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STANDARDIZATION AND HPTLC ANALYSIS OF *SHWASKUTHAR RASA* ANAYURVEDIC FORMULATION PREPARED USING DIFFERENT CONCENTRATIONS OF *PIPERINE*

Madhuri Pawar*[1]and Dr.Sheeba Varghese[2]

Professor, Department of Rasashastra Evam Bhaishajya Kaplana, Bharati Vidyapeeth [Deemed to be University] Collegeof Ayurved,Pune,Maharashtra

Email:madhuri.pawar@bharatividyapeeth.edu

1. Professor,Department of RasashastraEvamBhaishajyaKaplana,NangeliAyurvedamedicalcollegeKothamangalam, Kerala

Email:drsheeba@rediffmail.com

Correspondingauthor-**Dr. Madhuri Pawar**

Department of Rasashastra Evam Bhaishajyakalpana, Bharati Vidyapeeth [Deemed to beUniversity] Collegeof Ayurved, Pune, Maharashtra

Email:madhuri.pawar@bharatividyapeeth.eduMobile no: 9822661507

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Abstract

Shwaskuthar Rasa [SKR] is a herbo-mineral ayurvedic formulation indicated in the treatment of *Shwas, Tamakshwas*[~BronchialAsthma],*Kasa*[cough]andalliedrespiratorycondi tions.Specificmineral and herbal drugs along with *Marich* [*Piper nigrum]* are processed to formulate themedicine. However, variations in quantities of ingredients and preparation methods have been revealed in ayurvedic classical literature. In onepreparation method of SKR it is suggested toaddone one kernels of *Marich* in given quantity to the whole mixture of formulation. However, generally in pharmaceutical practice *Marich* fine powder is mixed directly. Thus, in present study the attempt is made to establish standard preparation method of SKR and to generate qualitystandards. Three samples of SKR were prepared with different quantities and forms of *Marich*.These samples and market sample of SKR were analyzed in laboratory using physico-chemical, phytochemical tests and HPTLC technique. Physicochemical and phytochemical analytical values of all four samples were nearby alike. However, S3 sample prepared with aqueous extract of*Marich* showed less percentage of Piperine and S1 sample showed comparative less percentageof Shogaols than three other samples. Slightly higher value of Piperine was noticed in S1 samplethan S2 and S4. Considering the property of Piperine as bio enhancer, S1 sample may be helpful to increase bioavailability and efficacy of the formulation. Thus, it is interpreted that the method stated in traditional formula could be useful toproducedesired effect.

Keywords: *Shwaskuthar Rasa*, *Marich* [*Pipernigrum*], HPTLC, Piperine, Shaogal, *Shwas* [Bronchialasthma]

Introduction:

Ayurvedic alchemy emphasized the use of herbo-mineral formulations, producing better effectsformanagementofdifferentaliments.Ayurvedicphysiciansareusingthesemedicinesforheal thcare in India wherein different forms of mineral and metals combined with herbal drugs areutilized in therapeutics. However, Ananad choudhary et al [2010] have interpreted that qualitycontrol and standardization is needed for herbo-mineral formulations prepared with differentmethods.[1]

ShwaskutharRasa,oneoftheherbo-mineralformulationscontainspurified*Vatsanabha*(*Aconitum ferox* Linn), detoxified *Parada* (Mercury), detoxified *Gandhaka*(Sulphur), popped*Tankana*(Borax) detoxified *Manahsila*(Arsenic disulfide). *Pippali*(*Piper longum* Linn), *Marich*(*Piper nigrum* Linn), *Shunthi*(*Zingiber officinalis* Linn), prescribed for the treatment of Cold,Asthma,Bronchitis,Anorexia,indigestion.[2]IndifferentformulaeofSKRquantityof*Marich* varies from one part to ten parts [3]. Similarly, quantity of *Pippali and Shunthi*also varies fromone part to six parts. It is also found that SKR available in market is prepared with mixing ofpowderof*Marich*insteadofaddingonebyoneseedsof*Marich*toallothercontentsofpowder.

Inpreviousstudies,characterizationof*ShwaskutharRasa*,XRD,FTIRanalysis[4]andeffectiveness in the treatment of respiratory disease conditions have been also reported [5,6,7].However, SKR prepared with variations in quantity of *Marich* ingredient, application of differentmethods and standardization using HPTLC techniques have not been explored till date. Hence,present study is proposed to develop standard preparation method of SKR, to provide qualityparametersandevaluatequantitativepercentageofPiperineinfoursamplesofSKRusingHPT LCtechnique.

