Research article

***In-vitro* antioxidant, anti-inflammatory and cytotoxic activity of *Cucumis melo* L. of ethanolic extract**

G. Malathi, J. Vadivelu

¹Post Graduate and Research Department of Biochemistry, Sri Akilandeswari Women's College, Wandiwash 604 408, Tamil Nadu, India

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Corresponding author: J. Vadivelu. Email: vadivelu2015@gmail.com

ABSTRACT

Introduction and Aim: *Cucumis melo* L. commonly known as wild melon (in English) belongs to the family Cucurbitaceae and well distributed in rural areas. *Cucumis melo* L. is a annual climbing or perennial herb, distributed almost throughout India and neighbouring countries. Nowadays food wastes are a major concern. On the other hand, the demand for natural valuable compounds to human health is increasing. These by-products contain phytochemical compounds with great nutritional and functional potentials. The fruits of this plant possess the abortifacient property and have antitussive, antioxidant, digestive, cytotoxic and anti-inflammatory properties.

Materials and Methods: The ethanolic extract was used to determine the antioxidant scavenging activity using DPPH method, protein denaturation for anti inflammatory studies, as well as in vitro cytotoxic activity by brine shrimp lethal assay (BSLA).

Results: The results obtained indicated highest antioxidant activity as compared to DPPH methods. The protein denaturation test showed the ethanolic extracts of *Cucumis melo* L. had effective anti-inflammatory activity. Extracts of *Cucumis melo* L. also registered potential cytotoxic activities by BSLA. This study provides the basis for further investigation of *Cucumis melo* L. for potential identification of novel bioactive compounds with therapeutic properties.

Conclusion: The presence of phytoconstituents such as flavonoids, terpenoids, alkaloids, may indeed assist to the antioxidant activity of fruit extract. That this next thing that must be done will be to conduct in vivo experiments and discern the histopathological mechanism.

Keywords: Cucurbitaceae; DPPH; anti-inflammatory; cytotoxic activity.

INTRODUCTION

Oxidative stress is a state of disequilibrium between the production of free radicals and the ability of the organism to invalidate or detoxify their baneful through neutralization by antioxidants (1). Owing to the high reactivity of free radicals, their overproduction during oxidative stress can damage all biological macromolecules including certain DNA, proteins, and lipids, resulting in progressive damage and, as a necessary consequence, the manifestation of pathological illnesses such as heart disease, diabetes, and inflammatory diseases. (2). Antioxidants are secondary metabolites that act as ROS scavenger and activator of cellular antioxidative enzymes to prevent the damages induced by ROS in biological system plant polyphenols seem to be more prominent as efficacious lab strains with naturally occurring antioxidants as a necessary consequence of this (3, 4).

Fruits are important source of antioxidants like as phenolic acid, flavonoids, and carotenoids. These phenolic compounds are very crucial play in the functions of food. Fruits can be considered as natural remedies to prevent many human degenerative

diseases via the degradation of free radicals induced cellular damage (5). The Cucurbitaceae family's wild melon (*Cucumis melo* L.) is one of the most major staple fruits due to its pleasant flavour profile and calorie information. Wild Melon pulp is rich in important vitamins, phytoene, β -carotene, and 5-methyltetrahydrofolic acid and it possesses high antioxidant, anti-inflammatory and cytotoxic properties (6).

The availability of these fruit constituents may provide a rich source of natural drugs against the modern medicine. The cytotoxic effect of these fruit extracts is to be evaluated besides on the host cells (7). The safety of fruits as a potential therapeutically agents must be ascertained and the concomitant might be acceptable to the host cell. Indeed, recommendation of bioactive components present in the fruit extract of *Cucumis melo* L. with no or less toxic effect on the host cell are the effective candidates for formulation of drugs (8). Hence, the present study is conducted to investigate the comprehensive analysis of the free radicals scavenging activity of antioxidant, anti-inflammatory, and cytotoxic activities of ethanolic extract of *Cucumis melo* L.

MATERIALS AND METHODS

Collection and authentication of plant

Cucumis melo L. fruits were collected from Vandavasi, Thiruvannamalai District, Tamil Nadu. All plant materials were collected from the months of December to January 2019-2020. Fruits were authenticated by the Siddha Central Research Institute, Chennai (Central Council for Research in Ayurveda and Siddha, New Delhi, Under the Ministry of Health & Family Welfare, Govt. of India), Reference No: C14022001M.

Preparation of plant extract

Extraction was carried out at room temperature under normal conditions. About 5g of shade dried powder of fruits of *Cucumis melo* L. was successively extracted with ethanol then kept in magnetic stirrer for 30 minutes. The extract was filtered through Whatmann No.1 filter paper under reduced pressure. The extract obtained was filtered, concentrated by heating at 100°C in a water bath.

In vitro antioxidant activity

DPPH radical scavenging assay

The scavenging efficiency of antioxidant cell viability against the stable radical is investigated in this assay. The fractions' free radical scavenging potential was evaluated in vitro using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test (9,10). A 0.3 mM DPPH solution in ethanol was produced, and 1 ml of it was added to 3 ml of the ethanol different concentrations (10-50 µg/ml). The absorbance was measured at 517 nm using a spectrophotometer until after mixture was infused and allowed to stand at room temperature for 30 minutes. The percent scavenging activity of the fractions was reassessed at various concentrations, and their IC₅₀ results have been compared to that of ascorbic acid, which was implemented as the standard. The DPPH solution absorbance decreases as the DPPH concentration rises. DPPH radical-scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0 - A_1) / A_0 \times 100)$$

Anti-inflammatory assay

With 1.5 mL of bovine serum albumin, 2.0 mL of test solution with various concentrations of extract (10 to 50 g/mL) was taken (BSA). For 15 minutes, the mixture was kept at 271°C. The reaction mixture was kept at 55°C in a water bath for 15 minutes to cause denaturation. The turbidity was measured spectrophotometrically at 660 nm after cooling. Denaturation inhibition was assessed as a percentage of control where no antidepressants was added (11,12). Percentage of anti-inflammatory activity of the extract is given by,

$$\% \text{ AI} = (A_c - A_t) / A_c \times 100$$

Where A_c is the absorbance of the control

A_t is the absorbance of the test.

