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A Comparative analysis of four 'Sariva' plants: HPTLC profiling and Antioxidant activity

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ABSTRACT

The 'Sariva', a drug having diverse uses in Ayurveda. Under the name 'Sariva' four different species have been mentioned and supplied viz. *Cryptolepis buchananii* Roem & Schult., *Decalepis hamiltonii* Wight. & Arn., *Hemidesmus indicus* (L.) R.Br., and *Ichnocarpus frutescence* R. Br. Vanillin is a key active constituent of these plants. Considering the availability of plant material in the market there is an ambiguity in supplying the authentic species as 'Sariva'. This study was planned to study the effective species as 'Sariva' using biological activity as well as content of Vanillin using HPTLC fingerprints, and antioxidant abilities. A precise method was developed for detecting vanillin concentrations in various 'Sariva' extracts, using suitable mobile phase. The method was suitable for routine examination of vanillin in 'Sariva' spp. providing reliable and consistent results. The highest peak area for vanillin was reported in *Hemidesmus indicus*, followed by *Decalepis hamiltonii, Ichnocarpus frutescence,* and *Cryptolepis buchananii* based on area under curve, indicating content variation in tested samples. The study also assessed the antioxidant capability of different 'Sariva' drugs using DPPH, FRAP, and ABTS assays. *Hemidesmus indicus* showed significantly higher antioxidant activity followed by *Decalepis hamiltonii*, *Cryptolepis buchananii* and *Ichnocarpus frutescence*. These findings suggest that *Hemidesmus indicus* and *Decalepis hamiltonii* have similar natural antioxidants with potential health benefits. Considering the less availability of *Hemidesmus indicus*, *Decalepis hamiltonii* can be potent substitute as 'Sariva'. Further research is needed to explore the specific mechanisms of action and potential applications of Vanillin in preventing oxidative stress-related diseases.

Key words: *'Sariva', HPTLC, Vanillin, Antioxidant potential, Oxidative stress.*

Introduction

Herbal remedies are gaining popularity worldwide due to its natural source and minimal adverse effects. Currently, there is a growing emphasis on herbal and Ayurvedic medicines worldwide because of the rising knowledge of the possible dangers and adverse consequences linked to synthetic pharmaceuticals. The reliability and trustworthiness of herbal medicine are enhanced in many cultures by the transmission of traditional knowledge and practices throughout generations (Mukherjee *et al.*, 2007; Jagtap *et al*., 2022).

'Sariva', a widely used medicinal plant in

Ayurveda, is renowned for its extensive traditional and experimental evidence supporting its use Indian sarsaparilla, also known as Indian 'Sariva'. *Hemidesmus indicus* (Asclepiadaceae) is officially reported as 'Sariva' in Ayurveda. It is a climber with aromatic roots in deep soil and popular medicinal herb distributed throughout the tropical and subtropical parts of India, especially in upper Gangetic plains, Bengal, Madhya Pradesh, and South India. It is widely used in traditional medicine and is officially part of the Indian pharmacopoeia (Pansare *et al*., 2018). Apart from *Hemidesmus indicus* regularly four different species have been supplied viz. *Cryptolepis buchananii*, *Decalepis hamiltonii*, and *Ichnocarpus frutescence* under the name 'Sariva'.

Decalepis hamiltonii (Asclepiadaceae) is a climbing shrub native to the Deccan Peninsula and Western Ghats of India, known for its aromatic tuberous roots used in traditional medicine and food. *Decalepis hamiltonii* plant roots are utilized in folk medicine as a vitalizer, blood purifier, and antimicrobial, aiding in treating infections and inflammatory conditions due to their anti-inflammatory properties. Roots have potential anticancer properties, making them valuable in modern medicine. It also promotes digestive health and have antioxidant effects (Jayaweera, 1981; Vedavathy, 2004). It is also used in traditional Ayurvedic preparations as a substitute for *Hemidesmus indicus* (Nayar *et al.,* 1977). Despite facing threats from habitat loss and overharvesting, the species is endangered and is used for medicinal purposes in Ayurveda, an ancient Indian system of medicine. Conservation efforts are proceeding to protect its populations and reserve its exclusive and aromatic roots (Ayurvedic Pharmacopoeia, 1979; Sharma *et al*., 2002).

