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A Study on Phytochemical Evaluation of Ardra and Shushka Dosage Formsof Eclipta Alba Hassk. (Bhringaraj) Panchanga According to Sharangdhar Samhita

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Abstract:

Pharmacological properties of the plant drug depend on their phytochemical constitution. *Sharangadharacharya* has quoted to use *ardra dravya* always *dviguna* (twice) in quantity to *shushka dravya* as there may be changes in properties or quantity of the phytochemical constituents.

Aims and objectivesare to evaluate physicochemical & phytochemical properties of *ardra*and*shushka* dosage forms of *Bhrungaraj - Eclipta alba* Hassk.*panchanga*.

Methods- *Ardra* and *shushka* dosage forms of self-collected *Bhrungaraj panchanga* were prepared as follows-1) *swaras* (*ardra*/wet form), 2) *anukalpa swaras* (*ardra*/wet form), 3) *shushka churna* (dry form) and 4) tablet form (*shushka*/dry form). Various phytochemical tests for solid and liquid forms as per applicability - Foreign matter test, Extractive values, ash values, HPLC, HPTLC, Spectrophotometry, Atomic absorption Spectrophotometry,etc.

Observation and results- All the tests like extractive values, ash values, and others were having equal results in all dosage forms but atomic absorption spectrophotometry showed that Iron content was highest in *anukalpa swaras ghan vati*(dry form)- 3060 ppm, and comparatively very less in spray dried tablet (370.137 ppm), fresh *swaras* (108.0 ppm), and *churna* (211.84 ppm). Similarly, content of wedelolactone was maximum in *churna*(dry form) and minimum in fresh *swaras* (wet form).

Key words: *Ardra*, *shushka*, *guru*, *tikshna*, phytochemical and physicochemical,

Introduction

Pharmacological properties of the plant drug are based on the different phytochemical constituents[1]present within these plants and these phytochemical constituents produce definite physiological or pharmacological action on the human body[2].

The chemical composition of the plant drug is dependent on species identity and harvest time, collection time, maturity of plant, soil composition, altitude, actual climate, processing, storage conditions. The transformation, processing and degradation may cause changes in the phytochemical of compounds[3].*Ayurveda* has considered all these factors since ancient times and accordingly formularies has been established which are based on some basic principles for the treatment as well as formulation of medicine. One such *ayurvedic* formulary is *Sharangdhar Samhita* in which *Sharangadharacharya*[4]has described to use s*hushka dravya* (dry drug) which is freshly collected from plant source and recently dried (not to use old *dravya* as *shushka dravya*) in the preparation of medicine. Similarly, when there is reference

of *ardra dravya* (fresh plant drug) always use freshly collected samples from plant source and it is also stated to use *ardra dravya* twice in quantity to that of *shushka dravya*. This is because there may be some difference in the properties or quantity of the phytochemical constituents present in the drug in *shushka* and *ardra avastha* (condition).

Therefore, a study was planned to understand this concept to evaluate the difference in physicochemical and phytochemical properties in *ardra* and *shushka dravya* with the help of various physicochemical and phytochemical analytical tests.

Eclipta alba is an annual herbaceous plant, commonly found and having a long history of traditional medicinal uses. A wide range of chemical compounds including coumestans,alkaloids, thiopenes, flavonoids, polyacetylenes, triterpenes[5,6,7,8]and their glycosides have been isolated from this species. High therapeutic and medicinal values are due to its chemical composition with wedelolactone, desmethylwedelolactone, 14-hepatocosanol, luteolin-7-0-glucoside, alkaloids and polypeptides as principle components[9].Because of its varied medicinal values, it has great commercial demand which calls for further investigation at the bimolecular level. For the same reason, this species needs prime attention for its future cultivation and conservation[10]. *Bhavaprakash Samhita*[11]has described that *Bhrungaraj* is especially effective in *yakrut vikruti, yakrut vruddhi, pandu, kamala, shotha* and other diseases related to *yakrut*. *Dhanvantari Nighantu*[12]*,Raj Nighantu*[13]*,Kaiyyadev* Nighantu[14]has also described that *Bhrungaraj* is indicated in *pandu, shotha, kamala* and *Vaghbhata* advocated its consumption for one month to have *rasayana* effect. So*Bhrungaraj* was selected for the *Shushka* and *ardra* dosage forms of *Bhrungaraj*. **Aims and objectives:** To study physicochemical and phytochemical propertiesof*shushka* and *ardra* dosage forms through physicochemical and phytochemical properties tests and to understand the difference in chemical constituents of ardra and shushka forms.

