RESEARCH ARTICLE

Evaluation of Antioxidant Potential of *Drakshavaleha*: A Polyherbal Ayurvedic Formulation

Sharma Alok[1], Mishra Dinesh Kumar[2], Shukla Karunakar[3]

[1]Department of Pharmaceutical Chemistry, Mahakal Institute of Pharmaceutical Studies, Ujjain, Madhya Pradesh, India, [2]Department of Pharmaceutics, School of Pharmacy and Technology Management, SVKM's NMIMS, Shirpur, Maharashtra, India, [3]Department of Pharmacognosy, Royal Institute of Management and Advanced Studies, Ratlam, Madhya Pradesh, India

Abstract

Aim: A semisolid (avaleha) herbal formulation *Drakshavaleha* (DKV) was prepared based on the method described in Ayurvedic Formulary of India**.** It contains *Vitis vinifera* (*Draksha*), *Piper longum* (*Pippali*), *Glycyrrhiza glabra* (*Yeshtimadhu*), *Zingiber officinale* (*Sunthi*), *Bambusa arundinacea* (*Vamshalochana*), *Emblica officinalis* (*Dhatri Phala* or *Amalaki*), and honey and sugar (*Sarkara*). **Materials and Methods:** Methanolic extract of the DKV formulations was used for this study. Methanolic extract of three marketed preparation of DKV also use for the comparative study of DKV. This formulation was known as DKV-1, DKV-2, and DKV-3. A sample of this marketed formulation also used for 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. **Result and Discussion:** The methanolic extract of DKV exhibited a maximum DPPH scavenging activity of 68.24% at 100 µg/ml, and marketed formulations of DKV (DKV-1, DKV-2, and DKV-3) exhibited maximum DPPH scavenging activity 67.90%, 68.35%, and 68.40%, respectively, at 100 µg/ml. With the standard ascorbic acid, it was found to be 84.75% at 100 µg/ml. The IC50 values of the methanolic extract of DKV were 63.27 µg/ml, and marketed formulations (DKV-1, DKV-2, and DKV-3) were 63.13 µg/ml, 62.82 µg/ml, and 62.91 µg/ml, respectively, and ascorbic acid was 40.05 µg/ml. **Conclusion:** Results obtained suggest the antioxidant and free radical scavenging activity potential of DKV, and further it may be used as an antioxidant in associated diseases.

Key words: Antioxidant, *Drakshavaleha*, 2, 2-diphenyl-1-picrylhydrazyl

INTRODUCTION

It is progressively more being realized that lots of today's diseases are due to the "oxidative stress" that consequences from an imbalance between formation and neutralization of free radicals. Oxidative stress is occurred by free radicals, which search for stability through electron pairing with biological macromolecules such as proteins, lipids, and deoxyribonucleic acid in healthier human cells and cause protein, and deoxyribonucleic acid damage besides with lipid peroxidation. These changes produce to the formation of cancers, atherosclerosis, cardiovascular diseases, many other inflammatory diseases, and aging.[1,2] All human cells shield themselves against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherol, and glutathione. Sometimes, these protective pathways are disrupted by various pathological processes. Hence, antioxidant supplements are vital to combat oxidative damage.[3]

In recent times, greatly attention has been directed toward the development of "Herbal formulations" that possess strong antioxidant properties with less or no toxicity. *Rasayana tantra* is a distinctive branch of Ayurveda and the drugs mentioned in this chapter have been described to both heal disease and also support health. In general, *Rasayana* drugs promote healthy longevity, memory and intellect, preserve

Address for correspondence:
Alok Sharma, Mahakal Institute of Pharmaceutical Studies, Ujjain, Madhya Pradesh, India.
Phone: +91-9755159404/9425108591.
E-mail: aalokpharma@yahoo.com

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youthfulness, luster of skin, and clarity of voice, and strengthen the all organs in the body.[4]