Cryptolepis buchanani, a large evergreen shrub, is found in sub-himalayan tracts, Bihar, Orisa, East Uttar Pradesh, Varanasi region, and Maharashtra, recognised as Jambupatra 'Sariva' in Sanskrit and Dudhi or Karanta in Hindi (Sharma *et al*., 2012). The plant is utilized in Indian folkloric medicine for treating diarrhea, ulcers, inflammation, coughs, infections, purifying blood, and promoting overall health (Kaul *et al*., 2003). It is also known as 'Black creeper', is a perennial herbaceous plant used in Ayurveda medicine to treat various diseases, including paralysis, rickets, skin diseases, and respiratory disorders like asthma and bronchitis (Datta *et al*., 1978). The plant is renowned for its vibrant flowers and aromatic leaves used in traditional medicine and food (Ashutosh *et al*., 2018)

Ichnocarpus frutescens, an evergreen climbing plant, commonly known as Black Sariva, is an important medicinal plant found throughout in India. It has been used to treat various diseases, including diabetes, demulcent, skin problems, fevers, nephrolithiasis, and liver abnormalities (Sini and Malathy 2006; Babita *et al.,* 2010).

Chemically, *Hemidesmus indicus*, a plant rich in therapeutic compounds, has shown promising results in traditional medicine for treating various ailments and has potential for developing new drugs and treatments (Austin, 2008; Aneja *et al.,* 2008). Benzoid derivatives are organic compounds with an aromatic structure, including vanillin, Benzaldehyde, Hydroxy-methoxy-benzaldehyde (HMBA), and 3-hydroxy-4-methoxybenzaldehyde (3H4MBA), with diverse biological actions like antibacterial, antioxidant, and cancer properties, making them crucial for drug discovery (Gupta *et al*., 1992). *Decalepis hamiltonii* root contains 0.68 % volatile oil. It has 96 % Hydroxy-methoxy-benzene and 0.45% vanillin, both of which are physiologically relevant for its medicinal properties (Nagarajan and Rao, 2003). Phenolic compounds, found in foods, are believed to have health benefits due to their antioxidant activity, which is influenced by factors like food origin[19,20] (Xi *et al*., 2014; Liu *et al*., 2016).

Cryptolepis buchananii and *Ichnocarpus frutescens* are intentionally adulterated with *Hemidesmus indicus* roots due to their similar appearance. These adulterants are often used as substitutes for genuine 'Sariva' (*Hemidesmus indicus*) in the form of roots and root powder, but they may not provide the same therapeutic benefits (Jadhav *et al*., 2022). Therefore, it's crucial for consumers and practitioners to ensure the authenticity of their medicinal materials to maximize desired effects and avoid potential health risks. The high demand for this plant has led to the importation of large quantities of its material and extracts to meet the needs of these alternative medicine practices (Aiyer, 1951; Prasad *et al*., 1964; Warrier *et al*., 2001). Additionally, inconsistent supply of 'Sariva' and its different extraction methods can cause confusion and misinterpretation, making it challenging for researchers and regulators to ensure the safety, effectiveness, and quality of herbal drugs in the agrochemical industry. Hence, this study planned to identify the health-promoting potential of suitable species under the name 'Sariva' which could lead to improved plantations of au-

thentic plant for commercial utilization.

Materials and Methods

Sample collection and permissions

'Sariva' roots were obtained from the Western Ghats of Maharashtra, India. The medications were verified at the Department of Dravyaguna, College of Ayurveda, Bharati Vidyapeeth University. The prior permission was taken from Maharashtra State Biodiversity Board (MSBB), Nagpur to access biological samples (MSBB/Desk-5/Research/Permission/641/23-24, dated December 19, 2023).

Chemicals

All chemicals and reagents utilized in the studies were of analytical grade. The compound's purity was confirmed using several analytical methods. Stringent quality control protocols were adhered to in order to guarantee precise and replicable outcomes in the studies. Methanol was obtained from SD Fine Chem Ltd. in Mumbai, India. Pre-coated HPTLC plates 60 F254 were purchased from Merck in Mumbai, India. Vanillin of standard quality was obtained from Sigma-Aldrich. The HPTLC plates were maintained in a cool, dry location shielded from direct sunlight to avoid deterioration.

