

## COMPARATIVE VALIDATION OF SELF COLLECTED AND MARKET 'SARIVA' ROOTS WITH RESPECT TO THEIR AMBIGUITY IN THE IDENTIFICATION USING *IN-VITRO* BIOCHEMICAL ASSAYS

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**Abstract** – Plant 'Sariva' has been used since long in Ayurveda and folk medicine. There are four different species used as 'Sariva' in various regions of India viz., *Cryptolepis buchanani* Roem. & Schult., *Hemidesmus indicus* (L.) R.Br. and *Decalepisha miltonii* Wight & Arn belongs to same family Asclepiadaceae, while *Ichnocarpus frutescens* (L.) R. Br. belongs to family *Apocynaceae*. Present study is designed to evaluate the ambiguity in market and authenticate samples of 'Sariva' root extract in terms of their polyphenolic contents and antioxidant profile. Market samples were collected from major markets from India viz., Bangalore, Delhi, Mumbai, Nagpur, Pune and West Bengal. While, authenticate species of 'Sariva' was collected from Western Ghats of Maharashtra, India. Antioxidant profile was evaluated by nitric oxide scavenging potential, BSA anti-denaturation assay and anti-lipid peroxidation assays. Total phenolic and flavonoid contents were studied by standard methods viz. Folin-Ciocalteu and aluminium chloride respectively. Methanolic extract of 'Sariva' market sample showed highest phenolic contents ( $0.295 \pm 0.023 \mu\text{g/ml}$ ) as compared to the flavonoid contents in Pune market sample. While, highest concentration of total phenol contents was observed in *Decalepisha hamiltonii* ( $0.346 \pm 0.034 \mu\text{g/ml}$ ) and flavonoids was observed in *Cryptolepis buchanani* ( $0.11 \pm 0.007 \text{mg/ml}$ ). The methanolic extract of West Bengal showed highest anti-lipid peroxidation activity ( $\text{IC}_{50}$  value of  $50.96 \mu\text{g/ml}$ ). While, lowest activity was observed in Delhi market sample ( $\text{IC}_{50}$  of 218.63). On the contrary, *Hemidesmus indicus* showed highest anti-lipid activity ( $28.88 \mu\text{g/ml}$  of  $\text{IC}_{50}$ ) followed by *Decalepisha miltonii*, *Cryptolepis buchanani* and *Ichnocarpus frutescens*.  $\text{IC}_{50}$  value indicates that among all the market samples Bangalore market sample ( $67.26 \mu\text{g/ml}$ ) showed more significant protein stabilizing activity (denaturation is inhibited). While, *Decalepisha hamiltonii* showed more significant protein stabilizing activity ( $32.73 \mu\text{g/ml}$ ) followed by *Hemidesmus indicus*, *Cryptolepis buchanani* and *Ichnocarpus frutescens*. Among market samples, Pune market sample showed highest nitric oxide scavenging activity ( $65.15 \mu\text{g/ml}$  of  $\text{IC}_{50}$ ) and in authenticate samples, *Decalepisha hamiltonii* showed highest nitric oxide scavenging potential ( $31.26 \mu\text{g/ml}$  of  $\text{IC}_{50}$ ). By evaluating the five market samples in comparison with authenticate 'Sariva' species, it is concluded that distinct variation observed in polyphenolic contents and antioxidant activity. Hence, present study suggests the need of awareness in the selection of the correct species and scientific efforts to solve this ambiguity.

### INTRODUCTION

Medicinal plant 'Sariva' is an eminent drug of Ayurveda and been in ample use since ancient times. It is also known as Indian Sarsaparilla. It is commonly found throughout India and is widely

recognized in traditional system of medicine. Two types of 'Sariva' are mentioned in ayurveda those are 'Shweta and Krushna Sariva' (Austin, 2008). 'Sariva' have been used in Ayurveda as a curative agent for variety of ailments like skin disorders, fever, leucorrhoea, urinary infections, dysentery,

dyspepsia, leukoderma, burning sensation, asthma, leprosy, loss of appetite, eye diseases, epileptic disorder, nutritional disorder, ulcer, rheumatism and blood purifier purpose etc. Due to its attributes of Madhur, Tikta Rasa; Madhur Vipak, Sheet Veerya and Guru, Snigdha Gunas; it performs actions such as Tridoshshamak but mainly Pittashamak, Varnya, Purishsangrahnaya etc. The root is presumed for its cooling and blood purifying action and is consequently used to make refreshing drinks. (Chopra *et al.*, 1956; Pansare, 2018).

