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Phytochemical evaluation of the marketed drakshasava formulation by spectroscopic & chromatographic methods

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Abstract--Background: Drakshasava is a general health tonic, carminative and blood purifier. It is used in the treatment of anemia, digestive & cardiac disorders. A review of the literature suggested that modern analytical methods are not reported for the phytochemical evaluation of drakshasava. Objective: To report phytochemical evaluation of Drakshasava Formulation by Spectroscopic & Chromatographic Methods. Materials and methods: Total phenolic, flavonoid, sugar and reducing sugar content in marketed drakshasava formulations were estimated by a UV-Vis spectrophotometer. The markers and extract of drakshasava formulations I & II were scanned by FTIR spectrophotometer individually to find the common bands of the vibrational spectra of markers and formulations. The markers and extract of drakshasava formulations I & II were subjected to HPLC analysis. Results: UV-Vis spectrophotometer analysis revealed that total phenolic, flavonoid, total sugar, & total reducing sugar of marketed drakshasava formulations were within the limit of ayurvedic pharmacopeia. The gradient mobile phase solvent A (HPLC grade water containing 0.85% w/v ortho phosphoric acid) and solvent B (acetonitrile) were optimized by considering the resolution, peak shape, and symmetry. Conclusion: The evaluation parameters reported in this article could be used as a standard reference for quality management of marketed drakshasava formulations.

Keywords--Phytochemical, Drakshasava, spectroscopic, chromatographic.

1. Introduction

Herbal medicine has a long history worldwide and was used for various therapeutic purposes in ancient Greek, Egyptian, Chinese and Indian medicine. As per the WHO, 80% population of the world depends on traditional medicines for their basic healthcare needs (1). Ayurveda is an ancient Indian medicinal system. Ayurveda places great emphasis on maintaining and promoting health and preventing the occurrence of disease (2). In ayurvedic medicine, the main sources of drugs are plants, metals & minerals origin. Single ingredient & multiple ingredients containing ayurvedic formulations are available in the ayurvedic medicine system (1). There are various forms of Ayurvedic medicines available in the market like ark (distillate), arista (fermented decoction), asava (fermented infusion), avaleha (semisolid), bhusma (calcinate), churnas (powder), ghritas (medicated ghee), kashayams (decoctions), taila (oil) and vatti (tablets), etc. Various dosage forms are mentioned for different disease conditions in different texts of ayurveda (2), (3), (4).

Asavas and arishtas are alcoholic preparations of ayurvedic medicine. Asavas are fermented infusions of plant material in which fresh herbal juices are used and arishtas are fermented decoctions of plant material in boiling water. In the fermentation process of asavas and arishtas flowers of *Woodfordia fruticosa* play a major role, in which approximately 10-12 % of self-generated alcohol is produced (5),(6),(7),(8), (9). Effective hydro-alcoholic extraction in the asava & arista enhances the rate of isolation of pure phytochemicals from medicinal plants, betters the efficacy profile and improve absorption. Self-fermented products may undergo continuous phytochemicals transformation that can lead to the emergence of new natural molecules with effective therapeutic activity (10). According to 'Charaka Samhita' cereals (Dhanyasava), fruits (Phalasava), roots (Mulasava), exudate (Sarasava), flowers (Pushpasava), branches (Kandasava), leaves (Patrasava), bark (Twagasava) and sugar (Sharkarasava) are the potential herbal sources for the preparation of asavas (11). Darakshasava is a Phalasava. Dried fruits of grape berries (draksha) are used as a main ingredient of drakshasava, other ingredients such as jaggery, honey, flowers of fire flame bush, flower buds of clove, fruits of long pepper & cubeb, seeds of nutmeg & cardamom, the bark of sandalwood & cinnamon, leaves of cinnamon are added during preparation (12). Drakshasava is a general health tonic, carminative and blood purifier. It is used in the treatment of anemia, digestive & cardiac disorders (13),(14),(15),(16). In the modern era of traditional medicines, the demand for ayurvedic formulations has been increase day by day. Quality control of ayurvedic formulations is important for their efficacy & safety as a medicament (17). The quality parameters of the ayurvedic formulation can be tested using modern analytical techniques.