Extract preparation

The roots of 'Sariva' were dried in the shade, ground into powder, and sifted using a sieve with a 40-mesh size. For extraction, 10 g of powder sample was reflux extracted using four different solvents: methanol at 50 °C for 60 min, with a drug-to-solvent ratio of 1:10 g/ml. The solid residue was filtered out after extraction, and the remaining solvent was concentrated using rotary evaporator.

Analysis of HPTLC fingerprint

Preparation of standard and sample solutions

A solution was prepared by dissolving 1 gram of dried 'Sariva' extract in methanol. A 1 mg/ml vanillin solution was prepared by dissolving 2 milligrams of vanillin in 2 ml of methanol. The standard and sample solutions underwent filtration using a 0.22 micrometer Millipore syringe filter from Burlington, MA, USA, before analysis.

Development of a solvent system

Solvent systems were used for the elution process. Chloroform: ethyl acetate: methanol: formic acid at a ratio of 1.5:7:1:0.5 (Das *et al*., 2017).

Sample application and development of chromatogram

The reference solution and sample solutions were applied as 6 mm bands on 20×10 cm plates using a 100 µl syringe and a critical glass chamber CAMAG Linomat 5 automated applicators from Muttenz, Switzerland. The application rate was set at 150 nL/s with the help of nitrogen gas. The small gaps between the bands measured 10 mm. The plate was prepared in a twin-trough CAMAG that was presaturated with the mobile phase. It was taken out when the solvent level reached 85 mm. To identify the bands, the plate was subsequently dried and baked at 60 °C in the oven for 5 minutes. The densitometric analysis was conducted with a CAMAG TLC scanner equipped with WinCATS software, operating at a wavelength of 254 nm (Das *et al.,* 2017).

Free radical scavenging assays

Free radical scavenging assays viz. DPPH, FRAP and ABTS were carried out. All the assays were done in triplicate.

DPPH assay (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) (Narkhede *et al.,* 2012)

DPPH is a stable free radical with a nitrogen core that can be easily neutralized by a free radical inhibitor and has a prominent absorption peak at 517 nm. The absorption band diminishes when the DPPH radical is lowered, serving as a valuable method for assessing antioxidant activity in different samples. The antioxidant capability of a material may be assessed by measuring the reduction in absorbance at 517 nm following the addition of the sample. The *in-vitro* DPPH-free radical scavenging experiment was conducted according to the established protocol. Ascorbic acid serves as a standard. The data were presented as IC_{50} values, indicating a 50% suppression of DPPH activity (Bhavamisra and Bhavaprakasha, 2012).

FRAP assay (Ferric Reducing Antioxidant Power Assay) (Abdul *et al*., 2012)

The hydrophilic antioxidants in plant extracts were identified according to Benzie and Strain (1996). Their antioxidant capability was determined based on their ability to decrease the TPTZ-Fe (II) complex

to TPTZ-Fe (II). The ability of all the extracts to reduce ferric ions was measured using a standard FeSO4 solution (0.1 to 1 mM). The results were analyzed using a standard curve of ferrous sulfate and reported as µM Fe/g of dry mass.

ABTS assay (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (Ahmed, 2011; Babita *et al.,* 2014)

The ABTS radical cation decolorization test was used to investigate the free radical scavenging activity of 'Sariva' plant samples. The process begins with the neutralization of a radical cation produced by the one-electron oxidation of the synthetic chromophore 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) test. The reaction is observed using spectrophotometry to track changes in the absorption spectra. Results acquired using this approach are often converted to Trolox® concentration and referred to as "Trolox® Equivalent Antioxidant Capacity" (TEAC). TEAC for chemically pure substances is the micromolar concentration of Trolox® equivalents that exhibits equal antioxidant activity to the tested molecule at a concentration of 1 mmol/L. TEAC values are frequently utilized as a relative measure of antioxidant capability for intricate samples like meals or biological fluids (Reddy and Murthy, 2013).

