Characterization of *Sahaj Vati* using Infrared and UV/Vis Spectroscopy

K. D. YADAV* AND A. K. CHAUDHARY

Department of Rasa Shastra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Yadav and Chaudhary: Infrared, UV/Vis study of *Sahaj Vati*

Sahaj Vati*, an Ayurvedic herbo-mineral formulation comprising of *Suddha Shilajeet*, *Suddha Guggul*, *Haridra* and *Chitrak*, has been used for treatment of obesity. Two types of *Sahaj Vati* was prepared namely I and II, *Sahaj Vati* I was prepared by *bhavana* of *Agnimanth kwatha* to the powder of *Suddha Shilajeet*, *Suddha Guggul*, *Haridra* and *Chitrak* whereas *Sahaj Vati* II was prepared by dissolving the *Suddha Shilajeet*, *Suddha Guggul* in *Haridra* and *Chitrak kwatha* followed by *bhavana* of *Agnimanth kwatha*. The characterization of *Suddha Shilajeet*, *Suddha Guggul*, *Haridra*, *Chitrak* and both *Sahaj Vati* was carried out using infrared spectroscopy and UV/Vis-infrared study. It was observed that IR absorption peaks of both *Sahaj Vati* did not absolutely match with its ingredients and some peaks of ingredients were totally absent in both *Sahaj Vati*. It was also observed that peaks of *Sahaj Vati* I and II differ significantly from each other. UV/Vis spectra of both *Sahaj Vati* showed absorption in region of 200-700 and 200-500 nm, respectively. Thus both *Sahaj Vatis* have different IR and UV/Vis spectra.

Key words: *Sahaj Vati*, IR spectroscopy, UV/Vis spectroscopy

Herbal medicines are widely accepted as therapeutic agents for the treatment of diabetes, arthritis and other disorders as adaptogens[1]. About 80 % of the world population uses herbs and traditional medicines for their primary health care needs[2]. The increasing use of herbal medicines and the growing demand of the global have raised concerns on the quality and the safety of herbal materials and final herbal products with the respective national health authorities. The quality of the finished herbal products is largely

***Address for correspondence**
E-mail: k.d.yadav1983@gmail.com

Accepted 13 October 2018
Revised 05 April 2018
Received 02 February 2017
Indian J Pharm Sci 2018;80(6):1165-1170

dependent and influenced by the quality of the raw materials used[3]. In current time of commercialization, medicines are prepared in large scale in industries so there are needs of authentication of raw materials as well as of final products. Quality control and the standardization of herbal medicines involve several steps. However, quality of raw materials used plays a pivotal role in quality of herbal/herbo-mineral formulations. The recent advances in the processes of purification, isolation and structure elucidation of naturally occurring substances necessitates to choose appropriate strategies for the analysis of quality and the process of standardization of herbal preparations in order to maintain high homogeneity. Spectroscopic techniques *viz*., UV/Vis and infrared spectroscopy (IR) alone or in combination can be successfully used for standardization and to control the quality of both, raw materials and finished herbal drug[4].

Obesity has risen tenfold in the past four decades in world[5] and United State Food and Drug Administration (USFDA) recommended developing plant-derived drugs as alternatives to synthetic drug as these can be developed at much faster rate and cheaper prices[6]. The ingredients of *Sahaj Vati* such as *Suddha Shilajeet*, *Suddha guggul*, *Haridra* and *Chitrak* and *Sahaj Vati* itself has antiobesity properties along with other beneficial properties[7].

Among the spectroscopic techniques, UV/Vis and IR is often used for characterization of plant and their ingredients. UV/Vis spectroscopic method is based on electronic absorption caused by the compound present in the plants and compound formed in chemical reactions whereas IR spectroscopic studies help in identifying the type of chemical bonds/functional groups present in the compound.

The present study was focused at evaluating the effect of different processing methods to characterize *Sahaj Vati* using techniques such as IR and UV/Vis spectroscopy. *Sahaj Vati* is comprised of *Suddha Shilajeet*, *Suddha Guggul* (*Commiphora mukul* (Stocks) Hook.), *Haridra* (*Curcuma longa* L) and *Chitrak* (*Plumbago zeylanica* Linn) and prepared as per pharmaceutical procedure of Ayurveda with different processing techniques[8]. Its characterization has been carried out to assess the possible differences in two *Sahaj Vatis* and to compare with their raw materials.