MaterialandMethodology:

Preparation of *Shwaskuthar Rasa* formulations: Three samples of SKR were prepared usingvariations informs andquantities of*Marich*and*Pipalli*asdepictedinTable 1.

StandardOperatingProceduresofSKR:

Identification and authentication of all herbal and mineral drugs was done using consensusmethod with ayurvedic as well as modern parameters. Selected *Parad* [mercury] was detoxifiedin the mixture of garlic paste and rock salt while powdered *Gandhak*[sulphur] was detoxified incow ghee and cow milk as per ayurvedic standard guidelines. Raw form of *Manashila*[realgar]was powdered and then triturated with ginger juice, this process was repeated for seven timestomakeitinnon-detoxifiedform.*Vatsanabh[Aconitumferox]*wasdetoxifiedincowmilk,boiledfor three hours, washed with hot water and then well dried. All other ingredients of plant basedwere reduced to 60 mesh size powder. Detoxified mercury and sulphurwas triturated for 72hours to formulate black colored lusterless intermediate product [*Kajjali*]. Powdered detoxifiedrealgar[120meshsize]andallotherherbaldrugswerethenmixedproperlywith*Kajjali*with drytriturationmethodandconvertedintohomogenous mixture**.**

Table1:Contentsandvariationsoffoursamplesof*Shwaskutharrasa*

Samplesreference	**Ingredients**						
	DetoxifiedMercury	**Detoxified Sulphur**	**DetoxifiedRealgar**	**Detoxified*Aconitum ferox***	**Detoxified *Piperlongum***	**Detoxified*Zingiberofficinale***	**Detoxified *Pipernigrum***
S1	1part	1part	1part	1part	1/3rdpart	1/3rdpart	8&1/3rd part

S2	1part	1part	1part	1part	1part	1part	2part
S3	1part	1part	1part	1part	1/3rdpart	1/3rdpart	8&1/3rd part
S4	1part	1part	1part	1part	2part	2part	10part

Specificationsinpreparationmethods:

Sample1andsample2werepreparedinaccordancetoclassicaltextsformulae.Slightmodification was done in sample 3, instead of *Marich* powder water soluble extract was used.Sample4was marketsamplewhichcontains differentquantity of*Marich* powder.

In sample 1, except *Marich*, all other ingredients were separately grinded and sieved, then allweremixedwitheachothertoformhomogenouspowder.Powderedsinglekernelof*Marich*wasmixed to the homogenous mixture. In the similar manner each kernel powder of *Marich* wasaddedonebyoneinagivenquantityandmixedwelltoreceiveuniformcompoundformulation.[SKR]

Insample2,*Marich*kernelsofgivenquantitywasconvertedintopowder[60meshsize]ata glance,whichwasmixedwellwiththemixtureofallother ingredients.

Insample3,powdereddryextractof*Marich*[60meshsize]wasmixedwiththemixtureofallotheringredients.

ThreesamplesofmixtureofSKRwerethensubjectedtopreparetablets[125mg]byfollowingstandardmethodof tableting.Tablets were thenlabelledandstoredinair tightcontainers.

Organoleptic,Physicochemical,phytochemicalandHPTLCanalysisofSKR:

Four samples of*ShawaskutharRasa*tablets were analyzedasfollowsorganolepticstests[colour,touch,odour,taste]wasdoneasperstandardmethod

Qualitycontroltests[shape,size,hardness,weightvariation,friability,disintegration]asperstandard protocolPhysicochemicalcharacters [moisture content, ash value, alcoholsoluble extractives, watersolubleextractives,andpH] were evaluatedinlaboratory usingAPIparameters.

Phytochemicaltestingwasdonetoassesschemicalentitiesorgroupspresentinfoursamplesof SKR.

HPTLCtechniquewasperformedinAnchorlaboratory,Mumbaitostudyqualitativeandquantitative values of *Pipeline* [active constituent of *Marich* and *Papilla*] and shoals, [activeconstituentof*Santi*].

Methods:

Organolepticstests:

Color,size,shape,textureofthetabletswasnotedandanalyzedbyfollowing APImethods.

Qualitycontrol tests:

Weight variation was calculated wherein 20 tablets of SKR were taken and weighed separately,then average weight was noted. Weight variation was calculated using percentage deviationmethod.

Hardness of SKR tablets was estimated using Monsanto hardness tester. One tablet of SKR wasplaced radially between spindle and anvil. Pressure was applied till it breaks. Hardness wascalculatedinKgsq.cm.

Friability was done in Roche Friabilator instrument. Twenty tablets of SKR were weighed, thenput in instrument, which rotates at 25rpm. Dropping of tablets were done from a distance of 6inches during each rotation. The test was continued for 100 rotations. Afterwards, tablets werereweighed,the difference inweightwas notedandpercentage differencewas calculated.