'Sariva' showed few pharmacological activities having the potential of anti-inflammatory, antimicrobial, antinociceptive, anti-enterobacterial, anti-oxidant, anti-atherogenic and anti-carcinogenic agent (Aneja *et al.*, 2008).

Presently, *Hemidesmus indicus* is referred as true 'Sariva' (Prasanna and Maruthi, 2019). According to literature, in different regions four species are being used as 'Sariva' i.e., *Decalepis hamiltonii* (Wight and Arn), *Cryptolepis buchanani* (Roem & Schult), *Ichnoparcs frutescens* (L.) R.Br. and *Hemidesmus indicus* (L.) R.Br. In Ayurveda, *Cryptolepis buchanani* and *Ichnocarpus* are also collectively known as 'Sariva'. In south region of India *Decalepis hamiltonii* is being used as substitute of 'Sariva' (Nayar, 1977). So, there is need to solve the ambiguity in different species coming under the name 'Sariva'.

In the present study, market and authenticate species of 'Sariva' were evaluated in terms of biochemical assays. Antioxidant activity was investigated using a variety of *in-vitro* methodologies, including an anti-lipid peroxidation assay, nitric oxide scavenging potential assay, and BSA anti-denaturation assay. Total phenol and total flavonoid content estimation were also detected. In present work, the efforts are made to study the ambiguity with respect to their biological activity and antioxidant activity of 'Sariva' samples collected from major markets of India and Western Ghats of Maharashtra, India.

## MATERIALS AND METHODS

### Chemicals and reagents

The chemicals and reagents used in the following biochemical assays are Potassium chloride (0.15M), Butylated hydroxyl toluene solution (0.05% BHT), Thiobarbituric acid (TBA) solution (1 mM), Ferric chloride anhydrous, Standard Ascorbic Acid, Folin-Ciocalteu reagent (1:10 diluted) 1M Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), Quercetin, 5% Sodium Nitrite

( $\text{NaNO}_2$ ), 10% Aluminium chloride ( $\text{AlCl}_3$ ).

### Plant collection and extraction

Root samples of the 'Sariva' were purchased in India's major marketplaces, including Bangalore, Delhi, Mumbai, Nagpur, Pune, and West Bengal. While, authentic species samples were collected from village Chavni of Raigad district from 'Sahyadri' Range of Maharashtra. Plants were identified and authenticated by Taxonomist, Dr. Suresh Jagtap, Associate Professor, IRSHA, Pune. Required State Biodiversity permission has been taken from MSBB, Nagpur, Maharashtra. Roots were pulverized to a fine powder in a mechanical blender. These fine powders were utilized for further experimental purposes.

### Polyphenolic content assays

Polyphenolic content of extracts was determined using the Folin-ciocalteu method. It is demonstrated as mg of Gallic acid equivalents (GAE) per gram of dry weight of the extract. Total flavonoid content was assessed using an Aluminum chloride colorimetric method and it is expressed as mg of Quercetin per gram of dry weight (Narkhede, 2016). All the experiments were carried out in triplicates.

### *In vitro* biochemical assays

#### Nitric oxide scavenging potential

Nitric oxide has been shown to be directly scavenged by flavonoids (Lakhanpal and Rai, 2007). *In vitro* quenching of NO radical is one of the methods that can be used to determine antioxidant activity (Nagmoti, 2011). The procedure is based on the Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent.

The percent inhibition was calculated using the formula:

$$\% \text{ Inhibition activity} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \quad \dots \text{Eq.2}$$

Where,  $A_{\text{Control}}$  is absorbance of Control and  $A_{\text{Sample}}$  is absorbance of Sample.

#### BSA anti-denaturation assay

Bovine Serum Albumin (BSA) is a water-soluble protein that denatures on heating. Denaturation of proteins involves the disruption and possible destruction of both the secondary and tertiary

structures. This assay applies for the detection of compounds that can stabilize the protein BSA from denaturation process at a physiological pH 6.5 (Williams *et al.*, 2008).

The percentage denaturation inhibition was calculated by the formula:

$$\% \text{ Inhibition activity} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \quad \text{.. Eq.1}$$

Where  $A_{\text{Control}}$  is absorbance of the control and  $A_{\text{Sample}}$  is absorbance of samples.