All the ingredients of *Sahaj Vati* were procured from Varanasi, Uttar Pradesh except *Guggul*, which was procured from Jaipur, Rajasthan. Plant materials like *Haridra*, *Chitrak* and *Guggul* were authenticated in the Department of Botany, Banaras Hindu University (BHU), Varanasi, India and voucher specimens vide no. Zinziber.2014/2, Plumbazina.2015/1 and

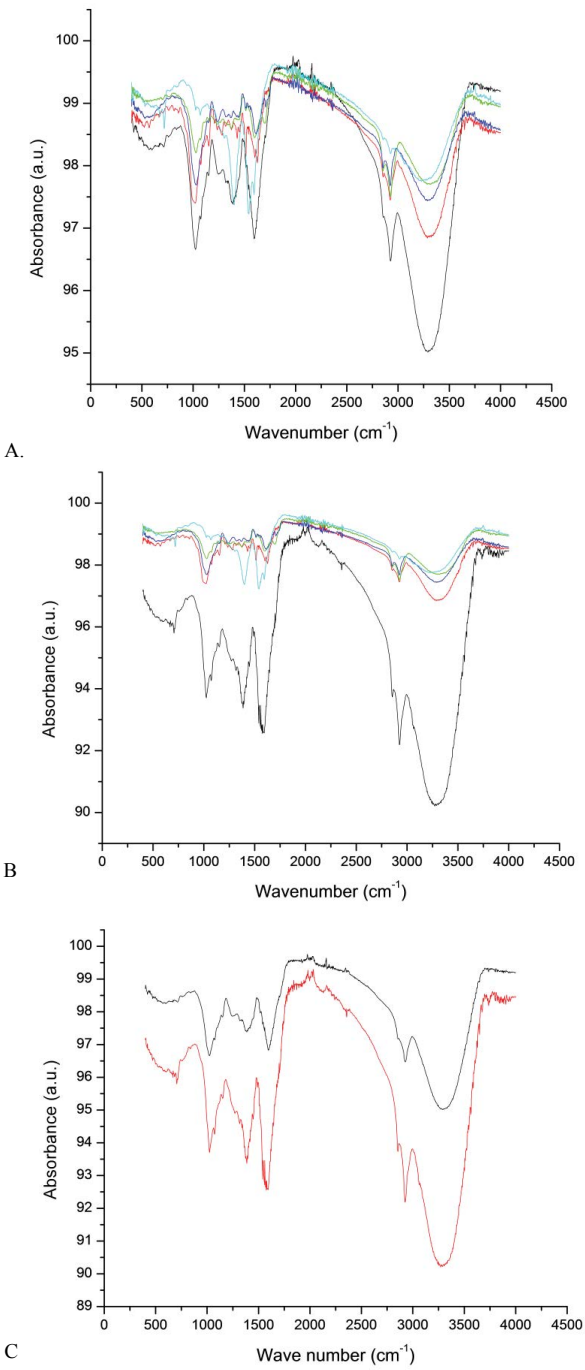

Fig. 1: FTIR spectra of *Suddha Shilajeet*, *Suddha Guggul*, *Haridra*, *Chitrak* and both *Sahaj Vati*

A: FTIR spectra of batch I of *Sahaj Vati* (——) with FTIR spectra of *Suddha Shilajeet* (——), *Suddha Guggul* (——), *Haridra* (——) and *Chitrak* (——), B: FTIR spectra of batch II of *Sahaj Vati* (——) with FTIR spectra of *Suddha Shilajeet* (——), *Suddha Guggul* (——), *Haridra* (——) and *Chitrak* (——). C: FTIR spectra of batch I (——) and II (——) of *Sahaj Vati*

Bursera.2015/1, respectively were kept in herbarium of Laboratory of Herbal Pesticides, Department of Botany. *Shilajeet* sample was authenticated in the Department of *Rasa Shashtra*, Faculty of Ayurveda, IMS, BHU, vide voucher specimen no. Shila.var.2013/3 was kept in museum of Department of *Rasa Shashtra*.

