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Research Article

PHYSICO CHEMICAL CHARACTERIZATION AND ANTI-OXIDANT ACTIVITY OF *MOMORDICA CYMBALARIA* TUBERS

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ABSTRACT

A study was conducted to evaluate the Physico chemical Characterization and anti oxidant activity of Momoradica cymbalaria tubers. The tubers were subjected to soxhalation using alcohol, water, and the extracts thus obtained were studied for Physicochemical Characterization screening various total ash value was found to be 14.26%w/w the acid insoluble value and sculpted ash values were found to be 1.45% w/w and 1.75 %w/w and The water soluble extractive value was found to be 22.12% w/w respectively, fluorescence analysis of different chemicals. The antioxidant activity of ethanol and analyzed for their free radical scavenging activity in different e.g. DPPH radical scavenging and Nitric oxide radical scavenging activity, Superoxide radical scavenging activity, ferric ion scavenging activity, were compared with standard antioxidant like ascorbic acid. All the extracts showed good antioxidant activity.

Key Words: *Momoradica Cymbalaria, Antioxidant, Tubers, Fluorescence.*

INTRODUCTION

Momordica cymbalaria fenzl belonging to the family Cucurbitaceae is a perennial herbaceous climber distributed over tropical parts of western peninsular, India and well known as athalakkai in Tamil. This plant possesses manifold folkloric claims [1]. Our research interest in this plant arose because of its myriad effects as substantiated in the legendary claims. Folkloric record reports its fruits as an antidiabetic, antihyperlipidemic and antiulcer agent [2, 3]. The root tubers are used by the natives of north interior Karnataka and Andhra Pradesh to treat gynecological ailments and to induce abortions [4]. Moreover, leaves are used to alleviate whooping cough in the legendary medicine [5]. The extracts of dried fruits of Momordica cymbalaria have been reported to possess hypoglycemic and hypolipidemic properties [6]. Ethanolic extract of root tubers are documented as anti-ovulatory, anti-implantation, abortifacient, cardioprotective and smooth muscle relaxant agents [7-9]. Thorough literature review indicated that, up till now the pharmacological property of the leaf has not yet been scientifically corroborated. Henceforth, our current study was attempted to investigate the in vitro antioxidant and antimicrobial ability of Momordica cymbalaria leaves extracts.

The plant is a perennial climber available only during the Kharif& Rabi season and is found in the south Indian states of Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, and Tamil Nadu. In Karnataka it is known by the name Karchikai, in Tamil as Athalakai, in Telugu as Kasarakai and in Marathi as Kadavanchi [10]. The crop is not cultivated by the farmer as a regular crop, even though it comes very well during Kharif and Rabi season mainly in the black soils where sorghum, Bengal gram and onion can be cultivated. Initially it was considered as a weed, but the tubers were used for medicinal purpose, from ancient times. Recently because of the nutritional value of the fruits, it is used as vegetable. It is reported as medicinal plant in India and various parts of plant are useful for treating the common ailments. Not only can the fruits even leaves also be used as a leafy vegetable [11]. Because of lack of awareness about the nutritional aspects, it is not commercially cultivated. Hence it is considered as an underutilized vegetable crop.

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MATERIAL AND METHODS

Collection of plant material:

The fresh Plant of *Momordica Cymbalaria* was Collected Chittoor (district) during the month of April 2016. The plant material was identified and authenticated at, Sri Krishna devaraya University, Botany Department by Dr. S.Thimma Naik. The fresh plant material was dried under shade. Dried plant material was powdered using mechanical grinder and passed through sieve no.60 to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Preparation of extracts:

A coarse powder of shade-dried tubers of M. tubers was extracted successively with petroleum ether (60-80), chloroform, ethanol and water [12]. Similarly, an ethanol (70%) extract of the tubers of M. tuber (TMT) was prepared after defatting the drug with petroleum ether. Ethanol (70%) is a well-documented solvent for the majority of polar constituents of a plant. The extracts were dried under reduced pressure using a Rota-flash evaporator and heating. All extracts were screened for phytoconstituents using simple chemical tests [12, 13].