Material and methods:

Samples of *Bhrungaraj* were self-collected gathered from its natural habitat from Naginaghat area of Nanded. The plants were botanically identified[15]and authenticated by local botanists of Botany Department, Department of Botany, Nanded Education Society's (NES) Science College, Nanded and confirmed as *Eclipta alba* Hassk.

Place of work:

Drug testing laboratory, Government Ayurved and Unani Pharmacy, Nanded and Qualichem laboratories, Nagpur.

Organoleptic andmicroscopic examination:

The sample of *Bhrungaraj* was examined for all its organoleptic characters and microscopic examination[16].

Preparation of study drug samples for physicochemical study

For the evaluation of physicochemical and phytochemical properties of *ardra* and *shushka* dosage forms of *Bhrungaraj panchanga* four different dosage forms were prepared such as –

1. *swaras* (*ardra*/wet form),
2. *anukalpa swaras* (*ardra*/wet form),
3. *shushka churna* (dry form) and
4. tablet form (*shushka*/dry form).

For this purpose, different dosage forms used were -

1. *Swaras,* (*ardra*/wet form)
2. Spray dried *swaras ghan vati,* (*ardra*/wet form)
3. *Anukalpa swaras* (*shushka*/dry form)*and*
4. *Ghan vati of anukalpa swaras*(*shushka*/dry form)

Procedure for preparation of the above dosage forms:

1. **Procedure for preparation of *swaras*:**The plant samples free from any pest, disease or decay and fresh were collected, cleaned and then crushed to paste like form which was tightly held in clean piece of cloth and the *Bhrungaraj swaras* was obtained after filtering through the cloth as per procedure described in *Sharangdhar samhita*[17].
2. **Procedure for preparation of *anukalpa swaras*:** Dried plant material was grinded to prepare *churna* and to one part of churna water was added twice in quantity (*dviguna*). The filtrate was kept overnight and was smashed with hands and then filtered with clean cloth this filtrate was used as *Anukalpa swaras* (*nishoshit swaras/ swaras* from *shushka dravya*)[18, 19].

3. Procedure for preparation of *ghan vati* of *anukalpa swaras*: As per the method described in *Siddha sara sangraha*[20]*churna* was taken once in quantity and water was added four times to it. The mixture was boiled until 1/4th of the mixture was left. This was then cooled and filtered with clean cloth and then the filtrate was kept for boiling again until it became thick paste and then it was cooled and passed to tablet making machine for preparation of tablets of 500 mg eachnamed as *anukalpa swaras ghan vati*. Tablets were obtained and dried, packing of tablets was done under hygienic condition.

4. Procedure for preparation of dried form of s*waras* (spray dry *swaras ghan vati*): The spray drying procedure of fresh *swaras*was conducted in five steps as per the standard operating procedure[21] - a).*Bhrungaraj swaras* was concentrated by heating and then introduced into the spray dryer. b).Evaporation of the introduced swaras was started using rotatory atomizer by exposing the concentrated swaras spray with the heated air. c). After atomization the atomized liquid was exposed to hot air at vacuum and here evaporation process was completed along with evaporation of water droplets. d). Temperature was maintained and complete moisture was evaporatedand resultant particles obtained in the form of irregularly shaped porous spheres.e). The obtained powdered particles were separated from the bottom of the instrument. *Ghan vati* of this spray dry powder was preparedand named as spray dry *swarasghan vati*.Tablets were obtained and dried, packing of tablets was done under hygienic condition.

Methodology for physicochemical studies:

To evaluate the difference in physicochemical and phytochemical properties of shushka (dry) and ardra (wet) dosage forms different tests were applied as per applicability.