The quality & purity of ayurvedic formulations are established as per ayurvedic pharmacopeial standards. The ayurvedic pharmacopoeia describes organoleptic, physical & phytochemical analysis of crude drugs & formulations (18). Phytochemical evaluation of the ayurvedic formulation detects the various bioactive components like alkaloids, carbohydrates, flavonoids, glycosides, tannins, phenols and saponin by color reaction (19),(20),(21).

The colorimetric methods are available to quantify the total phenolic, flavonoid, sugar and reducing sugar content of different plant extracts (22),(23). For quick, sensitive, simple & high throughput these methods are modified by using a UV-Vis spectrophotometer. The use of a UV Vis spectrophotometer can improve the speed & sensitivity of the analysis. UV-Vis spectrophotometric methods are more convenient and take less time for analyses of large numbers of samples. Marker-based fingerprint profiling by chromatographic methods have also been used to characterize the phytochemical composition of ayurvedic, herbal formulation (24),(25),(26). Similarly RP- HPLC method provides a sensitive method for quality control of phenolic compound analysis used for quality evaluation of ayurvedic & herbal formulation. Though many herbal formulations have been analyzed using chromatographic or spectroscopic methods, there are no reports of analysis of drakshasava by UV & HPLC. Thus the aim of the present research article is to analyze drakshasava by spectroscopic & chromatographic method for phytochemical evaluation.

2. Materials & Methods

2.1. Chemicals

Analytical grade reagents and chemicals were used for experiments. Methanol, acetonitrile and ortho phosphoric acid of HPLC grade were procured from Merck (Mumbai, India).

2.2. Markers

Catechin, ellagic acid, gallic acid, kaempferol, quercetin, resveratrol and piperine were obtained from Yucca Enterprises (Mumbai). A stock solution of each marker was prepared in methanol.

2.3. Samples

Drakshasava of Dhutapapeshwar (formulation I) & Baidhynath (formulation II) were purchased from the local market.

2.4. Equipment

Jasco UV V-630 was used to record the UV-Vis spectra, Shimadzu FTIR 8400S was used to record the FT-IR spectra by using KBr and chromatograph was recorded on HPLC (Jasco) system with a quaternary solvent pump, photodiode array detector (MD 4000) and an autosampler (AS 4050). ChromNav 2 software (Jasco) was used for data acquisition and processing.

2.5. Sample Preparation

Drakshasava (50 mL) was evaporated to remove self-generated alcohol by using a rotary evaporator. In the residue distilled water (50 mL) was added & vortex. Further n-hexane, chloroform and ethyl acetate were sequentially partitioned with the mixture (3 x 50 ml). The extract was concentrated and the remaining residue was stored in a cool place.

2.6. Experimental

2.6.1. Organoleptic evaluation

Organoleptic evolution of drakshasava formulation I & II was carried out for parameters viz. odour, appearance and taste.

2.6.2. Physical parameter evaluation

Standard ayurvedic pharmacopoeial procedures were used to analyze Drakshasava formulation I and II for physical properties such as pH, specific gravity, refractive index, viscosity and total solids (12).

2.6.3. Phytochemical evaluation

Drakshasava formulation I & II were subject to preliminary phytochemical evaluation. Preliminary evaluations of flavonoids, alkaloids, tannins & phenolic compounds, saponins, steroids, carbohydrates and triterpenoids were undertaken using several phytochemical procedures such as the alkaline reagent test, Dragendorff's test, ferric chloride test, froth formation test, Libermann burchard test, Molish's test and Salkowski test.