RESULTS AND DISCUSSION

Determination of Physicochemical Parameters: Various physicochemical constants like Total ash value, water and acid, soluble and insoluble ash value, and moisture content were determined as per Indian pharmacopoeia [14, 15].

Table No. 1: Physico chemical Parameters of *Momordica Cymbalaria*

S.No	Parameter	Ash value (%w/w)
1	Total ash value	14.26
2	Acid insoluble ash value	1.45
3	Water insoluble ash value	6.24
4	Alcohol soluble extractive	9.23
5	Water soluble extractive	22.12
6	Sulphated ash	1.75

Fluorescence Analysis:

The fluorescence analysis of drug of powder showed reddish brown color under white light and greenish brown color under UV light. The fluorescence analysis results of the extracts were presented in the **Table 2.**

As there is no standardization work on record of this much valued traditional drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. In other words, the pharmacognostic features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations.

Fig. 1: Fluorescence analysis of different chemicals

Table No. 2: Behavior of powdered *Momardica cymbalaria* tubers on treatment with different chemical reagents.

S.No.	Reagent	Day light	UV light (366 nm)
1	Drug Powder	Green	Green
2	Powder + water	Light brown	Cream
3	Powder + 50%HNO_3	Yellow	Brown
4	Powder + 5%Hcl	Light yellow	Dark Black
5	Powder + Con H_2So_4	Light black	Greenish blue
6	Powder + NaoH	Dark Black	Black
7	Powder +$Fecl_3$	Brown	Yellow

Anti oxidant activity:

DPPH radical scavenging activity:

DPPH scavenging activity was measured by the Spectrophotometric method [16, 17]. To an ethanolic solution of DPPH (1mM), equal volume **o**f test compounds dissolved in ethanol was added at different concentrations (2-1000μg/mL). An equal amount of ethanol was added to the control. After 20 min the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated.

$$\% \text{ scavenging activity} = \frac{\text{A Control} - \text{test or Std}}{\text{A control}} \times 100 \text{ Control}$$

Where,
A control= absorbance of control
A test or A Std= absorbance of test or std

Scavenging of nitric oxide radical:

Nitric oxide was generated from sodium nitroprusside and measured by griess reaction [18]. Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration (2-1000μg/mL) of the ethanol extract dissolved in phosphate buffer (0.025M, pH: 7.4) and the tubes were incubated at 25^0C for 5 h. Control experiments without the test compound but with equivalent amount of buffer were conducted in an identical manner. After 5 h, 0.5mL of incubation solution was removed and diluted with 0.5mL of Griess reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% napthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotion of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylene diamine was read at 546nm. The experiment was repeated in triplicate.
The percentage inhibition was calculated using the formula:

$$\% \text{ scavenging activity} = \frac{\text{A Control} - \text{test or Std}}{\text{A control}} \times 100 \text{ Control}$$

Where,
A control=absorbance of control; A test A Std=absorbance of test or std.

Scavenging of superoxide radical:

The scavenging activity towards the superoxide radical (O_2) was measured in terms of inhibition of generation of O_2 [19, 20]. The method was performed by using alkaline DMSO method. Potassium superoxide and dry DMSO were allowed to stand in contact for 24 h and the solution was filtered immediately before use. 200 μL of the filter was added to 2,8mL of an aqueous solution containing NBT (56μ M), EDTA (10μM) and potassium phosphate buffer (10mM). Test compounds (1mL) at various concentrations (2-1000μg/mL) were added and the absorbance was recorded at 560nm against a control in which pure DMSO was added instead of alkaline DMSO.
The percentage inhibition was calculated using the formula:

$$\% \text{ scavenging activity} = \frac{\text{A Control} - \text{test or Std}}{\text{A control}} \times 100 \text{ Control}$$

Where,
A control= absorbance of control: A test or A Std = absorbance of test or std.