Statistical Analysis

The data underwent one-way ANOVA analysis and were presented as mean and standard deviation.

Results and Discussion

Plant materials collection and extraction yield

All the collected samples were extracted in methanol, and the percentage yields were calculated (Table 1). The highest yield was observed in drug *Decalepis hamiltonii* followed by *Hemidesmus indicus, Ichnocarpus frutescence*, *Cryptolepis buchananii.*

HPTLC Analysis

HPTLC is a method that can be used to test the quality of herbal drugs. Additionally, it can be used to test the stability of herbal preparations and their purity (Shinde *et al*., 2018). It was observed that *Decalepis hamiltonii* is used as a substitute of *Hemidesmus indicus* in Indian drug market (Murthy *et al.,* 2006). The extracts were subjected to the qualitative HPTLC analysis and the Rf values was compared with standard Vanillin. Further area was determined for Vanillin concentration from different 'Sariva' plant extracts has been mentioned in table 2 (Figure 1). The highest peak area for vanillin was reported in *Hemidesmus indicus* (3858.623). Plant drug followed by *Decalepis hamiltonii* (3735.193), *Ichnocarpus frutescence* (3618.09) and *Cryptolepis buchananii* (3415.447) sample based on area under curve, indicating differential content variation in tested samples. The places were graded according to their vanillin content higher in *Hemidesmus indicus* and followed by *Decalepis hamiltonii*, *Ichnocarpus frutescence* and *Cryptolepis buchananii* (Table 2). Vanillin is a well-known bioactive marker present in the roots of *Hemidesmus indicus*, which is used in herbal formulations in India (Tiwari *et al*., 2012). HPTLC has been widely used as quality-control tool for phyto-chemical evaluation of herbal drugs. In this work a precise and accurate HPTLC method has been established for rapid and accurate analysis of isomer of vanillin in *Hemidesmus indicus* dried root

Table 2. Peak table with Rf values, height, and area of Vanillin from different 'Sariva' sample

Sr. No.	Track (ul)	Rf	Area
1	*Hemidesmus indicus*	0.44	3858.623
2	*Decalepis hamiltonii*	0.44	3735.193
3	*Ichnocarpus frutescence*	0.44	3618.09
4	*Cryptolepis buchananii*	0.44	3415.447

Table 1. Percent yield of different 'Sariva' sample

Samples	Quantity of plant material taken for extraction (gm)	Solvent	Weight of empty vials (gm)	Weight of vials+ extract (gm)	Actual weight of extract (gm)	Percent Yield (%)
Decalepis hamiltonii	10	Methanol	12.84	13.69	0.85	**8.5**
Hemidesmus indicus	10	Methanol	12.43	13.00	0.57	5.7
Ichnocarpus frutescence	10	Methanol	12.07	12.54	0.47	1.7
Cryptolepis buchananii	10	Methanol	12.47	13.12	0.65	1.5

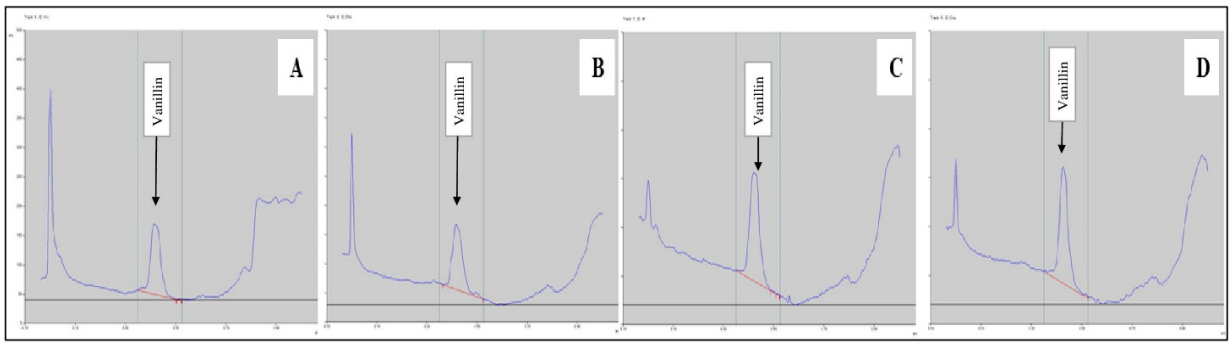

Fig. 1. HPTLC Peak of Vanillin A: *Hemidesmus indicus* B: *Decalepis hamiltonii* C: *Ichnocarpus frutescence* D: *Cryptolepis buchananii*