Disintegrationwasdoneintabletdisintegrationtester.Themovementofbasketwasadjustedto30 rpm and at 37 º C temperature. One tablet of SKR was kept in each six test tubes containingdistilled water. Apparatus was started and testing was done till no residue remained in basket.Time takenfor completedisintegrationofalltablets werenoted.

Phytochemicalanalysis:

Aqueous extract of 20 g of SKR powder was done using soxhlet apparatus and water bath.Concentratedextractsofallfour SKRsampleswere tested for phytochemical screening. Standard methods were followed for detection of alkaloids, carbohydrates, tannins, steroids, saponins,flavonoids,andtriterpenoids

HPTLC analysis for estimation of Shogaols:

Chromatography analysis was done for Shunthi [dry ginger] powder and four samples of SKR.Here N-hexane and diethyl Ether [4:6] was used as mobile phase, silica gel was employed as stationary phase. UV scanning was done at 254 nm for detection of Shogaols for its qualitative and quantitative calculation. The peaks were studied and recorded.

HPTLC analysis for Piperine estimation:

Standard Piperine (99 % pure) was procured from an authentic source. 0.70 mg Piperine was dissolved in 1 ml of methanol, to prepare stock solution.

Accurately weighed 5 g of powdered *Shwaskuthar Rasa* samples were taken in a conical flask and extracted with 10 ml Methanol for fifteen minutes. The liquid extract was filtered through whatman's filter paper, in the concentration 500 mg/ml. All the reagents were of AR grade and procured from local source. Chromatography of *Shwaskuthar Rasa* was performed on aluminum silica gel 60 F_{254} HPTLC plates. Mobile phase used was Toluene: ethyl acetate (7:3). Tank was saturated with mobile phase for 30 min. Samples and standards were applied as sharp bands by means of 'CAMAG Linomat 4' sample applicator.

After drying the plate in a current of hot air, the plate was placed in one trough of a CAMAG twin trough chamber. The plate was developed until the solvent front had traveled 7 cm distance above the position of sample application. It was removed from chamber and dried in a current of hot air. Then it was scanned immediately using CAMAG TLC Scanner 3 at a wavelength of 254 nm. *Piperine* spots were observed at Rf. 0.37 in the samples and standard. TLC software version 1.2.3 was used for the detection as well as evaluation of data.

In order to establish linearity, standard solution of *Piperine* having different concentrations (3.0, 4.0, 5.0, 6.0, 7.0 µg /ml) were prepared by suitably diluting the stock solution with methanol. 2µl of standard solution was spotted as sharp band on the pre-coated HPTLC plate. The chamber was saturated with mobile phase for 30 min. The plate was immersed in mobile phase and allowed to travel a distance of 7 cm above the band applied. After development the plate was removed and dried under hot air.

The plate was scanned using CAMAG TLC Scanner 3 at a wavelength of 254 nm. Spot of *Piperine* were observed at Rf. 0.37. The graph of drug concentration against peak area was found to be linear. Linearity range was observed between 3 – 8 nm. The standard solution of *Piperine* and sample solutions were spotted on HPTLC plate. The percentage of *Piperine* in each sample was calculated by comparison of the area measured for the sample to that for the standard.

To study accuracy and precision of the method, recovery experiment was performed by the method of standard addition. Recovery of added standards was studied in a manner similar to that described for the assay. Based on recovery studies the mean recovery in case of '*Shwaskuthar Rasa*' was found to be 99.03%.

RESULTS:

Analysis of *Shwas kuthar rasa* for organoleptic tests revealed that three samples prepared in laboratory have shown greycolor and agreeable odor, while S4 showed brown color. Shapes and sizes of S1, S2 and S3 were similar [biconvex with 8 mm diameter] while market sample of SKR[S4] possess flat shape and 6mm diameter size. Weight variation, hardness, friability,

Disintegration time of all four samples have shown values comparable to standard values

[Table2]. The data of all four SKR samples of physico- chemical values were enumerated in table 3. Itindicates that pH was similar for all samples. SKR 3 showed higher percentage of alcohol solubleextractives compared to other three samples. All samples showed alike values in phytochemicalscreening.[Table 4]

