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PHYTOCHEMICAL STANDARDIZATION AND ANTIOXIDANT EVALUATION OF NOVEL FORMULATION SAHAJ VATI

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ABSTRACT

The present investigation involves antioxidant and phytochemical evaluation of herbomineral formulation *Sahaj Vati*. The antioxidant activity was performed using 2,2-diphenylpicrylhydrazyl (DPPH) assay. The results of study proved prompt antioxidant activity of *Sahaj Vati* in DPPH assay. The concentration dependent antioxidant activity of formulation was observed up to an optimum level. The presence of specific phytoconstituents considered responsible for the antioxidant activity of *Sahaj Vati*. Phytochemical investigation of herbal formulation was also performed using HPTLC, IR and UV-Visible spectrophotometer. The establishment of standardization parameter by modern technique provides figure printing profiling of herbomineral formulation *Sahaj Vati*.

KEYWORDS: Herbomineral formulation, *Sahaj Vati*, Phytochemical, Antioxidant.

INTRODUCTION

Sahaj vati is one of the poly-herbal formulation utilized by some researchers for various therapeutic purposes.[1,2] The formulation consisted of ingredients such as; *Sudha Shilajeet, Guggul, Chitrak* and *Haridra* prepared by *bhavana* of *Agnimanth kwatha*.[2-4] *Suddha Shilajeet* & *Suddha Guggul* were mixed with powder of *Haridra* & *Chitrak* by seven *Bhavana* of *Agnimantha Kwatha*. Mixture processed in mechanical *Kharal* and after complete drying *Vati* was prepared in uniform size. The chief ingredients of formulation possess antioxidant, anti-obesity and anti-inflammatory properties.[2-5]

Herbs and herbal products contributed greatly towards the healthcare management and their uses increases day by day due to safety concern. However use of herbal products requires great care towards the quality standard of natural ingredients. The standardization of herbal formulation using various analytical techniques helps to establish quality parameter and composition of formulation may also be ensured. The quality standardization by analytical technique also helps to confirm marker compound of formulation in qualitative as well as quantitative manner. These modern analytical techniques such as HPLC, HPTLC and UV-visible spectroscopy offers great advantages towards the high quality standardization of herbal products.[6-8]

MATERIALS AND METHODS

Ingredients of *sahaj vati* were procured from local market while other chemicals and reagents were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

Preparation of *sahaj vati:* *Haridra* and *Chitrak* were powdered by milling process after drying; purification of *Shilajeet* and *Guggul* was done as per standard protocol. *Shilajeet, Guggul, Haridra* and *Chitrak* were mixed in equal proportion then processed with decoction of *Agnimantha* for seven times, finally mixture was processed in *Kharal* to prepare uniform particle size *Vati*.

Moisture content Analysis: Drug sample was dried at 100°C in hot air oven for 24 h and difference in weight was calculated as moisture content.

pH Analysis: Drug sample was grounded by mixer grinder and a suspension in 1:10 ratio (sample: distilled water, w/v) was prepared, stirred and then pH was measured using digital pH meter.

Preparation of Plant Extract: Air dried powder of grinded plant material was dissolved in petroleum ether in a container plugged with cotton wool and shaken continuously. The supernatant was discarded and remaining solvent allowed to evaporate, dry powder obtained after ether extraction dissolved in methanol and rotated frequently to obtained methanolic extract of defated plant material. These extracts were used further for evaluating antioxidant activity.[7,8]

Phenols and tannins: The 50 mg extract was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. Formation of bulky white precipitate observed as a presence of phenolic compounds. The presence of tannin tested by mixing 1 ml of 5% $FeCl_3$ solution with dried extract and formation of bluish black observed as confirmatory test of tannins.[9]

Antioxidant Activity: Antioxidant activity of *Sahaj Vati* was evaluated by measuring its potency to inhibit free radical produced by DPPH radical[10]. Different concentrations (100-500 µg/ml) of drug extract were added to 5 ml of 0.004% solution of DPPH in methanol. Incubated for 30 min. at room temperature in dark condition then absorbance was measured against blank at 517 nm using UV-Visible spectrophotometer. The antioxidant activity of drug extract confirmed by measuring reduction in absorbance which ensure inhibition of DPPH free radical by various aliquots of sample.