2.6.4. Total phenolic content

The extract of drakshasava formulations I & II was assessed for phenolic content by the Folin Ciocalteu method using a spectrophotometer (16), (17). A volumetric flask with a capacity of 10 mL was loaded with 0.2 mL of stock solution (1mg/mL) and 2.8 mL of distilled water. Then 0.5 mL of 2 N Folin-Ciocalteu reagent (diluted 10-fold in distilled water) was mixed into the volumetric flask. After waiting for 3 minutes at 35 °C, 2 mL of 20% of sodium carbonate was mixed. After 1 hour of dark storage, the mixture was tested for absorbance at 765 nm. The mean absorbance was calculated from triplicate samples analysis. Gallic acid standard curves were used to determine gallic acid equivalent concentrations in the extracts of the drakshasava formulations I and II. The findings were represented in mg per gram of gallic acid equivalent.

2.6.5. Total flavonoid content

The extract of drakshasava formulations I & II was assessed for flavonoid content by the Aluminium chloride method using a spectrophotometer (27),(28). A volumetric flask with a capacity of 10 mL was loaded with 1 mL of stock solution (1mg/ mL), and 0.1 mL of 10 % w/v aluminum chloride. The mixture was shaken, and 0.1 mL of 1 M potassium acetate was added to the volumetric flask. The mixture was kept aside for 1 minute, 2.8 mL of distilled water was poured into the volumetric flask. The mixture was incubated for 30 minutes at 30°C. The absorbance of the solution was measured at 510 nm. The mean absorbance was calculated from triplicate samples analysis. Quercetin standard curves were used to determine quercetin equivalent concentrations in the extracts of the drakshasava formulations I and II. The findings were represented in mg per gram of quercetin equivalent.

2.6.6. Total Sugar content

The extract of drakshasava formulations I & II was assessed for sugar content by the Phenol sulfuric Acid method using a spectrophotometer (23). A volumetric flask with a capacity of 10 mL was loaded with 1 mL of stock solution (1mg/ mL), and 1 mL phenol (5%). The mixture was shaken, and 5 mL of conc. sulfuric acid was poured into the volumetric flask. The volumetric flask was vortexed for 3 minutes and allowed to stand for 30 minutes, at 25°C. The absorbance of the solution was measured at 540 nm. The mean absorbance was calculated from triplicate samples analysis. D-glucose standard curves were used to determine D-

glucose equivalent concentrations in the extracts of the drakshasava formulations I and II. The findings were represented in mg per gram of D-glucose equivalent.

2.6.7. Total Reducing Sugar

The extract of drakshasava formulations I & II was assessed for reducing sugar content by the DNS reagent method using a spectrophotometer (29). A volumetric flask with a capacity of 10 mL was loaded with 1 mL of stock solution (1mg/ mL), 3 mL of distilled water and 3 mL of DNS reagent. The flask was kept in a boiling water bath for 5 minutes. The mixture was allowed to cool in a cold water bath. The absorbance of the solution was measured at 540 nm. The mean absorbance was calculated from triplicate samples analysis. The total reducing sugar of the extract of drakshasava formulations I & II was calculated by using D-glucose standard curves and the findings were represented in mg per gram of D-glucose equivalent.

2.6.8. UV Vis Spectrophotometric study

UV-Vis spectra were recorded (Jasco UV V-630) by dissolving 10 mg of gallic acid, catechin, ellagic acid, resveratrol, quercetin, kaempferol, piperine and extract of drakshasava formulations I & II separately in 100 mL of methanol. The UV spectra of markers and extract of drakshasava formulations I & II were recorded between the spectral range 200nm to 400 nm, to get maximum absorption wavelength.

2.6.9. FT-IR study

Infrared spectra were recorded by mixing powdered materials with potassium bromide. The markers and extract of drakshasava formulations I and II were separately scanned to detect the common bands in the vibrational spectra of the markers and formulations.

