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In-Vitro Antioxidant Activity Of *Cycas Beddomei*

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Abstract

The current study was to investigate antioxidant activity of methanol extract of whole plant of *Cycas beddomei* was administrated by utterly diverse models like DPPH radical scavenging assay, superoxide anion assay, nitric oxide radical inhibition assay, thiocyanate methodology, lipid peroxidation assay and hydroxyl radical scavenging assay. Methanol extract of *Cycas beddomei* showed effective antioxidant activity compared to straightforward antioxidants like ascorbic acid, rutin, curcumin, fat-soluble vitamin (Vitamin E) and -tocopherol. The antioxidant activity depends upon the concentration and it had been increased with escalating the concentration of extract. The antioxidant activity could also be endorsed to the occurrence of phenolic and flavonoids compounds gift within the methanol extract. The results obtained within the present study point out that the methanol extract of whole plant of *Cycas beddomei* area unit a possible supply of natural antioxidant.

Keywords: *Cycas beddomei,* -tocopherol, curcumin, vitamin E, Cycadaceae

Introduction

The use and search of medication and dietary supplements from plants are intense in recent year [1]. Medicative plants are a supply of great economic value within the Indian sub continent. For many of the unwellness, plant materials are used as medication due to its active compounds. In several disorders the free radical mediate injury could play a vital role. Free radicals are responsible for inflicting a good range of health issues that embrace cancer, aging, heart diseases and gastric issues [2] etc. Antioxidants cause protecting impact by neutralizing free radicals that are poisonous byproducts of natural cell metabolism. The mechanism of the action of those antioxidant compounds embrace suppression of reactive oxygen species formation either by inhibition of the enzymes or by chelating of trace parts concerned in radical production, scavenging of reactive species and up- control or protective antioxidant defense. *Cycas beddomei* Dyer (Cycadaceae) is an endemic and critically vulnerable spermatophyte confined to Seshachalam part Reserve, Eastern ghats, India [3]. This plant initially thought of as a rare species [4] and vulnerable in Indian Red Data [5]. *Cycas circinalis* var. beddomei (Dyer) J. Schust. could be a word of *Cycas beddomei* [6]. Historically male cones of this plant were accustomed cure atrophic arthritis, muscle pains and joint swellings [7]. Seeds are position to a stick with coconut oil and are used as a poultice to take care of skin complaints like wounds, sores and boils [4]. *Cycas beddomei* showed important pharmacologic activities like anti-inflammatory [8], antioxidant [9], antidiabetic [10], anthelmintic [10] and antibacterial activities [Alekhya et al., 2013]. *Cycas beddomei* consists of different active constituents like alkaloids, tannins, flavonoids, terpenoids, saponins, phenols, glycosides, lignins, 2-(hydroxymethyl)-2-nitro-1, 3-propanediol, methyl tetradecanoate, palmitic acid, methyl organic compound and methyl cis-7-octadecenoate [11] are a unit up to now according in *Cycas beddomei.* The target of the current study was to examine the antioxidant activity of the methanol extract of whole plant of *Cycas beddomei* by victimization in-vitro models.

Materials and Method

Collection of plant material

The whole plant of *Cycas beddomei* was collected from Tirumala Hills of chittoor district, Andhra Pradesh, India and the plant substance was taxonomically recognized and authenticated by the Dr. Madhava chetty (Research Officer) botany, Andhra Pradesh. Voucher specimen (KCP-104/2012) of this plant has been retained in the Karnataka College of Pharmacy, Bangalore, Karnataka, India.

Extraction of plant material

The whole plant of *Cycas beddomei* were dried below shade and so powdered with a mechanical grinder to get a rough powder. Equal amount of powder was passed through forty mesh sieve and extracted with methanol in soxhlet equipment at 60ºC. The solvent was utterly removed by rotary vacuum evaporator and focused. The extract was freeze dried and stored in a vacuum desiccators for further phytochemical and antioxidant studies.

Preliminary phytochemical analysis

The extract obtained as above was then subjected to qualitative tests for the identification of assorted plant constituents with

completely different chemical reagents [12, 13]. The check for tannins was administered by adding the extract to a quarter gelatin solution containing sodium chloride. The formation of white precipitate indicates the presence of tannin. The extract was subjected to frothing check for the identification of saponins. The frothing test was carried out by diluted extract with distilled water to 20ml and shaking the graduated cylinder for 15min. The formation of 1cm layer of froth indicates the presence of saponins. The check for alkaloids was administered by subjecting 1gm of extract into 10ml of 1% HCl so poached and filtered. The filtrates were treated with Mayer's reagent. The creation of yellow cream impulsive indicates the presence of alkaloids. Steroids were screened by adding 1mL of acetic anhydride to 0.25 g extract of every sample with 1ml H_2SO_4. The colour modified from violet to blue or green in some samples, indicating the presence of steroids. Sulfuric acid (Concentrated; 2 ml was superimposed to 2ml of extract solution. Then it had been treated with 15% ethanolic -naphthol (Molisch's reagent). There's no formation of a chromatic violet ring at the junction of 2 layers indicated the negative check for gums (Molisch's test). The presence of flavonoids resolve by dissolving the extract in fermentation alcohol and one piece of Mg turnings was superimposed followed by conc. HCl, superimposed drop wise that and heated. Look of magenta color indicated the presence of flavonoids.