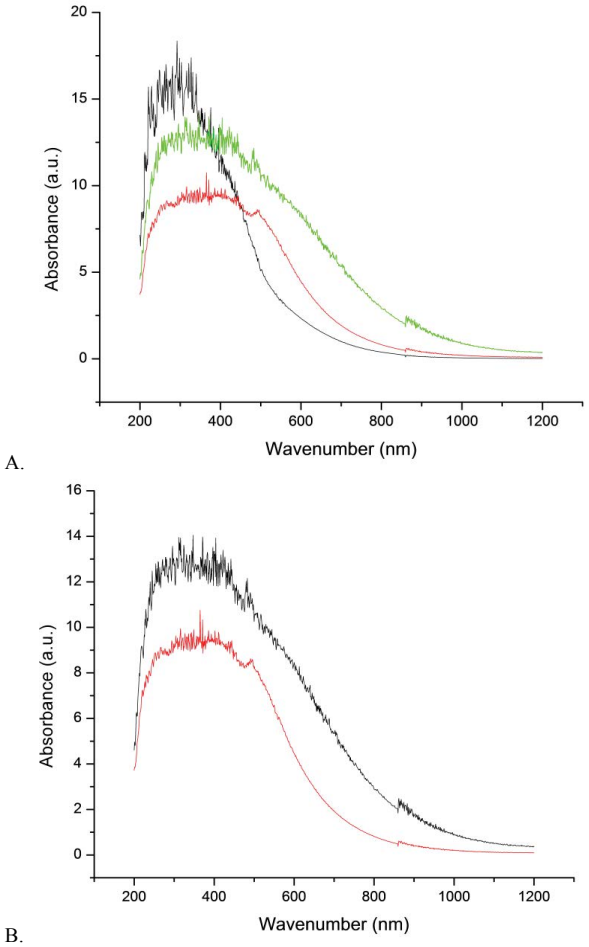

Fig. 2: UV/Vis analysis of *Shilajeet* and both batches of *Sahaj Vati*
A: UV/Vis spectra of *Suddha Shilajeet* (——), batch I (——) and II (——) of *Sahaj Vati*, B: UV/Vis spectra of batch I (——) and II (——) of *Sahaj Vati*

Before preparation of *Sahaj Vati*, *Haridra* and *Chitrak* were dried in oven at temperature of 40° and powdered by milling process. *Shodhan* of *Shilajeet* and *Guggul* was done as per Ayurvedic concepts. In this way *Suddha Shilajeet* and *Suddha Guggul* was obtained[9]. *Sahaj Vati* I was prepared as per standard operative procedures by using *Suddha Shilajeet*, *Suddha Guggul*, powder of *Haridra* and *Chitrak* as main ingredients and seven *bhavana* (levigation) of *Agnimantha kwatha* (decoction) with variation in pharmaceutical technique. And, *Sahaj Vati* II was prepared by dissolving the *Suddha Shilajeet*, *Suddha Guggul* in the *Haridra-Chitrak kwatha* after that seven *bhavana* of *Agnimantha kwatha*[8].

Fourier-transform infrared (FTIR) measurements were carried out using Spectrum 65 spectrophotometer (Perkin Elmer), and the spectra were collected at a resolution of 2 cm^{-1} in the region of 4000-400 cm^{-1}[10,11]. The IR spectrum was monitored for the individual ingredients and both *Sahaj Vati* using this instrument. The IR spectrograms of *Suddha Shilajeet*, *Suddha Guggul*, *Haridra*, *Chitrak* and both *Sahaj Vati* are shown in fig. 1.

UV/Vis absorption spectra of different samples were monitored using a Shimadzu UV-2600 UV/Vis absorption spectrophotometer (model A116650). The UV/Vis absorption spectra of *Suddha Shilajeet* and both *Sahaj Vati* are shown in fig. 2.