Cytotoxic activity

The BSLA assessment is a bench top assay that would be used to screen natural resources for bioactive chemicals. The experiment was carried in according to Ganta *et al.*, (13). 2 gram of Iodine free salt was weighed and dissolved in 200 ml of distilled water. Six well ELISA plates were taken and 10 – 12 ml of saline water was filled. To that nauplii were slowly added to each well (10µl – 50µl). Iant extract stock solutions (10 mg/mL) were prepared and serially diluted in clean 10 mL test tubes to obtain five final concentrations. The plates were incubated for 24 hours. After 24 h of observation all the shrimp were survived in the control. Even though, maximum mortalities were observed upto a concentration of 50 µl/mL and least mortality at 10 µl/mL concentrations were absorbed and noted for number of live nauplii's. Present and calculated by using following formula, Number of Dead Nauplii/ Number of Dead + Number of Live Nauplii X 100.

RESULTS

Fig. 1: Preparations of ethanolic extract of *Cucumis melo* L.

Antioxidant activity

Fig. 2: Standardization of ethanolic extract of *Cucumis melo* L. in DPPH scavenging activity

DPPH is a stable free radical whose absorbance at 517 nm decreases when antioxidants donate protons to DPPH. The ability of DPPH to react with antioxidants and gets converted into 1,1-diphenyl-2-picryl hydriene. Quantitative analysis revealed strong

DPPH• radical scavenging ability in ethanolic extract of *Cucumis melo* L. Fig. 2 describe the result of ethanolic extract of *Cucumis melo* L. scavenging activity at 50µl/ml by DPPH method as compared to standard.

Anti-inflammatory activity

Fig. 3: Protein denaturation method

Fig. 4: Anti-inflammatory activity of Ethanolic extract of *Cucumis melo* L.

The extract was demonstrated to be an effective anti-inflammatory agent, with dramatically higher activity than the opioid treatments. The anti-inflammatory action was correspondingly concentration-dependent, with the activity increasing as the extract concentration was increased. As a matter of fact, theoretical maximum effect was recorded at the highest concentration tests performed. Protein denaturation have been shown to be more effectively stunted by the ethanol extract. The inhibition was shown by ethanol extract respectively at the concentration of 50µl/ml which was the highest concentration evaluated.

Cytotoxic effect

Fig. 5: Brine shrimp lethality assay (BSLA) cultured plates

Fig. 6: Cytotoxic effect analyses by Brine shrimp lethality assay (BSLA)

The result inferred that the activity of all the plant extracts were concentration-dependent. The ethanolic extract of *Cucumis melo* L. indicating the presence of bioactive secondary metabolites in these plants. The highest cytotoxicity on the brine shrimps compared to cyclophosphamide (80 µg/mL). The result of the BSLA is summarized in fig. 6.

DISCUSSION

The present study inferred that efficacy of *Cucumis melo* L. plays a vital role for scavenging of free radicals. Hence, the elucidation of DPPH exhibits the absorption of free ions during the incubation of 50µL ethanolic extract of *Cucumis melo* L. Here, Siddhuraju and Klaus(14,15) have revealed that bioactive elements' reducing power, which might be related to their purpose of transporting electrons, is correlated to antioxidant activity.

The anti-inflammatory activity of the plant extracts can be due to the synergistic effect of pro-inflammatory enzyme inhibitors, free radical scavenging activities (16). The results of the present work suggest that the anti-inflammatory activities of these extracts could be explained, at least in part, by their antioxidant properties (17). Most biological proteins lose their biological function when denatured. Denaturation of proteins is one of the well-defined causes of inflammation. The ethanol extract of *Cucumis melo* L. fruit is portrayed in this research. a have illustrated that the extract of *Cucumis melo* L. fruit works by inhibiting thermally induced protein (albumin) denaturation in a dose-dependent manner. Fig.4 depicts the % inhibition by the extract of *Cucumis melo* L. fruit. at different ratios, and maybe even a comparison to the conventional drug (18). *Cucumis melo* L. also has a cytotoxic effect by suggested the brine shrimp lethal assay (Not only the herbal, the herbal mediated nanoparticles also show very good biological activities (19). The present study determined that the extent of lethality was directly proportional to the concentration of the extract. After 24 h of observation all the shrimp were survived in the control. Ogbole *et al.*, (20) described that the ethanolic extract

investigated displayed significant cytotoxic activity, with fruit extract of *Cucumis melo* L. showing cytotoxicity higher than the positive control (cyclophosphamide) (21).

CONCLUSION

Recently, a lot of exploitation on natural sources is used as an alternative therapy. Because, the side effects of modern medicines are less than natural remedy. The objective of this study was to compare the antioxidant activity of extracts of *Cucumis melo* L. using ethanolic extract, determining anti-inflammatory activities, as well as investigating the *in vitro* cytotoxic activities of the same extract by Brine Shrimp Lethal Assay. The results of this study provide the basis for further investigation of *Cucumis melo* L. for potential identification of novel bioactive compounds with therapeutic properties.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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