Percent inhibition with respect to concentration was calculated as follows:

% inhibition =

(Absorbance of the control – Absorbance of the test compound / Absorbance of the control)×100.

HPTLC analysis: HPTLC systems equipped with WinCATS-4 software having Linomat-5 applicator were used. Solvents used for HPTLC analysis were of required grade. The dried methanolic extract of formulation was expected to possess most chief phytoconstituents of plant therefore methanolic extract subjected for further evaluation. Solvents combinations with varying polarity were tested for selecting appropriate mobile phase using trial and error based approach. Combination of methanol: ethyl acetate (5.5: 4.5) was selected as mobile phase and precoated Silica Gel G-60 plate was used as stationary phase. The samples (10 µl) spotted in with a Camag microlitre syringe on pre-coated plate. The sample loaded plate was kept in TLC twin trough developing chamber saturated with mobile phase then mobile phase allowed to run up to the optimum level for plate development. Linear ascending development

was performed and developed plate was air dried to evaporate solvents. Finally plate was scanned by using UV Densitometric scanning Camag scanner to detect band spot.[11, 12]

The overall chromatographic conditions are as follows

- Band length 6 mm.
- Application rate 3 µl/sec.
- Distance between bands 4 mm.
- Distance from the plate side edge 8 mm.
- Distance from the bottom of the plate 2 cm.

Ultraviolet visible absorption (UV): The methanolic extract of formulation was analyzed in UV-Visible spectrophotometer (UV-1800, Shimadzu) for its characteristics spectrum record ranging between 200-780 nm.

Infra-red spectroscopy (IR): Infra-red spectroscopy of final extract was also performed to confirm presence of specific functional group. The absorption spectrum recorded over the frequency range from 4000-400 cm^{-1} and characteristic peaks of different functional group were identified.

RESULTS AND DISCUSSION

The result of moisture content analysis, pH Analysis, appearance of methanolic extract and total phenolic content were reported in **Table 1**. The pH of samples were found to be towards slightly acidic range since major ingredient of formulation such as; *Suddha Shilajeet* and *Suddha Guggul* having slightly acidic pH. Moisture content was found to be near about 1 % (0.78 %) for *Sahaj Vati* which was found to be slightly higher side as compare to their ingredient. Quantitative analysis confirmed presence of polyphenols and steroids; since chief ingredient also rich in polyphenols. The presence of polyphenols expected to impart antioxidant potential in formulation. Therefore methanolic extract of formulation was further evaluated for its radical scavenging activity.

Table. 1: Parameters and total phenolic content.

S. No.	Evaluation	Results
1	Moisture content analysis	0.78 %
2	pH Analysis	5.9
3	Appearance of methanolic extract	Brownish-gray
4	Total phenolic content	11.04 (g/100g TAE)

Free radical scavenging potentials of *Sahaj Vati* determined using DPPH assay and results were expressed as % inhibition of free radical by drugs. Formulation showed dose dependent antioxidant potential and radical scavenging potentials increases with concentration in non-linear manner. The 33 to 74 % inhibition was observed for different concentration range of formulation as shown in **figure 1**. Presence of polyphenols and other constituent may be considered responsible for antioxidant activity of *Sahaj Vati*.