IR peaks of *Shilajeet* (720.67, 1400.37 and 2929.69 cm^{-1}), *Guggul* (1226.44, 1235.45, 1374.38,

TABLE 1: PEAKS IN FTIR SPECTRA OF *SUDDHA SHILAJEET*, *SUDDHA GUGGUL*, *HARIDRA*, *CHITRAK*, *SAHAJ VATI* I AND *SAHAJ VATI* II

Peak	*Shilajeet*	*Guggul*	*Haridra*	*Chitrak*	*Sahaj Vati* I	*Sahaj Vati* II
500-800	720.67		570.74	551.75	708.68	715.68
800-1100	1067.67	1029.54	1012.54	1025.54	1018.54	1025.54
1100-1500	1400.37	1235.45, 1374.38	1228.43, 1374.38, 1443.36	1242.44, 1374.38, 1443.36	1226.54, 1243.44, 1321.41, 1383.38	1387.38
1500-1800	1546.31	1512.32, 1598.28, 1703.24	1512.32, 1598.28, 1622.27	1512.32, 1612.28, 1708.24	1593.29	1585.29
1800-2100						1994.11
2100-2400			2109.06			2126.05 2352.95
2400-2700	-	-	-	-	-	-
2700-3000	2849.73, 2925.69	2850.73, 2925.69	2921.70	2850.73, 2925.69	2929.69	2856.73, 2925.69
Above 3000	3243.55	3320.52, 3824.30	3290.53	3287.53	3287.53	3274.54, 3738.33

1598.28 and 2925.69 cm^{-1}), *Haridra* (1228.43 and 2921.70 cm^{-1}) and *Chitrak* (1242.44, 1374.38, 2925.69 and 3287.43 cm^{-1}) were corresponding to peaks of 708.68, 1226.54, 1243.44, 1383.38, 1593.29, 2929.69 and 3287.53 of *Sahaj Vati* I (fig. 1A).

Similarly IR peaks of *Shilajeet* (720.67, 1400.37, 2849.73 and 2925.69 cm^{-1}), *Guggul* (1029.54, 1374.38, 1598.28 and 2925.69 cm^{-1}), *Haridra* (1025.54, 1383.68, 1593.29, 2909.06 and 2921.70 cm^{-1}) and *Chitrak* (1025.54, 1242.44, 1374.38, 2925.69 and 3287.43 cm^{-1}) were corresponding to peaks of 715.68, 1025.54, 1387.38, 1585.29, 2126.05, 2856.73, 2925.69 and 3274.54 cm^{-1} of *Sahaj Vati* II. Furthermore, some new peaks like 1994.11, 2352.95 and 3738.33 cm^{-1} are seen in *Sahaj Vati* II (fig. 1).

IR peaks of *Sahaj Vati* I like 708.68, 1383.38, 1593.29, 2925.69 and 3287.53 cm^{-1} were corresponding to 715.68, 1387.38, 1585.29, 2925.69 and 3274.54 cm^{-1} *Sahaj Vati* II. However, the IR spectra of *Sahaj Vati* II does not have corresponding peaks to IR spectra of *Sahaj Vati* I at 1226.54, 1243.44, 1321.41, 1025.54 cm^{-1}, similarly *Sahaj Vati* I also does not have corresponding IR spectra of *Sahaj Vati* II at 1025.54, 1994.11, 2352.95 and 3738.33 cm^{-1} (fig. 1C). The UV-Vis spectrum of *Shilajeet* and both *Sahaj Vati* showed UV-Vis absorption in region of 200-500 and 200-700 nm, respectively (fig. 2A, B).

As it was found in the literature survey, curcumin and plumbagin are the major components of *Haridra* and *Chitrak*, respectively and these were partially soluble or insoluble in water[12]. With this information, a formulation design with same ingredients but prepared using a different processing technique was attempted. In this sequence, two types of *Sahaj Vati* were prepared having same ingredients like *Suddha Shilajeet*, *Suddha Guggul*, *Haridra* and *Chitrak*, *Sahaj Vati* I contents were *Haridra* and *Chitrak* as whole but *Sahaj Vati* II has *kwatha* (water extract) of *Haridra* and *Chitrak*.

The IR absorption spectrum may be discussed in two sets i.e. bands lying 4000-1500 cm^{-1} and bands lying below 1500 cm^{-1} regions and recognized as functional group region or fingerprint region, respectively[13] (Table 1). It was also reported that IR absorption in 2850-3000 cm^{-1} region is due to sp^3 C-H stretching, the absorption bands above 3000 cm^{-1} are due to sp^2 C-H stretching and sp C-H stretching[14,15] and broad bands in region of 3400 cm^{-1} are due to OH group. It has been observed that IR spectra of both *Sahaj Vati* extend from 2850-3300 cm^{-1}, which may be due to sp^3, sp^2 and sp C-H stretching.

