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MEDICINAL ATTRIBUTES OF *SOLANUM CAPSICOIDES* ALL.: AN ANTIOXIDANT PERSPECTIVE

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ABSTRACT: The study evaluates the medicinal efficacy of *Solanum capsicoides* fruits as an antioxidant. Fruit extracts were prepared using acetone, ethanol, HCl, and water for the measurement of antioxidant potential by reducing power, DPPH radical scavenging activity, and CUPRAC assay and for the spectrophotometric determination of total phenol, flavonoid, anthocyanin, and carotenoid content. Chemical profiling of the fruit extract through GC-MS was also conducted. The total phenolic contents in extracts ranged between 1.2-4.25mg GAE g^{-1} FW and flavonoid contents in the range of 3.89-22.96mg QE g^{-1} FW. Among the various solvents used, acetone extract showed maximum antioxidant activity, and the least was observed in the water extract. Correlation coefficient analysis between bioactive compounds and antioxidant property revealed that TPC and TFC were positively and significantly correlated with antioxidant activity. A negative correlation was observed between the pigments, anthocyanins, and carotenoids, with DPPH and CUPRAC activity. The correlation between DPPH radical scavenging activity and carotenoids as well as TFC was not significant. In the case of RP, except carotenoids, all others showed a significant and positive correlation. From the multivariate analysis, it was clearly observed that the antioxidant activity decreases from acetone extract to water extract. Among different variables, TPC was with the maximum antioxidant property and the least with carotenoids. From this study, it can be considered that the phenolics present in the fruits contribute to the characteristic antioxidant property. The present study demonstrated considerable antioxidant activity of *S. capsicoides* All.

INTRODUCTION: The genus *Solanum* is one of the largest and most diverse among the members of Solanaceae and is ethnobotanically very important. *Solanum capsicoides* All. is an ayurvedic drug used as an equally good substitute of *S. xanthocarpum* Schard. and Wendl. which is popularly known as Kantakari in Malayalam and is an important ingredient of many formulations [1].

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It is used in many formulations that were used to cure respiratory ailments like bronchial asthma [2]. The imbalance between free radicals and antioxidants in our body causes oxidative stress that results in respiratory diseases.

In such situations, antioxidant therapy is preferred [3]. Most of the medicinal plants are reported to have antioxidant properties [4-7] and are due to the presence of free radical scavenging molecules such as phenolic acid [4], flavonoids [7], anthocyanins [8], carotenoids [9] *etc*. Gas Chromatography-Mass Spectrometry is an analytical technique that is used to separate and identify volatile and thermally stable components in sample [10].

In recent years GC-MS analysis has been widely used for identifying phytoconstituents in plant extracts [11-16]. *Solanum xanthocarpum* is one of the major ingredients of Dasamoolarishtam, an important ayurvedic formulation to treat respiratory disorders [17]. Due to overexploitation, the availability of this raw drug has declined, and at the same time, another species of the same genus, *S. capsicoides* has been used by the traditional practitioners as a substitute. In the present study, the antioxidant potential of *S. capsicoides* has been reported, which is associated with its bioactivity in reducing respiratory ailments. Scientific reports on the bioactivity of *S. capsicoides* is scanty. In this context, the present investigation has been undertaken to scientifically validate the bioactive potential of *S. capsicoides* which is used as a substitute to *S. xanthocarpum*.

MATERIALS AND METHODS:

Plant Material: Healthy plants of *Solanum capsicoides* All. from Ernakulam District of Kerala were collected and identified using referral herbaria, and voucher specimens were deposited at Kerala Forest Research Institute (KFRI), Peechi, Kerala (herbarium number KFRI 13056). Ripened fruits of *Solanum capsicoides* All. were washed in running tap water and were directly used for the estimation of phenol, flavonoids, and pigments. The washed fruits were dried under shade, powdered, and used for solvent extraction to study the antioxidant property.

Solvents used for Extraction: 80% acetone, 80% ethanol, 1% hydrochloric acid (HCl) (Merck, Germany) and distilled water.

Estimation of Total Phenol, Flavonoid, and Pigment from the Fruit Peel: 1 gram of fresh fruit was homogenized with solvents using motor and pestle and kept overnight in the dark at 4 °C. Samples were centrifuged at 4000 rpm for 15 min. The supernatant was collected and used for further experiments.

Preparation of Fruit Extract for Antioxidant Studies: 10 gm of dried powder was suspended in 50ml of various solvents for 48 h. The extract was filtered and concentrated in a water bath at 50±5 ºC.

