

Free radical scavenging activity of leaves of *Cucumis sativus*

Pritesh Shah*, Yadunath Joshi, Priya Dongare, Swati Dhande and Vilasrao Kadam

Department of Pharmacology, Bharati Vidyapeeth's College of Pharmacy, Sector -8, C.B.D., Belapur, Navi Mumbai-India 400614.

***Correspondence Info:**

Pritesh Shah,

Department of Pharmacology,

Bharati Vidyapeeth's College of Pharmacy, Sector -8, C.B.D., Belapur, Navi Mumbai-400614, India.

E-mail: pritesht239@gmail.com

Abstract

Cucumis sativus commonly called as 'Cucumber' is commonly used plant throughout the world. The plant is attributed to various uses in Ayurveda. The methanolic extract of leaves of *Cucumis sativus* was screened for free radical scavenging activity properties using gallic acid as standard antioxidant. Free radical scavenging activity was evaluated using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical. Different concentrations of leaf extract ranging from 100- 1000µg/ml were subjected to DPPH assay. Leaf extract showed a maximum DPPH scavenging activity of 86.17% at 1000µg/ml, whereas for Gallic acid it was found to be 98.03%. The study reveals that antioxidant activity of plant would exert beneficial effects if consumed.

Key Words: Antioxidant, Cucumber, *Cucumis sativus*, Gallic acid

1.Introduction

Reactive oxygen species (ROS) generated by NADPH oxidase during oxidative phosphorylation, are normal components of healthy cell. ROS are also mediators of the first defensive actions of cells and involved in phagocytosis, apoptosis and detoxification^{1,2}. Recently, increasing evidence highlights that overproduction of ROS and oxygen derived free radicals may contribute to variety of pathological effects and induce many diseases like cancer, atherosclerosis, diabetes and rheumatoid arthritis^{3,4}. In order to reduce ROS-induced damage, both synthetic and natural antioxidants are used. However, synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene are considered for liver damage and carcinogenesis⁵. Therefore it is essential to develop natural non-toxic antioxidant to protect human body from free radicals and retard the progress of many chronic diseases.

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. A large number of herbs have been reported to exhibit antioxidant activity. The majority of antioxidant activity in plants is due to presence of phenolic compounds⁶. Phenolic compounds are group of compounds naturally present in plants⁷. Plant derived polyphenols receive considerable interest because of their antioxidant and antimicrobial properties. Polyphenolic compounds mainly include simple phenols, phenolic acids, coumarins, tannins and flavanoids. The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity⁸. Antioxidant based drug formulations are used for prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimers's disease and cancer. Radical scavenging activity is considered to be involved in aging process, anti-inflammatory, anticancer and wound healing activity. The development of antioxidants that scavenge reactive oxygen species (ROS) would support biological resistance to free radicals, retard the process of aging, and decrease the risk of age associated degenerative disease⁹.

Cucumis sativus commonly known as Cucumber is well known plant belonging to family Cucurbitaceae. The plant is widely cultivated in India and throughout the world. The fruit obtained from the plant is widely consumed

throughout the world. The plant is attributed to various uses in Avurveda. Seeds are highly nourishing. Leaves boiled in water and mixed with cumin seeds are used for throat infection. Seeds are used by Unani physicians in fevers. Seed oil is used for burning, insomnia and frontal headache. Plant is also used for jaundice, bleeding disorders and anuria. The seeds are used as diuretic, tonic, anthelmintic and also as taeniicide. The leaf juice is emetic and is used to treat dyspepsia in children.^{10,11}

Free radical scavenging activity of leaves of *Cucumis sativus* was evaluated in vitro using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical.

2. Materials and Methods

2.1 Chemicals

1, 1-diphenyl-2-picryl-hydrazyl (DPPH) was obtained for Sigma Aldrich Co USA. All other chemicals used were of analytical grade.

