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COMPARATIVE FREE RADICAL SCAVENGING AND ANTI-INFLAMMATORY POTENTIAL OF BRANDED MARKET SAMPLES OF AN *AYURVEDIC* FORMULATION: *DASHAMOOLARISHTA*

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ABSTRACT

Dashamoolarishta is a well known *Ayurvedic Rasayana* preparation comprising ten different roots. At least two of these plants are rare, compelling use of alternatives or substitute plant parts, which could results in pharmacological variation in marketed formulations. Moreover, studies on their anti-inflammatory properties are also lacking. This investigation evaluated antioxidant and anti-inflammatory potential of six selected eminent brands of *Dashamoolarishta* (A, B, C, D, E & F). Sample F stood out as highest (Total Phenolics: 21.67± 0.06 mg GAE/g dry mass; ABTS: 29.23 ±0.09 % and Anti Lipid Peroxidation: 91.94 ±0.02 %). Sample E scored lowest in all these values. Formulations F, B and E were further compared using carrageenan-induced rat paw edema model of inflammation. All formulations exhibited variations in anti-inflammatory activity. Maximum inhibition was in animals intervened with formulation F (44.16 %) comparable to that of standard Diclofenac (48.10 %, 10 mg/kg) at 6 h. Our findings support the *Ayurvedic* rationale of using *Dashamoolarishta* formulation and its therapeutic use in treatment of inflammatory diseases. It is reiterated that significant variation exists in biochemical and pharmacological potential among marketed formulations.

KEYWORDS: ***Ayurvedic***, ***Dashamoolarishta***, **Antioxidant, Inflammation.**

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INTRODUCTION

Free radicals are normal metabolic by-products, which in their normal course are of useful in various regulatory processes. However, their excess is required to be controlled by natural anti-oxidants to maintain equilibrium of pro and anti oxidants. When this equilibrium is disturbed due to environmental factors or perturbations in the metabolic processes, it leads to a cascade of changes associated with increased oxidative stress, tissue destruction and inflammation [1]. Imbalance in this homeostasis due to environmental factors or perturbations in the metabolic processes, leads to a cascade of changes associated with increased oxidative stress, tissue destruction and inflammation [1] and known to be the cause of disease conditions like atherosclerosis, hypertension, ischemic disease, Alzheimer's disease, Parkinsonism, cancer, diabetes mellitus and inflammatory conditions [2]. Chronic inflammation is considered to be a major etio-pathological phenomenon in variety of degenerative diseases [3]. Inflammation is considered to be a major etio-pathological phenomenon in variety of diseases. Modern system of medicine mainly emphasises use of Non-steroidal anti-inflammatory drugs (NSAIDs) to control symptoms of inflammation, but number of adverse effects are associated with their long term use. Not surprisingly, people nowadays are choosing Ayurvedic medicines with a great hope to alleviate their pain and ailments. Traditional *Ayurvedic* system has proven role in bio-prospecting natural medicine through various dosage forms [4]. *Ayurvedic* medicinal system has classified medicinal plants into different groups according to their pharmacological actions. Drugs in various '*Rasayana'* forms, which act by modulating the neuro-endocrino-immune systems, have found to be a rich source of antioxidants [5]. *Dashamool* is one amongst such groups comprises of roots of ten pharmacologically potent plants. *Dashamoolarishta,* a classical *Ayurvedic* formulation is one of the major *Sandhana Kalpa* (fermented liquid dosage form). According to *SarngadharaSamhita* [6], *Dashamoolarishta* "nourishes the lean, stimulates the production of *Ojas* and gives progeny to the childless." It helps to restore health and vitality and to recover the normal physiological state from inflammatory condition that has occurred due to vitiated *Vata* [7]. It is used by the *Ayurvedic* fraternity mainly as super-tonic for women during perinatal and perimenopausal phases [8] and has wide prophylactic properties such as antiarthritic, anti-osteoporotic and rheumatism [9, 10]. Considering the wide range of therapeutic efficacy, there are numerous manufacturers producing commercial variety of brands of *Dashamoolarishta*. Unfortunately, availability of all ten ingredient plant roots in *Dashamoolarishta* is limited as some of the plants like *Desmodium gangeticum*, *Oroxylum indicum*, *Uraria picta* are in short supply and on the verge of becoming rare. It is therefore of interest to study variation in pharmacological outcome of the marketed products especially in the light of the different substitute plants being used. The present study was carried out to evaluate this variation in branded market samples of *Dashamoolarishta* for their free radical scavenging activity using *in vitro* biochemical assays. Further, *in vivo* anti-inflammatory potentials of selected 3 samples were assessed in comparison with standard anti-inflammatory drug Diclofenac.

