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PHYTOCHEMICAL PROFILING AND ANTIOXIDANT POTENTIAL OF MARKETED BRANDS OF *DRAKSHASAVA*: A COMPARATIVE EVALUATION

Dushing Y. A.
U. G. Department of Botany, RFNS, Senior Science College, Akkalkuwa,
K.B.C.N.M.U., Jalgaon (MS)

Corresponding Author:
Yogesh A Dushing
Department of Botany,
RFNS, Senior Science College, Akkalkuwa,
Dist Nandurbar, Maharashtra, India
KBCNMU, Jalgaon

ABSTRACT:
Background: *Drakshasava* is a traditional Ayurvedic polyherbal formulation prepared through natural fermentation of grapes (*Vitis vinifera L.*) with medicinal herbs. Its therapeutic potential is attributed to bioactive phytochemicals, yet comparative scientific data on marketed brands are limited. **Objective:** To evaluate and compare the biochemical composition and antioxidant activity of four commercial *Drakshasava* formulations. **Methods:** Samples from Baidyanath, Sandu, Vaidyaratnam, and Rasashala (Pune) were analyzed for total phenolics, flavonoids, alkaloids, reducing sugars, pH, and alcohol content. Antioxidant activity was assessed using DPPH, superoxide, hydroxyl radical, hydrogen peroxide, nitric oxide scavenging, and ferric thiocyanate assays. **Results:** The Rasashala and Vaidyaratnam formulation showed the highest levels of phenolics (15.67 mg/ml and 14.66 mg/ml respectively) and flavonoids (13.35 mg/ml and 11.46 mg/ml respectively) correlating with stronger antioxidant activity, In DPPH and superoxide assays, Rasashala demonstrated the lowest IC_{50} (0.53 µL and 0.61 µL, respectively), while Vaidyaratnam was most effective in hydroxyl radical scavenging (0.43 µL). Both brands showed superior activity in hydrogen peroxide, nitric oxide, and lipid peroxidation assays compared to Baidyanath and Sandu Brothers. **Conclusion:** Considerable inter-brand variability was observed, highlighting the need for standardization and quality assurance in Ayurvedic formulations to ensure consistent therapeutic efficacy.

Keywords: *Drakshasava, Ayurveda, antioxidants, phytochemicals*

INTRODUCTION:

Antioxidants are bioactive compounds that interrupt oxidative chain reactions and protect against reactive oxygen species (ROS) such as superoxide anion ($•O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($•OH$)[1,2]. Excess ROS is implicated in chronic diseases including cancer, diabetes, arthritis, cardiovascular disorders, and accelerated aging[3]. Synthetic antioxidants such as BHA and BHT face safety concerns[4], driving demand for natural alternatives. Plant-derived compounds, particularly polyphenols and flavonoids, demonstrate strong antioxidant activity and protective effects against oxidative stress–related diseases [5,6].
Herbal medicine has shaped healthcare in ancient civilizations including Greece, Egypt, China, and India [7]. The WHO reports that nearly 80% of the world's population still relies on traditional medicine [8]. Ayurveda, one of the oldest medical sciences, prescribes diverse dosage forms such as *asava* and *ariṣṭa*—fermented formulations that enhance bioavailability through self-generated alcohol [9–11]. Fermentation, aided by

Woodfordia fruticosa, produces 10–12% endogenous alcohol and may generate novel bioactive metabolites [12].

Within this category, *Drakshasava* is a classical *phalasava* prepared from dried grapes (*Vitis vinifera*) with adjunct botanicals including jaggery, honey, *Woodfordia fruticosa*, and spices [13]. Traditionally used as a tonic, carminative, and hematinic for anemia, digestive disorders, and cardiovascular ailments, it is now in growing demand. Ensuring safety, efficacy, and consistency requires rigorous quality control [14]. Containing 5–10% self-generated alcohol, *Drākṣāsava* also holds promise as a natural antioxidant, warranting systematic phytochemical and pharmacological evaluation [15]. There are many views about the ingredients of this *ayurvedic* formulation. However, the formulation taken for the present work is as compiled in *Bhavaprakasha-Madhyakhanda* (Āsava–Ariṣṭa Prakaraṇa1896 edition) Sanskrit Śloka [16]