Ayurveda, which is known as the ancient science of long life, is at least a 5000-year-old Indian traditional system of medicine (1500–1000 BC) projected to encourage admirable health and long life rather than to fight ill health and was practiced by physicians and surgeons.[5,6] *Ayurveda* precisely means "the knowledge of life." In Sanskrit, the word *Ayurveda* consists of the words *āyu*, meaning "life" and *veda*, meaning "knowledge" or "science."[5] *Ayurveda* comprises various types of medicines including the formulations forms, namely, *Avaleha*, *Arishta* (fermented decoction) *Asava* (fermented infusions) these are used as important therapeutics due to their effectiveness and advantageous features.[7]

Avaleha or *Lehyam* is categorized under semi-solid dosage form of herbal drugs, prepared with addition of jaggery, sugar or sugar-candy and boiled with prescribed juices or decoction.[8,9] Essential ingredients used to prepare avaleha are:[10]

1. *Drava dravya*: Any other liquid preparation *(Kasaya, svarasa)*.
2. *Madhura dravya*: Sweetening agents *(Guda, sarkara, khanda sarkara,* etc.).
3. *Ausadha dravya*: Powder of herbal drugs or any fruits pulp.
4. *Ghrita and madhu*: Ghee and honey as mentioned in the preparation.

Drakshavaleha (DKV) formulation mentioned in *Brihat Nighantu Ratnakar* (as quoted in *Bharat Bhaishajya Ratnakara*), *Astanga Hridaya*, *Bharat Bhaishajya Ratnakar,*[11] *Bhaishajya Ratnavali*,[12] *Harita Samhita, Sahasra Yoga,*[13] and A.F.I.[14]

There are many views about the ingredients of this *ayurvedic* formulation. However, the formulation taken for the present work is as compiled in *Astanga Hridaya Chikitsa Sthana* 16th Chapter, *Pandu Roga Chikitsa,* and *Sloka* No.29-31.

drākṣāprasthaṃ kaṇāprasthaṃ sarkarārdhatulaṃ tathā ||29||
divpalaṃ madhukaṃ śuṇṭhīṃ tvakkṣīrīṃ ca vicūrṇitām |
dhātriphalarasadroṇe tatikṣaptvā lehavatpacet ||30||
śitānmadhuprasthayutād lihyātpāṇitalaṃ tataḥ |
halīmakaṃ pāṇḍurogaṃ kāmalāṃ ca niyacchati ||31||

Draksha and *kana* (Pippali) are added in sugar solution over a mild fire in a clean vessels then add *Yeshtimadhu* (mulethi), *Sunthi* and *Twakshira* (Vamshalochana) powder. Add *Dhatriphala* (Amla) *rasa* when kept on mild fire. Honey should be added after the preparation is fully cool and avaleha checked by whether the material sinks and settles at the bottom of water or not, which indicates the completion of preparation. It is used for treatment of anemia, Jaundice and for obstructive jaundice.[10,14]

DKV has been indicated to treat *Pandu* (anemia) and *Kamala* (jaundice). DKV is a semi-solid avaleha preparation (*Avaleha* is a semisolid preparation of drugs, prepared with addition of jaggery, sugar and boiled with prescribed juices or decoction) that mainly consisting of *Draksha*, along with other ingredients.[14] It contains *Vitis vinifera* (*Draksha*), *Piper longum* (*Pippali*), *Glycyrrhiza glabra* (*Yeshtimadhu*), *Zingiber officinale* (*Sunthi*), *Bambusa arundinacea* (*Vamshalochana*), *Emblica officinalis* (*Dhatri Phala* or *Amalaki*), and honey and sugar (*Sarkara*) as given in Table 1. Many studies demonstrated that the main content of DKV is *V. vinifera* (Draksha) which contains a chemical substance, i.e. resveratrol, a polyphenol and flavonoids which show antioxidant properties.[15] *G. glabra* (*Yeshtimadhu*) contain phenolic component exhibit antioxidant activity.[16] *Z. officinale* (*Sunthi*) contains many antioxidants such as beta-carotene, ascorbic acid, terpenoids, alkaloids, and polyphenols such as flavonoids, flavones glycosides, and rutin.[17] *B. arundinacea* (*Vamshalochana*) contains phenolic compounds such as phenolic acids, flavonoids, and tannins which shows the antioxidant activity of the plant.[18-20] The antioxidant activity of flavonoids results from the combination of their iron chelating activity and their ability to scavenge aging induced free radical. Flavonoids can inhibit oxidases such as lipoxygenase, cyclooxygenase, and xanthine oxidase, thus prevent *in vivo* development of reactive oxygen species and organic hydro peroxidase. Flavonoids also inhibit enzymes indirectly occupied in oxidative processes.[21]