Tests applied for solid form of drug

1. Physicochemical tests
2. Phytochemical tests
3. Chromatographic analysis
4. Spectrophotometry UV
5. Atomic-absorption Spectrophotometry

Tests applied for liquid form of drug

1. Specific gravity
2. Viscosity
3. Spectrophotometry
4. Chromatographic analysis
5. Atomic-absorption spectrophotometry

Determination of Foreign matter

The collected raw material of drug sample was spread on white paper and foreign matter was separated, weight was taken and the percentage of foreign matter present was calculated[22].

pH Value

Determination of pH– 1 gm of powdered drug was extracted in 100 ml of distilled water and the pH was measured using previously calibrated pH meter[23].

Determination of swelling index- 1 gm of powder was soaked in 25 ml of water in a glass topped measuring cylinder was shaken vigorously and kept standing for 3 hours, then the height of *churna* was calculated and difference calculated from initial height reading[24].

Determination of foaming index (F.I.)-1% water extract of *Bhringaraj churna* was diluted to 100 ml, this solution was then added to 10 stoppered test tubes as 1 ml, 2 ml, 3 ml, etc. up to 10 ml and made to 10 ml each by adding distilled water and shaken vigorously,height of foam was measured as FI = 100/a
Where a = the volume in ml of decoction[25].

Determination of moisture content / loss on drying -The instrument Halogen moisture content analyzer was switched on and initially weighed sample was added.After pressing the start key the drying process was started.At the end of the process drying temperature, weight after drying, total drying time and % of moisture content displayed[26].

Digital Colorimetry-5 % of water extract of *Bhrungaraj* was used for colorimetry analysis with reference to distilled water.The wavelength adjusted as per the color of solution. The sample of *swaras* and *anukalpa swaras* were placed in cuvette and the readings were taken[27].

Determination of Extractives - 5% alcohol extract of the *Bhrungaraj churna*was prepared in shaker machine by shaking for six hours and standing for 18 hrs.The extract was filtered in previously weighed empty petri dish and was allowed to evaporate till complete drying and weight taken. The difference in weight and the percentage was calculated. Same procedure applied to anothersolvents[28].

Determination of total ash value- 1 g of powdered sample taken in a previously weighed empty platinum crucible was burnt to ash.The difference in weight and % was calculated. Similarly,acid insoluble ash and water-soluble ash was determined as per method described in API[29].

Spectrophotometry- In UV visible double beam scanning spectrophotometer,5% water extract of *Bhrungaraj*powder was used for analysis. The wavelength (800-400, 400-200 nm), scanning, speed; absorbance, etc. were set and reference was taken as water of the compartment, scanning was started and the specific absorption spectrum was displayed on the monitor. The numbers of peaks, their absorbance of respective wavelength was recorded.
Wedelolactone, Ecliptaalbasaponin I and II were used as standards for comparison[30].

Thin Layer Chromatography analysis - 5% extract of *Bhrungaraj* samples were prepared by using different solvents.The cleaned glass plates were coated with Silica Gel –G slurry using the spreading device toa thickness of 0.25 mm.The plates were dried and activated. Thespots of samples were applied with the help of a micro capillary and dried[31].
The chromatography chambers were activated by the saturation of fumeso the solvent mixtures used as mobile phaseevaluated on the basis of the nature of the components by trial and error.Ascending technique was used and the level of the solventflow was marked and the observed spots were marked with needle in daylight and ultraviolet light. The retention factors(Rf value) were calculated as described in the observation and results.

Atomic absorption Spectrophotometry- The water extracts of all dosage forms were prepared,instrument was calibrated, the standard solutions were introduced into the flame and then the extracts were introduced, the steady readings were recorded.The apparatus was washed through with water after every trial. The concentrations of the elements were determined from the calibration curve[32].

High performance thin layer chromatography (HPTLC)- The methanolic extract of all dosage forms wereprepared.Wedelolactone, Ecliptaalbasaponin-I and Ecliptaalbasaponin-II were used as marker compounds; these markers were dissolved in methanol at a concentration of 1 mg/ml. Samples were applied above the lower edge of the plate and points were marked with a pencil.A twin trough chamber was filled with mobile phase solvent mixture and was left for saturation. The plates were kept in the chamber and removed whenthe mobile phase travelled the distance of 6 cm and dried. After that derivatization reagent was sprayed and the plates were visualized and chromatograms were observed and compared with monograph and standards used[33].