drākṣā dhātakīpuṣpaṃ madhu cha priyataṃ priyaḥ |
ilāyacī tvakpatraṃ cha dālacinī cha karkaṭiḥ ||
jaladrākṣāsamaṃ sarvaṃ kṣiptvā mṛdbhājanaṃ sthiram |
māsamātreṇa saṃyogād bhaved Drakshasavaḥ sudhīḥ ||

Meaning: Grapes (*Vitis vinifera*), dhātakī (*Woodfordia fruticosa*) flowers, honey, priyaṅgu (*Callicarpa macrophylla*), cardamom (*Elettaria cardamomum*), tejpatra (Cinnamomum tamala), cinnamon (*Cinnamomum verum*), long pepper (*Piper longum*) etc. are mixed with water (equal to the weight of grapes). Kept in a sealed earthen vessel for one month. By natural fermentation, *Drakshasava* is formed.

This polyherbal formulation is beneficial for maladies such as lethargy, weakness and heat exhaustion, helpful for gastro-intestinal tract, stimulate appetite, improves body's strength and energy. Excellent for digestion, eases gas and bloating after meals [11].

Despite the widespread application of these analytical approaches, limited research has focused on the detailed phytochemical characterization and antioxidant analysis of *Drakshasava*. The methods of preparation and amounts of plants used vary from formulation to formulation. These may influence the amounts of active ingredients and antioxidants in this *Drakshasava*. Hence, in present investigation four marketed brands of *Drakshasava* were assessed for their antioxidant potentials based on in-vitro assays.

MATERIALS:

Four marketed brands of *Drakshasava* were selected for the study: Baidyanath–Nagpur (BD), Rasashala–Pune (RD), Sandu Brothers–Mumbai (SD), and Vaidyaratnam–Kerala (VD). All formulations were procured from authentic sources and stored under refrigerated conditions until analysis. Different concentrations (0.2%, 0.4%, 0.6%, 0.8%, and 1.0% v/v) were prepared in double distilled water (DDW) for antioxidant assays. For phytochemical estimations, the samples were diluted 100-fold in DDW.

METHODS:

Phytochemical Analysis:

Total Phenolic Content: Phenolic constituents were determined using Folin–Ciocalteu reagent following the method of Farkas and Kiraly (1962) [17] with modifications. Diluted samples (0.2 mL) were mixed with 0.5 mL of Folin–Ciocalteu reagent and incubated for 3 min. Subsequently, 2.0 mL of 20% Na_2CO_3 solution was added and the mixture was incubated for 1 min in a boiling water bath. Absorbance of the blue-colored complex was measured at 650 nm using a UV–Visible spectrophotometer (Shimadzu-1700). Catechol was used as the standard reference.

Total Flavonoid Content: Flavonoids were estimated using the aluminum chloride method[18]. Each sample (0.5 mL) was mixed with 1.5 mL methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL distilled water. After incubation at room temperature for 30 min, absorbance was measured at 415 nm. Quercetin was used to construct the calibration curve.

Total Alkaloid Content: Alkaloids were determined according to Sreevidya and Mehrotra (2003) [19]. 10 mL of *Drakshasava* samples were oven-dried, extracted with 100 mL of 10% acetic acid in ethanol, and kept for 4 hours. The extract was filtered and concentrated to one-fourth volume. Alkaloids were precipitated by dropwise addition of ammonium hydroxide, collected, dried and weighed. The alkaloid content was expressed as mg/100 mL of the sample.

Ascorbic Acid Content: Ascorbic acid was estimated by titration with 2,6-dichlorophenol indophenol (DCPIP) [20]. Diluted samples (5.0 mL) were mixed with 4% oxalic acid, filtered and made up to 100 mL. An aliquot (5.0 mL) was titrated against standard DCPIP solution, with color change from colorless to faint pink taken as the endpoint. Results were calculated using the standard conversion factor, where 1 mL of dye corresponds to 0.05 mg of ascorbic acid.

