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Research Article

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Assessment of Antioxidant Activity of Methanolic Extract of *Momordica Charantia* L. Seeds

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ABSTRACT

Aim: The present investigation was aimed to evaluate the antioxidant activity of methanolic extract of *Momordica charantia* using albino rats.

Material and Methods: Anti-oxidant activity of *M. charantia* evaluated by using superoxide anion scavenging activity, reducing power, flavonoids content, hydrogen peroxide radical scavenging assay method were adopted for screening the antioxidant activity. Ranitidine was used as standard then comparison between standard and test was carried out.

Results: Due to the presence of saponins and flavonoids in *M. charantia* increases the antioxidant activity. The methanolic extract of *M. charantia* showed significant activity ($p < 0.05$).

Conclusion: The result indicates that the action of methanolic extract of *M. charantia* shows anti- oxidant activity.

Keywords: Anti-oxidant, *Momordica charantia*, Methanolic, Extract, Albino rats, Activity.

INTRODUCTION

Large number of herbal plants have been investigated for their antioxidant properties. Antioxidants in the form of natural extract of their chemical constituents are very effective to prevent destructive processes caused by oxidative stress.[1] Antioxidant in the food plays an important role. Primarily source of naturally occurring antioxidants are fruits, whole grains and vegetables. The main function of an antioxidant is its ability to reduce free radicals.[2] Antioxidants are substances in our foods which can prevent or slow the oxidative damage to our body. An oxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turns these radicals can start chain reaction that damage cells. Antioxidants terminate these chain reactions by free radical intermediates, and inhibit other oxidation reactions.[3,4]

The present study herbal drug was aimed to investigate the antioxidant effect of methanolic extract of *M. charantia* L. seeds. *M. charantia* Linn. belonging to family Cucurbitaceae is known as Bitter melon or Bitter gourd in English, Karela in Hindi. The plant is widely distributed throughout the India. The dried seeds of this plant are extensively available in market of India. The fruits and dried seeds of this plant reported to be used traditionally in various ailments. Phytochemicals like alkaloids, tannins, flavonoids, saponins and glycosides were also present.[5] Major active constituents responsible for antidiabetic,[6] antibiotic,[7] *in vivo* and *in vitro* studies to identify hypoglycemic activity.[8,9] It is observed for its flavonoids content, reducing power, superoxide anion scavenging activity, and hydroxyl radical scavenging activity by using suitable standard.[10,11]

MATERIALS AND METHODS

Plant Material and Preparation of Methanolic Extract

The plant material seeds of *M. charantia* were collected from the local market of Malegaon (BK), Baramati. The seeds were authenticated by Prof. Deshmukh, Head and Research Officer (Botanist), Department of Botany, SPMV, Sharada Nagar, Baramati. Seeds were washed properly then air dried and finally dried in the oven at 40-45°C. After ensuring the seeds were completely dried, they were powdered to a

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fine size. The fine powder was subjected to de-fated with petroleum ether and then extracted with hot methanol (60-65°C) by using soxhlet extractor, the methanolic extract of *Momordica charantia* (MEMC) obtained. The extract was concentrated by simple evaporation method. The semisolid extract was stored in airtight container in refrigerator below 10°C. The percentage yield of extract was 8%.

Chemicals

MEMC at dose level of 250 mg/kg and 500 mg/kg were given orally to animals for the study. Ascorbic acid was obtained from and used as standard anti ulcer drug at 50 mg/kg dose for the present study.

Animals

The Wistar albino rats of 150-200 g and swiss mice 20-30 g was used throughout the experimentation. The animals were procured from S.V.P.M. College of Pharmacy, Malegaon (BK) Baramati, Maharashtra, India. The animals were housed in propylene cages and were maintained at room temperature: 27±3°C, relative humidity: 65±10%, and 12: 12 hrs light: dark cycle. All animals were acclimatized to experimental room environment before one week and provided fed with rodent pellet diet (Gold Mohr, Lipton India Ltd) and water was allowed *ad-libitum* under strict hygienic condition. Ethical clearance for performing experiments on animals was obtained from Institutional Animal Ethical Committee (IAEC). They are divided into four groups of five each; Group-1: Control; Group-2: Standard; Group-3: MEMCS (250 mg/kg body weight); and Group 4: MEMCS (500 mg/kg body weight).