MATERIALS AND METHODS

A) Test Formulations

For the study purpose *Dashamoolarishta* samples, which are commonly prescribed by *Ayurvedic* fraternity and available in market were selected. Among these samples, 6 representative samples with pronounced use and with similar manufacturing date (±1 month) were procured to rule out maturation effect.

These samples were then coded A to F for the analysis to nullify evaluator bias in data generation. The decoding was done only when all the analysis was complete.

B) Chemicals

All chemicals used for *in-vitro* biochemical assay to evaluate free radical scavenging potential were of analytical grade. Carrageenan, for *in-vivo* anti-inflammatory study was obtained from HiMedia, India. Diclofenac (Reactin 50-Cipla) was obtained from local medical store.

C) Biochemical Evaluations

For the ease of performing the following biochemical assays, extractive values (% yield) of all formulations were calculated and the stock concentration of each formulation was adjusted to 10 mg/ml. All assays were done in triplicate and an average value was taken for final analysis.

(a) Determination of Total Phenol Content

The amount of total phenolic compounds in formulations was determined using Folin-Ciocalteu (FC) reagent [11, 12]. A standard curve (R^2 = 0.9) of gallic acid was used to measure the phenolic content.

(b) Anti-oxidant Assays

i) DPPH Free Radical Scavenging Assay

Antioxidant capacity of the formulations was confirmed by DPPH scavenging assay according to Brand-Williams *et al*., [13] with slight modifications. Different concentrations (10-100 µg/ml) of the formulations and ascorbic acid (standard) were mixed with 4 ml of methanolic DPPH solution and were incubated for 20 minutes at 37°C and OD was measured at 517 nm. The percentage radical scavenging activity was calculated by using the following formula:

% scavenging = $[(A_0-A_1)/ A_0]$ X100

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the samples.

ii) Total Anti-oxidant Capacity (ABTS assay)

A modified Method of Re *et al*., [14] was adopted for determination of $ABTS^{\cdot+}$ radical scavenging activity of the formulations. In brief, the $ABTS^{\cdot+}$ reagent was prepared by mixing 7 mM solution of ABTS in water with 2.45 mM potassium persulphate ($K_2O_8S_2$) (1:1). The mixture was kept in dark until the stable absorbance at 734 nm is achieved. This $ABTS^{\cdot+}$ solution was diluted with water in desired proportion to get stable absorbance of 0.7± 0.005 at 734 nm. The reaction mixture for assay included 980 µl $ABTS^{\cdot+}$ reagent and 20 µl test sample or standard and absorbance was measured after 6 min at 734 nm. As a standard, ascorbic acid (10-100 µg/ml) was used. The percentage of scavenging inhibition capacity of $ABTS^{\cdot+}$ was calculated using following equation;

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$$

iii) Inhibition of lipid Peroxidation Assay

Peroxidation of membrane lipids in the tissue leads to formation of Malondialdehyde (MDA) along with other aldehydes and enols as the end products, which react with thiobarbituric acid forming coloured complexes. The complex of TBA-MDA is detected at 532 nm spectrophotometrically [15]. The assay involved (i) perfusion of goat liver with 0.15 M KCl, (ii) initiation of lipid peroxidation by addition of 1 mM $FeCl_3$, (iii) terminating the reaction by adding ice-cold 0.25 N HCl containing TCA and TBA, (iv) addition of BHT and (v) finally measuring the OD at 532 nm against solutions without $FeCl_3$ (normal) and without drug (induced).

