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Research Article

# *In Vitro* ANTIOXIDANT ACTIVITY OF ETHANOLIC SEED EXTRACTS OF *MACROTYLOMA UNIFLORUM* AND *CUCUMIS MELO* FOR THERAPEUTIC POTENTIAL

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**ABSTRACT**

The present study was an endeavor to evaluate antioxidant activity of ethanolic seed extracts of *Macrotyloma uniflorum* and *Cucumis melo* for their therapeutic potential. In-vitro study of antioxidant activity was carried out by Nitric-Oxide radical Scavenging Assay, Hydroxyl radical Method and Phosphomolybdenum Reduction Assay with Ascorbic acid as the standard in all the three methods .The Ethanolic seed extract of *Macrotyloma uniflorum* was found to show significant scavenging activity of 64.01%±1.78at 500µg/ml in Nitric Oxide radical Scavenging Assay, 74.42%±2.37at 1000 µg/ml in Hydroxyl radical Method and 92.59%±2.05at 250 µg/ml in Phosphomolybdate method as compared to that of standard Ascorbic acid 69.42%±1.65at 500µg/ml in Nitric Oxide radical Scavenging Assay, 92.91%±1.24 at 1000 µg/ml in Hydroxyl radical Method, 99.38%±1.69at 250 µg/ml in Phosphomolybdate method. However, the ethanolic seed extract of *Cucumis melo* also showed significant scavenging activity of 60.65%±1.53at 500µg/ml in Nitric Oxide radical Scavenging Assay, 80.62%±1.46at 1000 µg/ml in Hydroxyl radical Method and 96.07%±2.48 at 250 µg/ml in Phosphomolybdenum Reduction Assay as compared to that of standard. The presence of phytochemicals like alkaloids, tannins, flavonoids, glycosides in both the extracts might contribute to the observed antioxidant activity.

**Keywords:** *Macrotyloma uniflorum*, *Cucumis melo*, Free radicals, Ascorbic acid, Flavonoids.

## 1. INTRODUCTION

Free radicals are produced by the body to aid in the metabolic processes, such as digestion and the conversion of food into energy. They are actually quite helpful in many of the body's natural functions. Excessive free radicals produced in our cells can attack the cell membranes (the outer coat of the cell) and cause cell and tissue damage.[1] Radicals can also break strands of DNA (the genetic material in the cell). This oxidative damage caused by the free radicals is considered to play a causative role in ageing and several stress related diseases including cataracts, cognitive dysfunction, cancer, myocardial infarction, diabetes and several heart disease.[2] Radical Oxygen Species and Radical Nitrogen Species are both playing a dual role as deleterious and beneficial species, since they can be either harmful or beneficial to living systems.[3] In low/moderate concentrations free radicals are involved in normal physiological functions but excess production of free radicals or decrease in antioxidant level leads to oxidative stress.[4] Our bodies try to protect us from free radical damage by producing enzymes that neutralize them. However, they are not capable of handling this function without antioxidants provided by our diets. Antioxidants are protective molecules also referred to as free radical scavengers and hence prevent and repair damage done by these free radicals.[5] Fruits and vegetables are the main source of antioxidants in the diet, are associated with

lower risk of degenerative disease.[6] Health problems such as heart disease, macular degeneration, diabetes, cancer are all contributed by oxidative damage. Antioxidants may also enhance immune defense and therefore lower the risk of cancer and infection. Many plant-derived substances, collectively termed "phytonutrients," or phytochemicals," are becoming increasingly known for their antioxidant activity. In plants, flavonoids serve as protectors against a wide variety of environmental stresses.[7]. Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and the future [8]. Demand for medicinal plants is increasing in both developing and developed countries due to growing recognition of natural products being non-narcotic, having no side-effects, easily available at affordable prices and sometime the only source of health care available to the poor.

*Macrotyloma uniflorum*, commonly known as horse gram (Fabaceae) is a herbaceous plant with annual branches, sub erect or twining, leaflets 2.5-5 cm and widely distributed throughout Asia, Africa and Australia. It is famous for its medicinal uses because different parts of the plants are used for the treatment of heart conditions, asthma, bronchitis, leucoderma, urinary discharges and for treatment of kidney stones. Literature survey showed that Dolichin A and B, pyroglutaminylglutamine along with some flavonoids were isolated from this plant.[9]*Cucumis melo (*Cucurbitaceae) is commonly known as wild melon, cantaloupe, small gourd; wild musk melon is an annual. The fruits can be used as a cooling light cleanser or moisturizer for the skin and has stomachic properties. They are also used as a first aid treatment for burns and abrasions. Seeds are antitussive, digestive, febrifuge and vermifuge. The extract of seed oil was reported for Antifungal activity.[10] further, the phytochemical studies of *Cucumis melo* seeds revealed the presence of flavonoids[11]. So our present study was carried out to evaluate the antioxidant activity of *Cucumis melo* seeds for their therapeutic potential.