2.6.10. HPLC Analysis

HPLC analysis of the markers and extract of drakshasava formulations I and II were performed by gradient elution of 0.85% ortho phosphoric acid in water (solvent A) and acetonitrile (solvent B), with a flow rate of 1 mL min⁻¹. Each sample & markers were injected (20 µL) into a BISCOF C-18 column (250×4.6 mm; 5 µm). The gradient mobile phase flow initiated with 0% B to 7% B from 0 to 10 minutes, then a gradient from 7% B to 35% B from 10 to 45 minutes, increasing to 65% B at 65 minutes. UV spectral range of the photodiode array detector was 200 nm-400 nm. The retention time of phytochemicals was compared to that of corresponding markers.

3. Results

3.1. Organoleptic evaluation

Organoleptic evaluation of the marketed Drakshasava formulations I & II was carried out for identification and results of the same are shown in table 1.

Table 1: Results of organoleptic Evaluation

Sr. No.	Particular	Marketed Formulation I (B)	Marketed Formulation II (S)
1	Appearance	Liquid	Liquid
2	Colour	Brown	Brown
3	Odour	Alcoholic	Alcoholic
4	Taste	Sour	Sweet

3.2. Physical evaluation

As per standard pharmacopoeial methods physical evaluations were carried out for marketed Drakshasava formulations for comparative studies as shown in table 2.

Table 2: Results of Physical evaluation

Sr. No.	Parameters	Specification as per Ayurvedic Pharmacopeia of India	Marketed Formulation I (B)	Marketed Formulation II (S)
1	pH	4.0-4.5	4.45±0.05	4.43±0.07
2	Specific gravity 25°C (g/mL)	1.08-1.20	1.10±0.07	1.12±0.09
3	Refractive Index	1.469 to 1.471	1.470	1.470
4	Viscosity (cp)	2.10-3.10	2.30	2.40
5	Total Solid (% w/w)	Not Less Than 25.0	26.65 ±0.17	27.45 ±0.21

3.3. Phytochemical evaluation

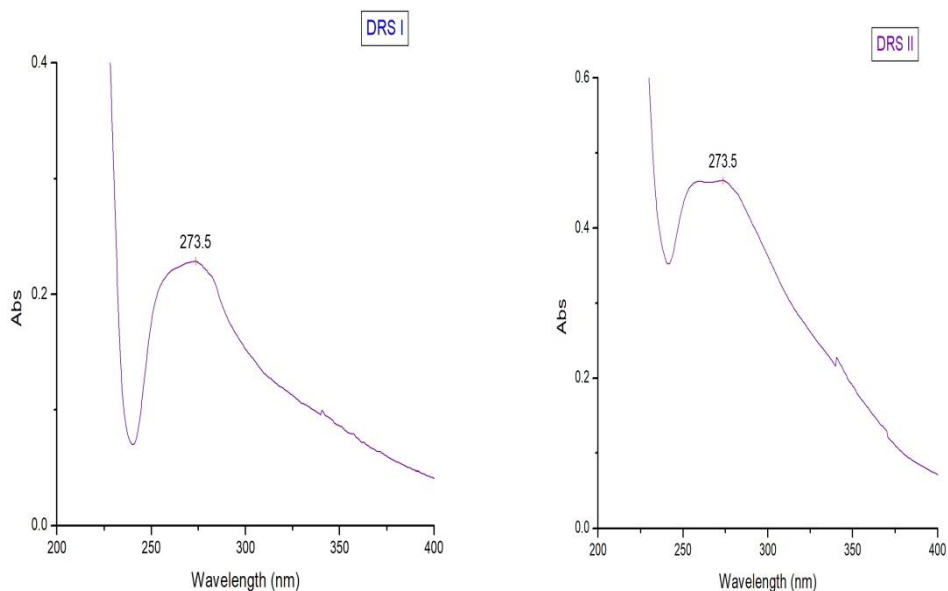
Preliminary phytochemical analysis of extract of drakshasava formulations I & II identified the alkaloids, carbohydrates, flavonoids, tannins & phenolic compounds in drakshasava. The phenolic, flavonoid, sugar, & reducing sugar content of marketed drakshasava formulations I & II were quantified by the linear regression analysis and reported in table 3.