powder and its extract. It has been noted that the roots of *Ichnocarpus frutescens* (Linn.) R.Br. (Apocynaceae), *Decalepis hamiltonii* Wight and Arn. (Asclepiadaceae), and *Cryptolepis buchanani* Roem. & Schult. (Asclepiadaceae) species are utilised as substitutes for *Hemidesmus indicus* (Sarin, 1996). Using HPTLC, the examination of four 'Sariva' species showed notable differences in vanillin content, which was used as a marker. *Hemidesmus indicus* has higher levels than the three alternative species, indicating its potential as a superior source of vanillin (Darekar *et al*., 2009).

Free radical scavenging activity

Recently, there has been a significant focus on the potential impact of diet in illness prevention. Antioxidants, particularly those obtained from natural sources like Indian medicinal plants and herbal medications, need special focus in this scenario. Antioxidants counteract harmful and unstable free radicals. Antioxidants have several potential uses, particularly in the context of human health, for both disease prevention and treatment (George *et al.*, 2008; Sies, 1997). Free radicals produced during metabolic activity can disrupt normal cell functions, leading to pathological changes. The present analysis confirmed the presence of DPPH-scavenging activity, FRAP activity, and ABTS activity in the studied extracts.

DPPH assay

The DPPH method evaluates ability of plant extracts to donate hydrogen ions, a neutral form of hydroxyl radical. This radical, generated from the breakdown of H2O2 by free metal ions like copper or iron, is the most reactive in biological systems (Block *et al.*, 1992). The antioxidant activity of *Decalepis hamiltonii* (IC_{50} of DPPH: 15.43 ±0.02), is significantly higher than that of other species of *Hemidesmus indicus* (IC_{50} of DPPH: 20.22 ±0.01), *Ichnocarpus frutescence* (IC_{50} of DPPH: 34.00 ±0.01), *Cryptolepis buchananii* (IC_{50} of DPPH: 23.29 ±0.01). This may be due to the amount of vanillin content the plants as *Decalepis hamiltonii* has reported higher content of vanillin than other studied plants (Figure 1). It has been reported earlier that vanillin is a good scavenger of DPPH radical and inhibits lipid peroxidation in microsomes, which is inconsistent with our results (Mandal *et al.*, 2009).

FRAP assay

The antioxidant can donate an electron to free radicals, leads to the neutralization of the radical which was visualized by forming the intense green colour

Table 3. Percentage inhibition of antioxidant assay of different 'Sariva' sample

Self-collected samples of 'Sariva'	DPPH assay (1mg/ml conc.)	FRAP Assay (1mg/ml conc.) μM Fe/g of dry mass	ABTS (1mg/ml conc.)
	Percentage of Inhibition (IC_{50})		
Cryptolepis buchananii	23.29 ±0.01	36.81 ±0.02	37.27 ±0.08
Decalepis hamiltonii	**15.43** ±0.02	32.28 ±0.01	31.26 ±0.02
Hemidesmus indicus	20.22 ±0.01	**31.95 ± 0.02**	**28.88** ±0.16
Ichnocarpus frutescence	34.00 ±0.01	37.12 ±0.01	39.80 ±0.10