Reducing Sugar Content: Reducing sugars were estimated by the DNSA method [21]. Diluted sample (0.2 mL) was mixed with 1.0 mL DNSA reagent and incubated in a boiling water bath for 10 min. The reddish-orange color was measured at 540 nm. D-glucose was used as the standard.

pH Determination: pH values of the samples were recorded using a calibrated digital pH meter.

Alcohol Content: Alcohol concentration was estimated using the dichromate oxidation method [22]. One mL of *Drakshasava* was treated with 25 mL dichromate reagent (prepared from $K_2Cr_2O_7$ and concentrated H_2SO_4) in a 50 mL volumetric flask. The mixture was incubated at 60 °C for 20 min, cooled, and absorbance recorded at 620 nm. A calibration curve was constructed using absolute ethanol.

Antioxidant Assays:

Radical Scavenging Activity (RSA): Free radical scavenging activity was assessed using DPPH assay [23]. Samples of varying concentrations were incubated with 0.1 mL DPPH solution in methanol. After 30 min, absorbance was recorded at 517 nm. Radical scavenging activity (%) was calculated as:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0 \times 100]$$

Where; A_0 is absorbance of control and A_1 is absorbance of test sample. IC_{50} values were also calculated from the data obtained.

Superoxide Anion Radical Scavenging Activity ($O_2^{\bullet-}$ RSA): The method of Nishimiki et al. (1972) [24] was followed. Reaction mixture containing (100mM, pH 7.4), NADH (468µM), NBT (156µM), PMS (60µM) was incubated with different dilutions of *Drakshasava* for 5 min under fluorescent light. Absorbance was recorded at 560 nm, and IC_{50} value was calculated from the values obtained for % Inhibition.

Hydroxyl Radical Scavenging Activity ($OH^{\bullet-}$ RSA): Hydroxyl radical scavenging was determined using the deoxyribose degradation assay [25]. Reaction mixture contained 2-deoxy-2-ribose (2.8mM), KH_2PO_4-KOH buffer (20mM, pH 7.4), $FeCl_3$ (100µM), EDTA (100µM), H_2O_2 (1.0mM), ascorbic acid (100µM) and different aliquots of *Drakshasava* (0.2-1% v/v) and reference compound were incubated with samples at 37 °C for 1 h. TBARS formation was measured at 532 nm after heating with thiobarbituric acid. Catechin was used as the standard.

Hydrogen Peroxide Radical Scavenging Activity ($H_2O_2^{\bullet-}$ RSA): The ability of *Drakshasava* to scavenge H_2O_2 was determined according to Jayaprakasha et al. (2004) [26]. Samples were incubated with 20 mM H_2O_2 in phosphate buffer (pH 7.4). Absorbance was measured at 230 nm after 10 min. Ascorbic acid was used standard as compound. Percentage of H_2O_2 scavenging of *Drakshasava* was calculated.

Nitric Oxide Radical Scavenging Activity ($NO^{\bullet-}$ RSA): Nitric oxide scavenging activity was assessed using sodium nitroprusside and Griess reagent [27]. Reaction mixtures contained sodium nitroprusside (5mM), PBS and various concentrations (0.2%, 0.4%, 0.6%, 0.8% and 1.0% v/v) of *Drakshasava*. Samples were incubated for 150 min at 25 °C, followed by addition of Griess reagent. Absorbance was measured at 546 nm. Percentage inhibition of NO generated was determined by comparing absorbance values of control and test compounds.

Ferric Thiocyanate Assay (FTA): Inhibition of lipid peroxidation was assessed by FTC method [28]. Samples were incubated with linoleic acid in phosphate buffer at 40 °C. At intervals of 48 hours, ferric chloride and ammonium thiocyanate were added and absorbance was measured at 500 nm. Vitamin C served as a positive control.

