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ASSESSMENT OF NUTRITIONAL CONSTITUENTS AND *IN VITRO* ANTIOXIDANT CAPACITY OF METHANOLIC STEM EXTRACT OF *CARALLUMA ATTENUATA*

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ABSTRACT: The main objective of the present study includes the assessment of nutritional constituents and anti-oxidant activity of methanolic stem extract of *Caralluma attenuata* by *in-vitro* methods. In the present investigation the author has identified the phytochemical constituents which have nutritional importance by specific chemical tests, thin layer chromatography, atomic absorption spectroscopy for Calcium, Zinc and Magnesium. *In vitro* antioxidant property was also performed by Free Radical Scavenging Assay, Reductive Ability and Nitric Oxide Method. Carbohydrates, proteins and amino acids, flavonoids and inorganic essential trace elements were identified and the results were tabulated. On the basis of the results observed the methanolic stem extract of *Caralluma attenuata* found to possess considerable amounts of nutritional constituents and anti-oxidant property

INTRODUCTION: Nutritional problems such as vitamin deficiencies, anaemia, and malnutrition affect cognitive ability and intellectual development. Free radicals are highly reactive and harmful which may also lead to above specified problems. These are capable of damaging almost all types of bio molecules such as proteins, lipids, carbohydrates and nucleic acids. The fact is that the free radicals would be formed as a chain reaction. Oxidative stress can arise when cell cannot adequately destroy the excess free radicals formed ^{1, 2}

Plants are the primitive source for medicinal principles. Various kinds of herbs possess distinct and specific therapeutic uses. Among them *Caralluma attenuata* ^{3, 4} is a perennial herb belongs to the family Apocynaceae and sub-family is Asclepiadaceae. According to the literature the folkal information reveals that the tribes generally chew the whole plant to treat peptic ulcer, diabetes, in weight reduction ⁵, to suppress hunger and also to produce endurance.

It has been reported for the presence of steroids, flavanoids, saponins, triterpenes and pregnane glycosides etc., several biological activities like antidiabetic ⁶, antihyperlipidemic ⁷ and anti ulcerogenic activities was reported. The present study is aimed to identify nutritional chemical constituents and antioxidant potential ⁸ of methanolic stem extract of *Caralluma attenuata*.

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MATERIAL AND METHODS:

Collection of plant material: The whole stem parts of *Caralluma attenuata* was collected in Ananthapuramu district, Andhra Pradesh. The plant was authenticated by Taxonomist Professor. J. Sreeramulu, Sri Krishna Devaraya University, Anantapuramu.

Preparation of extract: The dried and powdered stem part of *Caralluma attenuata* was passed through a sieve no.22 and each kilo gram was extracted successively by cold maceration with 2.5 litres of aqueous methanol (50:50). The extract was concentrated to dryness under reduced pressure using rotary vacuum evaporator.

Experimental Procedure: The extract was subjected for the nutritional constituent's investigations by chemical tests and TLC. The essential trace elements like calcium magnesium and zinc were quantified by AAS⁹. The extract was also utilised to evaluate *in vitro* antioxidant activity by Free Radical Scavenging Assay¹⁰, Reductive Ability and Nitric Oxide Method. The results obtained were tabulated.

Identification of flavanoids by using TLC: TLC technique became an important analytical tool for micro-analytical separation and determination of natural products. In the present study the methanolic stem extracts of *Caralluma attenuata* was subjected to TLC.

In this analysis pre coated aluminium plates (10 cm length) coated with silica gel were used and different solvent systems were employed depending upon the nature of the analyte. For saturation of TLC chamber a sheet of filter paper was laid so as to cover internal part of three sides

of the chamber which is soaked in the solvent system.

The chamber was left undisturbed to ensure saturation. A solution of stem extract of *Caralluma attenuata* plant was prepared in methanol. The spots of identical volume were applied 2cm away from the lower edge of plate with the help of microcapillary tube. The solvent was allowed to evaporate after each application by air drying. The spotted plate was then placed vertically in the chamber with the bottom edge immersed in developing medium. The solvent system was allowed to run approximately up to 8cm then the plates were taken out and solvent front was marked. The resolution of components of all extracts of plant of *Caralluma attenuata* was studied by locating the spots on the chromatogram. The spots were identified first by visual observation and then under UV lamp at 365 nm. Then the plates were developed in an Iodine chamber and the spots were located.