Table No. 3: Results of Phytochemical evaluation

Sr. No.	Parameters	Marketed Formulation I (B)	Marketed Formulation II (S)
1	Total phenolic content	10.11±0.00045mg GAE/g	12.11 ± 0.0020 mg GAE/g
2	Total flavonoid content	71.55 ± 0.49 mg QE/g	76.48 ± 0.37 mg QE/g
3	Total Sugar content	204.54 ± 2.34 mg Glu/g	196.38 ± 2.73 mg Glu/g
4	Total Reducing Sugar	151.71 ± 0.31 mg Glu/g	150.35 ± 0.027 mg Glu/g

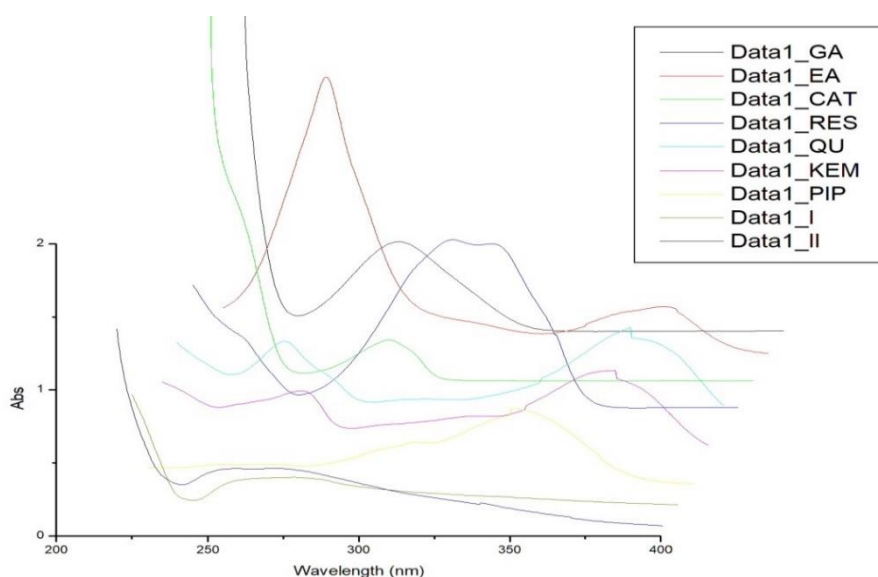
3.4. UV Vis Spectrophotometric study

A diluted solution of markers and extract of drakshasava formulations I & II (100 $\mu\text{g/mL}$) were irradiated between 200-400 nm. The markers like resveratrol, quercetin, kaempferol and Piperine showed strong absorbance peaks at 300-370 nm. The markers like gallic acid, catechin and ellagic acid showed strong absorbance peaks at 220-280 nm. The extract of drakshasava formulations I & II showed absorbance at 274.50 nm, 340 nm & 273.5, 340.5 nm respectively (Fig. 1).



a)

b)



c)

Fig.1. a) UV-Vis spectra of Drakshasava formulation I
 b) UV-Vis spectra of Drakshasava formulation II
 c) Overlay of UV-Vis spectra of marker, extract of Drakshasava formulation I & II