MATERIALS AND METHODS

The formulation was prepared based on the methodology described for *avaleha* preparation in Ayurvedic Formulary of India.[9]

Drakshavaleha preparation

In-house preparation of DKV was prepared by following strict norms of methodology as described in Ayurvedic Formulary of India. The drugs were cleaned, dried and tested for foreign matter. The drugs *V. vinifera* (*Draksha*), *P. longum* (*Pippali*), *G. glabra* (*Yeshtimadhu*), *Z. officinale* (*Sunthi*), *B. arundinacea* (*Vamshalochana*), and *E. officinalis* (*Dhatri Phala* or *Amalaki*) were then crushed and sieved through sieve no.80 to obtain fine powder. Jaggery, sugar, or sugar-candy (*Sarkara*) was dissolved in the liquid, and then the liquid was strained to remove the foreign particles. This solution was boiled over a moderate fire. When the *Paka* was thready (*tantuvat*) means when pressed between two fingers or when it sinks in water without getting easily dissolved, it was removed from the fire. Fine powders of drugs were then added in small quantities and preparation was stirred continuously and vigorously to form a homogenous mixture. Honey was added when the preparation was cool and this was mixed well. The Avaleha was then packed in an airtight

Table 1: Composition of *Drakshavaleha* (Ayurvedic formulary of India)

Name	Botanical name	Family	Part use	Quantity
Draksha	*Vitis vinifera*	Vitaceae	Fruit	768 g
Pippali	*Piper longum*	Piperaceae	Fruit	768 g
Sakura				2.800 kg
Licorice	*Glycyrrhiza glabra*	Leguminosae	Root	96 g
Sunthi	*Zingiber officinale*	Zingiberaceae	rhizomes	96 g
Banslochan	*Bambusa arundinacea*	Poaceae	Stem exudate	96 g
AmlaPhalRas	*Emblica officinalis*	Euphorbiaceae	fresh fruit pulp	12.288 L
Honey				768 g

container. This formulation is known as DKV. A sample of this formulation was used for this study. Three marketed preparation of DKV also use for the comparative study of DKV. This formulation was known as DKV-1, DKV-2, and DKV-3. A sample of this marketed formulation also used for the study.

Antioxidant activity

Chemicals

2, 2-diphenyl-1-picryl hydrazyl (DPPH, Lancaster, UK), riboflavin (Loba-India), methanol GR (99.8%) (Loba-India), and Double beam UV spectrophotometer-1700 (Shimadzu) were used for spectrophotometric analysis.

Preparation of methanol extract

As methanol is less polar in comparison to hydro-alcohol, to generate selectivity preference to flavonoid type, more methanol extract is selected here; methanol is totally evaporated from the extracted material. 25 g of the formulation was macerated with 300 ml of n-hexane for 24 h. To remove fats and waxes and then supernatant was decanted. Solid mass macerated with 300 ml of methanol (99.8%) in a conical flask by maceration followed by soaking for about 24 h. Then, the extract was filtered and heated in a water bath until methanol is evaporated and dried. The solvent selection is based to give preference to flavonoid type of compound group.

Free-radical scavenging activity (DPPH assay)[22]

Preparation of solutions

Sample stock solution

The dried extract was dissolved in methanol to a final concentration of 100 µg/mL. From stock solution 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL of the solution was taken in five test tubes and serially diluted, final volume of each test tube was made up to 10 mL whose concentration was then 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, and 100 µg/mL, respectively. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (100 µg/mL) of extract. The different concentrations of standard (20, 40, 60, 80, and 100 µg/mL) were prepared. 100 µM (3.95 mg) DPPH solution was prepared in methanol.

Evaluation of free radical scavenging activity

About 2 ml of the DPPH solution was mixed with 2 ml of sample solution and standard solution separately. These solution mixtures were kept in the dark for 30 min, and absorbance was measured at 517 nm using a spectrophotometer.