Flavanoids were detected using n-butanol: acetic acid: water (4: 1: 5 V/V) as mobile phase which has produced prominent fluorescent yellow colour spot. The TLC was shown in **Fig. 1**.



FIG. 1: TLC OF FLAVONOIDS

Identification of nutritional constituents by chemical tests: The following chemical tests were performed to identify various constituents present in plant *Caralluma attenuata*. The results were tabulated in **Table 1**.

TABLE 1: IDENTIFICATION OF NUTRITIONAL CONSTITUENTS

Tests for fibres			
S.no	Tests	Observations	Inference
1	Ignition test: (advance solely towards flame or heat in a crucible)	Rapid burning with flame. No foul odour or fumes, white ash.	Carbohydrate fibres are present
2	Boiling with aqueous picric acid solution.	No bead. Permanent yellow stain	Protein fibres are present
3	Million's test: (boil with millions reagent)	Red colour	Protein fibres are present
Test for carbohydrates:			
1	Molisch' test (general test) To 2-3 ml aqueous extract, add few drops of α -naphthol solution in alcohol shake and add concentrated H_2SO_4	Violet ring is formed at the junction of two liquids.	Carbohydrates are present.

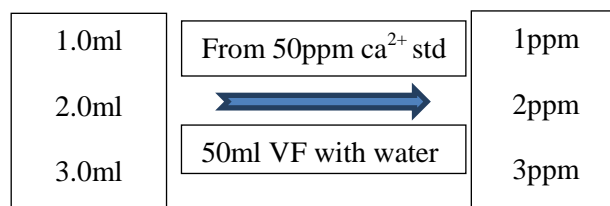
	from sides of the test tube.		
Tests for reducing sugars:			
1	Fehling's test: Mix 1ml of fehling's A and 2ml of fehling's B solutions, boil for 1 min, and add equal volume of test solution. Heat in boiling water bath for 5-10 min	First yellow, then brick red ppt is observed	Reducing sugars are present
2	Benedict's test: Mix equal volume of Benedict's reagent and test solution in test tube. Heat in boiling water bath for 5 min.	Solution appears green, yellow or red depending on amount of reducing sugar present in test solution	Reducing sugars are present
Test for hexose sugars			
1	Tollen's fluoroglucinol test for galactose: Mix 2.5 ml of conc.HCl and 4ml 0.5% fluoroglucinol. Add 1-2 ml of test solution heat.	Light green colour appears	Sugars are present
Test for amino acids:			
1	Ninhydrin test : Heat 3ml test solution and 3 drops 5% ninhydrin solution in boiling water bath for 10 min	Purple or bluish colour appears	Amino acids are presents
Tests for Vitamins			
1	Test for Vitamin A: Dissolve a quantity equivalent 10 -15 units in 1ml of chloroform and 5ml of Antimony trichloride solution.	Violet colour appeared	Vitamin A is present
2	Test for Vitamin D: Dissolve a quantity equivalent to about 1000 units of vitamin D activity in chloroform and add 10ml of antimony trichloride solution	Pinkish red colour appeared at once	Vitamin D is present
Tests for Inorganic ions:			
1	Tests for Iron: i. NH ₄ OH test: Sample solution + NH ₄ OH solution	Reddish brown colour appeared	Iron is present
	ii. Potassium ferrocyanide test: sample solution + K ₄ [Fe ²⁺ (CN) ₆] solution	Intense blue ppt formed and insoluble in dil.HCl	Iron is present
2	Tests for Phosphates: i. Ammonium molybdate test: sample solution + conc. HNO ₃ solution	Yellow ppt was formed	Phosphate is present
	ii. AgNO ₃ test: sample solution + AgNO ₃ solution	Light Yellow ppt was formed	Phosphate is present
3	Tests for Sulphates: i. BaCl ₂ test: sample solution + BaCl ₂ solution	White ppt insoluble in hot water and hot conc. HNO ₃	Sulphates present
	ii. Lead acetate test: sample solution + lead acetate solution	White ppt	Sulphates present

Estimation of Calcium, Zinc and Magnesium by AAS: Sample preparation: Weighed a 200mg of sample in to Teflon beaker then added 5ml of HNO₃ then the contents were mixed with spatula and heated the contents on hot plate at 200°C for 15min then cooled. Transferred the contents in to 50ml of volumetric flask, rinsed the beaker with few ml of water and transferred to volumetric flask and made up to the mark with water.