In-vitro antioxidant assays

Diphenyl-picryl-hydrazyl [DPPH] assay

The free radical scavenging capability of extract was tested by its ability to bleach the stable 2, 2 diphenyl 2-picryl hydrazyl radical [14]. A stock solution of DPPH [1.5 mg /ml of methanol] was prepared. This stock solution was used to calculate the antiradical activity. Diminish in absorbance within the presence of methanolic extract of bark of *Cycas beddomei* at totally different concentration [10-160µg] was noted once 15 minutes. IC_{50} was calculated from percentage inhibition. Ascorbic acid was used as reference standard.

Nitric oxide scavenging assay

The interaction of extract with nitric oxide was assessed by the radical detection methodology.

The chemical supply of NO was sodium nitroprusside [l0mM] in 0.5 M phosphate buffer, pH 7.4 that spontaneously produces nitric oxide in an aqueous solution. During this investigation, Griess Illosvoy chemical agent was changed by victimization naphthyl ethylene diamine dihydrochloride (0.1% w/v) rather than 1-napthylamine (5%). The reaction mixture (3mL) containing two mL sodium nitroprusside (10 mM), phosphate buffer saline (0.5 mL) and therefore the methanol extract of *Cycas beddomei* (10 µg to 320 µg) or standard solution (rutin) was incubated at 250C for a 150 min. A similar reaction mixture while not the methanol extract of sample however with equivalent quantity of methanol served as control [15,16]. Rutin was used as positive management.

Scavenging effect on Superoxide anion

Superoxide anion radicals were calculable by spectrophotometric measuring of the reduction products of nitroblue tetrazolium [NBT] generated in riboflavin-light system according the strategy of [17]. The reaction mixture consisted of EDTA [6pM; with 3pg NaCN], riboflavin [2 pm], NBT [50pM], completely different concentrations of methanolic extract and phosphate buffer [67 mM; pH seven.8] more in a very final volume of 3 ml. The tubes were uniformly illuminated with associate degree electric light for 15 min, then the optical density was measured at 530 nm before and once illumination [18]. Curcumin was used as a positive management.

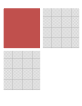
Inhibition of lipid peroxide formation induced by Fe^{2+}/ ascorbate system

The reaction mixture containing 0.1 ml of rat liver homogenate [25 the worries w/v] in Tris-HCl [30mM], ferric ammonia sulphate [0.16 mM], vitamin C [0.06 mM] and completely different concentrations of the extract [12.5 to 200 µg/ml] in a very final volume of 0.5 milliliter was incubated for one hour at 37 and therefore the ensuing thiobarbituric reacting substance [TBARS] was measured by the strategy of [19]. A 0.4 milliliter aliquot of the reaction mixture was treated with metal dodecyl sulphate [0.2 ml, 8.1%], thiobarbituric acid [1.5 ml, 0.8%], and ethanoic acid [1.5 ml, 20%, pH 3.5], made to a complete volume of four milliliter by adding distilled water, and kept in a very water bath at 95 C for one h. Once cooling, H_2O [1ml] and five milliliter of n-BuOH/pyridine 15:1 [v/v] were more. Once shaking and action, the organic layer was separated and therefore the absorbance measured at 532nm. Vitamin E was used as a reference compound.

Antioxidant assay by thiocyanate method

The antioxidant activity of methyl alcohol extract of *Cycas beddomei* determined in step with the thiocyante methodology [20; 21]. Briefly, five hundred µl of methyl alcohol extract of *Cycas beddomei* at entirely completely different concentrations (25µg to 200µg) were mixed with 2.5 mL of 0.02 M linoleic acid emulsion (contains equal weight of tween-20 in phosphate buffered saline, pH 7.4) and additionally the final volume was adjusted to 5ml with phosphate buffered saline during a test-tube and incubated at 37^0C in dark place. The number of peroxide determined by activity absorbance at 500 nm once coloring with 0.1ml of $FeCl_2$ (0.02M) and 0.1ml salt (30%) at intervals throughout incubation. The solutions whereas not the plant extracts were taken as blank for the study. -tocopherol was used as a reference compound.

Hydroxyl radical scavenging assay



The assay was performed as delineated by Halliwell [22] with minor changes. All solutions were prepared freshly. 1.0 ml of the reaction mixture contained 100 µL of twenty eight millimeter 2-deoxy-2-ribose (dissolved in phosphate buffer pH 7.4), 500µL resolution of various concentrations of the methyl alcohol extract of *Cycas beddomei* (10 to 320 µg), 200µL of 200µM $FeCl_3$ and 1.04mM ethylene diamine tetracetic acid (EDTA, 1:1 v/v), one hundred µL H_2O_2 (1.0 mM) and 100µL ascorbic acid (1.0mM). Once degree amount of time of 1hr at 37^0C, the extent of deoxyribose degradation was measured by the TBA reaction [23, 24]. The absorbance was measured at regarding 532 nm against the blank answer. Vitamin E was used as a positive management.