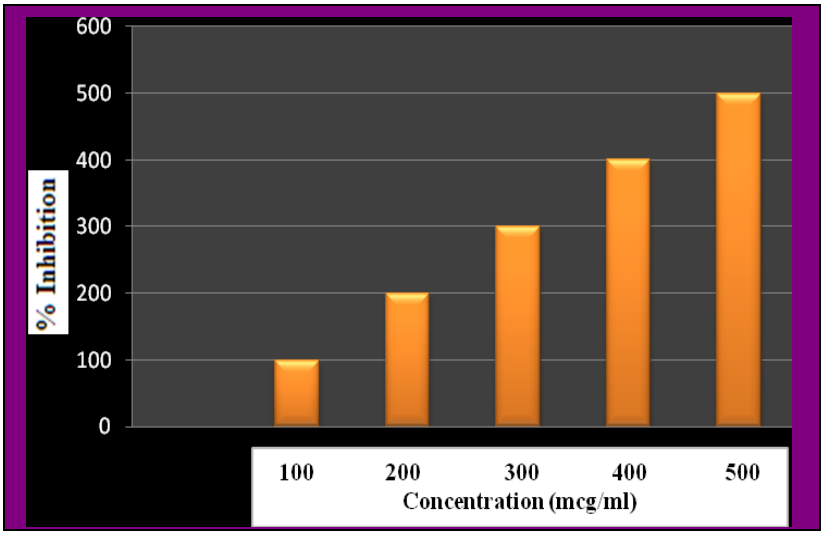

Figure. 1: Antioxidant activity of *Sahaj Vati*.

UV-Visible spectra of extract are shown in **figure 2**. The characteristic UV spectrum of *Sahaj Vati* showed absorption maxima at 274 nm.

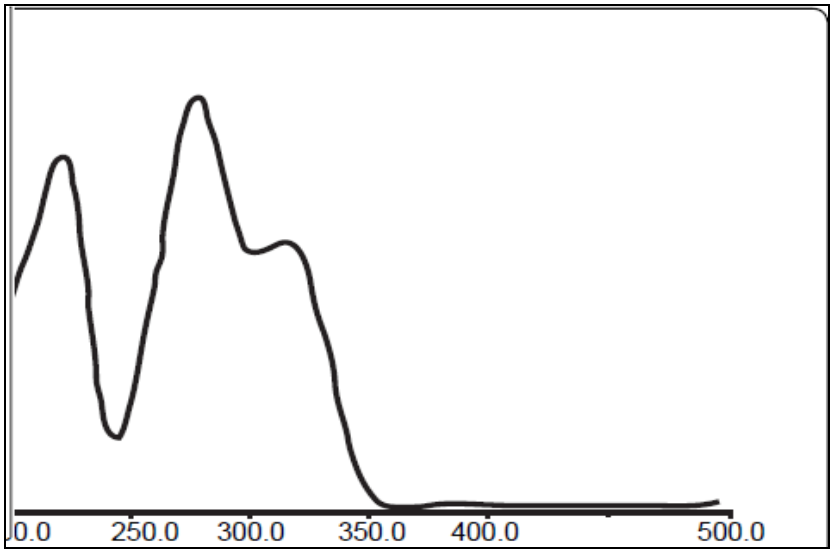

Figure. 2: UV-Visible spectra of *Sahaj Vati*.

IR spectra of formulation presented in **figure 3** and spectrum was recorded from 4000 to 400 cm^{-1}. Study confirmed presence of carbonyl group, aromatic & C-H stretching also observed along with characteristic peaks of alcoholic and phenolic group.

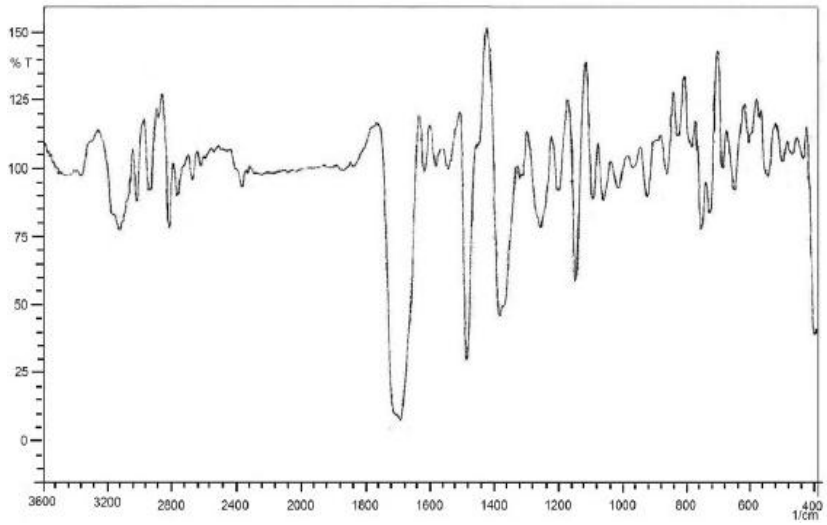

Figure. 3: IR spectra of *Sahaj Vati*.