Sample blank: The blank is prepared in the same manner as for the sample preparation but without addition of sample.

Standard preparation for Calcium, Zinc & Magnesium: 0.5ml of 1000ppm element ¹¹ (Calcium / Zinc / Magnesium) standard solution was transferred to 10ml volumetric flask and made up to the volume with water to contain 50 ppm of calcium / Zinc / Magnesium.

Calibration standards for Calcium:



Calibration standards for Zinc:

0.2ml	From 50ppm Zn ²⁺ std	0.2ppm
0.4ml	→	0.4ppm
0.6ml	50ml VF with H ₂ O	0.6ppm

Calibration standards for Magnesium:

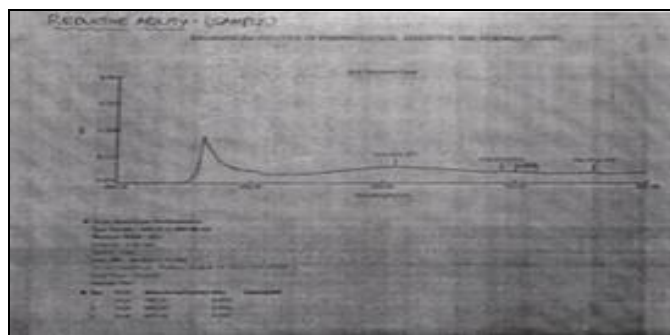
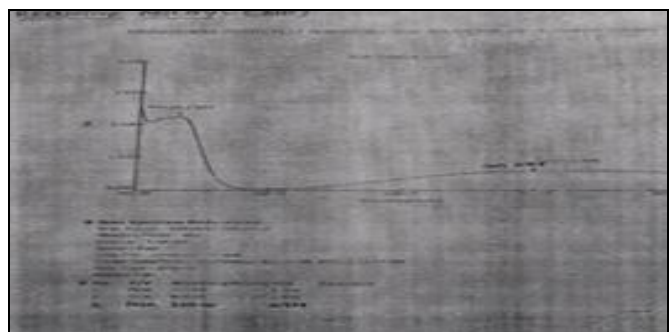
0.1 ml	From 50ppm Mg std	0.1 ppm
0.2 ml	→	0.2 ppm
0.3 ml	50ml VF with water	0.3 ppm

Standard blank: Water

AAS Procedure: Aspirated the calibration blank (standard blank), calibration standard, sample blank, (resultant blank) and sample solution. Then concentration of Ca, Zn & Mg in mg/g was calculated. The standard graphs obtained were shown in **Fig. 2, 3 and 4.**

**FIG. 2: AAS OF Ca²⁺****FIG. 3: AAS OF Zn²⁺****FIG. 4: AAS OF Mg²⁺**

Reductive Ability: The reductive ability¹³ of medicinal plants was determined according to the Oyaizu method. Methanolic stem extract (0.08-0.4 mg) of *Caralluma attenuata* was dissolved in 1ml of distilled water and then 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml of potassium ferric cyanide [K₃Fe(CN)₆] (1%) were added. The mixture was added to the mixture, which was then centrifuged at 3000rpm for 10min. 2.5ml of upper layer of the solution was mixed with 2.5ml of distilled water and 0.5ml of FeCl₃ (0.1%). Absorbance was measured at 700nm. Butylated hydroxyl toluene (BHT) was used as reference compound. All the analysis was performed in triplicate. The UV absorption spectrum was shown in **Fig. 5 and 6.**

**FIG. 5: UV SPECTRA OF SAMPLE****FIG. 6: UV SPECTRA OF CONTROL**

Free Radical Scavenging Activity (FRSA) using hydrogen peroxide: The hydrogen peroxide FRSA¹⁴ of the methanolic extracts was done as suggested by Czochra and Widwonsk. 2ml of hydrogen peroxide (43milli mole) and 1.0ml of methanolic sample [20-100 µl of methanolic extract (4mg/ml) in methanol] followed by 2.4ml of 0.1 M phosphate buffer (pH 7.4) were added. The resulting solution was kept for 10min and the absorbance was recorded at 230nm. All readings were repeated three times. Blank was prepared without adding hydrogen peroxide and control was

prepared without sample. Ascorbic acid was used as a standard compound. Free radical scavenging activity of hydrogen peroxide percentage was calculated as FRSA (%) = $[(V_0 - V_1) / V_0] \times 100$, V_0 = absorbance of control and V_1 = absorbance of sample. The UV absorption spectrum was shown in Fig. 7 and 8.

