International Journal of Pharmacology and Clinical Research (IJPCR)

IJPCR |Volume 5 | Issue 2 | Apr - Jun - 2021
www.ijpcr.net

Research article *Clinical research*

ISSN: 2521-2206

Preparation and standardization of ayurvedic polyhedral formulation; patoladi kvatha churna for skin diseases

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ABSTRACT

An Ayurvedic formulation of Patoladi kvatha Churna was selected for the present work. Developing a generation to prefer Ayurvedic medicines than the allopathic drugs. Patoladi kvatha churna has traditionally used to treat skin diseases which have not been proved scientifically. So, the patoladi kvatha churna has chosen to prove their activity with scientific evidence.

Keywords: Patoladi kvatha, Churna, Ayurvedic, skin diseases.

INTRODUCTION

Herbal Medicine[10-11],

Herbal and products containing herb(s) have been in trade and commerce and are currently used for variety of purpose. The WHO defined an herb as being fresh (or) dried, fragmented (or) powdered plant material; it can be used in this order state (or) further processed and formulated to become the final herbal product. Treatment of herbs by squeezing, steaming, roasting, detecting (or) infusing in water. Extracting with alcohol (or) sweetening and baking with honey can create herbal products such as juices, tinctures, decoctions, infusions, gums, fixed oil, essential oil and resins. These may be used medically (or) as the starting material for additional processing and as food ingredients.

Herbal formulation[5]

The herbal formulation consists of a selective combination of individual herbal ingredients that are formulated for a specific ailment or group of diseases conditions. When herbs combine together, they become more potent and effective within the body than individual herb due to their activating or catalyzing influence over one another. Herbal medicines are prepared from plant materials which are prone to contamination, deterioration and variation in composition. Therefore, quality control of herbal medicine offers a host of problems. To solve this problem, first and foremost task is the selection of the right kind of plant material which is therapeutically efficacious.

Advantages of Herbal Medicines

They have large amount of use they have better patient tolerance as well as acceptance the medicinal plants have renewable source of cheaper medicines. Improvements in the quality, efficacy and safety of herbal medicines with the development of science and technology.
Prolong and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy. They are cheap in cost. They are not harmful. They are more effective than any synthetic drug. Throughout the world herbal medicines have provided many of the most potent medicines to the vast arsenal of drugs available to modern medical science, both in crude form. In India, drugs of herbal origin have been used in traditional systems of medicines such as Unani, Ayurveda and Siddh

In order to identify the churna which is traditionally used in the treatment of skin disease a thorough literature survey was carried out on churnam used-traditionally for skin disease activity. Although many churnas are used to treat skin disease in Indian traditional systems of medicine, most of these churnas are not scientifically evaluated. If a systemic and intensive ethno pharmacological study is carried on one (or) more churnas used in traditional system, are sure to provide effective drug for skin diseases.

AIM AND OBJECTIVE OF THE STUDY

Thousands of floras have been studied and screened for various activities. But still most of them remain only in the textual form. No steps have been taken to apply those research works and make them useful to the poor and diseased.

The aim of the work is to uplift the traditional system of medicine by using the modern standardization techniques. The wealth of our mother nation should be utilized by us. Future research must focus towards herbal drugs and their improvement.

The main objective to choose a churnam formulation is to create awareness among the people regarding the usage of Ayurvedic Medicine. Strict guidelines to be brought to formulate any herbal medicine. All the methods of standardization given in the scriptures and books should be explored.

MATERIALS

Leaves of Trichosanthes dioica Roxb, Rhizomes of Picrorhiza kurroa Royle ex Benth, Heart Wood of Santalum album Linn, Roots of Marsdenia tenacissima Wight and Arn, Stem of Tinospora cordifolia (willd) Miers and Roots of Cissampelos pareira Linn were collected from various parts of the Indian states.

METHODOLOGY

METHOD OF PREPARATION

All the ingredients were taken as per Pharmacopoeial quantity as listed under table was roasted in a stainless steel pan at a low temperature till it becomes free from moister. All the ingredients were powdered individually in a pulverizer. Each ingredient was weighed separately, mixed together in specified ratio to obtain a homogenous blend. The churnam was packed in a tightly closed container to protect from light and moisture.

Dosage: 40ml two times a day for 21 days.

