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DETERMINATION OF ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF *CUCUMIS SATIVUS* LINN FRUIT EXTRACTS IN NON POLAR TO POLAR SOLVENTS

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ABSTRACT :
Cucumissativus (Linn) commonly known as cucumber. It is a trailing or climbing annual herb belonging to the family Cucurbitaceae, which bears elongated, thick, cylindrical fruits of varying sizes and forms. *C.sativus*originated in India but now cultivated in different parts of the world. Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical induced oxidative stress. The Present study was aimed to investigate the presence of different phytochemical constituents and to evaluate antioxidant activity of *C.sativus* fruit in petroleum ether, benzene, chloroform, acetone, ethanol and water extracts. All the extracts were testedfor 1-diphenyl-2-picryl hydroxyl (DPPH) radical scavenging activity and compared with L-Ascorbic acid as standard. The antioxidant activity of these extracts was investigated based on their ability to scavenge (DPPH) stable free radical. Phytochemical screening of *C.sativus* revealed the presence of carbohydrate, alkaloid, amino acid, tannin, flavonoid and Vitamin C. A higher percentage free radical scavenging was found for water extract as compared to all other extracts.
KEY WORDS-*Cucumissativus*, phytochemical screening, antioxidants, 1-diphenyl-2-picryl hydroxyl

INTRODUCTION

Naturally occurring antioxidants in vegetables and seeds such as ascorbic acid, vitamin E and phenolic compounds posses the ability to reduce the oxidative damage associated with many diseases.[1,2]

Oxidative stress (OS) is a general term used to describe the steady state level of oxidative damage in a cell, tissue or organ caused by the Reactive Oxygen Species (ROS) [3] Oxidative stress is a stress imposed on a biological system thatrequires oxygen to sustain a life. Oxidative damage is a result of oxidative stress. The extent of oxidative damage depends on many factors including rate of production of semi reduced oxygen species during aerobic metabolism as well as ability of biological system to withstand oxidative stress.[4] Free radical damage is what antioxidants are supposed to take care of either by stopping new damage or by reversing earlier damage caused by free radicals[5]

Cucumissativus (Linn) commonly known as cucumber[6]. It is a trailing or climbing annual herb belonging to the family Cucurbitaceae[7], which bears elongated, thick, cylindrical fruits of varying sizes and forms. *C.sativus*originated in India but now cultivated in different parts of the world[8].

Fruit contains an enzyme erepsin, Vitamin B1 and C, proteolytic enzymes, ascorbic acid, oxidise, succinic and malic dehydrogenases, rutin, palmitic, stearic and oleic acids.[9] The fruits are sweet, refrigerant, haemostatic. The Fruit juice is slightly purgative and diuretic internally. It is a source of vitamins B, and C, and of iron and calcium. The fruit juice is used for an emollient effect. It keeps skin soft and it gives cooling, soothing and healing effect. Seeds show presence of alkaloid, hypoxanthine.[10] Fruit flesh contains lipids and phospholipids.

Present study was carried out to analyze the presence of different phytochemical constituents and to evaluate antioxidant activity of *C.sativus*fruit in petroleum ether, benzene, chloroform, acetone, ethanol, and water extracts.

MATERIALS AND METHODS :

Plant materials and extraction

The fruits of*C.sativus*(Cucurbitaceae) were procured from the local market of Nagpur (Maharashtra) and authenticated in Department of Botany, RashtrasantTukdojiMaharaj Nagpur University, Nagpur.

Preparation of extracts

Fruits of *C.sativus* were washed and cut into very small pieces and then dried under the shade at room temperature for 8 days and later dried in an oven at 45^{0}C for complete removal of moisture to obtain constant weight then subjected to size reduction.200g of air dried powered fruit material was successively extracted in soxhlet assembly by using series of solvents in increasing order of polarity viz. petroleum ether, benzene, chloroform, acetone, ethanol and water.[11] Each extract was then

concentrated by distilling off the solvent and then evaporating the solvent to dryness and weighed[12]. Their percentage extractive values were recorded.