Preliminary Phytochemical Screening:

Preliminary phytochemical screening was carried out on MEMCS for detection of phytoconstituents present in it.[12]

Antioxidant activity

Reducing Power

Different doses of methanolic extract of *M. charantia* were mixed in 1 mL of distilled water so as to get 5 µg, 10 µg, 25 µg, 50 µg, and 100 µg concentration. This was mixed with phosphate buffer (2.5 mL, 0.2 mL, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 mL) of trichoroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power.[13]

Superoxide Anion Scavenging Activity

1 mL of nitroblue tetrazolium (NBT) solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4), 1 mL NADH solution (468 µM phosphate buffer, pH 7.4) and 0.1 mL of sample solution of aqueous and 70% methanolic extract of *M. charantia* and standard in water was mixed. The reaction was started by adding 100 µL of Phenazine Methosulphate (PMS) solution (60 µM PMS in 100 µM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 minutes and the absorbance at 560 nm was measured against blank. Decreased absorbance of reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition by extracts were evaluated comparing the absorbance values of control and experimental.[14]

Scavenging effect (%) = (1 – absorbance of sample / absorbance of control) × 100

Hydroxyl Radical Scavenging Activity

Hydroxyl radical generation by phenyl hydrazine has been measured by the 2-deoxyribose degradation in 50 mM phosphate buffer (pH 7.4), 1 mM deoxyribose, and 0.2 mM phenyl hydrazine were prepared. 0.6 mL of 1 mM deoxyribose and 0.4 mL of aqueous and methanolic extracts of *M. charantia* seeds and standard were taken 0.2 ml of phosphate buffer was added to makes reaction solution 1.6 mL after 10 minutes incubation 0.4 mL of 0.2 mM phenyl hydrazine was added. Incubation was terminated after 1 hr and 4 hrs and 1 mL each of 2.8% TCA and 1% (w/v) thiobarbituric acid were added to the reaction mixture and heated for 10 minutes in a boiling water bath. The tubes were cooled and absorbance was taken at 532 nm. Decrease in absorbance is indicating the increase in the hydroxyl free radical scavenging activity.[15,16]

Scavenging activity (%) = (1 – absorbance of sample / absorbance of control) × 100

Total Flavonoids Content

The total flavonoids content was determined with the aluminum chloride ($AlCl_3$) method using Quercetine as a standard. The plant extract (0.25 mL) was added to 1.25 mL distilled water followed by 75 µL, 5% $NaNO_2$. After 5 min at room temperature, $AlCl_3$ (0.15 mL, 10%) was added and further incubation at room temperature for 6 minutes. The reaction mixture was treated with 0.5 mL of 1 mM NaOH finally; the reaction mixture was diluted with 275 µL of distilled water. Further incubation for 20 minute at room temp was performed and the absorbance was measured at 510 nm. All tests were performed in triplets. The flavonoids content was calculated from Quercetine as a standard value.[17]

Statistical Analysis

Data were expressed as Mean±SEM. Statistical analysis of data was done by One-way ANOVA, followed by test for

comparison between control and test groups by using Graph Pad Prism v.6 software. The p-value of < 0.05 was considered to be statistically significant.[18]

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Result of the preliminary phytochemical investigation on methanolic extract of *M. charantia* seeds are shown in **Table 1**.

Table 1: Preliminary phytochemical investigation of MEMC.

Phytochemical Constituents	Inference
Flavonoids	++
Saponin glycosides	+
Tannins and Phenolic compounds	++
Steroides	+++

+ = indicates presence; ++ = More clarity; +++ = Better response

In-vitro antioxidant activity

Reducing power of MEMCS

It shows the dose for the reducing power of MEMCS (5-100 µg/mL). It was found that the reducing power increased with concentration of each sample.

Table 2: Reducing Power of MEMCS.

Treatment/ Group	Absorbance (nm)	% Increase in absorbance
Control	1.487	-
Control + MEMCS (5 µg/mL)	1.600	41.11%
Control+ MEMCS (10 µg/mL)	1.800	27.65%
Control+ MEMCS (25 µg/mL)	1.650	37.73%
Control+ MEMCS (50 µg/mL)	1.744	31.42%
Control+ MEMCS (100 µg/mL)	1.400	54.55 %

Superoxide anion scavenging activity

Superoxide radical is considered a major biological source of reactive oxygen species. Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen both of which contribute to oxidative stress. Superoxide radical scavenging effect of different doses was compared with the same doses of standard and MEMCS ranging from (25-500 µg/mL). When compared to standard with the MEMCS extract of Superoxide radical scavenging activity was found to below ($P < 0.05$).

Table 3: Superoxide Anion Scavenging Activity of MEMCS.

Treatment/ Group	Absorbance (nm)	% Decrease in absorbance
Control	0.940	-
Control + Standard (25 µg/mL)	0.210	77.65 %
Control+ Standard (50 µg/mL)	0.196	79.14 %
Control+ Standard (100 µg/mL)	0.150	84.04 %
Control+ MEMCS (100 µg/mL)	1.259	39.95 %
Control+ MEMCS (200 µg/mL)	1.096	22.60 %
Control+ MEMCS (300 µg/mL)	1.055	18.24 %
Control+ MEMCS (400 µg/mL)	1.263	40.36 %
Control+ MEMCS (500 µg/mL)	1.187	32.28 %

Hydroxyl radical scavenging activity

All doses showed antioxidant activity in dose dependant manner at concentration 25- 500 µg/mL. Hydroxyl radical scavenging activity was measuring the inhibition of the degradation of 2- deoxyribose by the free radical generated.