Table1 Composition of Patoladi Kvatha Churna (PKC) [3,5,10]

S.no	Name of the drug	Biological source	Biological source
1	Kambu pudalai	Leaves of Trichosanthes dioica Roxb.	5g
2	Kadugurohini	Rhizomes of Picrorhiza kurroa Royle ex Benth	5g
3	Chandanam	Heart wood of Santalum album Linn	5g
4	Perunkurinjan	Roots of Marsdenia tenacissima Wight and Arn	5g
5	Seenthilkodi	Stem of Tinospora cordifolia (Willd) Miers	5g
6	Stem of Tinospora cordifolia (Willd) Miers	Roots of Cissampelos pareira Linn	5g

RESULTS AND DISCUSSION

Table 2: Results of Macroscopical Studies

S.NO	PLANTNAME	COLOUR	ODOUR	TASTE	SURFACECHARACTERISTICS	SIZE ANDSHAPE
1	TrichosanthesdioicaRoxb	Upper surface green, lower surface dull green	Not specific	Slightly bitter	Both surfaces are very rough with rigid hairs. Sinuate and dentate margin, cordate base, acute to Acuminate apex.	Ovate-oblong Cordate
2	Picrorhizakurroa RoyleexBenth	Greyishbrown	Pleasant	Extremely bitter	Rough due to wrinkles, circular scales of roots, marked with numerous partially encircling closely arranged scaly leaves or scars of leaves, attached with a few roots Fracture short.	Rhizome about 5.4 cm inlengthand6.3mm in width.
3	SantalumalbumLinn	Reddish brown	Highly fragment	Bitter	Straight grains and splits easily longitudinally, heavy and solid.	Log and chips varying in width and length
4.	Marsdena tenacissima	Yellow to buff coloured with dark brown patches	Indistinct	Slightly bitter	Longitudinally ridges and furrow present. Fracture, fibrous in wood, granular in bark region and short.	Cylindrical, 0.6 to 2.6 cm thick and varying in length.

5.	Tinospora cordifolia (Wild. Miers.)	Dark brown	Odorless	Bitter	Wood light in weight, bark warty, numerous lenticels, perforated, dried sample conical pieces, bark papery, longitudin al ridges, difficult to fracture	Varying in length.
6.	Cissampelos pareira Linn.	Yellowish grey	Aromatic	At first sweetish and aromatic, latter slightly bitter.	Cylindrical tortuous pieces, entire or split longitudinally, on gitudinally wrinkled woody internally.	1 to 4 cm in diameter, 10 cm to 1.0 m long.

Table 3 Quantitative Physico-Chemical Analysis Of Pkc Foreign Organic Matter

S.NO.	Plant name	Foreign organic matter%	API Standard limit
1	Santalumalbum Linn	0.5 ± 0.05	NMT 1%
2	TrichosanthesdioicaRoxb	1.0 ± 0.08	NIL
3	CissampelospareiraLinn.	1.24 ± 0.005	NMT-2%
4	PicrorhizakurroaRoyleexBenth	1.1 ± 0.05	NMT-2%
5	Marsdeniatenacissima	1.2 ± 0.06	NMT 2%
6	Tinosporacordifolia	NIL	NIL

Table 4 Determination Of Ash Value

S.No.	Plant name	Determination of ash value(Total ash)(% w/w)	API Standard limit (% w/w)	Acid in soluble ash (% w/w)	API Standard limit (% w/w)	Water soluble ash (% w/w)
1	*SantalumalbumLinn*	0.7±0.03	NMT1%	0.06±0.04	NMT1%	NIL
2	*TrichosanthesdioicaRoxb*	13.36±0.02	NMT-7%	3.14±0.01	NIL	NIL
3	*CissampelospareiraLinn.*	6.5±0.1	NIL	0.8±0.09	NMT-1%	7.7±0.06
4	*picrorhiza kurroaRoyleexBenth*	5.33±0.01	NMT 7%	0.30±0.006	NMT-1%	1.35±0.008
5	*Marsdeniatenacissima*	4.5±0.09	NMT 5%	0.1±0.02	NMT0.5%	0.23±0.008
6	*Tinosporacordifolia*	7.5±0.09	NMT16%	1.16±0.009	NMT-3%	12.05±0.01

Table 5 Determinations Of Extractive Values

S.No.	Plant name	Water soluble extractive (% w/w)	API Standard limit	Alcohol soluble extractive (% w/w)	API Standard limit
1	*Santalumalbum Linn*2.5±0.09		NLT 1%	10.5±0.11	NLT8%
2	*Trichosanthesdioica Roxb*	12.5±0.09	NIL	2.1±0.12	NIL
3	*Cissampelospareira Linn.*	17.5±0.12	NLT13%	12.5±0.12	NLT11%
4	*picrorhizakurroa RoyleexBenth*	22.5±0.06	NLT-20%	11.5±0.15	NLT10%
5	*Marsdenia tenacissima* 23±0.05		NLT 14%	11±0.12	NLT7%
6	*Tinosporacordifolia*	12.05±0.01	NLT11%	7.27±0.01	NLT3%

Table 6 Determination Of Loss On Drying Values

S.No.	Plant name	Loss On Drying (%)
1	*Santalumalbum Linn*	NIL
2	*TrichosanthesdioicaRoxb*	7.8%
3	*CissampelospareiraLinn.*	17.9%
4	*picrorhizakurroaRoyleex Benth*	2.87%
5	*Marsdeniatenacissima*	6%
6	*Tinosporacordifolia*	2.31%

STANDARDISATION OF CHURNAM

Organoleptic Properties

In-house formulation of Patoladi Kvatha Churnam was alight brownc olour powder.
It was Bitter, Aromatic and Slightly Sweetish in taste. It has a characteristic odour.