complex. The FRAP method measures the capacity of plant extracts to reduce ferric iron to ferrous. The reduction ability increases with increase in concentration of the extract. In FRAP, the antioxidant activity of *Hemidesmus indicus* (IC_{50} of FRAP: 31.95 ± 0.02) is much higher than that of *Decalepis hamiltonii* (IC_{50} of FRAP: 32.28 ±0.01), *Cryptolepis buchananii* (IC_{50} of FRAP: 36.81 ±0.02), *Ichnocarpus frutescence* (IC_{50} of FRAP: 37.12 ±0.01). This indicates that they can help to prevent damage to cells caused by free radicals. The Fe3+/Fe2+ system catalyzes the formation of ferry-perferryl complexes or hydroxyl radicals, which break down lipid hydro-peroxides into peroxyl and alkoxyl radicals. Radicals harm biological molecules by interacting with polyunsaturated fatty acid components of cell membrane phospholipids, leading to the production of carbonyl compounds such as malondialdehyde (MDA), which produce a pink chromogen when combined with Thiobarbituric Acid (TBA). *Hemidesmus indicus* root extract's capacity to inhibit lipoperoxidation might be interpreted as the plant's antioxidant efficacy (Liu and Mori, 1993), as shown in Figure 2.

ABTS assay

ABTS•+ is a blue chromophore, is generated through the reaction between ABTS and potassium persulfate subsequent to a dark incubation period (Halliwell and Gutteridge, 1999). In ABTS assay, the antioxidant activity of *Hemidesmus indicus* (28.88 ±0.16) is much higher than that of *Decalepis hamiltonii* (31.26 ±0.02), *Cryptolepis buchananii* (37.27 ±0.08), *Ichnocarpus frutescence* (39.80 ±0.10). The ABTS activity of all the solvent extracts was relatively lower, and the IC_{50} was higher compared to the standards. In earlier studies, the ABTS scavenging activity of the extracts and standards increased with increasing concentrations indicating that standards and pharmaceuticals derived from the *Hemidesmus indicus* plant exhibit potent ABTS-scavenging capabilities when compared (Boominathan *et al*., 2019). A comparative study indicates that both standards and *Hemidesmus indicus* plant medicines exhibit notable ABTS scavenging capabilities. The interactions extract with the radical cation caused the blue chromophore to lose color as the concentrations increased.

Among all the species, *Decalepis hamiltonii* had the highest percentage yield of all the species, followed by *Hemidesmus indicus, Cryptolepis buchananii,* and *Ichnocarpus frutescence*. In HPTLC, the vanillin content in 'Sariva' plant sources varies, with *Hemidesmus indicus* having the highest concentration. The study highlights the potential of these plant species for pharmaceutical and commercial purposes, with *Decalepis hamiltonii* and *Hemidesmus indicus* standing out as particularly promising candidates considering the more or less similar results. However, the highest activity was observed in *Hemidesmus indicus* for its antioxidant profile but it closer to *Decalepis hamiltonii*. There is a positive relationship between these antioxidant tests, which indicates that *Hemidesmus indicus* and *Decalepis hamiltonii* is the best species to test for DPPH, FRAP, and ABTS antioxidant activities. This highlights the importance of continued study and exploration of *Hemidesmus indicus* and *Decalepis hamiltonii* for its potential health benefits. Overall, the results suggest that these plant species have promising antioxidant properties that could be beneficial for various indus-