Determination of Total Phenolic Content: Total phenolic content (TPC) of fruit extract was determined by Folin- Ciocalteu (FC) method described by Singleton and Rossi [18]. The results were expressed as mg gallic acid equivalent per g fresh sample of fruit (mg GAE g^{-1} FW).

Determination of Total Flavonoid Content: Total flavonoid content (TFC) was determined by aluminum chloride method[19,] and the results were expressed as quercetin equivalent per gram of fresh material (mg QE g^{-1} FW).

Pigment Analysis:

Total Anthocyanin: 3ml of the extract was taken in a cuvette, and absorbance was measured at 535nm. The total content of anthocyanin was calculated using Lee and Francis equation [20] and was expressed as mg cyanidin 3- glucoside equivalents per gram fresh weight of the sample (mg CGE g^{-1} FW).

Total Anthocyanin (TA) = Absorbance × dilution factor / 98.2

Total Carotenoid: The absorbance of the extract was measured at 480nm, and 510nm, and carotenoid content was calculated following the method of Duxbury and Yentach [21].

Carotenoid = 7.6 (A*R*480*R*) - 1.49 (A*R*510*R*) × V × W / 1000

V= Volume of extract, W= Weight of tissue

The result was expressed as mg per gram fresh weight of the sample (mgg^{-1}FW).

Antioxidant Studies:

Radical Scavenging Activity – DPPH Assay: The radical scavenging activity of fruit extracts was determined by DPPH assay following the method of Brand Williams *et al.* [22] Radical scavenging capacity was calculated using the ascorbic acid calibration curve and was expressed as mg ascorbic acid equivalent per gram of extract ($mgAAEg^{-1}$).

Reducing Power Capacity Assessment (RP): Reducing power of the extracts was studied by the potassium ferricyanide method as described by Oyaizu [23]. Ascorbic acid was used as the standard reference. The reducing ability was expressed as mg ascorbic acid equivalent per gram of extract ($mgAAEg^{-1}$).

Cupric Ion Reducing Antioxidant Capacity Assay (CUPRAC): The assay was conducted as prescribed by Apak *et al.* [24] Ascorbic acid was used

as the standard reference. Reducing the antioxidant capacity of extract was expressed in mg ascorbic acid equivalent per gram of extract ($mgAAEg^{-1}$).

GC-MS Analysis: To determine possible bioactive components of fruit extract, GC-MS (Gas Chromatography-Mass Spectrometry) analysis of acetone extract was conducted using Shimadzu GC-MS (QP2010S) equipped with Rxi-5Sil MS column (30-meter length, 0.25 mm ID, 0.25 µm thickness). GCMS solutions was the software used for analyzing the mass spectrum chromatograms, and the libraries used were NIST 11 & WILEY 8.

Statistical Analysis: Results were expressed as mean ± standard deviation (SD). The data were statistically analyzed using analysis of variance followed by Tukey's test ($p \leq 0.05$) to detect the significance between the treatments. To determine the relation between bioactive compounds and antioxidant capacity of samples, Pearson correlation coefficient analysis was done, and PCA (Principal Component Analysis) using biplot was drawn to interpret the relationship between bioactive compounds and antioxidant property. Biplot was performed using mean values.

Softwares used for Tukey's test and PCA Biplot were GraphPadInstat, 3.10 and XLSTAT 2018 respectively.

RESULTS:

Total Phenolic, Flavonoid, and Pigment Content: The result of total phenol, flavonoids, and pigment yield in each extract are tabulated in **Table 1**.

TABLE 1: TOTAL PHENOLIC AND FLAVONOID CONTENT

Samples	TPC (mg GAE g^{-1} FW)	TFC (mg QE g^{-1} FW)	ANTHOCYANIN (mg CGE g^{-1} FW)	CAROTENOIDS (mgg^{-1}FW)
AE	4.25±0.29	22.96±0.55	0.0035±0.0006	0.0514±0.002
EE	3.35±0.23	18.20±0.85	0.0033±0.0005	0.048±0.0008
HE	2.76±0.25	11.45±0.33	0.0011±0	0.011±0.0004
WE	1.2±0.19	3.89±0.04	0.0083±0.001	0.0915±0.001

Values are shown in mean ± standard deviation of the triplicate experiment. (Abbreviations: AE=Acetone Extract, EE=Ethanol Extract, HE=HCl extract, WE=Water Extract)

Antioxidant Studies: The variables TPC and TFC located on the right-hand side of the biplot showed more antioxidant activity than the variables such as carotenoids and anthocyanins on the left-hand side.