It was reported that the saturated and unsaturated hydrocarbons having carbon-carbon single bonds absorb IR spectra in the 800-1000 cm^{-1} region and carbon-carbon triple bond in the range of 2100-2260 cm^{-1}. Furthermore, strong skeletal bands for aromatics and hetero aromatics fall in the 1600-1300 cm^{-1} region of the spectrum[16] and arise from the stretching of the carbon-carbon bonds in the ring structure. Therefore, possibilities of carbon-carbon single bond present in *Sahaj Vati* I, which might be aromatic and hetero aromatic in nature whereas carbon-carbon triple bond in *Sahaj Vati* I. *Sahaj Vati* I has IR absorption peaks at 708.68, 1018.54, 1226.54, 1243.44, 1321.41, 1383.38, 1593.29, 2929.69 and 3287.53 cm^{-1}. Peak at 708.68 cm^{-1} corresponds to *Shilajeet* and 1018.54 cm^{-1} correspond *Shilajeet*, *Guggul*, *Haridra* and *Chitrak*. It was reported that the peak 708.68, 715.68 and 720.67 cm^{-1} fall in region of 715-725 cm^{-1} and was due to bending of -(CH_2)n present in alkane/alkyls[17]. IR absorption peak at 1226.54, 1243.44 cm^{-1} of *Sahaj Vati* I, occurred around the IR absorption peak of *Guggul*, *Haridra* and *Chitrak*, fall in region of 1180-1260 cm^{-1} was due to stretching of alcoholic C-O of aromatic compounds. Similarly the peak at 1383.38 cm^{-1} of *Sahaj Vati* I, correspond with *Shilajeet*, *Guggul*, *Haridra*, *Chitrak* comes in region of 1370-1390 cm^{-1} due to bending of CH_3C-H in alkane/alkyls groups. The peaks at 1593.29 cm^{-1} of *Sahaj Vati* I, was similar to *Haridra* and *Chitrak* that belong in region of 1580-1650 cm^{-1} arises due to bending of N-H in amine group. IR absorption peak at 2929.69 cm^{-1} of *Sahaj Vati* I would fall in the region of 2850-3000 cm^{-1} and corresponds to *Shilajeet*, *Guggul*, *Chitrak* and *Haridra* and was due to stretching of C-H stretch in alkanes and alkyls. The peak observed at 3287.53 cm^{-1} in *Sahaj Vati* I occur in region of 3200-3400 cm^{-1} similar to peaks of *Shilajeet*, *Guggul*, *Haridra* and *Chitrak*, arises due to stretching of N-H in amine group and symmetrical and asymmetrical stretching of N-H of amide group, respectively. It was interesting to note that the IR absorption peak of *Haridra* and *Chitrak* at 1443.36 cm^{-1} due to bending of C-H bond in alkane/alkyls and IR peaks of *Guggul* (1703.24 cm^{-1}), *Chitrak* (1708.24 cm^{-1}) present due to stretching of C=O of carboxylic compound totally disappeared in *Sahaj Vati* I.

IR absorption peaks of *Sahaj Vati* II at 715.68, 1025.54, 1387.38, 1585.29, 1994.11, 2126.05, 2352.95,