Statistical Analysis: All experiments were conducted in triplicates. Data were analyzed using one-way ANOVA, and results expressed as mean ± standard deviation (SD). Differences were considered statistically significant at $p < 0.05$.

RESULT:

Present investigation was carried out to evaluate phytochemical variations and antioxidant potentials of marketed brands of *Drakshasava* i.e. Baidyanath, Sandu Brothers, Rasashala and Vaidyaratnam.

Phytochemical profiling of commercially available *Drakshasava* formulations (Table 1) revealed significant inter-brand variability in bioactive constituents. Among the evaluated samples, RV exhibited the highest phenolic concentration (15.67 mg/mL), closely followed by VD (14.66 mg/mL) and SD (14.25 mg/mL). Flavonoid content peaked in RD (13.35 mg/mL) and VD (11.46 mg/mL), whereas BD (5.37 mg/mL) and SD (4.71 mg/mL) demonstrated comparatively lower levels. Alkaloid quantification showed RD (62.97 mg/100 mL) and VD (60.18 mg/100 mL) as dominant, with BD (53.93 mg/100 mL) and SD (51.51 mg/100 mL) trailing. Ascorbic acid was most abundant in RD (83.70 mg/100 mL), followed by VD (79.63 mg/100 mL) and BD (78.15 mg/100 mL), while SD recorded the lowest concentration (75.93 mg/100 mL). Reducing sugar levels remained relatively consistent across VD and BD, ranging between 11.60 and 16.47 g/100 mL. All samples exhibited acidic pH values, spanning from 4.00 (RD) to 4.26 (SD). Alcohol content varied from 10.63% to 12.60%, with RD presenting the highest concentration (12.60%), marginally exceeding VD

(12.06%), and lower levels observed in BD (11.30%) and SD (10.63%). These findings underscore the need for standardization and quality assurance across Ayurvedic formulations to ensure therapeutic consistency.

Antioxidant profiling of various marketed brands of *Drakshasava* (Table 2 and Fig 1) revealed marked differences in their radical scavenging capacities across multiple in vitro assays. In the DPPH assay, RD exhibited the highest free radical neutralization efficiency, requiring only 0.53 µL, followed by VD (0.58 µL), while BD (0.75 µL) and SD (0.71 µL) showed diminished activity. Superoxide radical scavenging was similarly most potent in RD (0.61 µL), outperforming VD (0.72 µL), BD (0.79 µL), and SD (0.84 µL). Hydroxyl radical inhibition was strongest in VD (0.43 µL), closely followed by RD (0.44 µL), with BD (0.51 µL) and SD (0.57 µL) demonstrating moderate efficacy. Hydrogen peroxide scavenging assays indicated comparable potency in RD (0.47 µL) and VD (0.52 µL), both significantly superior to SD (0.59 µL) and BD (0.64 µL). Nitric oxide scavenging was most effective in VD (0.56 µL), while RD (0.62 µL) and SD (0.67 µL) showed intermediate activity, and BD (0.82 µL) was least effective. In lipid peroxidation inhibition (ferric thiocyanate assay), VD again led with maximal activity (0.46 µL), followed by RD (0.49 µL), SD (0.55 µL), and BD (0.61 µL). Collectively, RD and VD consistently demonstrated superior antioxidant potential, suggesting their enhanced therapeutic efficacy and underscoring the need for standardization in Ayurvedic formulations. Overall, RD and VD consistently demonstrated superior antioxidant potential across multiple radical scavenging models, whereas BD and SD showed relatively lower efficacy.

DISCUSSION:

This study comparatively evaluated the phytochemical composition and antioxidant potential of four marketed formulations of *Drakshasava*. Rasashala (RD) and Vaidyaratnam (VD) consistently demonstrated higher phenolic, flavonoid, and alkaloid contents, which directly correlated with superior radical scavenging activity across multiple assays [29,30]. In contrast, Baidyanath (BD) and Sandu Brothers (SD) exhibited comparatively weaker antioxidant responses due to lower phytochemical abundance.