Scavenging of ferric ions:

Ortho substituted Phenolic compounds were found to be more active than un substituted phenol. Hence these compounds may exert pro-oxidant effect by interacting with iron similar to ascorbate and gallic acid. It is concluded that antioxidant property of the compound is due to Phenolic group, which can react with a free radical to from the phenoxyl radical. The reaction mixture containing *O*-phenanthroline, ferric chloride, and drug at different concentration ranging from 2g to 1000g/mL in a final volume of 5mL was incubated for 10 min at ambient temperature. The absorbance at 510nm was recorded. Blank was carried out without drug [21]. Experiment was performed in triplicate.

The percentage inhibition was calculated using the formula:

$$\% \text{ scavenging activity} = \frac{\text{A Control} - \text{test or Std}}{\text{A control}} \times 100 \text{ Control}$$

Where,
A control=absorbance of control; A test or A Std=absorbance of test or std

RESULTS AND DISCUSSION

DPPH model (colorimetric method):

The **Figure-1** shows concentration dependent DPPH scavenging of Ethanol fraction of ***Momaradica cymbalaria*** with maximum scavenging activity (102.12 %) at 200µg/mL extract, and the scavenging activity water extract the maximum scavenging activity (83.23%) was observed at 200µg/mL and it where as the standard reference substance ascorbic acid showed maximum scavenging activity of (115.24 %) at 200 µg/mL **(Table 1)**

Scavenging of nitric oxide radical:

Figure -2 shows concentration dependent scavenging of nitric oxide radical by ethanol fraction of ***Momaradica cymbalaria*** with maximum scavenging activity (68.24%) at 200µg/mL extract, and the scavenging activity water extract the maximum scavenging activity (58.25%) was observed at 200µg/mL and it where as the standard reference substance ascorbic acid showed maximum scavenging activity of (82.32%) at 200 µg/mL **(Table 2).**

Scavenging of superoxide radical:

Figure-3 shows concentration dependent scavenging of super oxide radical by the ethanol fraction of ***Momaradica cymbalaria.*** The maximum scavenging activity (55.45%) was observed at 200µg/mL and the scavenging activity water extract the maximum scavenging activity (45.18%) was observed at 200µg/mL and it and it was comparable to that of the standard reference substance ascorbic acid which was showed (85.42 %) of scavenging activity at 200µg/mL **(Table3).**

Scavenging of ferric ions:

Figure-4 shows concentration dependent ferric ion scavenging activity of ethanol fraction of ***Momaradica cymbalaria***. The maximum scavenging activity (48.25%) was observed at 200µg/mL and the scavenging activity water extract the maximum scavenging activity (38.16%) was observed at 200µg/mL and it was comparable to that of the standard reference substance ascorbic acid which was showed scavenging activity of (52.42%) at 200µg/mL **(Table-4).**

Table No. 3: Antioxidant activity of Alcohol and aqueous extract of *Momaradica cymbalaria* and Ascorbic acid (DPPH)

Conc. of extract (µg/ml)	% Inhibition *		
	Alcohol Extract	Water Extract	Ascorbic acid
20	38.26 ±1.4	26.12 ±0.5	46.24 ± 0.2
40	45.12 ±1.6	29.25 ±0.2	53.12 ±1.6
60	51.23 ±2.4	34.15 ±1.3	67.14 ±1.3
80	58.24 ±0.8	42.14 ±2.5	78.05 ±1.5
100	70.26 ±1.2	51.34 ±2.4	82.25 ±1.4
120	78.34 ±1.8	58.45 ±1.6	91.13 ±1.5
140	87.14 ±0.8	62.14 ±0.5	98.43 ±1.2
160	90.23±1.2	70.42±1.2	102.45±2.4
180	98.25 ±0.2	77.13 ±1.4	110.14 ±2.5
200	102.12 ±0.4	83.23 ±2.3	115.24 ±0.6