Methanol (2 ml) with 2 ml DPPH solution was used as a control. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. Methanol was used as a blank. The absorbance was recorded, and % inhibition was calculated using the formula.

Percent (%) inhibition of DPPH activity=A−B/A×100

Where, A=Absorbance of the control
B=Absorbance of the sample.

Constructed a plot between concentration versus % reduction in absorbance of DPPH and calculated the IC50. IC50 is the concentration of the sample required to scavenge 50% of DPPH free radicals.

RESULT AND DISCUSSION

Free radical scavenging effects of methanolic extract of DKV and three marketed formulation of DKV (DKV-1, DKV-2, and DKV-3). At different concentrations were measured with ascorbic acid as standard compound using DPPH method. The results are tabulated in Table 2.

DPPH assay: (DPPH)

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as:

(DPPH) + (H-A) → DPPH-H + (A)
(Purple) (Yellow)

Antioxidants react with DPPH, a stable free radical (purple color) which was reduced to DPPH-H (colorless) and as a consequence, the absorbance was decreased from the DPPH radical to the DPPH-H form. Hence, the more rapidly the absorbance decreases, the % of inhibition increases and the more potent the antioxidant activity of the extract.

The percentage of DPPH scavenging activity of methanolic extract of Ayurvedic formulation DKV was presented in Table 2. The methanolic extract of DKV exhibited a maximum DPPH scavenging activity of 68.24% at 100 µg/ml and marketed formulations DKV-1, DKV-2, and DKV-3 exhibit maximum DPPH scavenging activity 67.90%,68.35%, and 68.40%, respectively, at 100 µg/ml. Whereas for ascorbic acid (standard) was found to be 84.75% at 100 µg/ml. The IC50 values of the methanolic extract of DKV were 63.27 µg/ml, and marketed formulations DKV-1, DKV-2, and DKV-3 were 63.13 µg/ml, 62.82 µg/ml, and 62.91 µg/ml, respectively, and ascorbic acid was 40.05 µg/ml.

Table 2 and Figures 1-5 summarizes the DPPH radical scavenging activity of methanolic extracts of DKV DKV, DKV-1, DKV-2, and DKV-3 which is expressed in terms of IC50 value with respect to ascorbic acid as standard. Lower IC50 value shows more antioxidant potential. The IC50 value for methanolic extracts of DKV (DKV, DKV-1, DKV-2, and DKV-3) was approximately near 63 µg/ml which was comparatively lower than the IC50 value (40.05 µg/ml) of ascorbic acid.

CONCLUSION

The results obtained in the study indicate that the Ayurvedic formulation DKV shows significant antioxidant activity. This signifies that combination of herbs can make a good antioxidant herbal formulation, which can be used for the

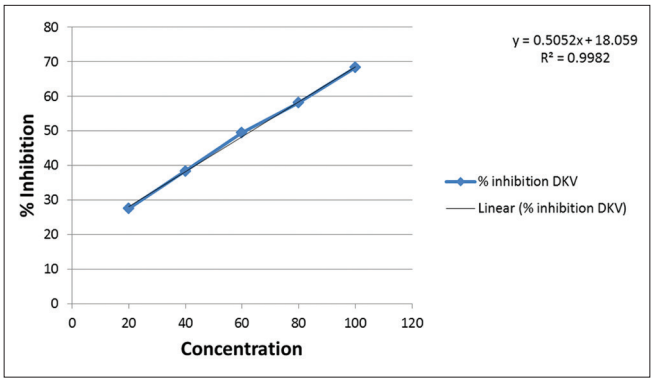

Figure 1: 2, 2-diphenyl-1-picrylhydrazyl radical scavenging effects of methanolic extract of *Drakshavaleha*

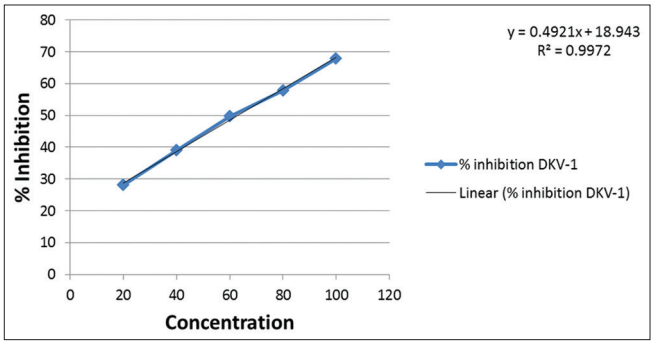

Figure 2: 2, 2-diphenyl-1-picrylhydrazyl radical scavenging effects of methanolic extract of *Drakshavaleha*-1