### Antilipid peroxidation assays

The lipid peroxidation was performed by TBARS (Thiobarbituric acid reactive substance) assay (Wade *et al.*, 1985). Decomposition of lipid membrane in the body leads to formation of Malondialdehyde (MDA) along with other aldehydes and enals as the end product. These react with Thiobarbituric acid to form colored complexes. Hence these are called as the Thiobarbituric acid Reactive Substances (TBARS). The measurement of lipid peroxidation inhibition enables an assessment of the protective effect of foods on oxidative stress and is the most applied methods. MDA + 2 TBA pink red complex (measured at 532 nm).

### Statistical Analysis

Data were analyzed by one-way ANNOVA and expressed as mean±standard error. *P* value less than 0.05 considered statistically significant.

## RESULTS

In present study, all the species of 'Sariva' were extracted by using methanol as a high extraction yield solvent (Das *et al.*, 2003 and Tabassum *et al.*, 2016). Highest yield was obtained in *Decalepis hamiltonii* extract of authentic samples while, in market sample group, Sample from Pune market gained highest yield (Table 1).

### Quantitative phytochemical screening

In present study, all the authentic and market samples were screened for their phytochemical screening (Table 2 and 3). Polyphenols are responsible for scavenging ability of the extract. Among polyphenolic content, phenolic and flavonoids are the major group of compounds which are believed to be responsible for antioxidant activity of the extract.

Authenticate samples of the 'Sariva' showed presence of both the polyphenols. Among them, highest concentration of phenol content was observed in all the species as compared to the flavonoids. Highest concentration of total phenol contents was observed in *Decalepis hamiltonii* (0.346±0.034) and flavonoids was observed in *Cryptolepis buchanani* (0.11±0.007). Among the methanolic extract of 'Sariva' market samples, Pune market sample showed highest phenolic contents (0.295±0.023) as compared to the flavonoid contents.

**Table 1.** The extraction yield of market and authentic samples of 'Sariva'

Authentic samples	Percent Yield (%)	Market samples	Percent Yield (%)
<i>Cryptolepis buchanani</i>	5.7%	Mumbai	5.6%
<i>Decalepis hamiltonii</i>	8.5%	Delhi	5.5%
<i>Hemidesmus indicus</i>	6.5%	Bengalore	5.5%
<i>Ichnocarpus frutescens</i>	4.7%	Nagpur	3.4%
		Pune	6.5%
		West Bengal	5.7%

**Table 2.** Total phenol and flavonoid content in authenticated species of 'Sariva'

Name of Authenticated species of Sariva	Total phenol content (50 µg/ml conc.) mg/g of GAE equivalent <sup>a,b</sup>	Total flavonoid content (1 mg/ml conc.) mg/g of Quercetin equivalent <sup>a,c</sup>
<i>Cryptolepis buchanani</i>	0.31±0.009	0.11±0.007
<i>Decalepis hamiltonii</i>	0.346±0.034	0.095±0.009
<i>Hemidesmus indicus</i>	0.334±0.03	0.10±0.007
<i>Ichnocarpus frutescens</i>	0.306±0.038	0.08±0.006

<sup>a</sup>=All values are mean±SD, n=3, <sup>b</sup>=Values are expressed as equivalent to Gallic acid (mg/gram of GAE), <sup>c</sup>=Values are expressed as equivalent to Quercetin.

Highest total phenol contents were observed in authentic samples as compared to the market samples.

### *In vitro* biochemical assays

Considering the results, all the 'Sariva' species able to reduce the stable radicals to 50% reduction, this termed as IC<sub>50</sub> value. In present work, the order of nitric oxide potential was observed highest in West Bengal followed by Pune, Nagpur, Mumbai, Bangalore and Delhi (Table 4). The methanolic extract of West Bengal showed highest free radical scavenging activity (IC<sub>50</sub> value of 50.96 µg/mL). While, lowest activity was observed in Delhi market sample (IC<sub>50</sub> of 218.63). Authenticate species viz. *Cryptolepis buchanani*, *Decalepis hamiltonii*, *Hemidesmus indicus* and *Ichnocarpus frutescent* which are known as 'Sariva' were also studied for their

antioxidant efficacy (Table 5). Results revealed that *Hemidesmus indicus* showed highest anti-lipid activity (28.88 µg/mL of IC<sub>50</sub>) followed by *Decalepis hamiltonii*, *Cryptolepis buchanani* and *Ichnocarpus frutescent*.