Table2:Qualitycontroltestsresultsoffoursamplesof*ShwaskutharRasa*

Sr.No.	Parameters	Standardvalues	SKR1	SKR2	SKR3	SKR4
1.	Color	-	Grey	Grey	Grey	Brown
2.	Touch	-	Smooth,hard	Smooth,hard	Smooth,hard	Smooth,hard
3.	Odor	-	Agreeable	Agreeable	Agreeable	Agreeable
4.	Taste	-	Pungent	Pungent	Pungent	Pungent
5.	Weight variation	7.5%	5%	5%	5%	5%
6.	Hardness	>_4 kgsqcm	5	5	5	6
7.	Friability	>_1.6%	1%	1%	1%	1%
8.	Disintegration	>_15min	9min	9min	9min	5min

Table3:Physico-chemicalanalysisoffoursamplesof*ShwaskutharRasa*

Sr.No.	Parameters	SKR1	SKR2	SKR3	SKR4
1.	Moisturecontent	5%	6%	5%	6%
2.	AshValue	20%	24%	26%	28.8%
3.	Alcohol soluble extractive	26.80%	24%	36%	28.8%
4.	Water soluble extractives	22.2%	20.2%	25.2%	23.6%
5.	pH	8	8	8	8

Table4:PreliminaryPhytochemicalScreeningoffoursamplesof*ShwaskutharRasa*

Sr.No.	Parameters	SKR1	SKR2	SKR3	SKR4
1.	Alkaloids	+ve	+ve	+ve	+ve
2.	Carbohydrates	+ve	+ve	+ve	+ve
3.	Tannins	-ve	-ve	-ve	-ve
4.	Steroids	-ve	-ve	-ve	-ve
5.	Saponins	+ve	+ve	+ve	+ve
6.	Falvonoids	+ve	+ve	+ve	+ve
7.	Caratinoids	-ve	-ve	-ve	-ve
8.	Triterpenoids	-ve	-ve	-ve	-ve

[+]indicatesPresentand [-]indicatesabsent

Qualitativeandquantitativeestimationof *Piperine*and*Shogaols*:

Quantitative evaluation of *Piperine* and *Shogaols* of four samples of SKR is depicted in Table 5.Theassayvalueof*Piperine*inS1,S2,S3andS4samplesof*ShwaskutharRasa*wasfoundtobe0.21 %, 0.19 %, 0.11 % and 0.20 % respectively. Percentage value of *Shogaols* in four samples of*ShawaskutharRasa*wasestimatedindividuallyforS1,S2,S3andS4samples[0.521,1.431,0.920.1. 203]. Figure 1 shows typical chromatogram of four samples of SKR and standard *piperine*, andFigure 2represents*Shogaols*of Shunthi andfoursamples ofSKR.

Table5:DeterminationofPiperineandShogaolsoffoursamplesof*Shwaskuthar Rasa*

Sr. No.	Sample	PercentageofPiperine	PercentageofShogaols
1.	S1	0.21%,	0.522%,
2.	S2	0.19%,	1.431%
3.	S3	0.11%	0.920%
4.	S4	0.20%,	1.203%.

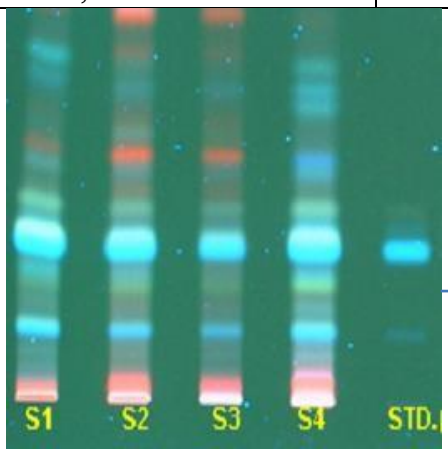

Figure1:IdentificationofPiperineinstandardpiperinesolutionandfoursamplesofShwaskutharrasathroughHPTLC