(c)Determination of Total Reducing Power

i) Ferric Reducing Antioxidant Potential (FRAP) Assay

The assay was performed according to standard method as described by Benzie and Strain [16]. The stock solutions included 300 mM acetate buffer, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. Fresh FRAP solution was

prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. Different concentration of formulations (150 µL) were allowed to react with 2.85 ml of the FRAP solution for 30 min in the dark and absorbance of the coloured ferrous complex was measured at 593 nm. The standard curve of $FeSO_4$ was plotted for 100-1250 µM $FeSO_4$. Results are expressed in µM Fe (II)/g dry mass from standard graph[17].

ii) Reducing Power Assay

A modified method described by Hazra *et al*., [18] was adopted for determination of ferric Fe^{3+}-reducing power of the formulations. Different concentrations (10-100 µg/ml) of the formulations were mixed with phosphate buffer (pH 6.6) and potassium hexacyanoferrate (0.1%), and then incubated at 50°C for 20 min. This reaction was arrested by adding 10% tricarboxylic acid (TCA) and distilled water (2.5 ml) and $FeCl_3$ solution (0.01%) to the upper portion of the reaction mixture. After 10 min. incubation, absorbance at 700 nm was measured. All tests were performed in triplicate and compared with Ascorbic acid as a standard.

D) In-vivo Carrageenan Induced Paw Edema Test

Based on the results obtained from *in-vitro* biochemical assays, 3 marketed *Dashamoolarishta* formulations, 2 samples for higher efficacies while 1 sample showing lowest efficacy were selected for evaluating *in vivo* anti-inflammatory potential. The anti-inflammatory activity was carried out in accordance with Carrageenan induced rat paw edema model by Winter *et al*., [19]. Female wistar rats weighing 180 ± 20 g were obtained from National Institute of Biosciences, Pune. The animals were housed in institute's animal house at an ambient temperature (25 ± 2°C) and day and night cycles of 12 hours. Access to food and water was *ad libitum*. Experimental protocol was approved by Institutional Animal Ethical Committee (Approval number; CPCSEA/10/PCP/2010) Animals were divided into 6 groups of 6 rats each; Group I: Healthy control (water); Group II: Inflammation control (induced by Carrageenan); Group III: Standard drug (Diclofenac, 10 mg/ kg); while Group IV to VI received selected *Dashamoolarishta* formulations B, E and F respectively (2.34 ml/kg with equal volume of water extrapolated from standard dose for human). The test drugs and vehicle were administered daily for consecutive 8 days. On the 8th day, the left hind paw volumes were recorded by using a plethysmometer (UGO Basile, Italy) [20]. Standard drug Diclofenac (10 mg/kg) was administered 1 h prior to carrageenan injection. 1 h after the drug administration, 0.1 ml of 1% carrageenan was injected into the sub-plantar aponeurosis of the left hind paw. Paw volumes were recorded at 1, 2, 3, 4, 5 and 6 h after the carrageenan injection. The difference between initial paw volume and paw volume at respective hours ensures increased edema. Percentage inhibition against edema formations is considered as an index of anti-inflammatory potential and was calculated by:

$$\% \text{ Inhibition of inflammation} = [(Vc-Vt)/ Vc] \times 100$$

Where Vc = mean paw volume of Inflammation control group

Vt = mean paw volume of drug treated group

E) Statistical Analysis

Statistical analysis was performed using Graph pad Prism (Version 6) software. For all the biochemical evaluations, all data are reported as the mean ± SD of triplicates. Student's t test for unpaired data has been used for analyzing the data generated during the study. For *in vivo* studies, analysis was carried out using Two-way ANOVA followed by *post hoc Bonferroni* tests. *P*- values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