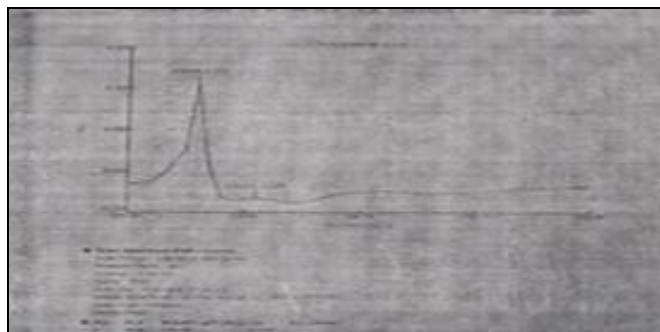


FIG. 7: UV SPECTRA OF SAMPLE

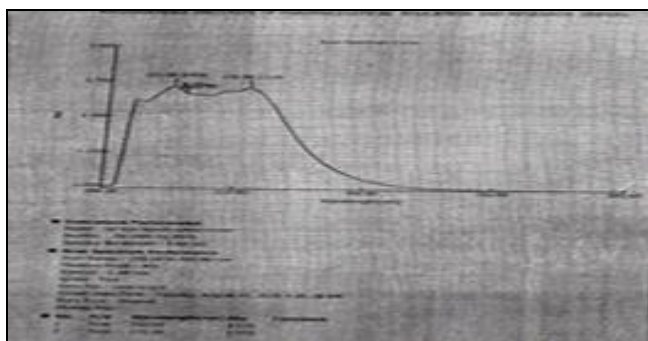


FIG. 8: UV SPECTRA OF CONTROL

Nitric oxide scavenging assay: Nitric oxide was generated from Nitroprusside and measured by the Greiss reaction sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen produce nitric ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide¹⁵ compete with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5 milli moles) in phosphate buffered saline (PBS) was mixed with 3.0ml of different concentrations of the drugs dissolved in the methanol and incubated at 25 °C for 150 min. The samples from the above were reacted with Greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm. The experiment was done in triplets.

Standard sodium nitrite (10mg/100ml) standard was processed in the same way and the inhibition percentage was calculated.

% inhibition = $(\text{absorbance of control} - \text{absorbance of sample}) \times 100 / \text{absorbance of control}$

RESULTS AND DISCUSSION: The methanolic stem extract of *Caralluma attenuata* found to posses flavanoids its R_f value is 0.73 by using specific mobile phase which is nearer to the standard 0.72. Various nutrients like carbohydrates, proteins, Vitamins were identified by qualitative tests. Inorganic essential trace elements^{16, 17} were notified by AAS and the percentage mg/g was identified as 0.324, 0.009, and 0.56 for calcium, zinc and magnesium respectively. The antioxidant^{18, 19} capacity of the extract was performed by Reductive ability, FRSA and Nitric oxide scavenging assay methods and the results observed as 94.18%, 89.6%, and 92.4% respectively. From the results obtained indicates that the methanolic stem extract of *Caralluma attenuata* contains considerable amount of nutrients and essential trace elements. The methods which were used for evaluation of anti-oxidant activity had shown appreciable percentage of activity.

CONCLUSION: The methanolic stem extract of *Caralluma attenuata* as it contains essential nutrients, flavanoids and anti-oxidants; it is useful to reduce malnutrition, anaemia, calcium deficiency and to enhance immunity. It also prevents the process of biological oxidation, it is the unchecked problem associated with free radical formation - oxidative stress. Severe oxidative stress can cause cell death and moderate oxidation triggers apoptosis & necrosis and cardiovascular diseases. Several medicinal herbs have been known as good nutritive and remedy for prevention of disorders and ailments. The present study reveals that methanolic stem extract of *Caralluma attenuata* enhances endurance, enzyme production and hepatoprotection. So the present research can be extended further to develop a new herbal nutrient for prevention of various diseases by proceeding *in-vivo* tests.

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