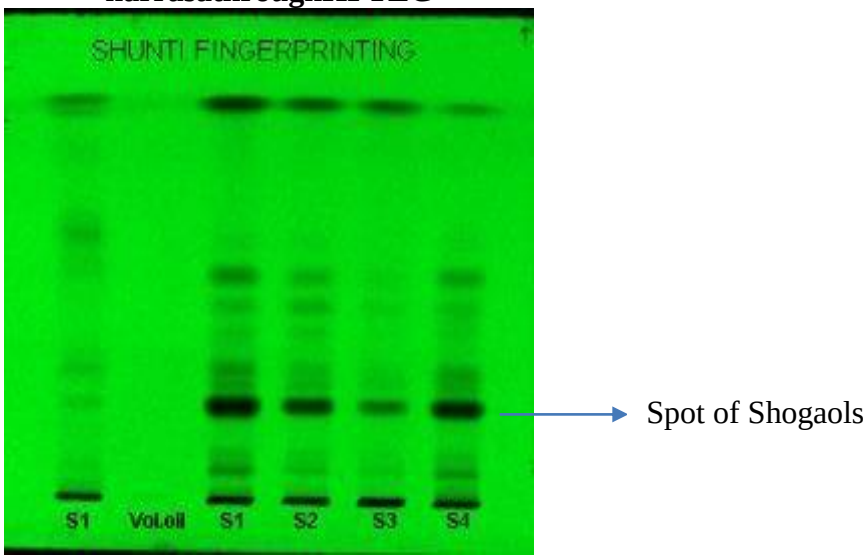

Figure2:HPTLCofShunthiandfoursamplesofShwaskutharrasa

DISCUSSION

Complexityinayurvedicherbo-mineralformulations,useofdifferentformulaeinpharmaceuticalpractice and unexplored standardization data, it is imperative to develop accurate methods foranalysisofherbo-mineralformulationsusingcurrentmoderntechniques.Onthesimilarthought,seeing the guidelines suggested for preparation of SKR and different method monitored incurrent practice, present study was planned to validate the preparation method and providestandardizationdataincludingHPTLCfor SKRformulation.

Present study reports show that S4 [market] sample had different color, size and shape, thisresult might be obtained as the source of contents and preparation method of formulation wasnotsimilarascomparedtootherthreesamples.Thoughfoursampleswerenotpreparedwith Similar proportions, still significant variation was not demonstrated in physico-chemical analysisof allsamples ofSKR.Itcouldnotbejustifiedinthepresentstudy.

Saponinsrevealedtohaveanti-inflammatory, antibacterial, antifungal, antiviral,

insecticidalactions[9]. Alkaloids are secondary metabolites, due to their presence in medicinal plants theyareusedinpharmaceuticalpreparationstoexhibitimportantbiologicalactionssuchasantioxidant musclerelaxantetc.[10].Medicinally,alkaloidsaremainlywellknownascardio protective,andanti-inflammatoryagents[11].

InphytochemicalscreeningofSKRsamples,differentgroupsoffunctionalentitiesfound[saponins,alkaloids]mayberesponsibletoproducecertain biological actions such as antioxidant, anti-inflammatory, antimicrobial, antiviral andusefultoprovide desired effectsintreatmentof respiratoryailments.

In HPTLC analysis, comparative lesser value of *piperine*in S3 sample [prepared with aqueousextract of *Marich]* was highlighted than other three samples. It is well known that *Piperine*alkaloid is sparingly soluble in water, [12]hence it can be inferred that addition of dry water-basedextract of*Marich*isnotworthwhile.

Inrecentstudies,itisclearlyrepresentedthat*Piperine*ofpipernigrumandpiperlongumusedinIndian and Chinese traditional medicines have shown anti-inflammatory, anti-infective, anti-microbial,anti-ulceractivities[13].Similarly,itisreportedthat*piperine*,isresponsibletodisplayantimicrobial,immune-modulatory,anti-proliferative,antioxidant,hepatic-protective,anti-allergic, anti-inflammatory and cardio-protective effects in experimental studies[14]. In recentstudies,bioavailabilityenhanceractionof piperineinthe treatmentof tuberculosisandinabsorbance of mercury of Shawaskuthar Rasa formulations were also reported[15,16].It is alsofoundinstudiesthatbioactivecomponentof*Shunthi*mitigateinflammatoryconditionsoccurredinasthma andshowed anti-inflammatoryaction.[17]

Therefore, it can be said that cumulative presence of piperine, shagol and alkaloids, saponins inSKR formulations synergistically might be exerting anti-inflammatory, anti-allergic, antioxidant,hepatoprotectiveactions.Highervalueof*piperine*alongwithpresenceofotherbio-componentsinS1sampleofSKRascomparedtootherthreesamplesprobablyshowencouragingtherapeuticeffectsintreatmentofShwas[Bronchial asthma].

Present study provides comparative physico-chemical values as well percentage of piperine andshagolforlaboratorypreparedandmarketedsamplesofSKR.However,toreachfortheselectionof appropriate formula for preparation of SKR formulation, bioavailability studies would befurtherplannedtounderstandtheabsorbanceofpiperineinrelationtoitspercentageconcentration.

Conclusion: Present study has generated laboratory analytical data including HPTLC assays for SKR sampleswhichwouldbeuseful toresearchers andpharmacists.TheHPTLCmethodusedforthedeterminationof*Piperine*of*ShwaskutharRasa*has beenfoundtobelinear,accurateandselectivefor the applicationinroutine quality controlanalysis.

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