The elevated phenolic and flavonoid concentrations in RD and VD likely contributed to their lower IC_{50} values in DPPH, hydroxyl radical, and lipid peroxidation assays, while alkaloids and ascorbic acid further enhanced their antioxidant efficacy. The moderate pH (4.0–4.26) and alcohol content (10.63–12.60%) suggest optimal fermentation, which aids in extraction, stability, and bioavailability of bioactives [31,32]. Controlled fermentation processes, including container selection [2], may explain the superior efficacy of RD and VD.

The observed consistency between phytochemical richness and antioxidant capacity is in agreement with previous studies on polyphenol-rich Ayurvedic formulations [33,34]. Since oxidative stress is central to cardiovascular, neurodegenerative, and metabolic disorders [35], these findings validate the classical description of *Drakshasava* as a *Rasayana* with cardiotonic and health-promoting effects. The marked inhibition of lipid peroxidation further highlights its protective potential against membrane damage and cellular aging.

Overall, this analysis underscores the importance of raw material quality, fermentation practices, and phytochemical standardization in ensuring the therapeutic consistency of Ayurvedic formulations. Although all brands exhibited measurable antioxidant activity, Rasashala and Vaidyaratnam emerged as superior, emphasizing the need for modern quality control measures to maintain efficacy and safety.

CONCLUSION:

This study demonstrates that *Drakshasava* exhibits significant phytochemical richness and antioxidant potential across different marketed formulations, validating its traditional therapeutic claims described in classical Ayurvedic texts. Among the brands analyzed, variations in phenolic, flavonoid, and alkaloid content correlated strongly with antioxidant efficacy, highlighting the importance of raw material quality and preparation methods.

With the rapid growth of the Ayurvedic drug industry, ensuring the safety, efficacy, and consistency of such formulations is critical. Conventional quality assessment methods are inadequate for complex polyherbal preparations. Advanced analytical approaches, including chromatographic fingerprinting and multicomponent quantification, provide a rational framework for standardization and quality control.

Future research should emphasize the development and validation of hyphenated techniques to authenticate and standardize multi-drug *asava–ariṣta* formulations, particularly where marker compounds exist only in trace amounts. Establishing robust and internationally harmonized protocols will not only strengthen the scientific validation of Ayurvedic medicines like *Drakshasava* but also enhance their global regulatory acceptance and clinical applicability.

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Table 1: Phytochemical analysis of selected brands of *Drakshasava*.

Brands	Total Phenols (mg/mL)	Total Flavonoids (mg/mL)	Total Alkaloids (mg/100 mL)	Ascorbic Acid (mg/100 mL)	Reducing sugar (g/100 mL)	pH	Alcohol content (%)
BD	12.07^{b}	05.37^{b}	53.93^{c}	78.15^{c}	16.33^{c}	4.16^{ab}	11.30^{b}
RV	15.67^{a}	13.35^{a}	62.97^{a}	83.70^{a}	16.77^{a}	4.00^{a}	12.60^{a}
SD	14.25^{a}	04.71^{b}	51.51^{d}	75.93^{d}	16.26^{d}	4.26^{b}	10.63^{c}
VD	14.66^{a}	11.46^{a}	60.18^{b}	79.63^{b}	16.47^{b}	4.06^{ab}	12.06^{a}
SEm	0.395	0.531	0.195	0.234	0.014	0.051	0.112
CD (0.05)	1.751	2.469	0.894	1.162	0.067	0.229	0.554

Values in each column with different superscript are significantly different at $p<0.05$.

Table 2: Antioxidant analysis of selected brands of *Drakshasava*.