The present findings exhibited an inhibition of protein (albumin) denaturation by all the test extracts (Table 3 and 4). IC<sub>50</sub> value indicates that among all the market samples Bangalore market sample (67.26 µg/mL) showed more significant protein stabilizing activity (denaturation is inhibited) followed by Nagpur, Mumbai, West Bengal, Pune and Delhi. Authenticate 'Sariva' species exhibited highest inhibition of protein (denaturation) as compared to the market samples (Table 3 and 4). Among them *Decalepis hamiltonii* showed more significant protein stabilizing activity (32.73 µg/mL) followed by *Hemidesmus indicus*,

**Table 3.** Total phenol and flavonoid content in market 'Sariva' drug

Name of Market sample	Total phenol content (1µg/ml conc.) mg/g of GAE equivalent <sup>a, b</sup>	Total flavonoid content (1 mg/ml conc.) mg/g of Quercetin equivalent <sup>a, c</sup>
Mumbai	0.286±0.017	0.08±0.01
Delhi	0.144±0.010	0.06±0.01
Bangalore	0.206±0.026	0.07±0.006
Nagpur	0.289±0.022	0.08±0.013
Pune	0.295±0.023	0.09±0.010
West Bengal	0.235±0.018	0.08±0.008

<sup>a</sup>=All values are mean±SD, n=3, <sup>b</sup>=Values are expressed as equivalent to gallic acid (mg/gram of GAE), <sup>c</sup>= Values are expressed as equivalent to quercetin.

**Table 4.** Antioxidant activity of market sample extracts

Market samples of Sariva drug	Anti-lipidperoxidation (ALP) Assay (1mg/ml conc.) Percentage of Inhibition (IC <sub>50</sub> µg/ml)	Anti-denaturation assay (BSA) Percentage of Inhibition (IC <sub>50</sub> µg/ml)	Nitricoxide scavenging potential (NO)
Mumbai	69.22	93.77	89.07
Delhi	218.63	106.85	199
Bangalore	161.08	67.26	82.52
Nagpur	67.10	78.12	83.43
Pune	57.96	95.77	65.15
West Bengal	50.96	92.33	94.75

**Table 5.** Antioxidant activity of authenticate sample extracts

Authenticated Sample	Anti-lipid peroxidation (ALP) assay (1mg/ml conc.) Percentage of Inhibition (IC <sub>50</sub> µg/ml)	Anti-denaturation assay (BSA) Percentage of Inhibition (IC <sub>50</sub> µg/ml)	Nitricoxide scavenging potential (NO)
<i>Cryptolepis buchanani</i>	37.95	41.44	37.27
<i>Decalepis hamiltonii</i>	30.18	32.73	31.26
<i>Hemidesmus indicus</i>	28.88	34.42	32.28
<i>Ichnocarpus frutescent</i>	39.80	46.07	49.115

*Cryptolepis buchanani* and *Ichnocarpus frutescent*.

In present work methanolic extract of both market and authenticate 'Sariva' plant extract were studied and were exhibited nitric oxide scavenging potential (Table 3 and 4). Among market samples Pune market sample (65.15 µg/ml of IC<sub>50</sub>) showed highest nitric oxide scavenging activity followed by Bangalore, Nagpur, Mumbai, West Bengal and Delhi. While, among authenticate 'Sariva' samples *Decalepis hamiltonii* showed highest nitric oxide scavenging potential (31.26 µg/ml of IC<sub>50</sub>) followed by *Hemidesmus indicus*, *Cryptolepis buchanani* and *Ichnocarpus frutescent*. Amongst market and authenticate samples of 'Sariva' authenticate samples showed more significant activity for all the studied antioxidant assays.

## DISCUSSION

Bioactive compounds present in medicinal plant extract usually occur in low concentration. Extraction technique is a physical transfer of the original plant material into another phase which can be separated by filtration and which is able to obtain high yield, minimum alteration in their functional properties etc. Variation in the biological activities of the plant extract, which were prepared using different techniques have been reported by several researchers (Kaker *et al.*, 2020). Hence, it is necessary to select the suitable solvent along with extraction method based on sample properties, chemical properties of the analytes, matrix-analyte interaction, efficiency and desired properties (Hayouni *et al.*, 2007; Ishida *et al.*, 2001). In present work, highest yield was obtained in *Decalepisha miltonii* extract of self collected samples and from market sample group Pune. Phytoconstituents present in plant extract contribute significantly towards the biological activities of medicinal plants such as antioxidant, anti-inflammatory, immunomodulatory etc. (Ahamad *et al.*, 2017). This is a need of the hour in order to develop novel therapeutic agents with improved efficacy. Authenticate 'Sariva' samples showed presence of both the polyphenols. Highest concentration of phenol content was observed in all the species as compared to the flavonoids. Highest concentration of total phenol contents was observed in *Decalepis hamiltonii* (0.346±0.034) and flavonoids was observed in *Cryptolepis buchanani* (0.11±0.007). Among the methanolic extract of 'Sariva' market samples, Pune market sample showed highest phenolic contents