3.5. FT-IR study

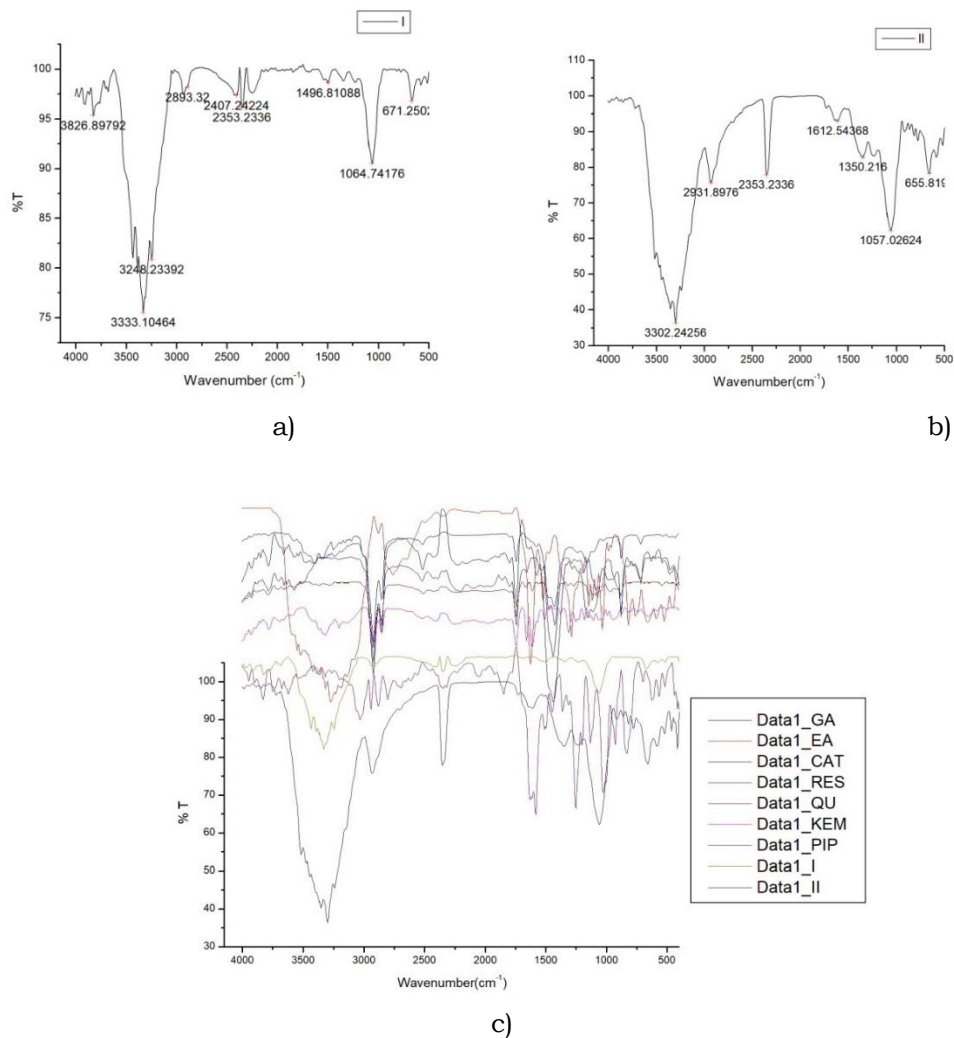


Fig.2. a) FTIR spectra of extract of Drakshasava formulation I
 b) FTIR spectra of extract of Drakshasava formulation II
 c) Overlay of FTIR spectra of marker, extract of Drakshasava formulation I & II

IR (KBr) spectra of markers & extract of Drakshasava formulation I & II reveal a characteristic OH group stretch between 3520 to 3200cm⁻¹, C-H stretch between 3000 to 2900 cm⁻¹ and carbonyl stretch between 1725 and 1600 cm⁻¹. The stretching vibrations C=C aromatic ring stretch bands between 1610 to 1560 cm⁻¹.