1) Determination of Total Phenolic Content

Secondary metabolites including poly-phenols are known to play pivotal role in the free-radical quenching properties of plants [21, 22]. Total phenolic content (expressed in mg/g of dry mass of Gallic acid equivalent) in marketed *Dashamoolarishta* samples is shown in Table 1. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species [23]. Highest content of total phenols was found in sample F (21.67 ±0.06 mg/g of dry mass of GAE).

Comparative chart of Total phenol content of all marketed formulations

Formulation	mg GAE/g dry mass
A	17.65 ±0.07
B	12.27 ±0.25
C	14.72 ±0.15
D	13.19 ±0.02
E	10.10 ±0.10
F	21.67 ±0.06

Table 1
Total phenolic content (mg/g of dry mass of Gallic acid equivalent) showing presence of highest phenolics in sample F and lowest in sample E

2) In-vitro Antioxidant Assays

In vitro anti-oxidant activity of *Dashamoolarishta* formulations indicated that the selected formulations have potent free radical scavenging capacity. Also, *Dashamoolarishta,* the *Rasayana* formulations have potent antagonistic actions on the oxidative stressors, which give rise to the formation of different free radicals [3]. In the present study, all samples of *Dashamoolarishta* formulation showed significant free radical scavenging activity at a concentration of 100 µg/ml in a dose dependent manner.

DPPH Free Radical Scavenging Assay

Dashamoolarishta formulations were also found to be potent DPPH-free radical scavenger (Table 2). Comparatively higher activity was observed in sample F (94.29 ±0.11 %) and B (94.02 ±0.29 %) while sample E (84.9 ±0.32 %) showed lowest activity at concentration 100 µg/ml. The free-radical scavenging activities of all samples were more than standard ascorbic acid (82.04 ±0.07 %) clearly indicating that the formulations are potent in scavenging free radicals *in vitro*, but there is wide variation among screened marketed formulations.

Total Anti-oxidant Capacity (ABTS assay)

All *Dashamoolarishta* formulations under investigation were efficient in scavenging the radical cation $ABTS^{\cdot+}$, which eventually reduced to ABTS'. Highest radical-scavenging activity was observed by formulation F (29.23 ±0.09 %) followed by C (25.73 ± 0.13 %) at 100 µg/ml concentration, while standard ascorbic acid showed 50.55 ±0.03 % activity at 100 µg/ml (Table 2). Proton radical scavenging is an important attribute of antioxidants. A characteristic absorbance maximum at 734 nm is observed for protonated radical 2, 2'-azinobis-3- ethylbenzothiazoline-6-sulfonic acid (ABTS), which decreases with the scavenging of proton radicals [24].

Anti-lipid peroxidation assay

Free radical is known to abstract a hydrogen atom from a methylene carbon of an unsaturated long chain fatty acid and can initiate a lipid peroxidation reaction, which increases in many oxidative damage-induced diseases and it reflects irreversible change of membranes [25, 26]. In the anti-lipid-peroxidation assay, formulation F exhibited highest anti-lipid peroxidation activity (91.94 ±0.02 %) at 100 µg/ml concentration while the lowest activity was observed in sample E (20.67 ±0.16 %). Formulation A, B, C and D showed equivalent protection against lipid peroxidation and was comparable to that of standard ascorbic acid activity, which was 72.19 ±0.05 %. The percent values of all formulations as compared to standard are given in Table 2.