Brands	RSA (Ic_{50} µl)	$O_2^{\bullet-}$ RSA (Ic_{50} µl)	OH^- RSA (Ic_{50} µl)	$H_2O_2^{\bullet-}$ RSA (Ic_{50} µl)	$NO^{\bullet-}$ RSA (Ic_{50} µl)	FTA (Ic_{50} µl)
BD	0.75^{d}	0.84^{b}	0.51^{b}	0.64^{d}	0.82^{d}	0.61^{d}
RV	0.53^{a}	0.61^{a}	0.44^{a}	0.47^{a}	0.62^{b}	0.49^{b}
SD	0.71^{c}	0.79^{ab}	0.57^{c}	0.59^{c}	0.67^{c}	0.54^{c}
VD	0.58^{b}	0.72^{ab}	0.43^{a}	0.52^{b}	0.56^{a}	0.46^{a}
SEm	0.005	0.006	0.013	0.009	0.010	0.008
CD (0.05)	0.017	0.022	0.042	0.03	0.033	0.029

Values in each column with different superscript are significant at $p<0.05$.

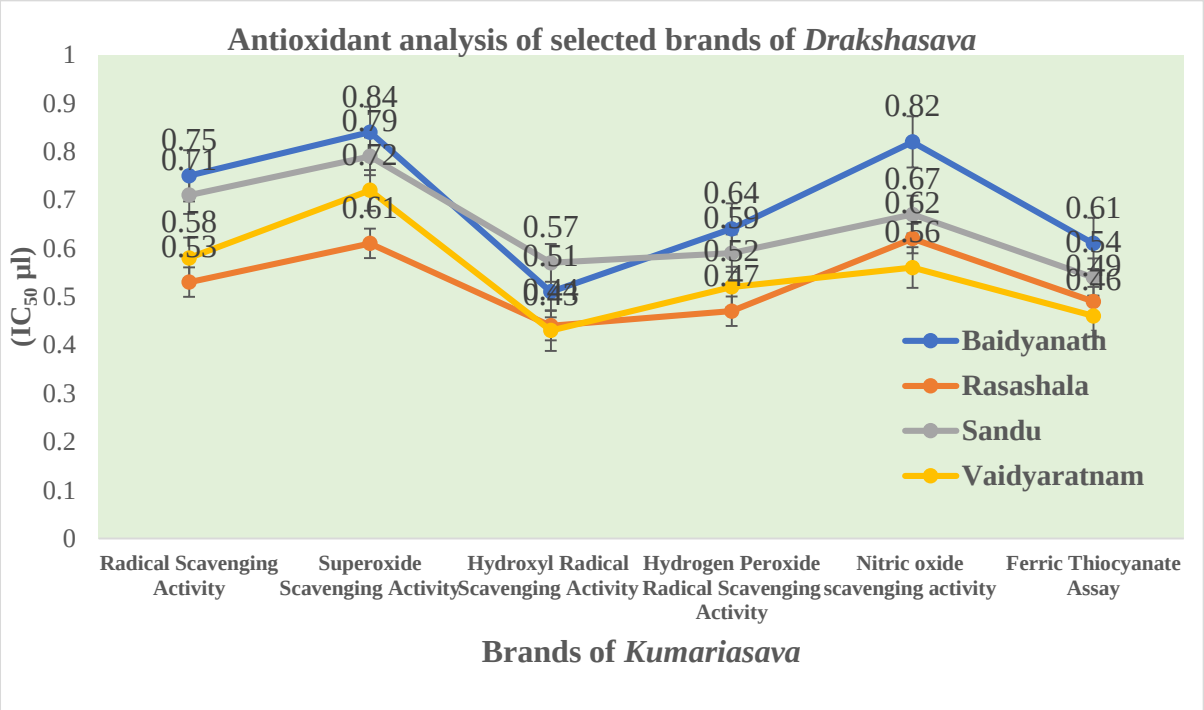

Fig 1: Antioxidant analysis of selected brands of *Drakshasava*.