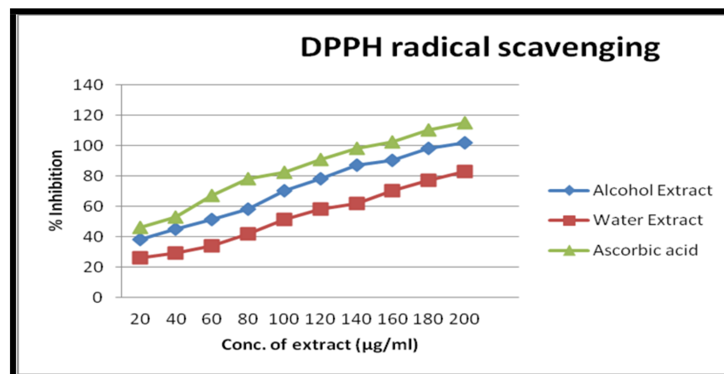

Fig. 1: DPPH radical scavenging activity of Standard aerobic acid alcohol extract and water extract of *Momaradica cymbalaria* Tubers.

Table 2-Antioxidant activity of Alcohol and aqueous extract of *Momaradica cymbalaria* and Ascorbic acid (Nitric acid)

Conc. of extract (µg/ml)	% Inhibition *		
	Alcohol Extract	Water Extract	Ascorbic acid
20	22.14 ± 0.2	18.18 ±0.5	38.86 ±1.2
40	28.16 ± 1.5	26.12 ±0.4	42.82 ±1.4
60	34.12 ±1.4	30.25 ±1.2	48.12 ±1.5
80	40.24 ±1.2	36.12 ±2.5	52.25 ±2.4
100	47.22 ±1.5	40.15 ±0.5	56.32 ±1.8
120	50.15 ±1.2	44.24 ±1.4	60.26 ±1.5
140	58.13 ±1.5	48.45 ±1.2	66.14 ±1.8
160	62.16 ±4.5	52.16 ±2.4	70.32 ±0.6
180	65.12 ±2.3	55.23 ±1.2	75.25 ±1.4
200	68.24 ±1.7	58.25 ±2.5	82.32 ±1.8

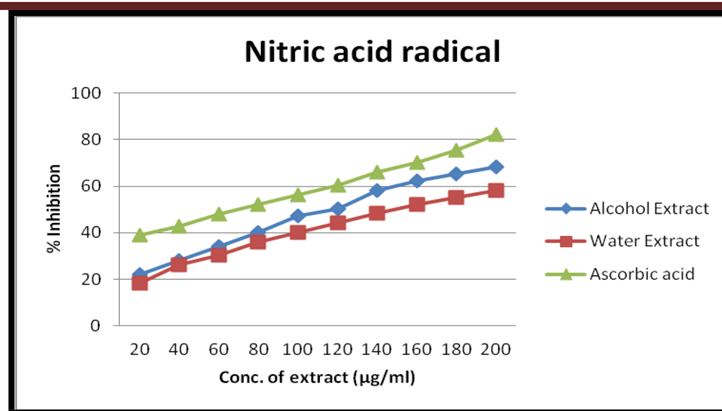

Fig. 2: Nitric acid radical scavenging model activity of Standard aerobic acid alcohol extract and water extract of *Momaradica cymbalaria* Tubers.

Table 3: Antioxidant activity of Alcohol and aqueous extract of *Momaradica cymbalaria* and Ascorbic acid (Superoxide radical)

Conc. of extract (µg/ml)	% Inhibition *		
	Alcohol Extract	Water Extract	Ascorbic acid
20	20.24± 2.3	13.28 ±1.5	30.24 ±0.5
40	24.32± 1.5	15.23 ±2.6	35.16 ±2.5
60	28.45±2.2	17.35 ±1.8	40.23 ±1.6
80	32.25±1.4	20.32 ±2.4	45.14 ±2.3
100	36.15±2.8	23.34 ±2.5	51.25 ±1.4
120	40.35±2.4	27.14 ±2.1	60.45 ±2.3
140	43.26±1.2	32.18 ±3.2	65.14 ±2.5
160	47.54±1.7	35.28 ±1.6	71.45 ±3.6
180	50.24 ±2.2	40.25 ±4.2	80.25 ±2.1
200	55.45 ±1.8	45.18 ±4.3	85.42 ±1.8