Anti-oxidative Potentials of market samples of Dashamoolarishta demonstrated by In Vitro biochemical assays

Antioxidant Assays	Conc. µg/ml	% Scavenging Activity						
		Ascorbic acid	Formulations					
			A	B	C	D	E	F
DPPH	20	8.49 ± 1.02	37.45 ±0.15	47.74 ± 1.05	57.45 ±0.05	39.40 ± 0.17	21.98 ±2.08	58.19 ±0.22
	40	23.46 ±0.31	68.29 ±0.29	69.74 ±1.07	74.89 ±0.97	54.99 ± 1.34	35.76 ±0.74	70.13 ±0.67
	60	49.11 ±0.61	83.64 ±1.01	81.50 ± 2.07	86.84 ±0.07	63.70 ± 1.03	57.28 ±2.17	81.41 ±0.40
	80	67.71 ±1.09	87.42 ±0.48	90.68 ± 1.23	92.63 ±0.68	70.43 ± 0.39	72.89 ±1.08	89.28 ±0.44
	100	82.04 ±0.07	92.11 ±0.09	94.02 ± 0.89	93.84 ±0.02	92.82 ± 0.38	84.93 ±0.32	94.29 ±0.11
ABTS	20	13.46 ±0.29	11.99 ±0.41	12.56 ±0.06	9.58 ± 0.12	7.37 ± 0.08	13.83 ±1.05	13.31 ± 0.05
	40	20.94 ±0.15	13.46 ±0.05	13.74 ±0.27	13.79 ±0.31	8.92 ± 0.73	14.12 ±2.15	17.04 ± 0.23
	60	31.62 ±0.52	16.43 ±1.05	16.62 ± 0.46	20.16 ±1.29	13.22 ± 1.05	14.68 ±0.64	21.48 ± 0.18
	80	41.17 ±0.17	19.88 ±0.08	18.93 ± 0.06	23.51 ±0.65	15.06 ± 0.39	17.14 ±0.25	25.87 ± 0.11
	100	50.55 ±0.03	21.95 ±0.37	20.40 ± 1.16	25.73 ±0.13	18.18 ± 0.41	17.89 ±0.82	29.23 ± 0.09
ALP	20	17.79 ±0.97	23.13 ±1.07	23.06 ± 0.04	27.05 ±1.27	27.79 ± 2.07	6.22 ±1.23	47.31 ± 1.05
	40	41.65 ±1.03	35.51 ±1.05	44.37 ± 0.47	36.31 ±0.57	36.25 ± 1.2	10.98 ±0.21	60.25 ± 0.07
	60	53.85 ±0.02	52.61 ±0.53	66.90 ± 1.12	46.33 ±1.34	48.98 ± 0.75	16.25 ±0.29	78.20 ± 0.62
	80	64.89 ±0.29	61.47 ±0.30	72.56 ± 0.08	54.56 ±0.33	51.37 ± 0.81	20.22 ±0.07	86.08 ± 0.09
	100	72.19 ±0.05	78.98 ±0.14	83.51 ± 0.03	79.16 ±0.21	65.62 ± 0.13	20.67 ±0.16	91.94 ± 0.02

Table 2

(a) % scavenging activity for DPPH and ABTS radicals and % inhibitory potential against lipid peroxidation showing highest activity for sample F at 100 µg/ml (94.29 ± 0.11, 29.23 ± 0.09 and 91.94 ± 0.02) as compared to Standard Ascorbic acid (82.04 ±0.07, 50.55 ±0.03 and 72.19 ±0.05)

All *Dashamoolarishta* formulations except sample E were found to be potent free-radical scavenger, *in vitro*. Thus the anti-lipid peroxidation activity of these formulations may be due to strong free-radical quenching potentials of the plants used in it or due to their

synergistic effect and also can be a possible clue for their protection offered against lipid peroxidation, which in turn reflect on in combating or preventing the inflammatory diseases.