Results

Percentage yield

The percentage yield (table No: 1) of the methanol extract of *Cycas beddomei* was found to be 21.6% given.

Table No 1. Percentage yield of methanol extract of *Cycas beddomei*

Extract name	% Yield (w/w)
methanol extract of *Cycas beddomei*	21.6

Preliminary phytochemical screening of *Cycas beddomei*

It was observed that the preliminary phytochemical screening of *Cycas beddomei showed* the presence of alkaloids, flavonoids, steroids, carbohydrates, phenolics, proteins, lignans, saponins and anthocyanidins, where as absence of reducing sugars and gums. The preliminary phytochemical screening for various functional groups was tabulated as Table No. 2.

Table No. 2. Qualitative analysis of methanol extract of *Cycas beddomei*

phytochemical test	observation
alkaloids	+
flavonoids	+
steroids	+
carbohydrates	+
phenolics	+
proteins	+
lignans	+
saponins	+
anthocyanidins	+
reducing sugars	-
gums	-

"+" indicates present; "-" indicate absence

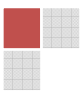
In-vitro antioxidant studies

DPPH radical scavenging activity

The methanol extract of *Cycas beddomei* of decreased the concentration of DPPH radical due to free radical scavenging ability. The result was shown in table No 3 and Figure No: 1. The methanol extract of *Cycas beddomei* had a well hydrogen donating ability with an IC_{50} value of 33.0 µg/mL and the value was found to be higher than that of standard, ascorbic acid (IC_{50} value of 19.0 µg/mL).

Table No. 3: DPPH radical scavenging activity

	% of inhibition	
Concentration	Standard- Ascorbic acid	Methanol extract of *Cycas beddomi*
10 µg/ml	11.4	6.5
20 µg/ml	53.6	22.8
40 µg/ml	104.4	69.4
80 µg/ml	136.8	82.8
160 µg/ml	158.8	111.8



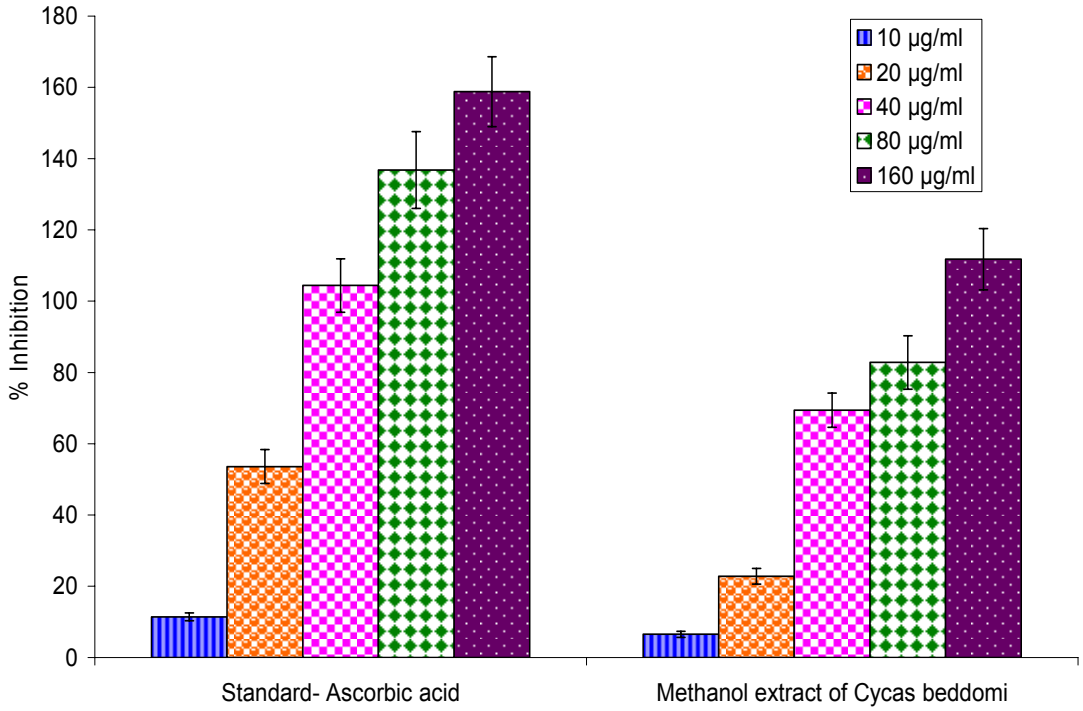


Figure No 1. Scavenging effect of methanol extract of *Cycas beddomei* and standard ascorbic acid on 1, 1'-Diphenyl-2-picryl hydrazyl (DPPH) radical. Results are mean ± S.D of five parallel measurements

Nitric oxide radical inhibition assay

The scavenging of nitric oxide by the methanol extract of *Cycas beddomei* was increased in a dose-dependent manner as illustrated in table No 4 and Figure No: 2. At concentration of 171.6µg/mL methanol extract of *Cycas beddomei* 50% of nitric oxide formed by incubation was scavenged. This IC_{50} value of methanol extract of *Cycas beddomei* was found to be more than that of standard, rutin (IC_{50} 96.0 µg/mL).

