis

Primary metabolites like carbohydrates, proteins, fixed oils, fats, gums and mucilage were analyzed for their presence as per the standard procedures [9, 10, 11]. Likewise the secondary metabolites of alkaloids, flavonoids, saponins, phenolics, glycosides were also performed in the formulation. The results are shown in Table 4.

Fluorescence analysis

1mg of the powdered sample was placed on a micro slide and observed under UV at 366 and 254 nm. The sample was treated individually with the various solvents such as NaOH (1N) in H_2O, HCl (1N), NaOH (1N) in MeOH, 50% KOH, 50% H_2SO_4, 50%HNO_3, Con. HNO_3, Con. H_2SO_4 and Iodine in H_20. Observations were carried out under UV at 366 and 254 nm. Fluorescence analysis of various extracts such as Petroleum ether, Benzene, Chloroform, Ethyl acetate, Methanol, Ethanol and Water also performed under UV at 366 and 254 nm [12]. The results are shown in table 5 and 6.

Physico-chemical analysis

Physico-chemical analysis such as water soluble extractive, alcohol soluble extractive values and ash values such as total ash, acid insoluble ash, water soluble ash and moisture content determination using IR moisture balance (Sartorius MA 150) were carried out [13]. The pH of KKC of 1%w/v and 10% w/v of water soluble portions were determined using digital pH meter (Systronics MK VI). The results are shown in table 7.

Determination of Physical characteristics

Physical characteristics like bulk density, tap density, angle of repose and Carr's index were determined. The term bulk density refers to packing of particles or granules. The equation for determining bulk density (Db) is Db = M/Vb where M is the mass of particles and Vb the total volume of packing. The volume of packing was determined in an apparatus consisting of graduated cylinder build upon mechanical tapping apparatus. It has a specially cut rotating can. 100gm of weighed formulation of KKC was taken and added to cylinder with the support of a funnel. Before tapping the initial volume was noted. Then, the sample was tapped until no further reduction.

The final volume was noted. The initial volume is called as bulk density value. The final reduced volume of sample after tapping is called as value of tapped density.

The powder flow ability was quantified indirectly by the method called as Angle of repose. In this method, the fixed funnel and the free standing cone used. It contains a tip at a given height (H) above the glass paper that is positioned on a flat horizontal surface. Sample was carefully poured through the funnel until the tip of the conical pile just touched the tip of funnel. When R is the radius of the conical pile, tan a = H/R where a is the angle of repose.

The powder flow ability was quantified indirectly by another method called as Carr's index. The powder flow was measured form bulk density. The equation for measuring Carr's index is I=(Df-D0/Df) × 100. Where Df is the tapped density and D0 the bulk density [14, 15]. The results are shown in table 7.

RESULT AND DISCUSSION

All the results generated from the present study are represented in the respective tables. Organoleptic evaluation of KKC was performed for the identification of sensory characteristics like appearance, colour, odour, taste and texture. The results are tabulated in table 2. The extract characteristics such as colour, consistency and percentage yield of KKC using different solvents were carried out. The consistency was found to be sticky in the non polar to not so polar solvent extracts while the polar solvent extracts were found to be non sticky. The percentage yield (w/w) of the different extracts was analysed in which the highest yield was found to be in the ethanolic extract (18.23%w/w). The results of preliminary phytochemical analysis of different extracts revealed the presence of both primary metabolites like carbohydrates, proteins, fixed oils. And secondary metabolites like alkaloids, phenols, flavonoid, phytosterols, terpenes, saponins in which major metabolites were observed in both methanolic and ethanolic extracts. The quantitative analysis of Total ash, Water soluble ash, Acid insoluble ash, Sulphated ash, Water soluble extractive, Alcohol soluble extractive, pH, Loss on drying at 105^0C were carried out in triplicate of KKC according to the prescribed method. The ash value of the formulation indicated the presence of minerals and earthy materials. The result of acid insoluble ash indicated the presence of siliceous matter present in the formulation. But it was very negligible amount (0.258%w/w). The results of water soluble extractive indicated the presence of carbohydrates, inorganic compounds and the alcohol soluble extractive value indicated the presence of polar constituents like phenols, glycosides, flavonoids. Deterioration time of the herbal formulation depends upon the amount of moisture present in formulation. If the moisture content is high, the product can be easily deteriorated due to contamination by fungal colonies. The Loss on drying at 105^0C was found to be 4.423%. The results of pH from 1% w/v and 10% w/v solution revealed that the sample was slightly acid. Fluorescence analysis was carried out for both powder and extracts of KKC under UV and ordinary light. The visibility of varying colours indicated that the presence of various phytoconstituents [16-17]. Physical characteristics like bulk density, tap density, angle of repose and Carr's index were determined.