(0.295±0.023) as compared to the flavonoid contents. Phenolic compounds act as antioxidants due to their redox properties, which allow them to act as hydrogen donors, reducing agents and singlet oxygen quenchers (Kasote *et al.*, 2015). Total phenol content could be used as a basis of their most potent antioxidant properties and their free radical scavenging ability eased by their hydroxyl groups (Baba *et al.*, 2015).

Antioxidant activity measured by nitric oxide scavenging potential, BSA anti-denaturation assay and anti-lipid peroxidation assays with comparative evaluation among market and authenticates samples. In present work, the order of nitric oxide potential was observed highest in West Bengal followed by Pune, Nagpur, Mumbai, Bangalore and Delhi. The highest free radical scavenging activity (IC<sub>50</sub> value of 50.96 µg/ml) was observed in methanolic extract of West Bengal sample. While, lowest activity was observed in Delhi market sample (IC<sub>50</sub> of 218.63). Lipid peroxidation refers to the oxidative deterioration of lipids, mainly by the effect of different ROS. Similarly, Singh *et al.*, 2012 reported highest lipid peroxidation potential (66.5 µg/ml) in methanolic extract of *Hemidesmus indicus* collected from Dawa bazaar market of Mumbai. Moreover, authenticate species *Cryptolepis buchanani*, *Decalepisha miltonii*, *Hemidesmus indicus* and *Ichnocarpus frutescent* which are known as Antioxidant activity results revealed that *Hemidesmus indicus* showed highest anti-lipid activity (28.88 µg/ml of IC<sub>50</sub>) followed by *Decalepis hamiltonii*, *Cryptolepis buchanani* and *Ichnocarpus frutescent*. To establish the mechanism of anti-inflammatory activity of traditionally used 'Sariva' drug, protein denaturation and stabilization of human red blood cell membranes were studied. Inflammation is usually associated with the denaturation of proteins. Bangalore market sample (67.26 µg/ml) showed more significant protein stabilizing activity (denaturation is inhibited) followed by Nagpur, Mumbai, West Bengal, Pune and Delhi. While, authenticate 'Sariva' species exhibited highest inhibition of protein (denaturation) in *Decalepisha miltonii* showed more significant protein stabilizing activity (32.73 µg/ml) followed by *Hemidesmus indicus*, *Cryptolepis buchanani* and *Ichnocarpus frutescent*. The lysosomal constituents of neutrophils consist of protease and bactericidal enzymes, which upon extracellular release cause more damage and tissue inflammation (Chou, 1997). In present study, extracts of *Decalepis*

*hamiltonii* might inhibit the release of the lysosomal content of neutrophils at inflammation sites but this would need to be further investigated.

Nitric oxide (NO) is a potent pleiotropic mediator of physiological process like smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation etc. (Begam and Muthukumar, 2014). Although over production of nitric oxide and superoxide radicals contribute to the pathogenesis of some inflammatory diseases (Guo *et al.*, 1999). Methanolic extract of both market and authenticate 'Sariva' plant extract were studied and were exhibited nitric oxide scavenging potential. Among market samples, Pune market sample (65.15 µg/ml of IC<sub>50</sub>) showed highest nitric oxide scavenging activity and among authenticate 'Sariva' samples *Decalepis hamiltonii* showed highest nitric oxide scavenging potential (31.26 µg/ml of IC<sub>50</sub>). On the basis of evaluation, present work reveal variation in market samples sold as the drug 'Sariva'. So, there is need to selection of authentic raw materials in the market on the basis of correct botanical identification.

### CONCLUSION

Sariva is most commonly used in Ayurveda for its various therapeutic uses. After evaluating the 'Sariva' market samples and authentic samples collected from Western Ghats of Maharashtra, it is concluded that, there is variation among both samples in terms of their polyphenolic contents and antioxidant profile. *Hemidesmus indicus* is a botanical name of true 'Sariva' where as *Decalepis hamiltonii* can be used as potent substitute for the same. Therefore, there is an urgent need in research community to aware in the selection of authentic species of 'Sariva' and need further investigation to ascertain most potent species as 'Sariva'.

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