3) Determination of Total Reducing Power

Ferric Reducing Antioxidant Potential (FRAP) Assay

The FRAP values are represented in terms of µM Fe (II) /g of dry mass of sample. The values for sample A, B C D E and F were determined using equation y = 0.158x + 0.037 obtained from the standard graph of Ferrous Sulphate. Considering the FRAP values, all formulations exhibited significant FRAP activity (Table 3). Highest FRAP value was found in Sample F (5.98 ±0.04 µM Fe (II)/g of dry mass). The FRAP antioxidant potential of formulations was estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) which may be due to the presence of poly-phenols.

FRAP value of market samples of *Dashamoolarishta*

Formulation	FRAP µM Fe (II)/g of dry mass
A	4.05 ± 0.44
B	5.06 ± 0.12
C	4.29 ± 0.32
D	3.3 ± 0.18
E	3.15 ± 0.63
F	5.98 ± 0.04

Table 3

FRAP expressed in µM Fe (II)/g of dry mass showing highest value for formulation F

Reducing Power Assay

Presence of antioxidants in the formulations would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex can be monitored by measuring the formation of Perl's blue at 700 nm. Increasing absorbance indicates an increase in reductive ability [18]. All *Dashamoolarishta* samples showed increased absorbance in a dose dependent manner at 700 nm, from 10-100 µg/ml concentration (Fig. 1), thus showing higher *in vitro* ferric reducing potential than that of standard Ascorbic acid (OD 0.434). Sample F showed maximum activity (OD 0.927) and there was significant variation observed in the activity of remaining formulations.

Comparative graph for determining Reducing power

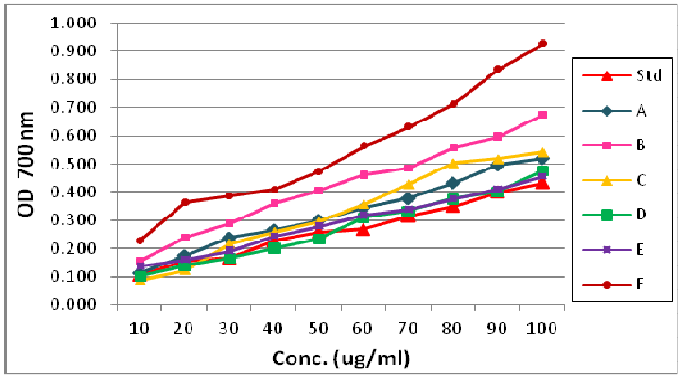

Figure 1
Dose dependant Graph showing comparative reducing potential of all market Dashamoolarishta formulations; sample F showing highest potential as compared to standard Ascorbic acid

Thus, our results showed that free-radical scavenging activities of all *Dashamoolarishta* formulations were significant, wherein formulation F showed the highest values as well as formulation E exhibited lowest values for all assays presenting a broad range of variation in their anti-oxidant potential which positively correlates with the presence of phenolic compounds in these formulations.

***In vivo* anti-inflammatory study**

Carrageenan-induced rat paw edema is a suitable experimental model that represents a classical tool to estimate acute inflammation and anti-inflammatory potential. This model has been extensively used for evaluating an antiedematous effect of herbs and herbal extracts having anti-inflammatory properties [27]. The paw edema developed serves as an index of acute inflammatory changes, which is determined from differences in the paw volume measured immediately after carrageenan injection and then every hour for 6 hours. Edema induced by carrageenan is believed to be biphasic: the first phase (~2 h) involves the release of chemical mediators such as serotonin and histamine and the second phase (over 2 h) is mediated by prostaglandins, the cyclooxygenase products. Continuity between the two phases is provided by kinins [28, 29] and these inflammatory changes can be well observed in Carrageenan-induced rat paw edema model of acute inflammation. Amongst all branded formulations, sample F and B were selected based upon their best scores shown in biochemical assays. Sample E was selected for the lowest efficacy to bring forth the entire spectrum of variability. These were compared with the standard anti-inflammatory drug Diclofenac. Results obtained from *in vivo* study showed that all formulations exhibited significant anti-inflammatory activity between 3-6 hours against carrageenan-induced rat paw edema. Maximum inhibition was observed in animals treated with *Dashamoolarishta* formulation B at 3rd h (13. 47 %) and sample F at 6th h (44.16 %) after carrageenan injection, suggesting that formulations produced maximum anti-edematous effect during the second phase. Standard Diclofenac (10 mg/kg) inhibited the edema formation to an extent of 26.18 % and 48.10 % at 3rd h and 6th h respectively (Fig. 2). Data pertaining to the effect of the test formulations on carrageenan-induced hind paw edema is furnished in Table 4.