POWDER MICROSCOPY

The formulated Churnam was then viewed and the following Results were found to contain all the characters seen the individual powders.

Characters from Patha -*Cissampelospareira*L.–Root

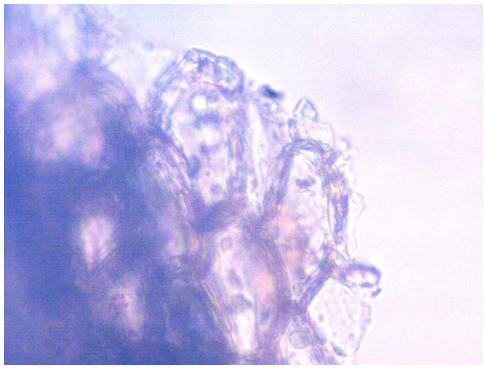


Fig1Thickwalled parenchyma

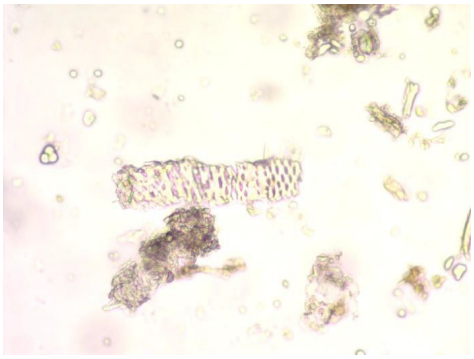


Fig2 Pitted vessel fragment

Characters from Patola-*Trichosanthesdioica* Roxb.–leaf

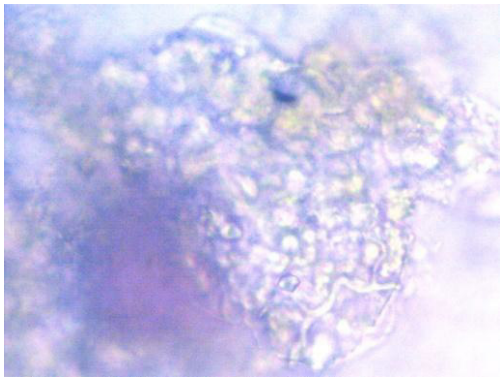
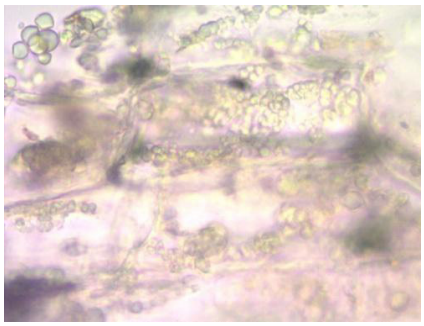


Fig 3 Epidermalcellsin surface view

Fig 4 Covering trichomes

Characters from Murva-*Marsdeniatenacissima* (Roxb.)Moon. –Root



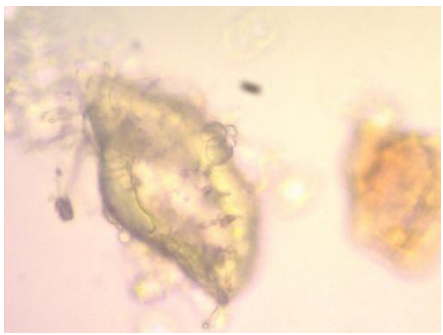


Fig 5.Parenchymawithstarch grains Characters from Guduci-*Tinosporacordifolia*(Willd.) Meirs.-Stem