1. The OH group bending was detected at 1379 cm^{-1} . C–O stretch between 1260 to 1030 cm^{-1} was detected (Fig 2).

3.6. HPLC analysis

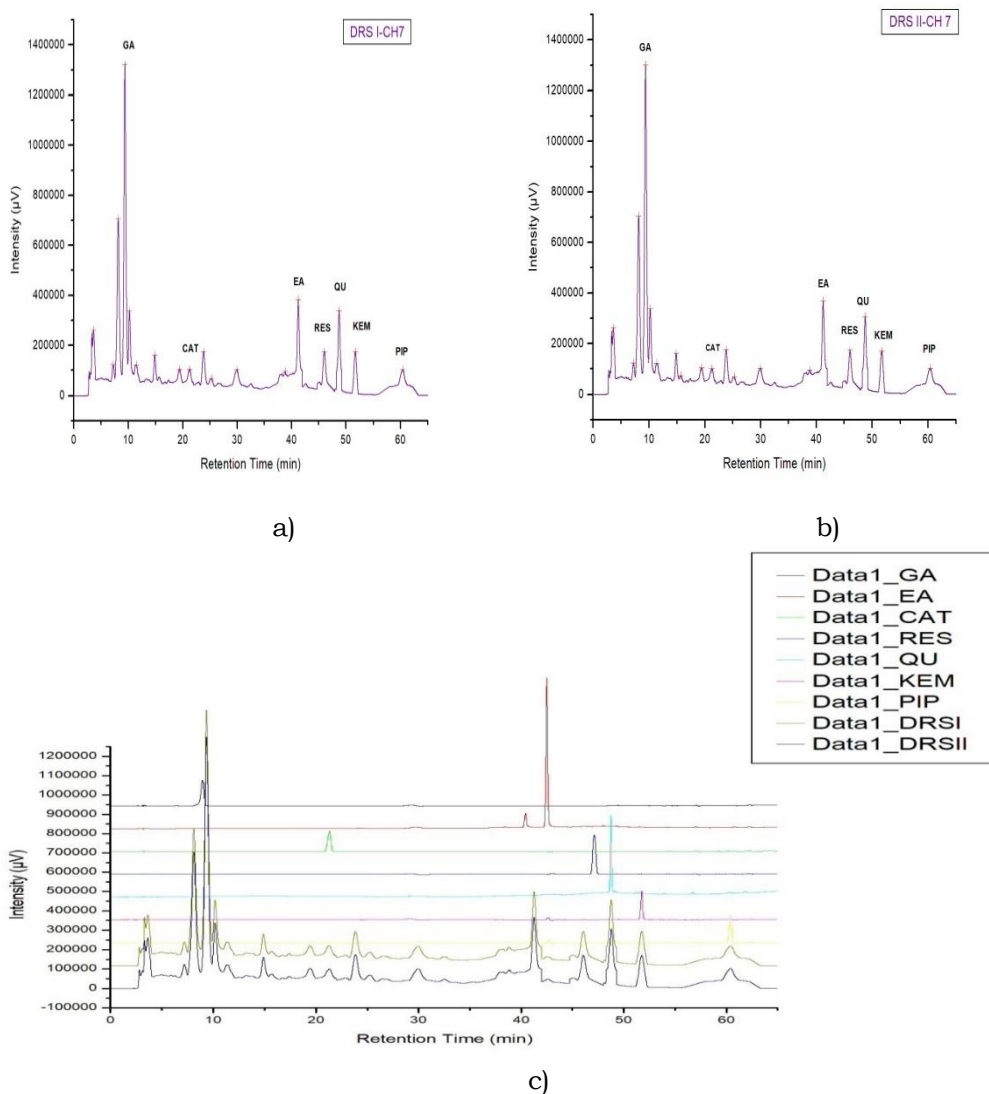


Fig.3. a) RP-HPLC Chromatogram of extract of Drakshasava formulation I
 b) RP-HPLC Chromatograms of extract of Drakshasava formulation II
 c) Overlay of RP-HPLC Chromatograms of marker, extract of Drakshasava formulation I & II

Analysis of markers and extract of drakshasava formulations I & II were carried out by using RP-HPLC system. The gradient mobile phase system described in section 2.6.10 was optimized by taking into account of peak shape, symmetry and resolution. Chromatograms of standards and markers [Figure 3] revealed that phytochemicals were completely separated in 65 minutes. The chromatographic

peaks of the marketed formulations were correlating with the markers. The RT for gallic acid, catechin, ellagic acid, resveratrol, quercetin, kaempferol and piperine were 8.91, 21, 42.4, 46.6, 48.6, 51.1 and 59.1 minutes respectively.