Table 4: Hydroxyl Radical Scavenging Activity of MEMCS.

Treatment/ Group	Absorbance (nm)	% Decrease in absorbance
Control	0.085	-
Control + Standard (25 µg/mL)	0.431	80.27 %
Control + Standard (50 µg/mL)	0.092	7.60 %
Control+ Standard (100 µg/mL)	0.057	32.94 %
Control+ MEMCS (100 µg/mL)	0.671	78.10 %
Control+ MEMCS (200 µg/mL)	0.445	51.51 %
Control+ MEMCS (300 µg/mL)	0.234	26.68 %

Total flavonoids content

The content of flavonoids expressed as mg equivalents/g extract. The MEMCS showed the highest amount of flavonoids content. The present study evaluates the *in vitro* antioxidant activity of methanolic extract of *M. charantia* Linn seeds. In recent time there is an increasing interest in the role of free radical-mediated damage in the etiology of human diseases. In the status of normal metabolism, the levels of oxidant and antioxidants in humans are maintained in balance, which is important for sustaining optimal physiological conditions. Overproduction of oxidants in certain conditions can cause an imbalance, leading to

oxidative damage to large bimolecules such as lipid, DNA, and proteins. Oxidative damage to body cells and molecules has been widely postulated to be involved in the causation and progression of a range of chronic disease, such as cardiovascular disease, neuronal disease. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test. Result of this study suggests that the plant extract contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage.

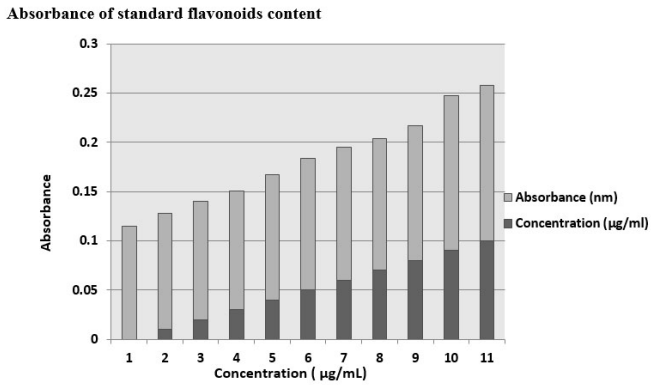

Figure 1: Standard flavonoids curve shows absorbances of MEMCS.

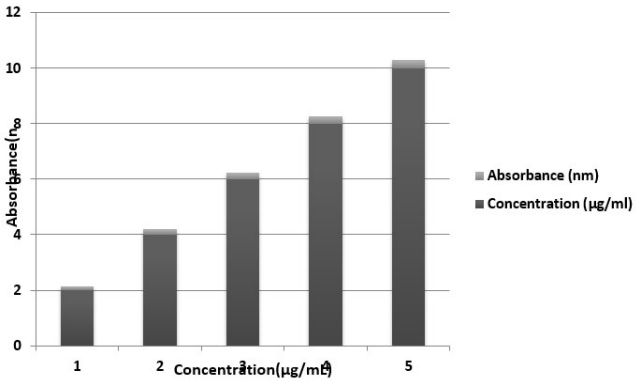

Figure 2: Test flavonoids curve shows absorbances of MEMCS.

CONCLUSION

The present study comprehensively evaluates the antioxidant potential of the methanolic extract of *M. charantia* L. seeds through various in vitro assays. The findings indicate that the extract exhibits significant free radical scavenging activity, demonstrating its potential as a natural antioxidant. The presence of bioactive phytoconstituents, such as flavonoids, phenolics, and alkaloids, may contribute to the observed antioxidant effects. The assays collectively confirm the efficacy of the extract in neutralizing oxidative stress, which is a crucial factor in the prevention of numerous chronic diseases, including cardiovascular disorders, neurodegenerative conditions, and cancer. Furthermore, the results suggest that *M. charantia* seed extract could be explored as a natural alternative to synthetic antioxidants in pharmaceutical and nutraceutical formulations. However, further investigations, including *in vivo* studies, mechanistic evaluations, and clinical trials, are essential to establish its therapeutic relevance, bioavailability, and safety profile. Additionally, isolating and characterizing the specific bioactive compounds responsible for the antioxidant activity would provide deeper insights into their pharmacological potential. Overall, this study highlights the significant antioxidant properties of *M. charantia* seed extract, reinforcing its potential application in health-promoting formulations and functional foods. Future research should focus on exploring its synergistic effects with other natural antioxidants and assessing its efficacy in disease models to validate its pharmaceutical and therapeutic benefits.

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AUTHORS' CONTRIBUTION

Sonali Deokate: Concept, Writing manuscript, Formatting,

Vinod Pawar: Literature Review, Grammar correction, Editing, Checking

Madhuri Hole: Analysis, Plagiarism correction, Initial revisions

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