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

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ABSTRACT

Momordica dioica Linn is a perennial climbing creeper belonging to the family Cucurbitaceae. *M.dioica* fruit is pungent, bitter, hot, alexiteric, stomachic and laxative. Fruit is rich in ascorbic acid and contains flavonoids, glycosides, alkaloid and amino acid. 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 undertaken to analyze the presence of different phytochemical constituents and to evaluate antioxidant activity of *M.dioica* fruit in petroleum ether, benzene, chloroform, acetone, ethanol and water extracts. All the extracts was tested for 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 *M.dioica* revealed the presence of carbohydrate, protein, alkaloid, amino acid, flavonoid and Vitamin C. A higher percentage free radical scavenging was found for ethanol extract as compared to all other extracts.

Key words: *Momordica dioica*, Phytochemical screening, Antioxidants, 1-diphenyl-2-picryl hydroxyl.

INTRODUCTION

The skin antioxidant defence system (ADS) is essential in protecting the epidermis from damage by free radicals generated by environmental and endogenous factors. The antioxidants counteract free radicals by removing them from body [1]. Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reaction can produce free radicals which start chain reactions that can damage cells. Antioxidant may terminate the chain reactions either by removing radical intermediates or by inhibiting other oxidation reaction by being oxidized themselves [2]. In process of aging, oxidation plays an important role in human and other animals [3]. 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)[4] Oxidative stress is a stress imposed on a biological system that requires 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 [5]. 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 [6].

Momordica dioica is perennial climbing creeper. In Maharashtra the local name for fruit is "Kartule" and belonging to the family Cucurbitaceae available in the month of April-July [7, 8]. Generally found in the forest of Southern India, Bengal, Maharashtra and Madhya Pradesh. The fruit contains alkaloid, flavonoids, glycosides and amino acids [9].

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M.dioica is used in prophylaxis of various disorders like Brain Cancer, Inflammatory disorders, Hepatic disorders, Diabetes's. Reducing agents have a lower redox potential and readily get oxidized and are effective against oxidizing agents [10].It is stimulant and astringent juice of root is antiseptic [11].

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

MATERIALS AND METHODS

Plant materials and extraction

The fruits of *M.dioica* (Cucurbitaceae) were procured from the local market of Nagpur (Maharashtra) and authenticated in Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur.

Preparation of extracts

Fruits of *M.dioica* 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 45^0C 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 [12]. Each extract was then concentrated by distilling off the solvent and then evaporating the solvent to dryness and weighed [13]. 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 [14, 15, 16].

Determination of antioxidant activity of *M.dioica* fruit extract by DPPH method [17]

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* [18].The DPPH is reacted with methanol or absolute ethanol to yield purple colour. The presence of antioxidants in the sample scavenge the formed DPPH radical and decrease in colour is observed which is Spectrophotometrycally measured at 517nm [19, 20]. 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 [21]. 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

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

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

RESULT

Extractive Value

The extractive value of petroleum ether, benzene, chloroform, acetone, ethanol and water extracts were found to be 2.2%w/w, 0.80%w/w, 1.15%w/w, 2.16%w/w, 3.55%w/w and 3.02%w/w respectively as recorded in Table no.1.Percentage yield of ethanol extract (EEMD) was found to be maximum i.e. 3.55%w/w as compared to other extracts.

Abbreviation : MD- *Momordica dioica* ; PE-Petroleum ether extract ; BE-Benzene extract ; CE-Chloroform extract ; AE-Acetone extract; AE-Alcohol 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, protein, alkaloid and flavonoid in ethanol and water extracts and Vitamin C in ethanol extract which is recorded in table no.2

DPPH free radical scavenging activity

DPPH free radical scavenging activity of PEMD, BEMD, CEMD, AEMD, EEMD, and WEMD is depicted in fig.1.It was observed that ethanol extract of *M.dioica* 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 ethanol extract of *M.dioica* fruit showed highest whereas benzene extract showed lowest DPPH free radical scavenging activity.

Table 1. Extractive Value % (W/W)

Sr.No.	*M.dioica* Fruit Extracts	% (W/W)
1	PEMD	2.2
2	BEMD	0.80
3	CEMD	1.15
4	AEMD	2.16
5	EEMD	3.55
6	WEMD	3.02

Table 2. Preliminary phytochemical screening of *M.dioica* fruit extracts.