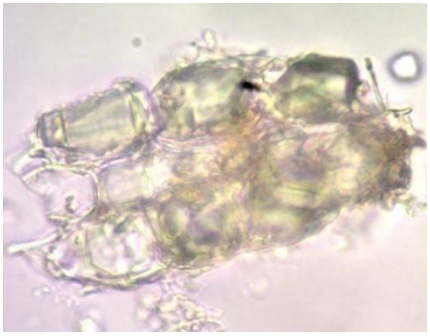


Fig 6 Parenchyma with crystals

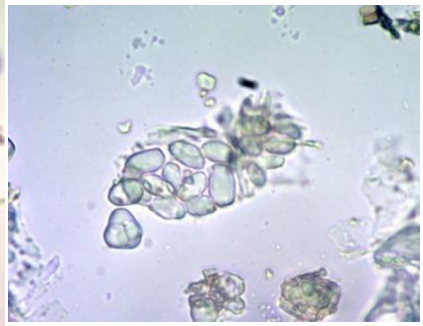


Fig 7 Elongated starch grains

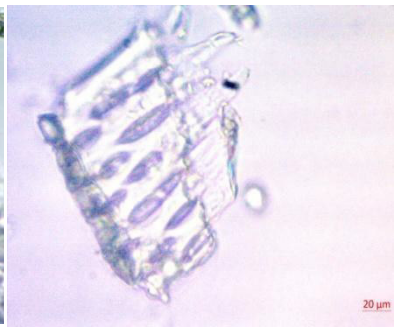


Fig 8 Vessels with bordered pits

Characters from *Santalum album* Linn.–Wood

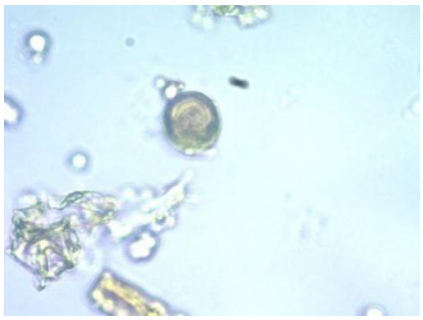


Fig 9 Volatileoil drop

Fig 10 Fibre bundle

Characters from Katuki-*Picrorhiza kurroa* Royleex Benth.-Rhizome

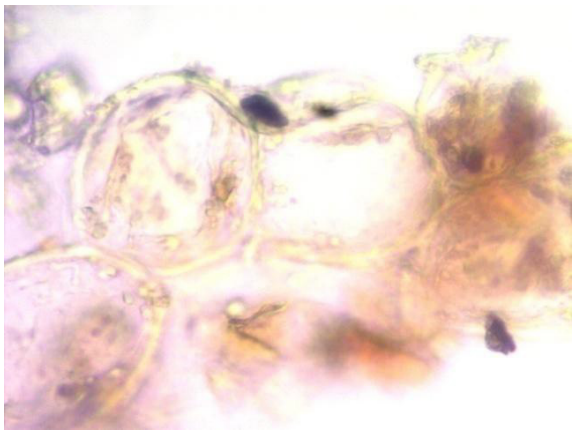


Fig11 Reticulate vessel

From the observations seen in the formulation, it is very clear that the Churnamposses all the ingredients in the light ratio as mentioned in the API. All the characteristic features matches with the powder microscopy of the individual powder samples.

Table 7: Flourescence Analysis of PKC

S.No.	Experiment	Visible/Daylight	UV light (254nm)	UVlight(366 nm)
1	Drug powder as such	Yellowish Brown	Brown	Fluorescent Yellow
2	Powder+1NNaOH	Yellow	Greenish Yellow	Yellow
3	Powder+1 N HCl	Greenish Brown	Yellowish	Brown

			Brown	
4	Powder+50%H_2SO_4	Greenish Brown	Yellow	Green
5	Powder+50% HNO_3	Reddish Brown	Yellow	Fluorescent Blue

Table 8: Physico-Chemical Analysis Of PatoladiKvatha Churna

S.NO	PARAMETERS	RESULTS
1	Total ash	9.8±0.09
2	Acid insoluble ash	1.8±0.08
3	Water soluble ash	8.2±0.09
4	Water soluble extractive	20.5±0.12
5	Ethanol soluble extractive	10.2±0.06
6	Loss on drying	10.58±0.02
7	Foreign organic matter	0.9±0.008
8	P_H	6.78±0.009

TOXICOLOGICALEVALUATION

Evaluation Of Safety Profile Of The Formulation

Determination of microbial contamination in PKCF.

The microbial load of formulation were determined and compared with WHO specified limits

Table 9: Results of microbial contamination of PKCF.

S.NO	Test	Observation	Limit As per (API)
1	Total Viable aerobic count(bacteria)	Nil	NMT10^7cfu/g
2	Total Viable aerobic count(Fungi)	Nil	NMT10^4cfu/g
3	*Escherichiacoli*	Nil	NMT10^2cfu/g
4	*Salmonellaspp*	Nil	AbsencePer Gram

Determination of heavy metal contamination in PKC

S.NO.	Heavy Metal	Observation(ppm)	Permissible limits (ppm)	As per (API)
1	Mercury	*<1*	*1*	
2	Cadmium	*<0.1*	*0.3*	
3	Lead	*<3*	*10*	
4	Arsenic	*<2*	*3*	

Table 10:Resultforthe estimation of Aflatoxins

S.No.	Aflatoxins	Observation(ppm)	Permissible Limit (ppm)	As per (API)
1	B1	0.11±0.01	0.5	
2	G1	0.11±0.01	0.5	
3	B2	0.06±0.02	0.1	
4	G2	0.06±0.01	0.1	

Table 12 Assay of sodium by Flame Photometry

S.No.	Total sodium content(mg/g)	Limit(mg/g)	As per(API)
1	41.57±0.20	NLT30	

PHYTOCHEMICALST AND ARDISATION

Table 13 Determination Of Total Flavanoid Content Of PKC

Concentration(µg/ml)	Absorbance
Quercetin10	0.50
20	0.55
30	0.61
40	0.81
50	0.83
Ethanol	0.91