Moreover, from the biplot it was very clear that the antioxidant property declines from acetone extract to ethanol, HCl, and the least in water with the attributes DPPH and CUPRAC. But the attribute RP showed the least activity in HCl rather than in water.

The result of the DPPH assay, reducing power, and CUPRAC assay were given in **Table 2**.

TABLE 2: DPPH, RP AND CUPRAC CONTENT OF FRUIT EXTRACTS

Sample	DPPH ($mgAAEg^{-1}$ DW)	RP ($mgAAEg^{-1}$DW)	CUPRAC ($mgAAEg^{-1}$ DW)
Ascorbic acid	922±8.48	805±24.74	1381.25±146.72
AE	83.66±1.15	58.45±1.35	239.54±2.37
EE	11.35±1.35	53.85±2.35	81.75±4.85
HE	19.9±1.5	18.5±1	40.2±9.1
WE	6.3±0.8	30.7±0.8	8.9±2.5

Values are shown in mean ± standard deviation of triplicate experiment

GC-MS Analysis: The GC-MS analysis identified 14 compounds from the acetone extract of fruit **Fig. 1**. The compounds with their biological activities reported were tabulated in **Table 3**.

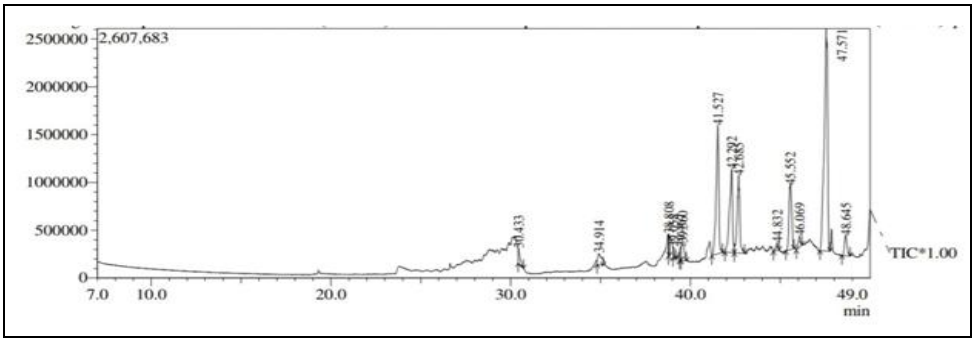

FIG. 1: CHROMATOGRAM OF ACETONE EXTRACT OF *S. CAPSICOIDES* FRUIT

TABLE 3: COMPOUNDS IDENTIFIED IN ACETONE EXTRACT OF *S. CAPSICOIDES* FRUIT

S. no.	Compound	Retension Time	Area (%)	Activity
1	Hexacontane	30.433	1.40	Not reported
2	Lup-20(29)-en-3-one	34.914	2.19	Antidiabetic, stimulate melanogenesis [25, 26]
3	2,7-dimethyl-4-isopropyl tetrahydrobenzo furan	38.808	0.82	Not reported
4	1,2-benzenedicarboxylic acid	39.058	0.63	Antimicrobial activity, antitumor activity [27, 28]
5	Lanostane	39.467	0.93	Anticancerous activity [29]
6	Methyl commate B	39.560	2.53	Antimicrobial [12]
7	Celidoniol, deoxy	41.527	17.18	Not reported
8	Stigmast-4-en-3-one	42.292	12.46	Antiprostatitic [30]
9	Pentacosane	42.685	10.44	Not reported
10	Spiro[4.4]nonan-2-ol, 1,1,6,6-tetramethyl-, cis-	44.832	0.84	Not reported
11	Friedelan-3-one	45.552	9.18	Antimicrobial [31]
12	2-methyloctacosane	46.069	1.23	Not reported
13	Tetratetracontane	47.571	37.32	Not reported
14	2-pentacosanone	48.645	2.83	Not reported

Statistical Analysis: There was a strong positive correlation between the antioxidant property (DPPH, RP, and CUPRAC) with phenols and flavonoids, whereas anthocyanins and carotenoids were positively correlated only with RP **Table 4**.