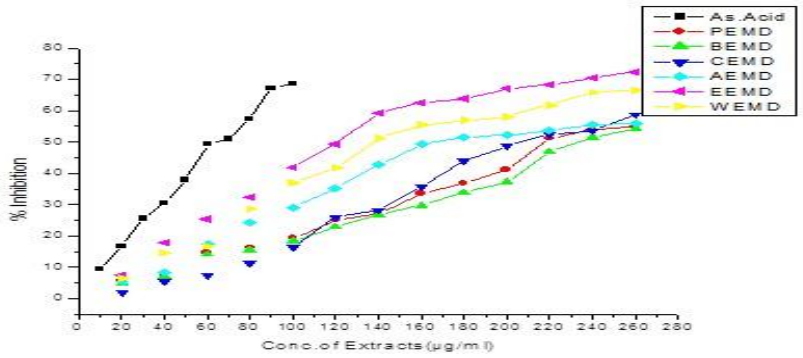
Sr.no.	Phytochemical	Test	PEMD	BEMD	CEMD	AEMD	EEMD	WEMD
1	Carbohydrate	Fehling test	-	-	-	-	+	+
2	Protein	Biuret test	-	-	-	-	+	-
		Xanthoprotein	-	-	-	-	+	+
3	Amino acid	Ninhydrin test	-	-	-	-	+	+
4	Alkaloid	Hager's Reagent	-	-	+	-	+	+
		Wagner's Reagent	-	-	-	+	+	+
5	Tannins	Ferric chloride reagent	-	-	-	-	-	-
		Lead acetate Test	-	-	-	-	-	-
		Potassium dichromate Test	-	-	-	-	-	-
6	Fat and Oil	Spot Test	-	-	-	-	-	-
7	Flavonoid	Shinoda Test	-	-	-	-	+	+
8	Vitamin C		-	-	-	-	+	-

Table 3. DPPH free radical scavenging activity of *M.dioica* fruit in Non-Polar to Polar Solvents

Conc. of Extracts (µg/ml)	PEMD % Inhibition	BEMD % Inhibition	CEMD % Inhibition	AEMD % Inhibition	EEMD % Inhibition	WEMD % Inhibition
Control	-	-	-	-	-	-
20	5.80	4.97	2.002	5.49	7.51	6.53
40	7.39	6.98	5.69	8.24	17.98	14.64
60	14.78	14.28	7.48	17.33	25.39	16.85
80	16.36	15.55	11.38	24.41	32.48	28.55
100	19.32	18.30	16.43	29.17	41.90	36.98
120	25.02	22.96	26.02	35.30	49.41	41.83
140	27.24	26.77	28.24	42.81	59.25	51.10
160	33.57	29.73	35.72	49.26	62.53	55.42
180	36.85	33.86	43.94	51.58	63.80	56.90
200	41.18	37.24	48.78	52.21	66.98	58.06
220	51.21	46.87	52.37	53.69	68.35	61.74
240	54.06	51.53	53.63	55.39	70.58	65.85
260	54.80	54.28	58.69	55.81	72.38	66.49
IC_{50}	**210µg/ml**	**235 µg/ml**	**200 µg/ml**	**160µg/ml**	**120µg/ml**	**135µg/ml**
IC_{50} (Std.)Ascorbic acid - 60 µg/ml						

Figure 1. Fruit of *Momordica dioica* Linn

Figure 2. DPPH free radical scavenging activity of *M.dioica* fruit in Non-Polar to Polar solvents



DISCUSSION

The phytochemical study of different extracts of *M.dioica* showed the presence of flavonoids in ethanol and water extracts and Vitamin C only in ethanol extract.

Antioxidant activity of different extracts was found to be Ethanol > Water > Acetone > Chloroform > Petroleum ether > Benzene.

Maximum antioxidant activity of ethanol extracts could be contributed to presence of Vitamin C and flavonoid.

CONCLUSION

Thus it can be concluded that *M.dioica* possesses antioxidant activity. Ethanol extract possesses maximum activity while benzene extract possesses minimum activity.

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