Table No. 4: Nitric oxide radical inhibition assay

	% of inhibition	
Concentration	Standard Rutin	Methanol extract of *Cycas beddomei*
10 µg/ml	7.46	1.89
20 µg/ml	11.14	5.02
40 µg/ml	24.68	16.09
80 µg/ml	44.07	30.42
160 µg/ml	67.16	47.23
320 µg/ml	121.46	86.01

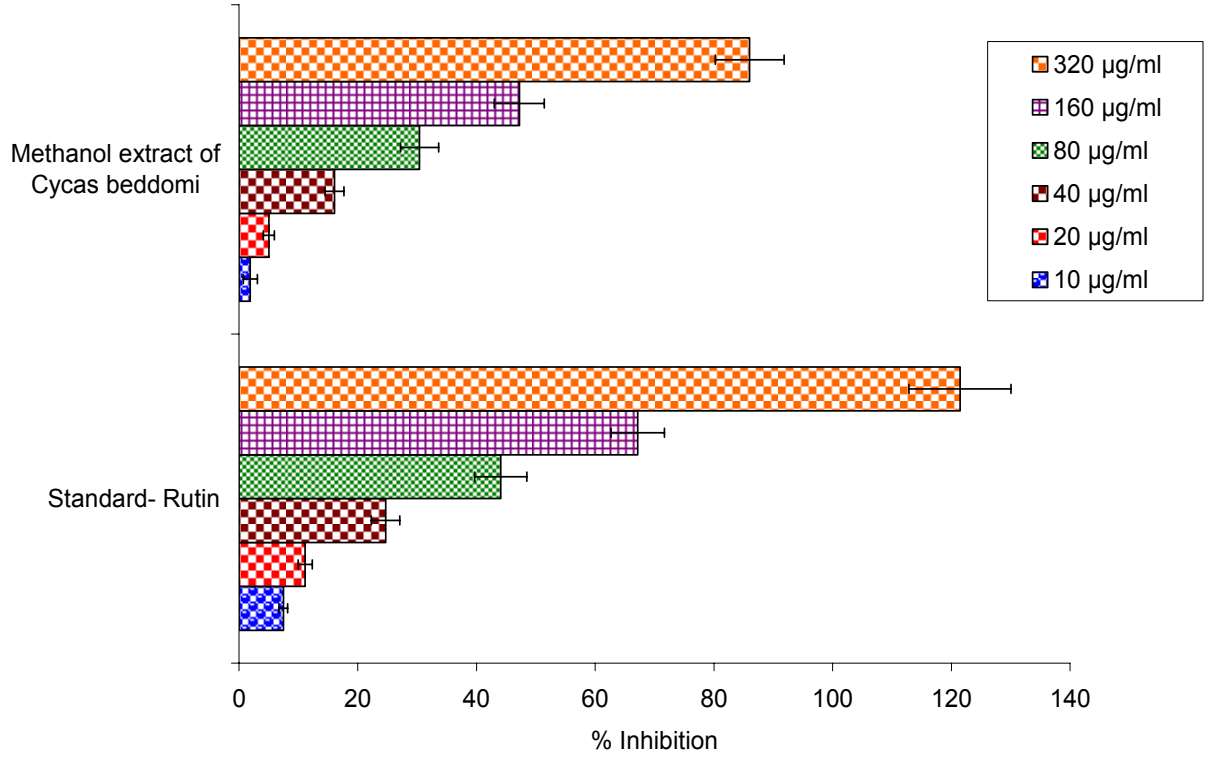


Figure No 2. Scavenging effect of methanol extract of *Cycas beddomei* and standard rutin on Nitric oxide radical. Results are mean ± S.D of five parallel measurements.

Superoxide anion scavenging activity

The superoxide anion resulting from dissolved O_2 by phenazine methosulphate/NADH coupling reaction reduces nitro blue tetrazolium. The decrease in the absorbance at 530 nm with the methanol extract of *Cycas beddomei* indicated the consumption of superoxide anion in the response mixture. As mentioned in Figure No 3 & table No 5, the extract of plant as well as curcumin showed the scavenging activity with the IC_{50} values, 32.0µg/ml and 19.0µg/ml, respectively.