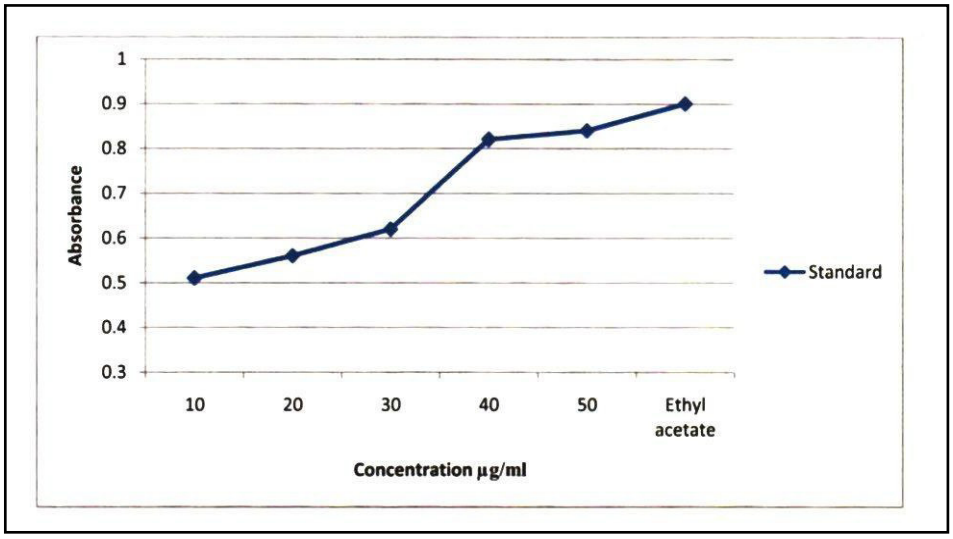


Fig 12 Phyto –analytical standardisationtlcstudies of rawmaterial& PKC

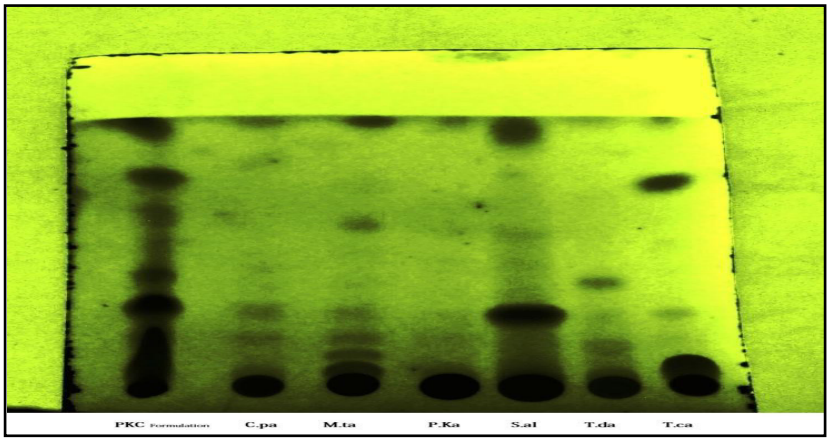


Fig 13:TLC Identification of Six plants extract compared with PKC formulation
GC-MS analysis of churnam