Table 1. Composition of Karisalai Karpa Chooranam

S.No	Botanical name	Family	Parts used	Each 100 gm Contains
1	Eclipta Prostrata	Asteraceae	Leaf, stem, root, flower and seeds	16.66 gm
2	Wedelia chinensis	Asteraceae	Leaf, stem, root, flower and seeds	16.66 gm
3	Acalypha indica	Apiaceae	Leaf, stem, root, flower and seeds	16.66 gm
4	Centella asciatica	Euphorbeaceae	Leaf, stem, root, flower and seeds	16.66 gm
5	Indigofera tinctoria	Fabaceae	Leaf, stem, root, flower and seeds	16.66 gm
6	Sphaeranthus indicus	Asteraceae	Leaf, stem, root, flower and seeds	16.66 gm

Table 2. Organoleptic characteristics of Karisalai Karpa Chooranam

S.No	Parameters	In-house formulation
1	Appearance	Powder
2	Colour	Green
3	Taste	Mild bitter
4	Odour	No Characteristic
5	Texture	Moderately coarse powder

Table 3. Extract characteristics of various extracts of Karisalai Karpa Chooranam

S.No	Extract	Colour	Consistency	% yield
1	Pet. Ether	Green	Sticky	6.45
2	Benzene	Dark green	Sticky	3.87
3	Chloroform	Dark brown	Sticky	6.28
4	Ethyl acetate	Green	Sticky	4.26
5	Methanol	Dark green	Sticky	9.65
6	Ethanol	Dark green	Sticky	18.23
7	Aqueous	Dark brown	Non Sticky	10.65

Table 4. Qualitative Phytochemical screening of various extracts of KKC

Metabolites	Tests	PE	B	C	EA	M	E	AQ
Alkaloids	Mayer's test	-	-	-	-	+	+	+
	Wagner's test	-	-	-	-	+	+	+
	Hager's test	-	-	-	-	+	+	+
	Dragendroff's test	-	-	-	-	+	+	+
Carbohydrates	Molish's test	+	+	+	+	+	+	+
	Fehling's test	+	+	+	+	+	+	+
	Benedict's test	+	+	+	+	+	+	+
Glycosides	Borntrager's test	-	-	-	-	+	+	+
	Legal's test	-	-	-	-	+	+	+
Saponins	Froth test	-	-	-	-	+	+	-
	Foam test	-	-	-	-	+	+	-
Proteins and Amino acids	Xanthoproteic test	+	+	+	+	+	+	+
	Biuret test	+	+	+	+	+	+	+
	Ninhydrin test	+	+	+	+	+	+	+
Phytosterols	Liebermann-burchard test	+	+	+	+	+	+	+
	Salkowski test	+	+	+	+	+	+	+
Diterpenes	Copper acetate test	-	-	+	+	+	+	+
Fixed oils and fats	Spot test	-	-	-	-	+	+	-
	Saponification test	-	-	-	-	+	+	-
Phenols	Ferric chloride test	-	-	-	-	+	+	+
Tannins	Gelatin test	-	-	-	-	+	+	+
Flavonoids	Lead acetate test	-	-	-	-	+	+	+
	Alkaline reagent test	-	-	-	-	+	+	+
Gums & Mucilages	Alcohol 95% test	-	-	-	-	-	-	-

PE-Petroleum ether; B-Benzene; C-Chloroform; EA-Ethyl acetate; M-Methanol; E-Ethanol; AQ-Aqueous.

Table 5. Fluorescence characteristics of different extracts of KKC

S.No	Extract	Under ordinary light	Under UV light (366)
1	Petroleum ether	Green	Yellowish green
2	Benzene	Dark green	Brown
3	Chloroform	Dark green	Brown
4	Ethyl acetate	Dark green	Brown
5	Methanol	Dark green	Brown
6	Ethanol	Dark green	Brown
7	Water	Dark brown	Blackish brown

Table 6. Fluorescence characteristics of KKC using various solvents

S.No		Day Light	UV 254 nm	UV366 nm
1	Powder as such	Green	Greenish Brown	Dark Green
2	Powder + NaOH(IN) in H20	Greenish Yellow	Yellowish Green	Greenish Yellow
3	Powder + In HCl (1N)	Brownish Green	Greenish Yellow	Brownish Green
4	Powder + In NaOH(IN) in MeOH	Greenish Yellow	Dark Green	Green
5	Powder + 50% KOH	Brownish Green	Brownish green	Greenish Brown
6	Powder + 50% H2SO4	Greenish Brown	Dark Brown	Green
7	Powder + 50% HNO3	Reddish Brown	Dark Green	Dark Brown
8	Powder + Conc.HNO3	Reddish Brown	Greenish Red	Dark Green
9	Powder + Conc.H2SO4	Greenish Black	Dark Green	Greenish Yellow
10	Powder + Iodine in H2O	Blackish brown	Brownish Red	Dark reddish brown

Table 7. Physico chemical characteristics of KKC

S.No	Parameter	Mean (n=3)±SD
1	Total ash(% w/w)	2.21±0.06
2	Water soluble ash(% w/w)	1.32±0.15
3	Acid insoluble ash(% w/w)	0.25±0.02
4	Sulphated ash(% w/w)	1.09±0.05
5	Water soluble extractive(% w/w)	18.58±0.17
6	Alcohol soluble extractive (% w/w)	54.69±1.33
7	pH of 1%w/v solution	5.4±0.25
8	pH of 10%w/v solution	5.6±0.10
9	Loss on drying at 105^0C (% w/w)	4.42±0.09

Table 8. Physical characteristics of KKC

S.No	Parameter	Mean (n=3)±SD
1	Tapped density	0.34± 0.02
2	Bulk density	0.35±0.01
3	Angle of repose	35.47±0.26
4	Carr's index	24.82±0.21

CONCLUSION

The present research work on preliminary photochemical evaluation of Karisalai karpa choornam could be used as the diagnostic tool for the standardization of the polyherbal formulation in accordance to WHO norms and standard laboratory methods. Various parameters such as phytochemical, physiochemical and fluorescence analysis were investigated and reported. The research outcome can be used to evaluate the quality and purity of the polyherbal formulation.

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