Table No. 5: Superoxide anion scavenging activity

	% of inhibition	
Concentration	Standard- Curcumin	Methanol extract of *Cycas beddomei*
5 µg/ml	15.32	3.23
10 µg/ml	27.47	11.24
20 µg/ml	55.32	30.68
40 µg/ml	99.46	76.82
80 µg/ml	138.42	89.41
160 µg/ml	176.62	121.76

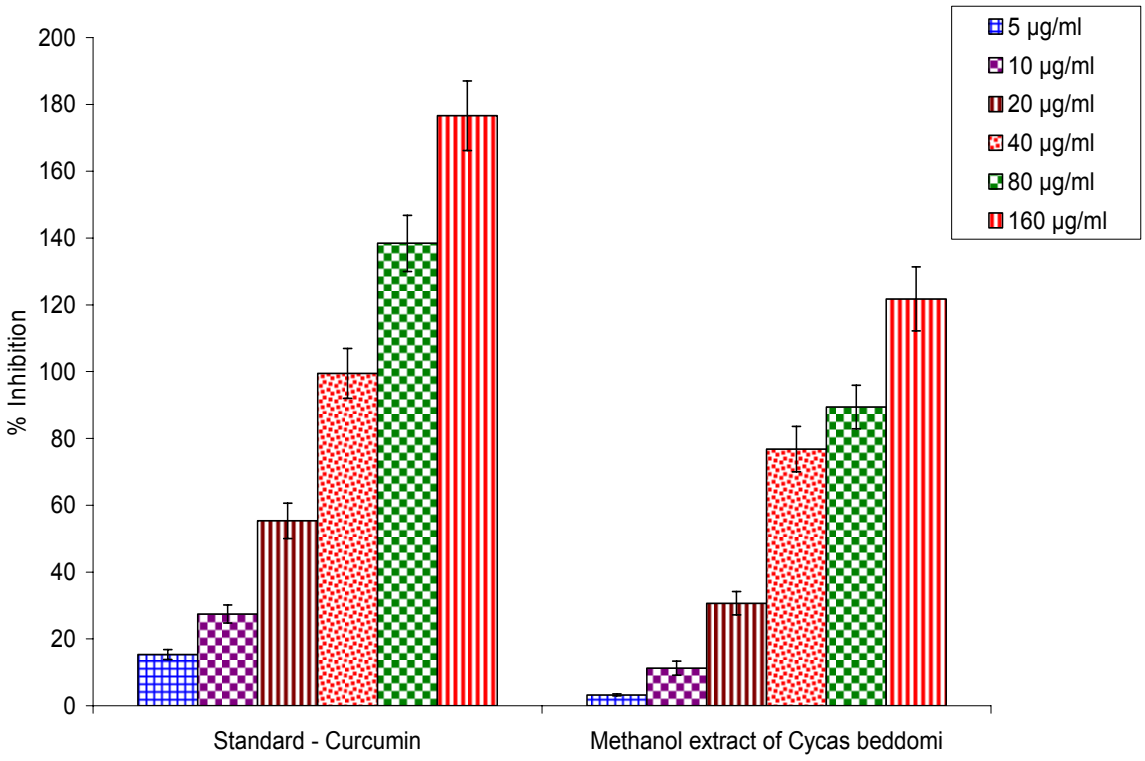


Figure No 3. Effect of methanol extract of *Cycas beddomei* and curcumin on scavenging of superoxide anion radical formation. Results are mean ± S.D of five parallel measurements.

Lipid peroxidation assay

The activity of methanol extract of *Cycas beddomei* against non-enzymatic lipid peroxidation in rat liver microsomes was shown in Figure No: 4 and table No 6. Addition of Fe^{2+}/ascorbate to the liver microsomes caused increase in lipid peroxidation. The extract of *Cycas beddomei* showed inhibition of peroxidation effect in all concentrations, which showed 50% inhibition effect at 190.0µg/ml. The extract inhibition value was found to be more than the standard, vitamin E (IC_{50} value 88.0µg/ml).

Table No. 6: Lipid peroxidation assay

	% of Inhibition	
Concentration	Standard-Vitamin E	Methanol extract of *Cycas beddomei*
12.5 µg/ml	9.6	4.1
25 µg/ml	19.8	13.5
50 µg/ml	36.88	16.12
100 µg/ml	52.64	31.43
200 µg/ml	81.88	51.61