4. Discussion

The preliminary quality of Drakshasava formulations I & II was analyzed by organoleptic evaluation. It observed that brown colour, alcoholic odour and sour & sweet taste were the characteristic of both drakshasava formulations. Physical evaluation can be utilized for routine analysis for further study and to facilitate the identification of formulation. The physical evaluation like pH, specific gravity, refractive index, viscosity and total solid of both drakshasava formulations were within the permissible limit as per the official book. The preliminary phytochemical analysis confirmed that both drakshasava formulations contained metabolites such as alkaloids, carbohydrates, flavonoids, phenolic & tannins compounds.

Plant-derived secondary metabolites could provide more protective and beneficial effects. Secondary metabolites with anti-aging, antimicrobial, antibacterial and antioxidant activities can suppress carcinogenesis at various stages and serve as efficient chemotherapeutic agents (14),(15). The property of absorption of UV-Vis radiation by the phenolic ring is exploited to quantify the phenolic compounds. The Folin-Ciocalteu method was employed for quantification of the total phenolic content of both drakshasava formulations. Flavonoids are bioactive compounds that have several categories like flavonols, flavones, isoflavone and flavanols. The aluminum chloride method was employed to determine the total flavonoid content of both drakshasava formulations. The total sugar content of both drakshasava formulations was determined by reaction with concentrated sulfuric acid to form furfural derivatives, which further reacts with phenol to developed detectible color by UV Vis spectrophotometer.

The total reducing sugar content of both drakshasava formulations was quantified by oxidation of free carbonyl group (aldehyde and the ketone) of reducing sugars using the DNS reagent method. This further developed a reddish brown coloured complex by UV Vis spectrophotometer.

The UV-Vis spectra of extract of both drakshasava formulations confirm the presence of phenolic compounds. Flavones & their glycosides have UV absorb between 300–380 nm and 240–280 nm (30). Phenolic acids have absorption peaks between 290–350 nm (31).

The FTIR spectra of extract of both drakshasava formulations showed peak between 3520 to 3200 cm^{-1} confirmed the presence of phenol functional group. The peak between 3000 to 2900 cm^{-1} confirmed aliphatic C-H group. The peak between 1725 and 1600 cm^{-1} confirmed carbonyl groups and stretch between 1260 to 1030 cm^{-1} confirmed to C-O-C glycoside linkage. HPLC-PDA analysis of both drakshasava formulations revealed that phenolic compounds. The chromatographic peaks of both drakshasava formulations showed flavonoid like resveratrol, quercetin, kaempferol, catechin and phenolic acids like gallic acid & ellagic acid were also identified.

5. Conclusion

In the present research work, drakshasava formulations were evaluated by organoleptic, physical, phytochemical, spectroscopic and chromatographic analysis. The UV-Vis spectrophotometric methods were successfully developed for estimation of the total phenolic, total flavonoid, total sugar and total reducing sugar content of both drakshasava formulations. Fourier transform infrared transmission revealed that both drakshasava formulations contained complex phytochemicals by confirmation of the functional group. RP- HPLC method was successfully developed for fingerprint profiling of both drakshasava formulations. HPLC fingerprint of catechin, ellagic acid, gallic acid, kaempferol, quercetin & resveratrol represents the simultaneous pharmacological markers analysis. The evaluation parameters discussed in this research article may be used as a reference for the quality management of drakshasava formulations.

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