TABLE 4: CORRELATION ANALYSIS BETWEEN BIO-ACTIVE COMPOUNDS AND ANTIOXIDANT PROPERTIES

	DPPH	RP	CUPRAC
TPC	0.754^{b}	0.677^{a}	0.859^{a}
TFC	0.744^{d}	0.784^{a}	0.882^{b}
ANTHOCYANIN	$^{-}0.256^{b}$	0.036^{a}	$^{-}0.283^{a}$
CAROTENOID	$^{-}0.120^{d}$	0.255^{d}	$^{-}0.105^{a}$

Statistical significance $p<0.001$ as a, $p<0.01$ as b, $p<0.05$ as c, $p>0.05$ as d

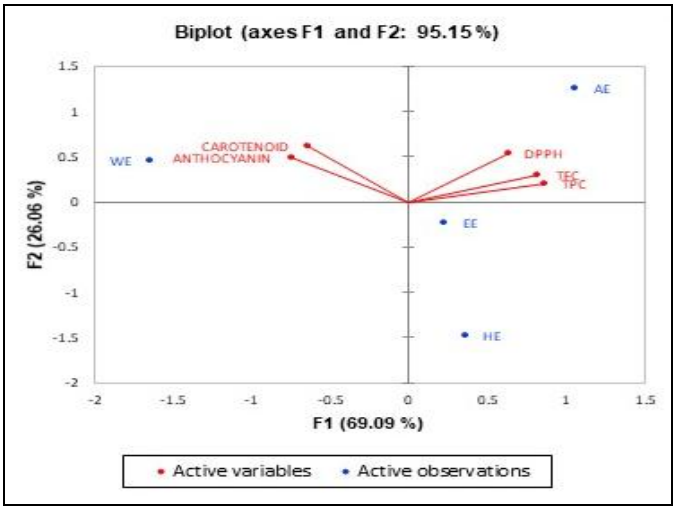

FIG. 2: BIPLOT OF RELATIONSHIP BETWEEN BIOACTIVE COMPOUNDS AND DPPH

Multivariate Analysis: In the biplot graph **Fig. 2,** the variables and attributes TPC, TFC, and DPPH respectively are close to each other and in the same geometric plane and hence interrelated. The principal components represented in F1 showed greater correlation to each other, of which TPC showed the greater distance from the origin of the axis and hence more positively correlated. F1 and F2 explained 95.15% of the total data variability. The variables carotenoid and anthocyanins were distributed away from the attribute DPPH activity and so are negatively correlated.

In the case of RP, the biplot **Fig. 3** also showed similar results with DPPH, but TFC was more positively correlated than TPC. Carotenoids and anthocyanins were correlated positively with RP, and the total data variability was 99.36%.

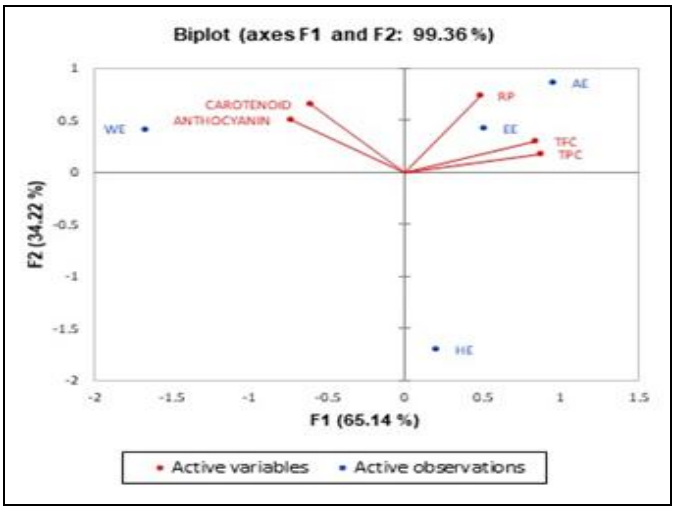

FIG. 3: BIPLOT OF RELATIONSHIP BETWEEN BIOACTIVE COMPOUNDS AND RP

In **Fig. 4**, the biplot gave 98.36% data variability, and TFC was more positively correlated with CUPRAC activity. Carotenoids and anthocyanins were negatively correlated with antioxidant property. The variables TPC and TFC located on

the right-hand side of the biplot showed more antioxidant activity than the variables such as carotenoids and anthocyanins on the left-hand side.