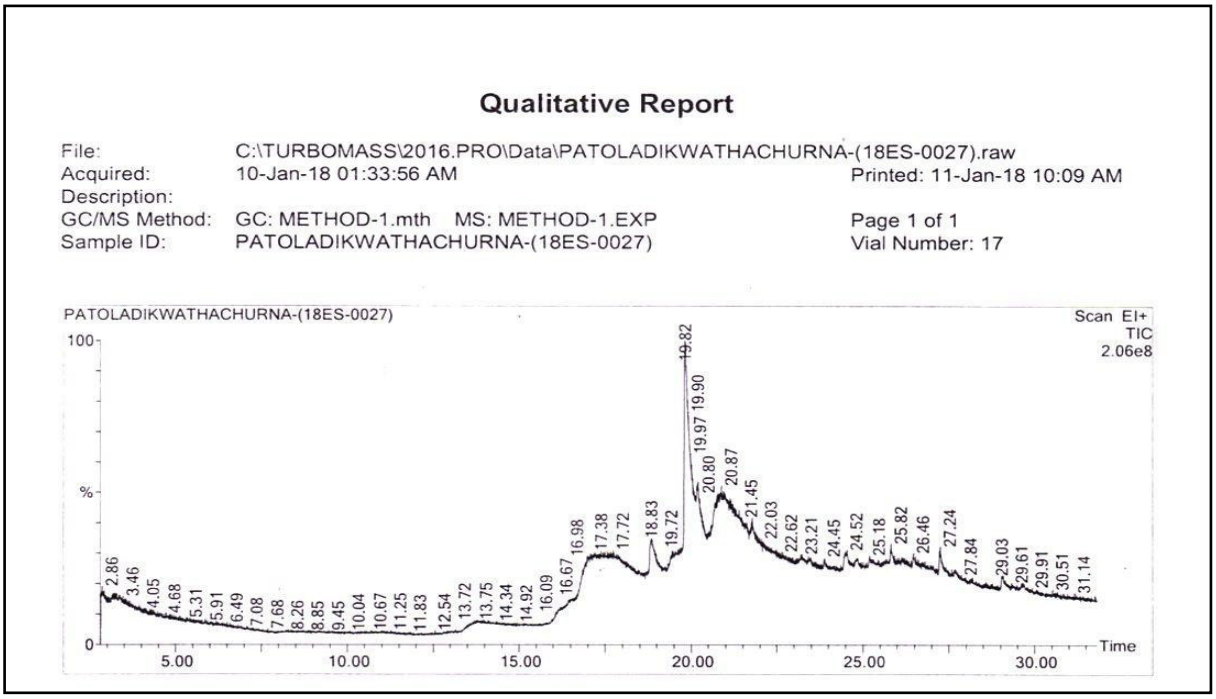


Fig 14 HPTLC finger printingof phyto-constituents of patoladikvatha churnam formulation

Table 14 Solvent system in HPTLC

S.No.	Sample	Solvent System	SaturationnTime	Visualization mode/ Spray reagent
1	QuercetininPKC	Toluene: Ethyl acetate: Formic acid(6:4:1)	1 hour	UV 254nm, UV366 nm
2	Individual plant extract in PKC	Toluene: Ethylacetate: Formic acid:Methanol (12:9:4:0.5)	1 hour	UV254nm, UV366 nm
3	*Santalumalbum* with Formulation PKC	Toluene : Ethyl acetate(9.3:0.7)	1 hour	UV 366 nm

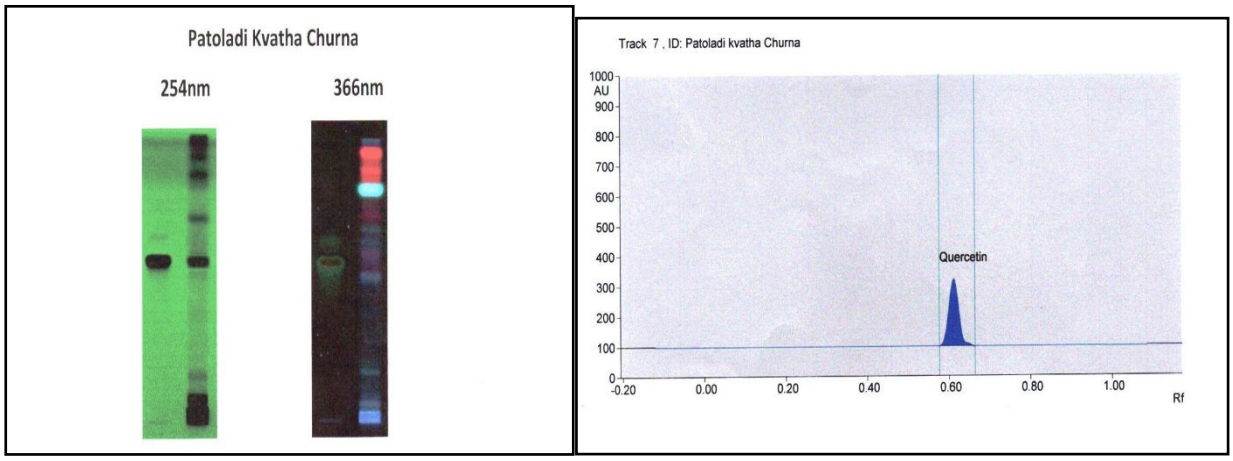


Figure 15 Finger printing of Patoladi Kvatha Churna Containing Quercetin

HPTLC For Patoladi Kvatha Churna Formulation Extract Compared With Individual Plant Extract