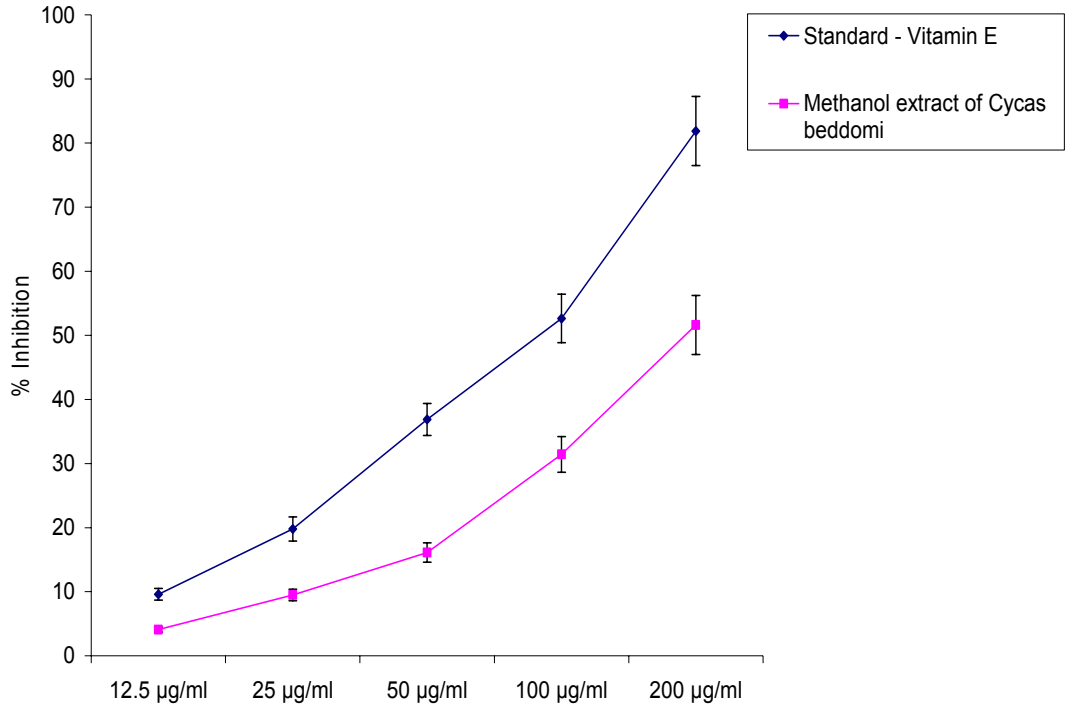


Figure No 4. Effect of methanol extract of *Cycas beddomei* and vitamin E on lipid peroxidation of liver microsome induced by Fe^{2+}/ascorbate. Results are mean ± S.D of five parallel measurements.

Antioxidant assay by thiocyanate method

The effects of methanol extract of *Cycas beddomei* on peroxidation of linoleic acid emulsion were shown in table No 7 and Figure No:5. From this method, the amount of peroxides formed in emulsion during incubation was determined spectrophotometrically by measuring absorbance at 500 nm. High absorption is an indication of high concentration of peroxides formed. Therefore, low absorbance indicates high antioxidant activity. The IC_{50} values were found to be 96.0µg/ml and 38.0µg/ml for methanol extract of *Cycas beddomei* and standard, - tocopherol, respectively.

Table No. 7: Antioxidant assay by thiocyanate method

	% of Inhibition	
Concentration	Standard- -Tocopherol	Methanol extract of *Cycas beddomei*
25 ug/ml	14.4	8.6
50 ug/ml	74.4	27.6
100 ug/ml	82.6	51.3
200 ug/ml	92.8	72.1