High performance liquid chromatography (HPLC) - Luna 5-micron NH_2 100 angstrom column was used for performing reverse phase high performance liquid chromatography using the above prepared dosage form sample extracts prepared previously and wedelolactone, Ecliptaalbasaponin I and II were used as standards[34].

Phytochemical analysis

Qualitative phytochemical analysis of the extracts was carried out such as-

1) Test for tannins[35],
2) Test for resins[36]
3) Test for saponins[37]
4) Test for glycosides/carbohydrates[38]
5) Test for proteins[39]
6) Test for sterol[40]
7) Test for amino acid[41]
8) Test for alkaloids[42]
9)Test for starchnon-reducing polysaccharides[43]

Observation and Results:

Macroscopic and Microscopic examination

The macroscopic examination of *Bhrungaraj* entire fresh plant as shown in figures 1-6.

Organoleptic characters:

Table 1: Organoleptic characters of *Bhrungaraj*

Sr no.	Characters	Bhrungaraj powder	Spray dry powder
1	Colour	Dark green	Dark green
2	Odour	Mild	Mild
3.	Taste	Sour and bitter	Sour and bitter
4.	Structure	Smooth	Smooth
5.	Colour of water extract	Dark green	Dark green

Physicochemical standardization

Table 2: Test for determination of foreign matter

Samples	Weight of foreign matter collected	Percentage
Entireplant (dried)	54 gms	1.5 % W/W

Table 3: pH values

Samples	pH	pH after 24 hours
Bhrungaraj Churna	4.2	4.2
Bhrungaraj Swaras	4.1	4.1
Bhrungaraj Tablet	4.2	4.2
Bhrungaraj Anukalpa Swaras	4	4

Table 4:Swelling index of *Bhrungaraj churna*

Observation	*Bhrungaraj Churna*	Spray Dry Drug
Mean	1.03	1.03

Table 5: Moisture content

Sr. No.	Sample	Initial Wt in gms	Wt. Afterwards (gms)	Loss (In Gm)	% M.C.
1.	*Bhrungaraj churna*	1.004	0.988	0.016	1.5936
2.	Spray Dry Powder	1.009	0.992	0.017	1.6848

Table 6: Foaming index

Sr. No.	*Swaras*	Spray Dry Drug	Tablet	*Churna*
Foaming Index	<100	<100	<100	<100

Table 7: Optical density at (670 nm) (Colorimetry analysis)

Sample	Optical density
Swaras (partial free)	0.41
Anukalpa Swaras (partial free)	0.25

Table 8: Extractive values of *Bhrungaraj churna*

Type of extractives	*Bhrungaraj Churna*	*Bhrungaraj* spray dry drug
Alcohol soluble	9.8	12.5
Water soluble	26.00	26.6
Methanol soluble	14.7	19.63
Petroleum ether	2.80	2.8666
Benzene soluble	13.0	13.6
Chloroform soluble	10.79	11.4
Ethanol soluble	9.5	10.6

Table 9: Ash values

Observation	*Bhrungaraj Churna*	*Bhrungaraj* spray dry drug
Total ash	14.4	4.4
Acid insoluble	3.06	1.7333
Water soluble	12.4	3.2

Tests applied to *Bhrungaraj swaras*

Table 10: Refractive indices

Samples	Fresh *swaras*	*Anukalpa Swaras*
RI	1.3434	1.3312

Table 11: Spectrophotometry analysis

Sample	Wavelength in nm	Absorbance
Fresh *swaras*	215.0, 330.0	1.76, 1.00
Churna	215.0, 330.0	1.76, 1.40
Spray dry *ghan vati*	390.0, 330.0	3.50, 2.50
Anukalpa swaras ghan vati	230.0, 330.0	2.270, 1.15

Table 12: Thin layer chromatographic values of *Bhrungaraj churna*

Rf values: S.F. = 15.8 cm

Extract	Mobile phase	I/ Vis	Under UV light	In Iodine chamber
Petroleum ether	Mobile phase used- Benzene: Chloroform	0.02	--	--
Benzene	Chloroform: Ethanol ((9.5:0.5)	0.8	0.53	0.42
Chloroform	Chloroform: Ethanol ((9.5:0.5)	0.8	0.53	0.4
Ethanol	Chloroform: Ethanol ((8:2)	--	--	--
Water	Benzene: Acetic acid: water (4: 1.1:4.9)	--	--	--

All spots in visible light were of yellowish brown color and UV spots of blue and fluorescent color.