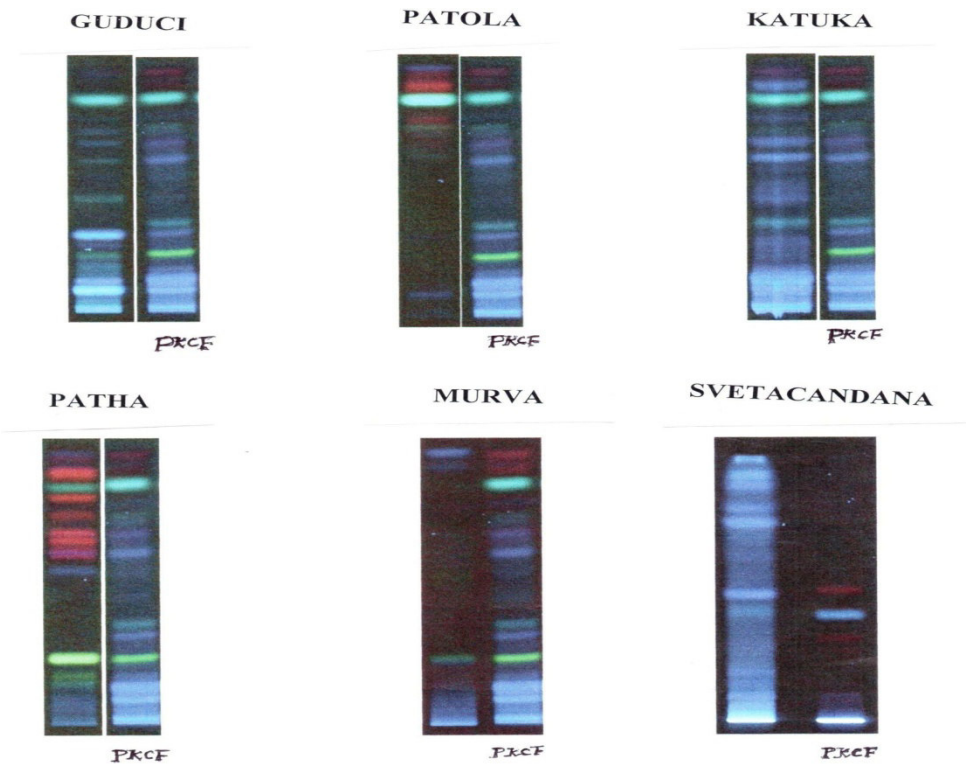
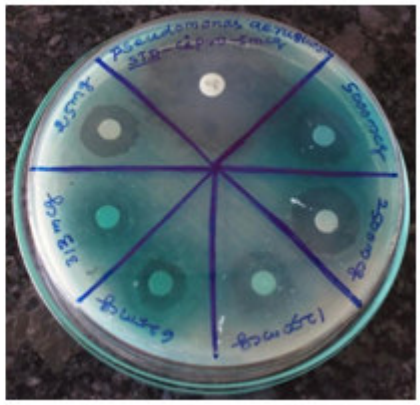


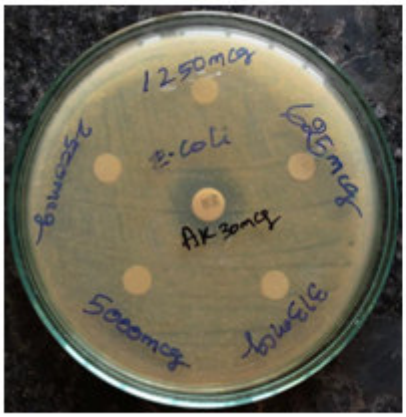
Fig 16 Invitro skin diseases screening of pkc

STD– Standard

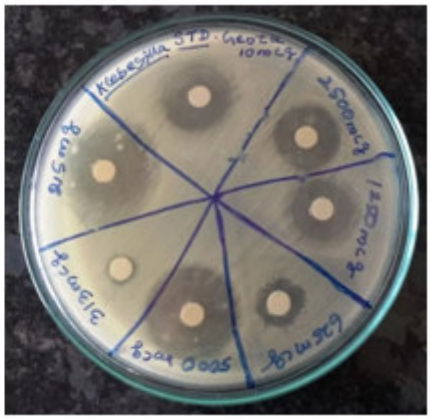
Zone of Inhibition of *ProteusVulgaris*



Zone of Inhibition of *Pseudomonasaureginosa*



Zone of Inhibition of *Escherichia coli*



Zone of Inhibition of *Klesiellapneumonia*

Fig 17 Disc Diffusion Method of Antibacterial activity of PKC Churnam Extract compared with Standard

Zone of inhibition for PKC Churnam extract compared with standard

The Methonolic extract demonstrated antibacterial activity to four of the tested bacteria. Highest inhibition was demonstrated toward *Klebsiella pneumonia (22 mm)*, and *Pseudomonas aeruginosa (20mm), Escherichia coli, Proteus vulgaris* were not sensitive to the extract

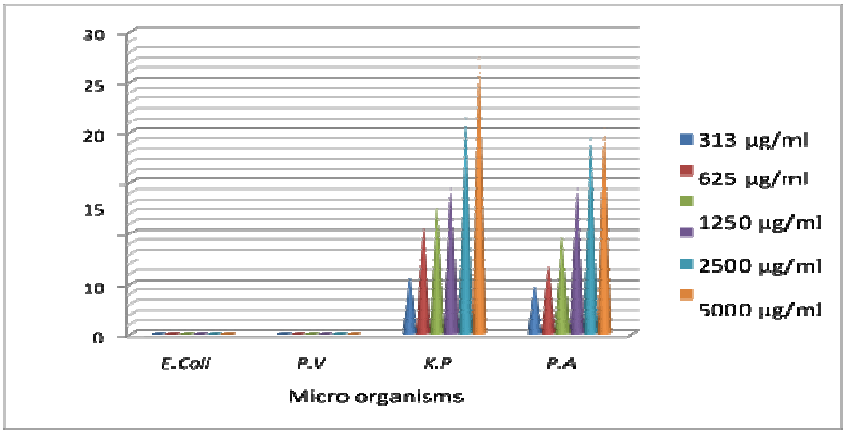


Fig 19 Antifungal Activity result for the Ethanolic Patoladi KvathaChurnam extract