Effects of selected Dashamoolarishta formulations on rat paw edema induced by λ-carrageenan

Table 4
Inhibitory effects of Dashamoolarishta formulations and Diclofenac on paw edema from 0-3 hrs. and 0-6 hrs.

Treatment	Dose (mg/kg)	Paw Volume at 3 hrs. (ml)	% Inhibition from 0-3 hrs.	Paw Volume at 6 hrs. (ml)	% Inhibition from 0-6 hrs.
Carrageenan		4.79±0.14***	-	4.69±0.31***	-
Diclofenac	10 mg/ kg	3.51±0.12 ns	26.18	2.60±0.10***	48.10
Dashamoolarishta B	2.34 ml/kg	4.11±0.13 ns	13.74	3.33±0.15*	25.99
Dashamoolarishta E		4.41±0.13 ns	7.57	2.79±0.17***	37.87
Dashamoolarishta F		4.47±0.09*	6.19	2.52±0.10***	44.15

Results are represented as mean ± SEM, (n=6), *p < 0.05, **p < 0.01, ***p < 0.001 compared with Carrageenan control, Two-way ANOVA followed by post hoc Bonferroni tests

Comparative % Inhibition of Carrageenan Induced Rat Paw Edema

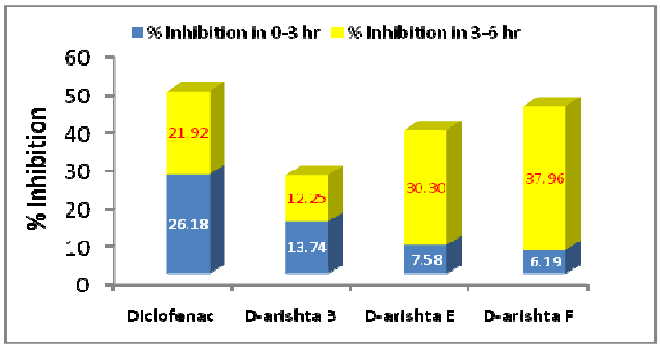

Figure 2
Graph showing % inhibition by formulations as compared to standard Diclofenac from 0-3 hrs. and 0-6 hrs.

CONCLUSIONS

Dashamool is considered to be an important *Rasayana* preparation in *Ayurveda*, which is recommended in various ailments. *Dashamoolarishta* is widely marketed formulation even today and leading manufacturers have this formulation in the market. Out of the ten plants described in the classical literature, at least two have become rare and their market availability is significantly low. In this scenario alternative plants or plant parts other than root are used. The objective of the current study was to compare the marketed samples of *Dashamoolarishta* based on their antioxidant and anti-inflammatory potential. All *Dashamoolarishta* formulations showed significant antioxidant activity and anti-inflammatory potential in the carrageenan-induced rat paw edema model providing rationale of their use in inflammatory conditions. However, there were remarkable

differences in the activity, which may be due to the differences in the process as well as in differences in substitution of rare plants. This reiterates the need for optimization of procedures and uniformity in use of raw drugs that alone will enable uniformity and predictability in their pharmacological action. Further studies are planned to prepare a formulation with all the prescribed plants after authentication and optimise the standard operating protocol so as to minimise the variation in activity and thus the clinical outcome of *Dashamoolarishta* formulations.

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CONFLICT OF INTEREST

Conflict of interest declared none

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