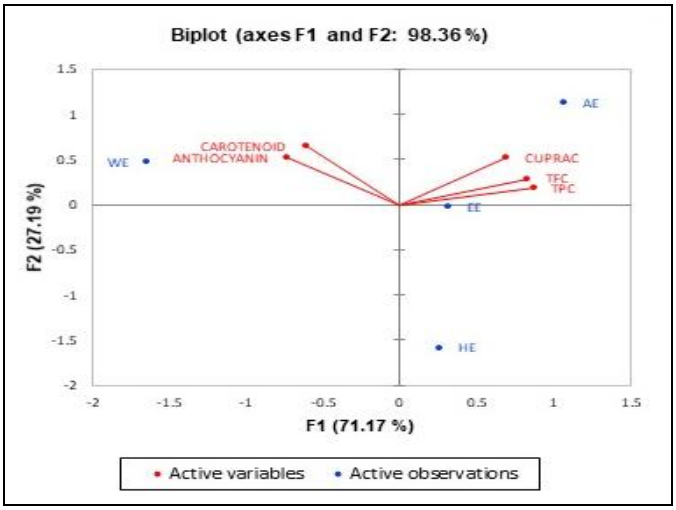

FIG. 4: BIPLOT OF RELATIONSHIP BETWEEN BIOACTIVE COMPOUNDS AND CUPRAC

Moreover, from the biplot it was very clear that the antioxidant property declines from acetone extract to ethanol, HCl, and the least in water with the attributes DPPH and CUPRAC. But the attribute RP showed the least activity in HCl rather than in water.

DISCUSSION: Antioxidant properties of medicinal plants play an important role in Ayurvedic treatments due to their multifaceted action. The significant positive correlation between phenols, flavonoids with different antioxidant assays such as DPPH, RP, and CUPRAC indicated that these bioactive compounds play a role in the antioxidant property of the plant [32, 33]. Earlier reports suggested that phenolic compounds concentrated in the fruit skin contribute to their antioxidant property. The contribution of phenolic compounds to the antioxidant property of various other *Solanum* species was already reported[34-36]. According to Mai *et al*., the redox property of phenolic compounds allows them to act as reducing agents, hydrogen donors, heavy metal chelators, and radical scavengers and was correlated with antioxidant property [37]. Flavonoids are other bioactive compounds that enable the antioxidant activity of medicinal plants. According to previous reports, flavonoids chelate copper and iron thus reducing reactive oxygen species (ROS) [38]. The positive correlation between flavonoids and antioxidant activity in the present study also indicated the same. As a high amount of flavonoids was found in *Solanum capsicoides* it can be used as a source of flavonoids. These compounds vary in their contribution to total antioxidant potential, and different assays that differ in their principle and experimental conditions will give diverse results [39]. At this juncture, three different methods were employed in the present study to measure the antioxidant capacity of *S. capsicoides.* DPPH was reported as the most effective assay for evaluating the radical scavenging activity of plant extracts [40]. Strong and significant positive correlation between phenol content and DPPH activity of the fruit extract was observed. RP assay indicated that *S. capsiocides* can be a good electron donor and can reduce the free radicals to form more stable products. CUPRAC is another widely used method, and the result obtained can be compared to in vivo antioxidant reactions as the experiment is carried in physiological pH [20]. Similarly, antioxidant property in relation to phenolic, flavonoid, and pigment contents has been reported recently in *Solanum lycopersicum* var. *cerasiforme* [40], *Dioscorea alata* [41, 42], *Litsea quinqueflora* [43], *Solanum betaceum* [44], *Morus* spp. [45] and in other fruits and vegetables [46].

Medicinal attributes of *Solanum xanthocarpum* as revealed by the antioxidant property was reported[47-50]. *S. capsicoides* and *S. xanthocarpum,* which were interchangeably used as the source of raw drug for many Ayurvedic preparations, can be attributed to the same properties as evidenced from the present study. Antioxidants also show antitumour, antimicrobial, and cytotoxic effects [51-55]. A study on the antioxidant potential in relation to total phenolics, flavonoids, anthocyanins and carotenoids was reported in the whole kernels of different wheat genotypes [56].

From the PCA Biplot analysis, it was indicated that the variables TPC and TFC showed more activity to DPPH, RP, and CUPRAC. A similar analysis was reported by Koley *et al.* [57] Multivariate analysis using PCA biplot has been used in genotype selection based on multiple traits [58]. GCMS analysis of acetone extract of *S. capsicoides* fruits revealed tetratetracontane as the major compound. Though its biological activity was not yet reported, its presence was noticed in various plant extracts, which exhibited antioxidant and antibacterial properties [59, 60].

Many other compounds with anticancerous [29], antimicrobial [27], and hypoglycaemic [25] properties were found in the chromatogram in addition to other compounds whose properties were yet to be established [61, 62]. From this, it can be assumed that the remarkable antioxidant property exhibited by *S. capsicoides* could be mainly attributed to the phenolic content, and the possibility of a synergistic effect of phenols, flavonoids, anthocyanins and carotenoids cannot be overruled.

CONCLUSION: *S. capsicoides* is an ethnobotanically important plant whose pharmaceutical properties were not evaluated so far. From the results obtained, it can be concluded that the fruit of *S. capsicoides* can be a potential source of natural antioxidants. Isolation and characterization of active components in the fruit extract are essential to determine its therapeutic applications.

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