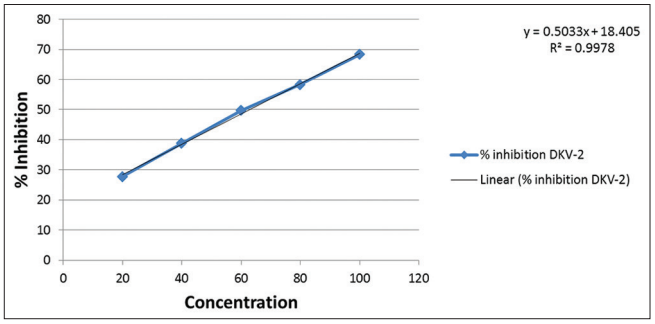

Figure 3: 2, 2-diphenyl-1-picrylhydrazyl radical scavenging effects of methanolic extract of *Drakshavaleha*-2

Table 2: DPPH radical scavenging activity of DKV

Conc. (µg/ml)	DPPH radical scavenging activity (% inhibition)				
	DKV	DKV-1	DKV-2	DKV-3	Ascorbic acid
20	27.60	28.01	27.75	27.65	38.84
40	38.38	39.05	38.85	38.45	48.13
60	49.49	49.69	49.75	49.78	62.53
80	58.13	57.68	58.30	58.40	75.26
100	68.24	67.90	68.35	68.40	84.75
IC_{50} (µg/ml)	63.27	63.13	62.82	62.91	40.05

DKV: *Drakshavaleha*, DPPH: 2, 2-diphenyl-1-picrylhydrazyl

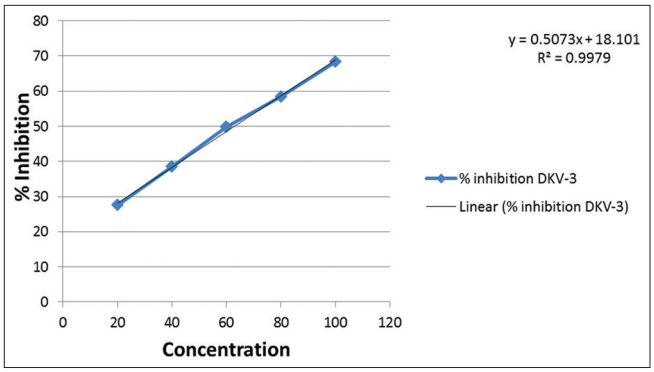

Figure 4: 2, 2-diphenyl-1-picrylhydrazyl radical scavenging effects of methanolic extract of *Drakshavaleha*-3

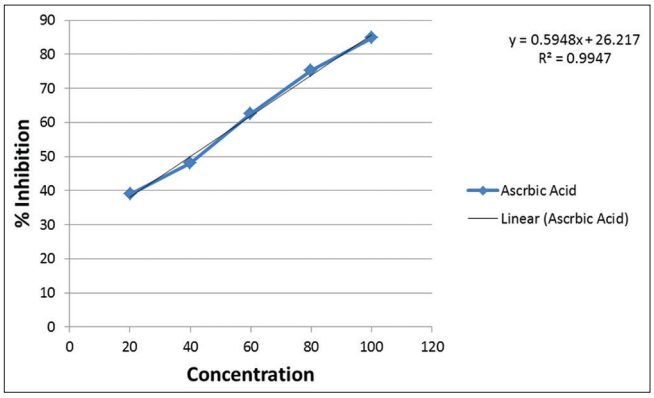

Figure 5: 2, 2-diphenyl-1-picrylhydrazyl radical scavenging effects of ascorbic acid

treatment of many diseases associated with free radicals generation. However, more detailed pre-clinical and clinical evidence are required to establish its potency.

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