Table 13: Atomic absorption spectrophotometry

Sr.no.	Samples	Elements (concentration in ppm) Iron (Fe)
1.	Fresh juice (swaras)	108.0
2.	Dry Powder (churna)	211.84
3.	Spray dry form (spray dry tablet)	370.137
4.	Tablet	3060.0

Table 14: HPTLC analysis to determine content of wedelolactone

Markers used for comparison: wedelolactone, ecliptaalbasaponin I and ecliptaalbasaponin II

Ecliptaalbasaponin I and Ecliptaalbasaponin II could not be detected in all the samples of fresh juice, spray dry tablet, *anukalpa swaras* and *anukalpa swaras ghan vati*.

Sr. no.	Samples (formulations)	Content in % w/w
1.	Fresh juice	0.005
2.	Dry powder	0.25
3.	Spray dry tablet	0.014
4.	Tablets	0.023

Table 15: HPLC analysisto determine content of wedelolactone by HPLC

Markers used for comparison: wedelolactone, ecliptaalbasaponin I and ecliptaalbasaponin II

Ecliptaalbasaponin I and ecliptaalbasaponin II could not be detected in all the samples of fresh juice, spray dry tablet, anukalpa Swaras and Anukalpa swaras ghan vati.

Sr. No	Samples (formulations)	Content In % W/W
1.	Fresh Juice	0.007
2.	Dry powder	0.25
3.	Spray dry tablet	0.014
4.	Tablet	0.017

Phytochemical analysis

Table 16: Preliminary qualitative analysis of alcoholic extract of powder of *Bhringaraj* for the presence of various functional groups

Sr.	Reagent	Functional group	observation	Result
1	Alcohol	Resins	Turbidity	absent
2	Sodium bicarbonate	Saponin	Frothing	present
3	Biurets test	Proteins	Yellow ppt	present
6	Wagner's reagent	Alkaloids	Brown ppt	present
7	Dragendroff's reagent	Alkaloids	Brown ppt	present
8	Salkowinskii's reaction	Sterols	Green ppt	absent
9	Liebermann's Buchard	Sterols	Green ppt	absent
10	Dil. $FeCl_3$ Test	Tannin	Blue ppt	present
11	Lead acetate test	Tannin	No ppt	present
12	Benedict's reagent	Glycosides	Violet colour	present
13	Fehling's reagent	Glycosides	ppt formation	present
14	Neutral $FeCl_3$	phenols	Violet colour	present

Discussion

Sharangadharacharya had described that in the drug formulation, *shushka* form[4] (dry but freshly collected and dried) should be taken in single quantity and while using the fresh drug or during the unavailability of the fresh drug, the green fresh plant drug should be taken twice in quantity to that of dry drug as the dry drug is *guru* and *tikshna* due to the lack of moisture and less concentration of chemical constituents[44]. This rule is applicable to all the drugs exceptfew enlisted[45,46].Various dosage forms of *Bhrungaraj panchanga i.e. ardra* and *shushka* dosage forms were prepared; these were analyzed by various physicochemical and phytochemical tests. *Ardra* forms were prepared as, a) fresh juice and b) spray dried powder of fresh juice.*Shushka* forms were prepared as, a) *anukalpa swaras* from *churna* of *shushka* drug and b) *ghan vati* of *anukalpa swaras*.