2856.73, 2925.69, 3274.54, and 3738.33 cm^{-1}. Among them the peak at 715.68 cm^{-1} fall in the region 715-725 cm^{-1} and corresponds to peak of *Shilajeet* and due to bending mode of -(CH_2)n present in alkane/ alkylase. The peak at 1025.54 cm^{-1} correspond to peak of *Shilajeet*, *Guggul*, *Haridra* and *Chitrak*, comes in the region of 1020-1075 cm^{-1} and belong to symmetric and asymmetrical stretching of =C-O-C bond. Peak at 1387.38 cm^{-1} was found in region of 1370-1390 cm^{-1} corresponds to *Shilajeet*, *Guggul*, *Haridra* and *Chitrak*, due to bending of CH_3C-H in alkane/ alkyls groups. The peak of *Sahaj Vati* II at 1585.29 cm^{-1} was similar to *Haridra* and *Chitrak,* lies in the region 1580-1650 cm^{-1}, was due to bending of N-H in amine group. IR absorption peak at 2925.69, 2856.73 cm^{-1} of *Sahaj Vati* II was due to *Shilajeet*, *Guggul*, *Chitrak* and *Haridra* was due to stretching of C-H bond of alkanes and alkyls because these bonds lies in the region of 2850-3000 cm^{-1}. Another peak at 3274.54 cm^{-1} fall in the region 3310-3350 and 3200-3400 cm^{-1} was similar to *Shilajeet*, *Guggul*, *Haridra* and *Chitrak*, arise due to stretching of N-H in amine and symmetric and asymmetric stretching of N-H of amide, respectively. Furthermore, three new absorption peaks i.e. 1994.11, 2352.95, 3738.33 cm^{-1} were observed in *Sahaj Vati* II, are characteristic of *Sahaj Vati* II, corresponding to asymmetric stretching of C=C of alkanes, carbon dioxide and hydroxyl group respectively[16,17]. It is possible due to pharmaceutical processing of *Sahaj Vati* II and interaction between ingredients. *Sahaj Vati* I, having peaks at 1226.54, 1243.44 cm^{-1} and 1321.41 1383.38 cm^{-1} whereas *Sahaj Vati* II, has only one peak at 1387.38 cm^{-1} in this region, this indicated that *Sahaj Vati* I, has more compounds than *Sahaj Vati* II*,* which might be due to *Haridra* and *Chitrak*.

It was observed that maximum absorption peaks of *Sahaj Vati* II, were found in the region of 2700 cm^{-1} whereas maximum absorption peaks of *Sahaj Vati* I, falls in region below 1500 cm^{-1}. This indicated a big difference between the both *Sahaj Vati* for as carbon skeleton and functional groups concerned, which might be due to variation of pharmaceutical processing of *Sahaj Vati* in spite of using same ingredients, so these are different from each other. In the preparation of *Sahaj Vati* II, *kwatha* of *Haridra* and *Chitrak* was used, whereas powder of *Haridra* and *Chitrak* in *Sahaj Vati* I, it could be assumed that heat application during preparation of *kwatha* might be responsible for changing carbon skeleton as well as functional groups of *Sahaj Vati* II, these changes in carbon skeleton may facilitate formation of new functional group in *Sahaj Vati* II.

The electronic spectra of *Sahaj Vati* I and II were monitored and observed that *Shilajeet* and both *Sahaj Vati* shows UV/Vis absorption in the region 200-500 and 200-700 nm, respectively. It was reported that humic acid, is one of the major component of *Shilajeet* and exhibit slight hump near 260-280 nm[18,19] that might be due to the absorption of radiation by the double bonds C=C, C=O and N=N of the aromatic or unsaturated components of humic substances[20]. So, it could be assumed that humic acid is present both *Sahaj Vati*.

Further, it was also investigated that curcumin, which is a major component of *Haridra*, shows UV/Vis absorption in region of 300-500 nm mainly due to the presence of phenolic groups[21]. The organic compound 2-methoxy-1,4-naphthoquinone a well-known constituent of *Chitrak* also shows UV/Vis absorption in region of 200-450 nm[22]. In present study it was observed that, a little bit more extended in *Sahaj Vati* II. This might be due to an additional pharmaceutical processing (*bhavana*) or due to the presence of other ingredients. In *Sahaj Vati* II, water extract of *Haridra* and *Chitrak* was used while in *Sahaj Vati* I powder of *Haridra* and *Chitrak* was used. So there is possibility of additional organic compounds in *Sahaj Vati* II due to which the UV/Vis absorption extends up to 700 nm. The change in the intensity of the absorption bands in *Sahaj Vati* I and *Sahaj Vati* II might be attributed to the processing in water.

IR spectrogram of *Sahaj Vati* I and II were quite different from each other. However, one new peak was observed in *Sahaj Vati* I and three new peaks in *Sahaj Vati* II. UV/Vis absorption band of *Sahaj Vati* II was extends more than *Sahaj Vati* I. These indicate that new chemicals may occur during *bhavana* and *kwatha* process, which needs to be analysed through more advanced techniques.

Acknowledgements:

Authors thank Prof. S. B Rai, Department of Physics, Institute of Science, BHU and his team for providing FTIR and UV/Vis data. They also thank Prof. M Shai, Department of Medicinal Chemistry, Faculty of Ayurveda, IMS, BHU for recording thin layer chromatography spectra. Authors also acknowledge Prof. N. K. Dubey, Department of Botany, BHU

for authenticating samples of *Haridra, Chitrak* and *Guggul* and Prof. A. K. Chaudhary, Department of *Rasa Shashtra*, Faculty of Ayurveda, IMS, BHU for authenticating sample of *Shilajeet*.