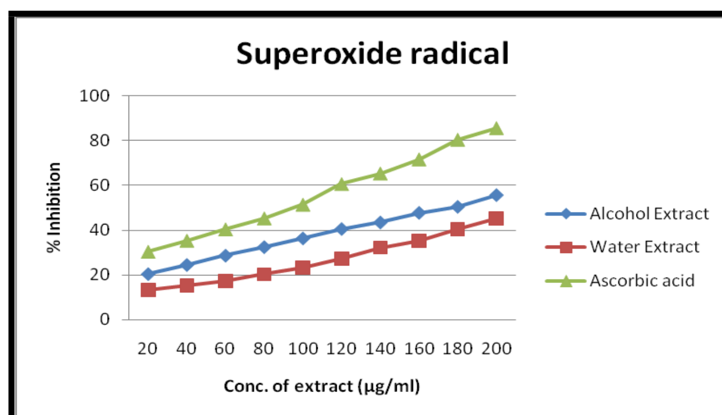

Fig. 3: Superoxide radical scavenging model activity of Standard aerobic acid alcohol extract and water extract of *Momaradica cymbalaria* Tubers

Table 4: Antioxidant activity of Alcohol and aqueous extract of *Momaradica cymbalaria* and Ascorbic acid (ferric ion scavenging)

Conc. of extract (µg/ml)	% Inhibition *		
	Alcohol Extract	Water Extract	Ascorbic acid
20	15.14± 0.6	8.28 ±0.6	18.34 ±0.5
40	18.18± 1.6	11.12 ±1.6	21.26 ±0.5
60	22.28±1.2	14.45 ±1.8	24.54 ±1.6
80	25.25±1.5	18.32 ±1.4	28.14 ±1.3
100	28.45±1.8	22.24 ±1.5	32.12 ±1.4
120	32.35±2.4	25.14 ±2.4	35.25 ±2.3
140	36.26±1.2	28.28 ±1.2	39.34 ±1.5
160	40.24±1.7	32.28 ±1.4	43.15 ±2.6
180	43.14 ±2.2	35.15 ±1.2	48.25 ±2.6
200	48.25 ±0.8	38.16 ±2.3	52.42 ±1.8

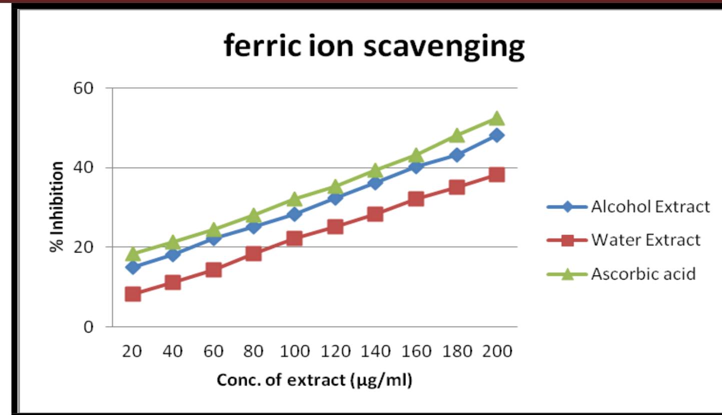

Fig. 4: ferric ion scavenging model activity of Standard aerobic acid alcohol extract and water extract of *Momaradica cymbalaria* Tubers

CONCLUSION

The present study demonstrated that ethanol and aqueous extract tubers of ***Momaradica cymbalaria*** Showed promising antioxidant and radical scavenging activity, from the observation it can concluded that the tubers of ***Momaradica cymbalaria*** are the good sources of natural antioxidants and might be useful in treating the diseases associated with oxidative stress.

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