Preliminary Phytochemical Screening

All the extracts were subjected to preliminary phytochemical screening for evaluation of phytochemical constituents such as carbohydrate, protein, amino acid, alkaloids, tannins, fats and oil, flavonoids and Vitamin C using standard procedure of analysis.[13,14,15]

Determination of antioxidant activity of *C.sativus* fruit extract by DPPH method.[16]

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compound .This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the non radical form DPPH-H*[17].The DPPH is reacted with methanol or absolute ethanol to yield purple color. The presence of antioxidants in the sample scavenge the formed DPPH radical and decrease in color is observed which is Spectrophotometrycally measured at 517nm.[18,19] In one cuvette 3ml of methanol was taken and kept as a standard for all the extracts. In other cuvette 3ml of DPPH was taken. Absorbance for the blank samples at 517 nm was determined.[20] Cuvette of methanol was not disturbed. Now in another cuvette 3ml of DPPH was kept aside for 5min.To this cuvette ascorbic acid was added in microlitre in various concentrations. Absorbance at 517nm was read for each concentration. Scavenging activity was expressed as the % inhibition. Now ascorbic acid was replaced by extracts and followed same procedure.

The percentage of inhibition can be calculated using the formula

Inhibition (%) = $(A_0-A_1/A_0) \times 100$

Where; A_0 is the absorbance of control and A_1 is the absorbance of test

RESULT &DISCUSSION :

Extractive Value

The extractive value of petroleum ether, benzene, chloroform, acetone, ethanol and water extracts were found to be 1.35%w/w, 0.66%w/w, 0.8%w/w, 1.46%w/w, 3.03%w/w and 3.75%w/w respectively as recorded in Table no.1.Percentage yield of water extract (WECS) was found to be maximum i.e. 3.75%w/w as compared to other extracts.

Table No.1.Extractive Value % (W/W)

Sr.No.	C.sativus Fruit Extracts	% (W/W)
1	PECS	1.35
2	BECS	0.66
3	CECS	0.8
4	AECS	1.46
5	EECS	3.03
6	WECS	3.75

Abbreviation : CS-*Cucumissativus* ; PE-Petroleum ether extract ; BE-Benzene extract ; CE-Chloroform extract ; AE-Acetone extract; EE-Ethanol extract; WE- Water extract

Preliminary phytochemical screening

All the extracts were screened for presence of carbohydrate, protein, amino acid, alkaloid, tannin, fat and oil, flavonoid and Vitamin C. Preliminary phytochemical screening showed the presence of carbohydrate, amino acid, alkaloid, tannin, vitamin C and flavonoid in water extracts and alkaloid, tannin and flavonoid in ethanol extract which is recorded in table no.2

Table No.2.Preliminary phytochemical screening of C.sativus fruit extracts.

Sr.N	Phytochemical	Test	PECS	BECS	CECS	AECS	EECS	WECS
1	Carbohydrate	Fehling test	-	-	-	-	-	+
2	Protein	Biuret test	-	-	-	-	-	-
		Xanthoprotein	-	-	-	-	-	-
3	Amino acid	Ninhydrin test	-	-	-	-	-	+
4	Alkaloid	Hager's Reagent	-	-	+	-	+	+
		Wagner's Reagent	-	-	-	-	+	+
5	Tannins	Ferric chloride reagent	-	-	-	-	+	+
		Lead acetate Test	-	-	-	-	+	+
		Potassium dichromate Test	-	-	-	-	-	+
6	Fat and Oil	Spot Test	+	-	-	-	-	-
7	Flavonoid	Shinoda Test	-	-	-	-	+	+
8	Vitamin C		-	-	-	-	-	+

DPPH free radical scavenging activity

DPPH free radical scavenging activity of PECS, BECS, CECS, AECS, EECS, and WECS is depicted in fig.1.It was observed that water extract of *C.sativus* showed highest DPPH free radical scavenging activity then other extracts. Different concentrations of L-ascorbic acid were used as standard antioxidant.