Physicochemical and phytochemical tests

Various tests were appliedas per applicability of solid and liquid form and results were as follows- Foreign matter [22]contentwas (table no 2) complying with Ayurvedic pharmacopoeia of India (API) standards. pH value [23]of all the samples (table no 3) was in the range of 4-4.5.Swelling index [24]of *Bhrungaraj churna* and spray dry drug was not significant as the drug was not having any mucilage content (table no 4).The test for foaming index[25]suggestive of saponin content was >100 in all samples and the saponin test (foam test) was found positive for all samples suggesting that *Bhrungaraj churna* was having some saponin content (table no.6).Percentage of moisture content[26] (table no 5) for *Bhrungarajchurna* and spray dry drug complied the API standards for *churna*.Colorimetry (test for determination of optical density)[27] showed optical density of the fresh swaras was more (0.41) than the *anukalpa swaras* (0.25) indicating that the fresh juice was more concentrated than the *anukalpa swaras* (table no.7).Extractive values [28] of *Bhrungarajchurna* and *Bhrungaraj* spray dry powder were evaluated - Alcohol soluble extractive value for *Bhrungarajchurna,* 9.8 %, was not less than 5 percent which complied the API standards and for the spray dry powder it was 12.5. The aqueous extractive value for *Bhrungaraj churna* was 26.00 % which was not less than 15 percent described in API, so it complied the API standards and for the spray dry powder it was 26.6%.Other extractives such as Methanol extractive, Petroleum ether soluble extractive value, Benzene soluble extractive value were also evaluated.(table no 8)From the above observations, it was clear that solubility of *Bhrungaraj churna* and spray dry form was more in water, than other solvents.

Test for determination of ash value[29]revealed that total ash value of *Bhrungaraj churna* was 14.4 which was not more than 22 percent, thus complied the API standards. (table no.9)

Acid insoluble ash of *Bhrungaraj churna* was 2.4 which was not more than 11 percent and thus complied the API standards and acid insoluble ash value of *Bhrungaraj* spray dry powder was 1.7.Water soluble ash of *Bhrungaraj churna* was 12.4 and total ash value of *Bhrungaraj* spray dry powder was 3.2.

It was observed that ash values of *Bhrungaraj churna* was more than the ash values of *Bhrungaraj* spray dry powder, all the values of *churna* complied the API standards.

Refractive index test suggestive of uniformity of solvent (oils, liquids, etc.), thickness or thinness and admixture was evaluated (table no 10) for freshly prepared *swaras* which was 1.3434 and that of *anukalpa swaras* was 1.33. Refractive index for both the solvents was nearly same which showed their similar texture or thinness and homogeneity.

Spectrophotometry analysis[30] showed common peaks at 220 nm, 330 nm in all the samples at the absorbance of 1.750 and 1.000 respectively. (table no 11). Thin layer chromatography[31]was carried out using various mobile phases, the spots were observed in UV chamber and Rf (factor of retention) values were calculated. (table no.12)

a. Petroleum ether extract of *Bhrungaraj churna* and spray dry powder using mobile phase, benzene:chloroform (1:1) showed similar spots of Rf value, 0.02 in visible light (200-400nm).

b. Benzene extract and Chloroform extractof *Bhrungaraj churna* and spray dry powder using mobile phase, chloroform:ethanol ((9.5:0.5) showed spots of Rf value, 0.8 (visible light), 0.53 (UV) and 0.42 (iodine chamber)and many other phases were tried.

Separation of constituents of *Eclipta alba Hassk.* was found maximum in chloroform and benzene extract using the mobile phase of chloroform: ethanol (9.5:0.5). Spots of TLC using wedelolactone were similar in both Bhrungaraj churna and spray dry drug but spots using Ecliptaalbasaponin I and II could not be detected.

Atomic absorption spectrophotometry[32]analysis determined that the iron content was- fresh*swaras*- 108.0 ppm elemental iron, *churna*- 211.84 ppm, spray dried tablet- 370.137 ppm, *anukalpa swaras ghan vati*- 3060 ppm. (table no.13) The *anukalpa swaras ghan vati* determined that iron content was 10 times more as compared to the spray dry drug, *churna* and fresh *swaras of Bhrungaraj*. In the process of spray drying the fresh juice was exposed to high temperature and high pressure while passing through the chambers, the exposure to heat might have affected the iron content in the spray dry drug.

The fresh juice is dilute due to the presence of water contentwhile *Bhrungaraj anukalpa swaras ghan vati* was prepared by processing of adding water to *churna* and boiling until the preparation of paste like thick slurry form to make tablets. So, evaporation of moisture, might have increased the iron content in *anukalpa ghan vati* as compared to *churna*.