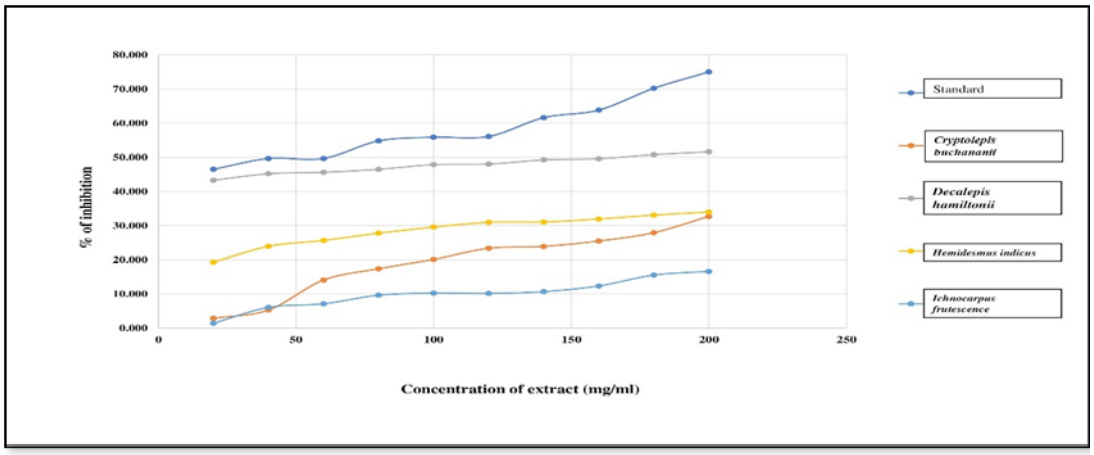

Fig. 2. DPPH activity of the 'Sariva' extract and their IC_{50} values

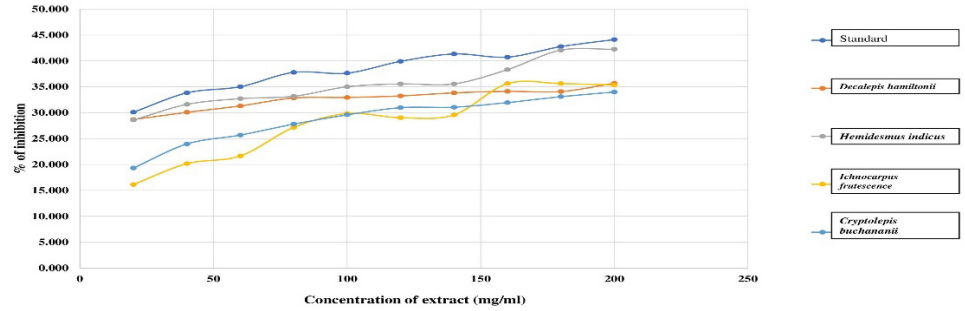

Fig. 3. FRAP activity of the 'Sariva' extract and their IC_{50} values

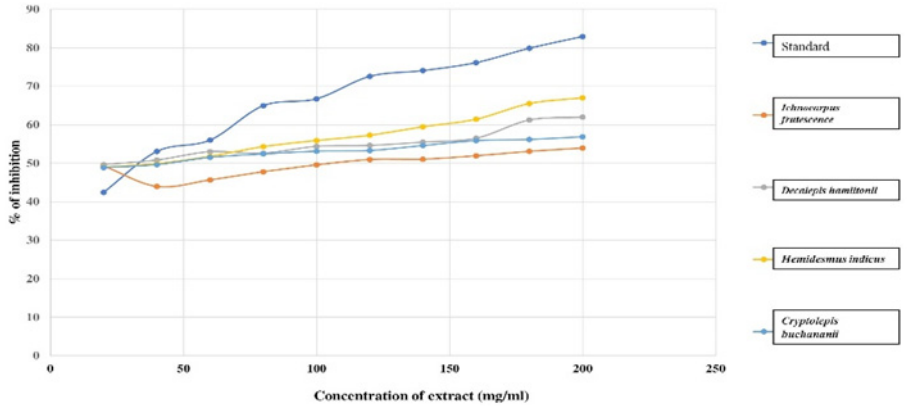

Fig. 4. ABTS activity of the 'Sariva' extract and their IC_{50} values

tries. Further studies should focus on isolating and identifying the specific compounds responsible for this activity to better understand their potential applications.

Conclusion

The study explores the antioxidant potential, and concentration of vanillin in the methanolic extract of selected 'Sariva' plants using HPTLC. The results suggest that *Hemidesmus indicus* and *Decalepis hamiltonii* roots could be natural sources of bioactive compounds that could be used as therapeutic agents to reduce or prevent oxidative stress-related ailments. *Hemidesmus indicus* and *Decalepis hamiltonii* are the optimal species for testing DPPH, FRAP, and ABTS antioxidant activities, emphasizing the need for further research for potential health benefits. Considering the availability, cultivation potential and yield of *Hemidesmus indicus* the *Decalepis hamiltonii* could potential alternative as a 'Sariva'. The study also highlights the need for further research to understand the mechanisms of action and potential use of compounds from *Hemidesmus indicus* and *Decalepis hamiltonii*, known for their anti-inflammatory properties.

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Conflict of interest

Authors declare that no conflict of interest occurs. The authors alone are responsible for the content and writing of this article.

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