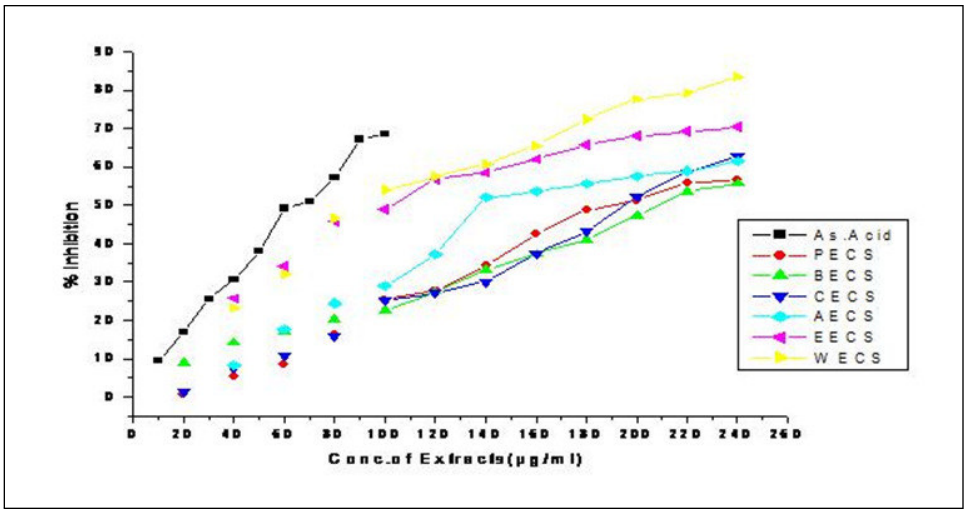
IC_{50} Value for Antioxidant activity

IC_{50} value (Table no.3) states the amount of concentration of extract required to produce 50% free radical scavenging activity. Hence IC_{50}value is inversely related to the free radical scavenging activity. Here result clearly states that water extract of *C.sativus*fruit showed highest whereas benzene extract showed lowest DPPH free radical scavenging activity.

Table No.3: DPPH free radical scavenging activity of *C.sativus*fruit in Non-Polar to Polar Solvents

Conc. of Extracts (µg/ml)	PECS % Inhibition	BECS % Inhibition	CECS % Inhibition	AECS % Inhibition	EECS % Inhibition	WECS % Inhibition
Control	-	-	-	-	-	-
20	0.74	9.0	1.37	5.60	12.38	14.49
40	5.29	14.19	7.70	8.35	25.71	23.28
60	8.46	17.16	10.77	17.54	34.17	32.06
80	16.19	20.12	15.73	24.52	45.82	46.56
100	25.39	22.45	25.34	28.96	48.88	53.96
120	27.83	27.54	27.13	37.20	56.82	57.46
140	34.28	33.15	30.09	52.00	58.51	60.84
160	42.64	37.28	37.48	53.69	62.11	65.60
180	48.78	41.10	43.18	55.60	65.71	72.38
200	51.32	47.24	52.27	57.61	68.04	77.77
220	55.76	53.60	58.71	58.98	69.20	79.36
240	56.71	55.72	62.82	61.52	70.37	83.59
IC_{50}	180 µg/ml	210 µg/ml	190 µg/ml	130 µg/ml	100 µg/ml	85 µg/ml
IC_{50} (Std.)Ascorbic acid - 60 µg/ml						

Fig. No.1.DPPH free radical scavenging activity of *C.sativus*fruit in Non-Polar to Polar solvents



RESULT AND DISCUSSION :

The phytochemical study of different extracts of *C.sativus*showed the presence of tannin, and flavonoids in ethanol and water extracts and Vitamin C only in water extract. Antioxidant activity of different extracts was found to be Water>Ethanol>Acetone >Petroleum ether> Chloroform >Benzene. Maximum antioxidant activity of water extracts could be contributed to presence of Vitamin C, tannin and flavonoid.

CONCLUSION :

Thus it can be concluded that *C.sativus* possesses antioxidant activity. Water extract possesses maximum activity while benzene extract possesses minimum activity.

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