High performance thin layer chromatography[33]was carried out taking all the four formulations (table no 14) against the above mentioned three markers and the observations were as follows, content of wedelolactone was only identified and the other markers used i.e. ecliptaalbasaponin I and ecliptaalbasaponin II were not detected in the HPTLC analysis. Percentage of wedelolactone was also maximum in *churna* and minimum in the fresh *swaras*.

High performance liquid chromatography[34]carried out for all four samples against three markers (table no 15). Wedelolactone content was nearly similar to that of the results obtained by HPTLC. The Ecliptaalbasaponin I and Ecliptaalbasaponin II could not be obtained in all the four samples or formulations.

The Wedelolactone is the important active coumestan derivative of *Eclipta alba*. Percentage of Wedelolactone was found more in the *Bhrungaraj churna* (0.25%w/w), *anukalpa swaras ghan vati* (0.017%w/w) and comparatively less in spray dry powder *ghan vati* (0.014%w/w) and fresh juice (0.007%w/w).

The concentration of this constituent might be more in the dried state (churna) than in fresh juice and in spray dry powder *ghan vati* form. As per the reference of *Sharangdhar Samhita*[44]dry drug is *guru in guna and tikshna* due to the lack of moisture and its *tikshna guna* may be due to the increased concentration of its active constituents in dry state.

The stability of wedelolactone might be more in dried form than in the fresh or wet form, so this may be the reason for more percentage of wedelolactone in *churna* than in fresh juice. Moreover, the spray dry drug was prepared by passing the fresh juice through hot air under pressure while churna is prepared by shade drying of raw material and then grinding. The processing in hot air may have altered the wedelolactone content of the drug in spray dried form.

Causes of changes in phytochemical and physicochemical studies:

The possible reasons might be, during the spray drying procedure, fresh juice was directly exposed to heat and so the active constituents may have evaporated during the direct heating process. In *Bhavprakash Nighantu, Acharya* KC Chunekar[47] had described to use fresh swaras of *Bhrungaraj* and no heating to be done as the active chemical constituents may get evaporated due to exposure to heat.

All the *ardra* and *shushka*formulations or dosage forms were evaluated for their physicochemical and phytochemical studies which studies revealed that there was little quantitative difference in the contents of the drugs in HPLC and HPTLC peaks and absorption spectrums (in Wedelolactone content), but atomic absorption spectrophotometry analysis revealed that elemental iron content was found more in the *anukalpa swaras tablet* form (*shushka* form)as compared tothe spray dry tablet (*ardra* form).

Conclusion

Present study revealed that *shushka* form of *Bhrungaraj i.e. anukalpa swaras ghan vati* had more percentage of wedelolactone and Iron content as compared to spray dried *swaras* tablet i.e. the *ardra* form of *Bhrungaraj* in double dose in iron deficiency anemia. So, the concept of using shushka drug twice in quantity to that of ardra drug to obtain equivalent results or contents was not observed in this study.

Limitations/recommendations

1. As *swaras* was difficult to prepare and restore for longer duration spray dried *swaras* was used in which the fresh juice is directly exposed to high temperature under high pressure to convert into powder form. This process could have caused variation in results.
2. HPTLC analysis were carried out by dissolving the formulations in different solvents. So, the concentration of the chemical constituents may differ due to different solubilities. So, the activity of *Bhrungaraj swaras* has to be further explored.

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Figure 1: Entire plant of *Bhrungaraj*

Figure 2: Inflorescence

Figure 3: Root of Eclipta

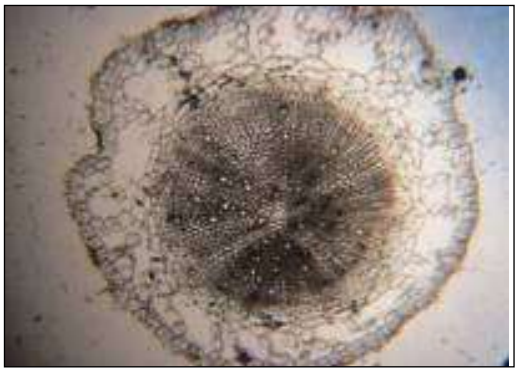

Figure 4: Root TS of *Eclipta*

Figure 5: Eclipta Flower

Figure 6: T. S. of stem