High Performance Thin Layer Chromatography (HPTLC) is also performed to separate and identify major phytoconstituents present in formulation with better precision and accuracy. The HPTLC fingerprinting of study are shown in **figure 4 & 5** with characteristics HPTLC fingerprinting of extract. Figure 4 represents densitometric chromatograms which suggested about concentration level of major constituent present in formulation. The HPTLC fingerprinting may be recommended for qualitative identification of ayurveda formulation *Sahaj Vati*.

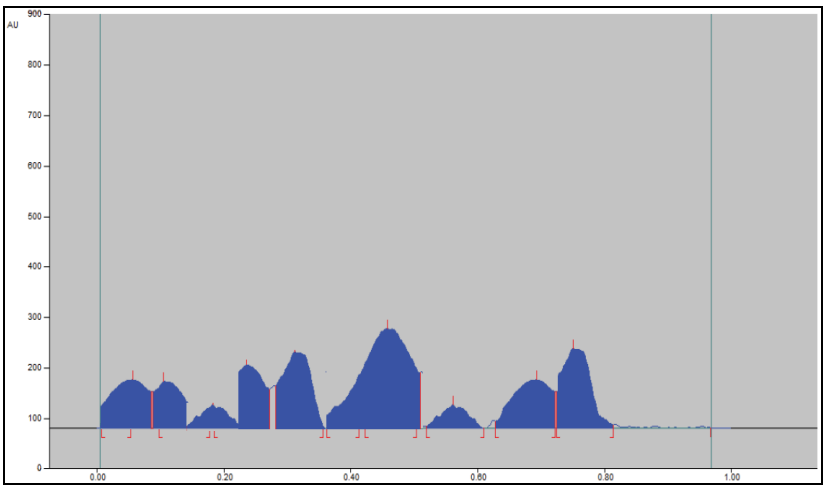

Figure. 4: Densitometric chromatogram of Methanolic Extract.

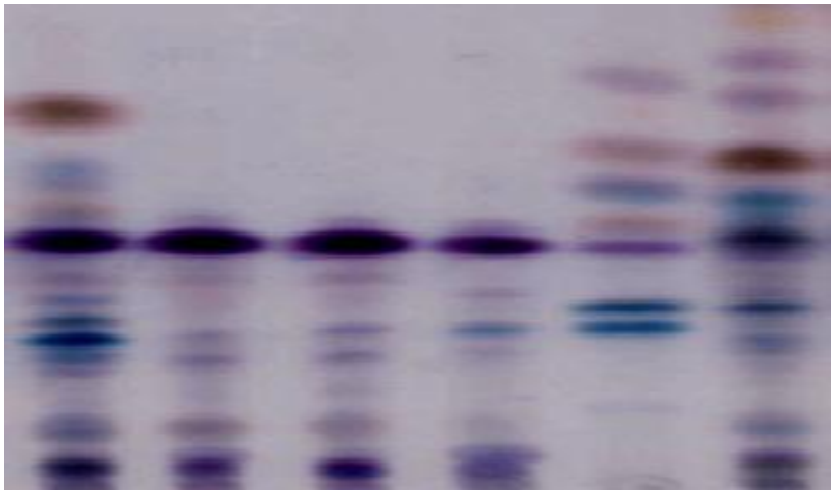

Figure. 5: HPTLC Chromatogram of Methanolic Extract.

CONCLUSION

The present investigation reported evaluation of herbomineral formulation *Sahaj Vati*. The antioxidant activity was performed using 2,2-diphenylpicrylhydrazyl (DPPH) assay and it was found that this formulation offers prompt radical scavenging effect especially at higher concentration. The presence of specific polyphenols may be considered responsible for the antioxidant activity of *Sahaj Vati*. The establishment of standardization parameter by modern techniques such as; HPTLC, IR and UV-Visible spectrophotometer provides finger printing profiling of herbomineral formulation *Sahaj Vati*.

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