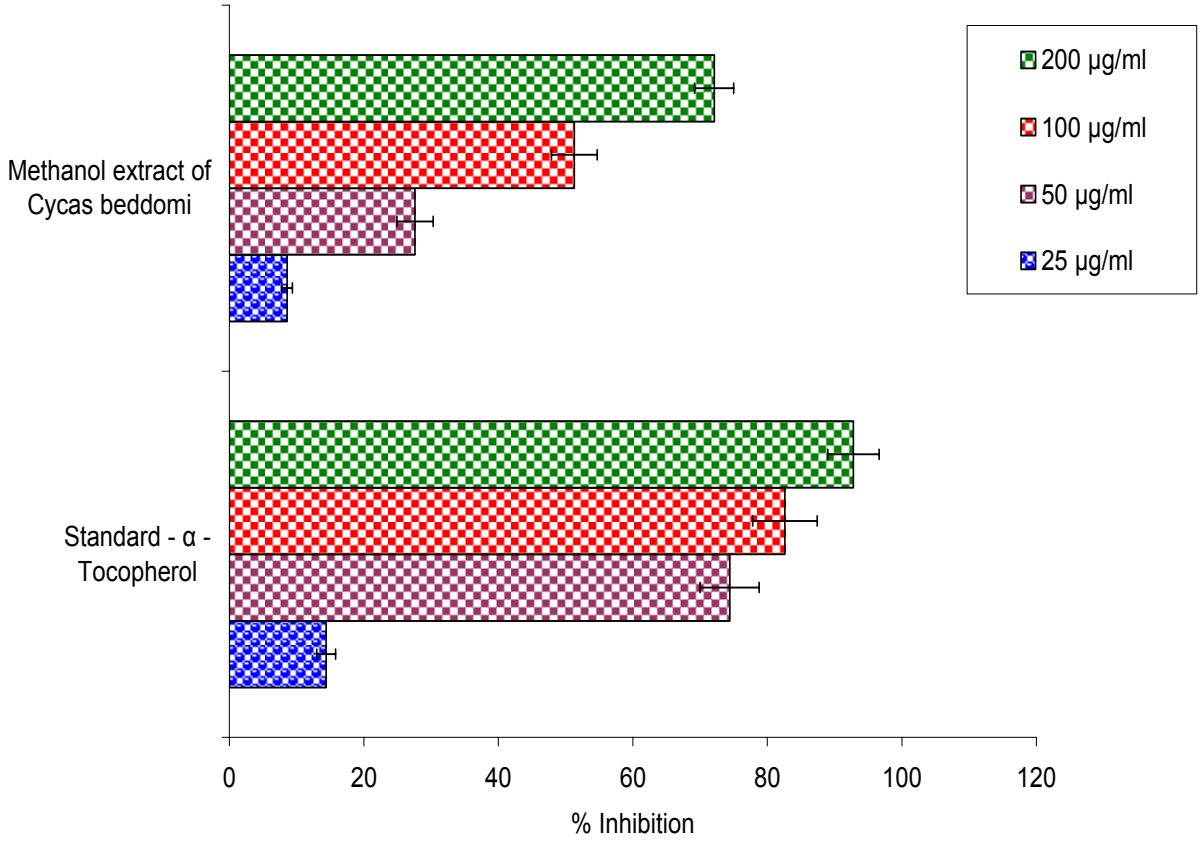


Figure No 5. Percentage inhibition of lipid peroxidation using the standard (α- tocopherol) and using methanol extract of *Cycas beddomei* in linoleic acid emulsion. Results are mean ± S.D of five parallel measurements.

Hydroxyl radical scavenging assay

To harass the substrate deoxyribose hydroxyl radicals were generated by response of Ferric-EDTA together with H_2O_2 and ascorbic acid. When the methanol extract of *Cycas beddomei* was incubated with the above reaction mixture, it could prevent the damage against sugar. The results was shown in Figure No 6 and table No 8, the concentrations of 50% inhibition were found to be 44.0µg/ml and 36.0µg/ml for the methanol extract of *Cycas beddomei* and standard compound, vitamin-E, respectively. The IC_{50} value (44.0µg/mL) of the extract of plant name was found to be more than the standard.

Table No. 8: Hydroxyl radical scavenging assay

	% of Inhibition	
Concentration	Standard-Vitamin E	Methanol extract of *Cycas beddomei*
10 µg/ml	14.04	9.78
20 µg/ml	31.07	21.04
40 µg/ml	59.14	47.2
80 µg/ml	87.13	72.1
160 µg/ml	101.76	90.01
320 µg/ml	124.13	101.62

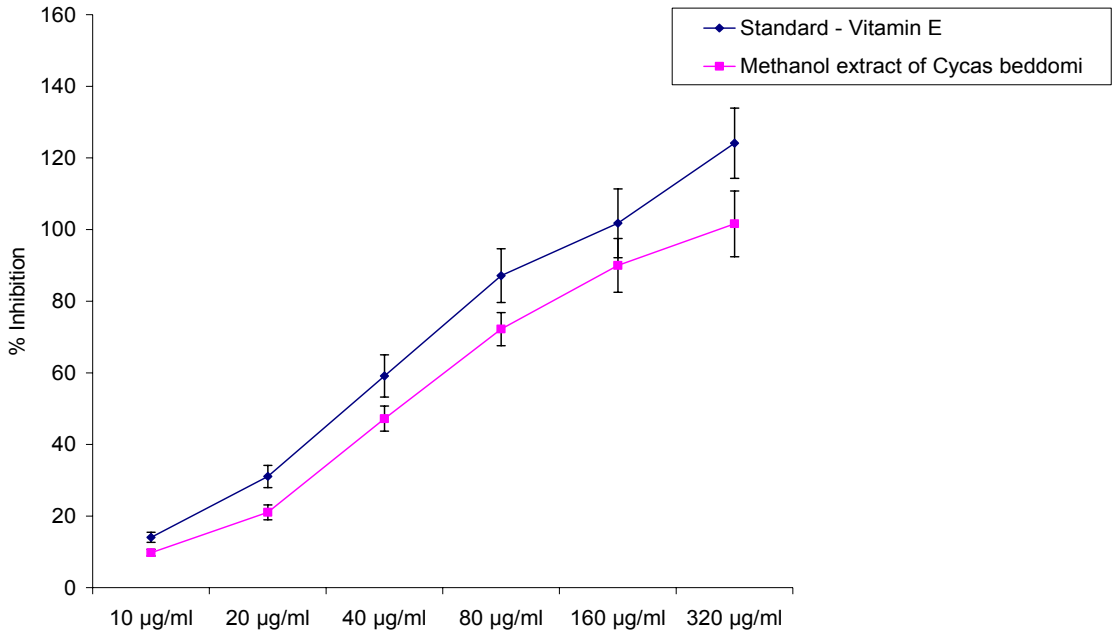


Figure No 6. Effect of methanol extract of *Cycas beddomei* and vitamin E on deoxyribose degradation assay. Results are mean ± S.D of five parallel measurements.