## 2. MATERIALS AND METHODS

### 2.1. Raw Materials and Extraction

The dry seeds of horse gram were obtained from Jeevan Ayurvedic and Medicinal Stores, Main road, Kakinada, A.P. The seeds were washed off from dust before extraction. The seeds of musk melon were collected from the fruit and dried under shade. The dried seeds of both the plants were individually macerated in ethanol for a period of 7 days and later subjected to hot-percolation for 8 hours. The extracts obtained were subjected to solvent evaporation for complete drying.

### 2.2 Chemicals and Instruments

Sodium nitroprusside, Sulphanilic acid, Phosphoric acid, Glacial acetic acid, Hydrogen Peroxide, Ferrous Sulphate, Ammonium molybdate, Sodium Salicylate, Sulphuric acid, Sodium Phosphate and Ascorbic acid used were of Analytical grade. The instruments used were UV-visible double beam spectrophotometer (Elico SL 210), pH meter, Shimadzu electronic balance.

### 2.3 In-Vitro anti-Oxidant Study

### 2.3 (A): Nitric Oxide Radical Scavenging Assay

At physiological pH, nitric oxide generated from aqueous sodium nitroprusside (SNP) solution interacts with oxygen to produce nitrite ions, which may be quantified by the Griess Illosvoy reaction .The reaction mixture contained 10 mM SNP, phosphate buffered saline (pH 7.4) and various doses (100µg/ml, 250 µg/ml, 500 µg/ml) of the both extracts in a final volume of 3 ml. After incubation for 150 min at 25°C, 1 ml sulfanilamide (0.33% in 20% glacial acetic acid) was added to 0.5 ml of the incubated solution and allowed to stand for 5 min. Then 1 ml of napthylethylenediamine dihydrochloride (NED) (0.1% w/v) was added and the mixture was incubated for 30 min at 25°C. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with NED was measured spectrophotometrically at 540 nm against a blank sample. All tests were performed six times.

**Percentage inhibition = $\{(A_0-A_1)/A_0\}*100$**

Where $A_0$ is the absorbance of the blank (containing all reagents except the sample extract), and $A_1$ is the absorbance of the sample extract.

### 2.3 (B) Hydroxyl Method

The scavenging ability of the extracts on hydroxyl radicals was determined according to the method described by Smirnoff and Cumbes (1989) with some modifications. Briefly, individual sample extract (1 mL) at different concentrations (250µg/ml, 500 µg/ml, 1000 µg/ml) was added to the reagent containing 1 mL 1.5 mM FeSO4, 0.7 mL 6 mM H2O2 and 0.3 mL 20 mM sodium salicylate.

After incubation for 1 h at 37°C, absorbance of the reaction mixture was read at 562 nm. The scavenging ability on hydroxyl radicals was calculated using the following equation:

**Scavenging ability on hydroxyl radicals (%) = [(A1 - A2) / A1] x 100**

Where A1 is the absorbance of the blank (containing all reagents except the sample extract), and A2 is the absorbance of the sample extract. Ascorbic acid was used as standard.

**2.3(C): Phosphomolybdenum reduction assay**

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method .The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. 0.3 ml extracts were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank.

**Percentage increase in absorbance = {($A_1$-$A_0$)/$A_1$}*100**

Where $A_1$ is the absorbance of the blank (containing all reagents except the sample extract), and $A_0$ is the absorbance of the sample extract.

**3. STATISTICAL ANLAYSIS**

Tests were carried out in triplicate for both the extracts in all the three methods. Results were expressed graphically. Statistical analysis was performed through One-way analysis of variance (ANOVA) ($p < 0.01$).

**4. RESULTS**

The antioxidant activity of Horse gram and Cantaloupe seed extracts was calculated according to the percentage inhibition in Nitric Oxide Assay and Hydroxyl Method at 545nm and 562nm respectively and percentage increase in absorbance in Phosphomolybdate Assay at 695nm. The results are tabulated as below:

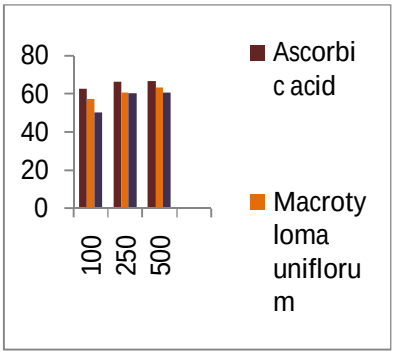
**Table 1: effect of extracts on free radicals with respect to standard**

| | Hydroxyl radicals Scavenging % Inh at conc. | | | Nitric oxide radicals Scavenging % Inh at conc. | | | Phosphomolybdate assay % increase in absorbance at conc. | | |
|---|---|---|---|---|---|---|---|---|---|
| | 250 µg/ml | 500 µg/ml | 1000 µg/ml | 100 µg/ml | 250 µg/ml | 500 µg/ml | 50 µg/ml | 100 µg/ml | 250 µg/ml |
| ***Macrotyloma uniflorum*** | 62.47 % ±2.31 | 66.03 % ±1.36 | 74.42% ±2.37 | 57.50% ±1.89 | 60.64% ±2.05 | 64.01% ±1.78 | 75 % ±1.98 | 88.23% ±2.98 | 92.59% ±2.05 |
| ***Cucumis melo*** | 62.11 % ±2.78 | 68.66 % ±1.98 | 80.62% ±1.46 | 50.33% ±1.78 | 60.61% ±1.69 | 60.65% ±1.53 | 80 % ±1.24 | 84.61% ±2.43 | 96.07% ±2.48 |
| **Ascorbic acid** | 68.11 % ±1.34 | 77.64% ±1.38 | 92.91% ±1.24 | 62.95 % ±1.65 | 66.64% ±2.81 | 69.42% ±1.65 | 94.35% ±2.08 | 98.23% ±1.92 | 99.38% ±1.69 |

All the results are expressed as Mean ± SD

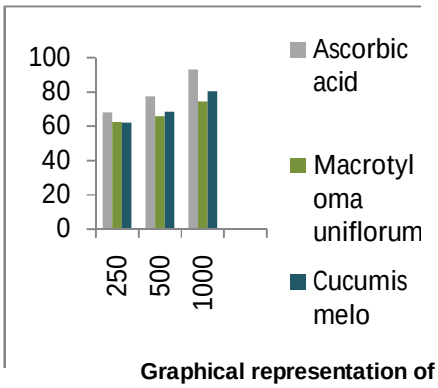
## 4.1. REPRESENTATION OF RESULTS

**(A): Nitric Oxide Radical Scavenging**



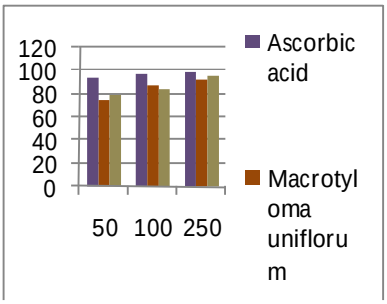

**Graphical representation of concentration (µg/ml) on x-axis and percentage inhibition on y-axis**

**(B) Hydroxyl method**




**Graphical representation of concentration (µg/ml) on x-axis and percentage inhibition on y-axis**

**(C)Phosphomolybdenum reduction**




**Graphical representation of concentration (µg/ml) on x-axis and percentage increase in absorbance on y-axis**

Free radicals contribute to more than one hundred disorders in human. Due to negative effects of synthetic antioxidants nowadays, much attention has been placed on phytoconstituents. Many of the phytoconstituents are beneficial and many of them are acting as natural antioxidants.[12]The results of the present investigation were suggestive of the potential of solvent extracts in scavenging free radical. According to our study, the presence of phytoconstituents such as flavonoids and phenolic compounds, triterpenoids, carbohydrates, proteins and glycosides [9,11] in the ethanolic extract of *Macrotyloma uniflorum* and *Cucumis melo* was responsible for the radical scavenging activity. Further in-vivo antioxidant activity was Nitric-Oxide radical Scavenging Assay, Hydroxyl method and Phosphomolybdate Assay by considering Ascorbic acid as the standard in all the three methods .The ethanolic seed extract of *Macrotyloma uniflorum* was found to show significant scavenging activity of 64.01%±1.78 at 500µg/ml in Nitric Oxide radical Scavenging Assay, 74.42%±2.37at 1000 µg/ml in Hydroxyl method and 92.59%±2.05at 250 µg/ml in Phosphomolybdate method as compared to that of standard. However, the ethanolic seed extract of *Cucumis melo* also showed significant scavenging activity of 60.65%±1.53at 500µg/ml in Nitric Oxide radical Scavenging Assay, 80.62% ± 1.46 at 1000 µg/ml in Hydroxyl method and 96.07%±2.48 at 250 µg/ml in Phosphomolybdate method as compared to that of standard. *Macrotyloma uniflorum* exhibited significant scavenging activity against Nitric oxide radicals where as *Cucumis melo* exhibited significant scavenging activity against hydroxyl radicals and phosphomolybdates which are comparable with that of standard.

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