Well Diffusion Method Assay of Antifungal activity of PKC Churnam Extract compared with Standard

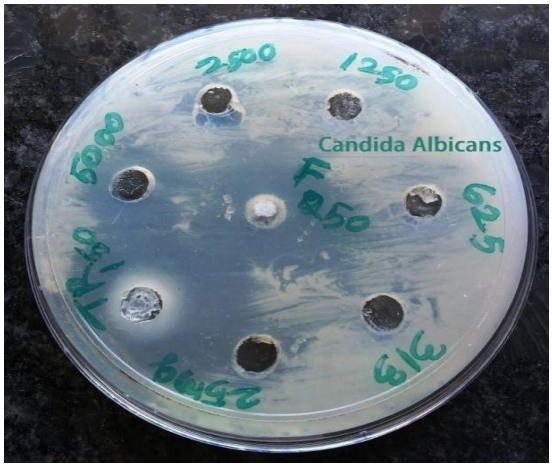
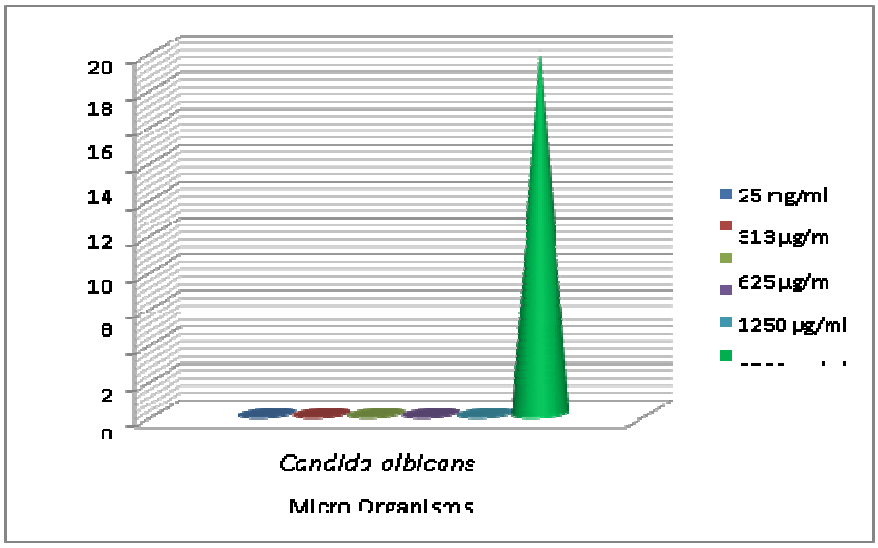


Fig 20 Zone of inhibition for PKC churnam extract compared with standard

The Methonolic extract demonstrated antifungal activity to one of the tested bacteria.
Highest inhibition was demonstrated toward *CandidaAlbicans* (20mm)



SUMMERY AND CONCLUSION

Raw materials standardization was done to confirm the absence of adulterants in the crude drugs and thereby it ensures a quality product.

Experiments for all physicochemical parameters were performed for both the raw materials as well as the finished products as per API and results obtained were verified with the limits and proved to be successful.

An extensive toxicological evaluation was done using the latest equipments to ensure the safety profile of the formulation.

Total bacterial count and total fungal count was almost nil. This showed that the formulation is free of pathogenic organisms.

The levels of heavy metals, pesticides, Aflatoxins were also determined. The results obtained were appreciable.

Estimation of sodium content in the formulation revealed that the percentage of sodium is within the specified limits.

Qualitative and quantitative phytochemical screening was performed for the raw materials as well as the formulation. It was found to contain almost all the major Phytoconstituents.

TLC studies were performed for individual crude drug. The Rf values of formulation are compared with the Rf value of crude drug, thereby ensuring the presence of the individual herb in the formulation.

GC-MS analysis was done to identify the presence of various chemical structures with the help of the peaks obtained.

HPTLC studies were also done to get the finger printing of the formulation. Quercetin used as bio marker. Finger printing shows the excellent presence of quercetin in the PKC.

In vitro anti-bacterial and anti-fungal activity was performed by Disc Diffusion and Well Diffusion method. Since five of the raw material contains flavanoidal content which was revealed by preliminary phytochemical screening and HPTLC studies? The formulation posses an excellent anti-bacterial and anti-fungal activity.

Thus an Indian traditional official Ayurvedic formulation "PATOLADI KVATHA CHURNA" has been standardized Pharcognostically, phytochemical and even pharmacologically. These studies will be helpful to increase the confidence among the people to use this formulation for various disorders.

ACKNOWLEDGEMENT

The Authors are thankful to Sura Labs, Dilshukhnagar, and Hyderabad for providing the necessary facilities for the research work.

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