REFERENCES:

1. Velioglu YS, Mazza G, Gao L and Oomah BD. Antioxidant activity and total phenolics in selected fruit vegetables and green products. J. Agric. Food Chem. 1998; 46: 4113-4117 https://doi.org/10.1021/jf9801973
2. Dushing Yogesh, A., & Laware Shankar, L. (2012). Antioxidant Assessment of Ashokarishta, A Fermented Polyherbal Ayurvedic formulation. *Journal of Pharmacy Research*, *5*(6), p3165.
3. Sanchez-Moreno C, Larrauri JA and Saura-Calixto F. Free radical scavenging capacity an inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Res Int. 1999; 32:407-412 https://doi.org/10.1016/S0963-9969(99)00097-6
4. Moein MR, Moein S and Ahmadizadeh S. Radical Scavenging and Reducing Power of Salvia mirzayanii Subfractions. Molecules. 2008; 13: 2804-2813. doi: 10.3390/molecules13112804
5. Pyo YH, Lee TC, Logendrac L and Rosen RT. Antioxidant Activity and Phenolic Compounds of Swiss Chard (Beta Vulgaris Subspecies Cycla) Extracts. Food Chem. 2004; 85: 19–26. DOI:10.1016/S0308-8146(03)00294-2
6. Cao G, Sofic E abd Prior R. Antioxidant Capacity of Tea and Common Vegetables. J. Agric. Food. Chem. 1996; 44: 3426-3431. https://doi.org/10.1021/jf9602535
7. Nigade, G. B., Deodhar, M. N., & Chavan, R. S. Phytochemical evaluation of the marketed *Drakshasava* formulation by spectroscopic & chromatographic methods. International Journal of Health Sciences, 2022 6(S9), 2969-2981. https://doi.org/10.53730/ijhs.v6nS9.13187
8. Parasuraman S, Thing G, Dhanaraj S. Polyherbal formulation: Concept of ayurveda. Pharmacogn Rev. 2014;8(16):73-80. DOI: 10.4103/0973-7847.134229
9. Ghosh S, Murthy PN, Joshi H. A Literature Review on Various Ayurveda Dosage Forms. 2018;5(3):19-23. https://doi.org/10.37591/rrjoasyn.v5i3.361.
10. Purnendu P, Das B, Bhuyan G.C, Meher SK,, Rao M. Ayurvedic Pharmaceutical Dosage Forms-A Review. 2017;3(5):926-934.
11. Paul B., Masih I., Deopujari J. and C. Charpentier. Occurrence of resveratrol and pterostilbene in age-old *Drakshasava*: An ayurvedic medicine from India. *J Ethnopharmacol.* 1999, 68(1):71-6. DOI:10.1016/S0378-8741(99)00044-6
12. Randive D, Sayyad S, Bhinge S, Bhutkar M. Preparation of Arjunarista-Using Microbes Isolated from Woodfordia fruticosa Flowers (Dhayati). Anc Sci Life. 2016;36(1):42-47. doi: 10.4103/0257-7941.195405
13. Asava and Arishtra. In: Vaidya Yoga Ratnavali. 1st ed. Madras: IMPCOPS; 1968. p. 5-47.
14. Mukherjee P, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J Ethnopharmacol. 2006;103(1):25-35. DOI: 10.1016/j.jep.2005.09.024
15. Dawane J. S, Dhande P. P. Study the Effect of *Drakshasava* on Dopamine, Serotonin and Cortisol Levels and Behavioural Changes in Acute and Chronic Stress Model in Wistar Rats. Biomed Pharmacol J 2024;17(3). DOI : https://dx.doi.org/10.13005/bpj/2967
16. Bhavamisra. *Bhavaprakasa Saṃhita*. Madhyakhanda, *Asava–Ariṣṭa* Prakaraṇa. 1896 ed. Varanasi: Chaukhambha Sanskrit Series Office; 1896.
17. Farkas, G. L., & Kiraly, Z. Role of phenolic compounds in the physiology of plant diseases and disease resistance. Phytopathology, 1962. 44, 105–150.
18. Chang, C., Yang, M., Wen, H., & Chern, J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 2002, 10(3), 178–182.
19. Sreevidya, N., & Mehrotra, S. Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. Journal of AOAC International, 2003, 86(6), 1124–1127.
20. Ghosh, A., Gupta, M., & Majumder, K. Determination of ascorbic acid in plant tissues. Journal of Experimental Biology, 1966,4, 123–126.
21. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. Journal of Biological Chemistry, 1944, 153, 375–380.
22. Saritha, V., Suresh, P., & Anilakumar, K. R. Alcohol determination in herbal formulations. Journal of Food Science and Technology, 2010, 47(2), 190–193. DOI:10.22270/jddt.v9i1-s.2339
23. Yamaguchi, T., Takamura, H., Matoba, T., & Terao, J. HPLC method for evaluation of free radical-scavenging activity of foods by using DPPH radical. Bioscience, Biotechnology, and Biochemistry, 2001, 62(6), 1201–1204. DOI: 10.1271/bbb.62.1201