Conflict of interest:

There is no conflict of interest, financial or otherwise associated with this project.

REFERENCES

1. Patel PM, Patel NM, Goyal RK. Quality control of herbal products. Indian Pharm 2006;5:26-30.
2. Traditional, complementary and integrative medicine. Available from: https://www.who.int/traditional-complementary-integrative-medicine/en/.
3. Voluntary Certification Scheme for Ayush Products [cited 2015 Oct 20]. Available from: http://www.qcin.org/pdf/ayush_project/certification_scheme_ayush-who4.pdf.
4. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz J Med Biol Res 2000;33:179-89.
5. Tenfold increase in childhood and adolescent obesity in four decades: new study by Imperial College London and WHO [cited 2015 Oct 20]. Available from: http://www.who.int/mediacentre/news/releases/2017/increase-childhood-obesity/en/.
6. Nimmi OS, George P. Anti-obesity anti-oxidant effect of new polyherbal formulation (PHF) in obesity induced Wister rats. Indian J Tredit Know 2017;16(2):297-302.
7. Yadav KD, Singh A, Chaudhary AK. Dose finding study of *Sahaj Vati* (comprised of *Shilajeet*, *Haridra*, *Guggul* and *Chitrak*) in validated animal model of obesity. Indian J Tredit Know 2018;17(2);376-80.
8. Yadav KD, Chaudhary AK. Pharmaceutical postulates for standard operative procedures to prepare *Sahaj Vati*. IJPBA 2015;6:37-41.
9. Anonymous. Rasatantrasara and Siddhiprayoga Sangrah. Part I. Ajmer, Rajasthan: Krinshna Gopal Ayurveda Bhawan (DT); 2000. p. 38.
10. Wartewiga S, Reinhard HH. Pharmaceutical applications of Mid-IR and Raman spectroscopy. Adv Drug Deliv Rev 2005;57:1144-70.
11. Stuart BH. Infrared spectroscopy fundamentals and applications, analytical techniques in science. Hoboken, New Jersey, United States: John Wiley & Sons, Ltd.; 2004. p. 113-34.
12. Modasiya. Studies on solubility of curcumin. Int J Pharm Life Sci 2012;3:1490-7.
13. Basic Organic Chemistry Laboratory Course, Identification Reactions and Qualitative Analysis. Available from: http://reagenssi.kemia.helsinki.fi/nettilabra/tiedostot/introductory_course_analysis.pdf.
14. Infrared Spectroscopy: A key to organic structure [cited 2015 Oct 25]. Available from: http://www.yale.edu/ynhti/curriculum/units/1999/5/99.05.07.x.html.
15. Infrared Spectroscopy [cited 2015 Oct 28]. Available from: https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/Spectrpy/InfraRed/infrared.htm.
16. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd ed. New Delhi, India: Springer; 1973.
17. Characteristic Infrared Absorption Bands of Functional Groups [cited 2015 Oct 28]. Available from: https://www.colorado.edu/chembio.
18. Prost R, Dameme E, Driard J, Leydecadsker JP. Infrared study of structural OH in Kaolinite, Dickite, Nacrite, and Poorly Crystalline Kaolinite at 5 to 600 K. Clays Clay Miner 1989;37:464-68.
19. IR Absorption Bands of Functional Groups in Organic Compounds [cited 2015 Oct 28]. Available from: https://www.rose-hulman.edu/~brandt/Chem252/IR_and_NMR_tables.pdf.
20. Domeizel M, Khalil A, Prudent P. UV spectroscopy: a tool for monitoring humification and for proposing an index of the maturity of compost. Bioresour Technol 2004;94:177-84.
21. Kim H, Kim D, Karthick SN. Curcumin dye extracted from *Curcuma longa* L. used as sensitizers for efficient dye-sensitized solar cells. Int J Electrochem Sci 2013;8:8320-28.
22. Joshi DD. U-Vis spectroscopy: herbal drugs and fingerprints evidence based herbal drugs. New Delhi, India: Springer India; 2012. p. 101-20.