Discussion

Free radicals, species with one (or) additional odd electrons, square measure a unit created in ancient (or) pathological cell metabolism from xenobiotics, (or) through radiation. Electron acceptors like molecular substance react merely with free radicals to become radicals themselves ROS (Reactive substance species). The first derivatives of substance (O_2, H_2O_2, .OH, O_2) play a vital role in mediating ROS connected effects. Short lived reactive species generated in state of affairs will react with non radicals and manufacture chain reaction [25]. Free radicals have aroused important interest among scientists among the past decade. Their broad vary of effects in biological systems has drawn the eye of the many experimental works. There are several reports that support the employment of antioxidant supplementation in reducing the extent of aerobic stress and in retardation or preventing the event of complications related to diseases [26]. Many manmade matter elements have shown poisonous and/or agent effects that have shifted the attention towards this antioxidants. Varied plant constituents have tested to purpose radical scavenging or antioxidants activity [27]. There's increasing evidences that advocate that consumption of natural matter contained in vegetables, fruits and medicative herbs unit of mensuration helpful in preventing the hurtful consequences of aerobiotic stress [28].

This investigation incontestable that DPPH could also be a radical, stable at temperature that produces a purple color answer in alcohol. It's reduced among the presence of associate degree inhibitor substance molecule, giving rise to achromatic methanolic solutions. Figure No.1 illustrates the decrease among the concentration of DPPH radical as a results of radical scavenging ability of alcohol extract of *Cycas beddomei* and water-soluble vitamin, that's reminiscent of the reportable value of Thabrew [29].

Gas radical inhibition study proved that alcohol extract of *Cycas beddomei* was a potent scavenger of gas. This gas generated from metal nitroprusside reacts with element to nitrate cluster. The extract of plant inhibits nitrate formation by competitive with element to react with gas directly and additionally to inhibit its synthesis. Scavengers of gas manage element leading to reduced production of gas [15, 16]. From the gas take a glance at, rutin was used as a typical. The IC_{50} worth of the rutin is of an identical kind to the reportable worth of Badami [30].

Among the PMS/NADH –NBT system, anion derived from dissolved part by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 530nm with antioxidants therefore indicates the consumption of anion among the reaction mixture. Addition of assorted concentrations of alcohol extract of *Cycas beddomei* even so as curcumin (standard) in additional than coupling reaction showed decrease in absorbance.

The liver grain fraction undergoes speedy non-enzymatic peroxidation once incubated with FeCl3 and antioxidant. The utilization fe (III) among the presence of a reducer like ascorbate produces .OH [31] that they attack the biological material. This finally lands up among the formation of MDA (malonodialdehyde) and varied aldehydes that forms a pink compound [32] with TBA, absorbing at 532nm. The methyl alcohol extract of *Cycas beddomei* and vitamin E exhibited sturdy scavenging impact of

hydroxyl which will inhibit molecule injury at whole fully completely different concentration. The scavenging impact of vitamin E is in accordance with the report of Hemanth [33]. The extract inhibits molecule injury caused by hydroxyl radicals and in addition the inhibition values mentioned in Figure No 4. The antioxidant impact of the extract is additionally as results of its flavonol content Haribal [34].

Among this study, in-vitro antioxidant activity of methyl alcohol extract of *Cycas beddomei* determined by exploitation the thiocyanate technique. From this methodology, the quantity of peroxides fashioned in emulsion throughout incubation determined spectrophotometrically by activity absorbance at 500nm. High absorption could also be a symbol of high concentration of peroxides fashioned. Consequently, small absorbance indicates elevated antioxidant activity [20]. The methyl alcohol extract of *Cycas beddomei* and customary of –tocopherol were shown to inhibit the formation of peroxides at varied concentrations.

The extract was examined for its aptitude to act as .OH radical scavenging mediator. Ferric EDTA was incubated with H_2O_2 and antioxidant at pH -7.4; radicals of hydroxyl were created in free resolution and were detected by their ability to degrade 2-deoxy-2-sugar into fragments that on heating with TBA at low ion concentration type a pink compound [35]. Once alcohol extract of *Cycas beddomei* and vitamin E were extra to the reaction mixture, they removed hydroxyl radicals and disallowed the degradation of 2-deoxy-2- sugar as mentioned over the IC_{50} discovered values of every plant extract and vitamin E were analogous to the reportable values of Sen [36].

Conclusion

The current study proved hopeful antioxidant prospective of methanol extract of whole plant of *Cycas beddomei* against a variety of free radicals. It is reported that phenolics and flavonoids are natural products which have been shown to acquire different biological properties associated to antioxidant mechanisms. Thus the antioxidant activity of *Cycas beddomei* may be attributed to the presence of these compounds as confirmed by qualitative phytochemical analysis. Hence these results maintain the vision that several traditionally used Indian medicinal plants are capable source of prospective antioxidants.

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