24. Nishimiki, M., Rao, N. A., & Yagi, K. Superoxide anion in autooxidation of hydroxylamine and an assay for superoxide dismutase. Biochemical and Biophysical Research Communications, 1972, 46(2), 849–854.
25. Elizabeth, K., & Rao, M. N. A. Oxygen radical scavenging activity of curcumin. International Journal of Pharmaceutics, 1990, 58(3), 237–240. https://doi.org/10.1016/0378-5173(90)90201-E
26. Jayaprakasha, G. K., Singh, R. P., & Sakariah, K. K. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. Food Chemistry, 2004, 73(3), 285–290. DOI:10.1016/S0308-8146(00)00298-3
27. Marcocci, L., Maguire, J. J., Droy-Lefaix, M. T., & Packer, L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Biochemical and Biophysical Research Communications, 1994, 201(2), 748–755.
28. Osawa, T., & Namiki, M. Natural antioxidants isolated from eucalyptus leaf waxes. Journal of Agricultural and Food Chemistry, 1981, 29(4), 807–811.
29. Hussen, E.M., Endalew, S.A. In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. *BMC Complement Med Ther*, 2023, 23, 146 . https://doi.org/10.1186/s12906-023-03923-y
30. More, G.K., Makola, R.T. *In-vitro* analysis of free radical scavenging activities and suppression of LPS-induced ROS production in macrophage cells by *Solanum sisymbriifolium* extracts. *Sci Rep*, 2020, 10, 6493. https://doi.org/10.1038/s41598-020-63491-w
31. Sekar S, Vinothkanna A. Polyherbal and submerge fermented medicines of Ayurveda: Convergence of tradition with scientific trends and needs. South African J Bot. 2019,121(March):410–7. DOI:10.1016/j.sajb.2018.12.009
32. Sushruta M, Anubha K. Asavarishtas Through Improved Fermentation Technology. Int J Pharm Sci Researh]. 2011;2(6):1421–5. Available from: https://ijpsr.com/bft-article/asavarishtas-through-improved-fermentation-technology/?view=fulltext
33. Suresha Patil, Gazala Hussain, Veeresh Fattepur, Anirudh MR and Maitreyi Nechiyil. Physico-chemical analysis of *Drakshasava* prepared using two different sandhana dravya in two different containers. J Biol Sci Opin 2024;12(6): 63-67. http://dx.doi.org/10.7897/2321-6328.126100
34. C. Das, G. Ghosh, A. Bose And D. Das. Analytical Methods for Standardization of Ayurvedic *Asavas* and *Aristas*; A Review. Indian J Pharm Sci 2019;81(3):396-405.
DOI: 10.36468/pharmaceutical-sciences.523
35. Deng Y, Wang M, Tian T, Lin S, Xu P, Zhou L, Dai C, Hao Q, Wu Y, Zhai Z, Zhu Y, Zhuang G, Dai Z. The Effect of Hexavalent Chromium on the Incidence and Mortality of Human Cancers: A Meta-Analysis Based on Published Epidemiological Cohort Studies. Front Oncol. 2019 Feb 4; 9:24. DOI: 10.3389/fonc.2019.00024 PMID: 30778374; PMCID: PMC6369173.