2.2 Preparation of crude extract

500 grams of leaves of plant *Cucumis sativus* were collected from local market. The leaves were authenticated by H.M. Pandit (Department of Botany) Khalsa College, Mumbai, with voucher number BV/COP/NM/350/2012-2013. The leaves were washed properly and dried at room temperature and thereafter they were powdered in grinder. 80gms of powder leaf were extracted in Soxhlet apparatus using methanol as solvent. Yield of the leaves was 21.3%. The leaves extract was then concentrated using Rotary evaporator under reduced pressure. The stock solution of crude extract (1mg/ml) was prepared by dissolving a known amount of dry extract in methanol. The working solutions (100, 200, 400, 600, 800 and 1000 µg/ml) of the extract were prepared from stock solution using suitable dilution.

2.3 Preliminary phytochemical screening¹²

The methanolic extract of *Cucumis sativus* was tested for preliminary phytochemical screening for the presence of alkaloids, glycosides, fixed oils, flavanoids, steroids and terpenoids, and tannins.

2.4 Antioxidant activity (DPPH free radical scavenging activity) of methanolic extract of *Cucumis sativus* leaves¹³

The antioxidant activity of the plant extract, silymarin and the standard was assessed on basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical activity. The DPPH radical scavenging activity was assayed according to method of Shimada *et al.* with some modifications. The diluted working solutions of the test extracts were prepared in methanol. Gallic acid was used as standard in 100-1000µg/ml. Silymarin was also used as standard as plant was further investigated for hepatoprotective action. Silymarin was used in concentrations of 100-1000µg/ml. Antioxidant activity of *Cucumis sativus* were compared with gallic acid and silymarin. 2ml of DPPH solution (0.2mM DPPH in methanol) was mixed with 2ml of sample, gallic acid and silymarin separately (100-1000µg/ml). The mixtures were shaken vigorously and were kept in dark for 30mins and optical density was measured at 517nm. Methanol 2ml with 2ml DPPH solution (0.2mM) was used as blank. The optical density was recorded and DPPH radical scavenging activity was calculated using following formula:

$$\text{Scavenging activity (\%)} = [1 - (A_I - A_2) / A_0] \times 100$$

Where A_0 is the absorbance of the control, A_I absorbance of the sample with DPPH and A_2 is absorbance of the sample only.

3. Results and Discussions

Preliminary phytochemical screening of the methanolic extract of *Cucumis sativus* of leaves is given in the table below,

Table 1: Preliminary phytochemical screening of methanolic extract of *Cucumis sativus* leaves extract

Phytoconstituents	Present/Absent
Alkaloids	Present
Glycosides	Present
Flavonoids	Present
Steroids and Terpenoids	Present
Tannins	Present
Fixed oils	Absent

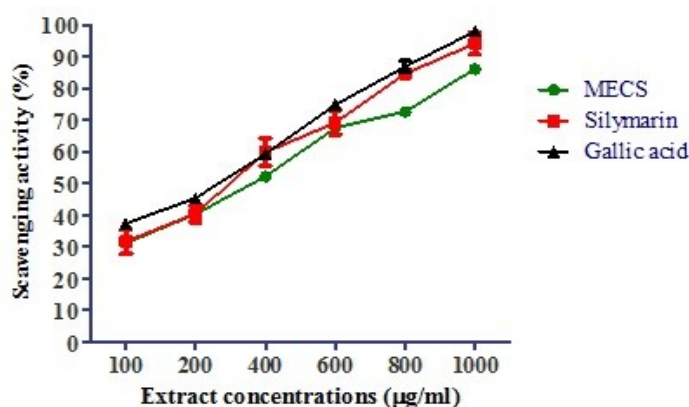
The DPPH radical scavenging model is a widely used method to evaluate the free radical scavenging activity of antioxidants.¹⁴ In the DPPH assay, the antioxidants are able to reduce the stable DPPH radical (purple) to non-radical form DPPH-H (yellow). The DPPH scavenging activities of antioxidants are attributed to their hydrogen donating abilities.

Natural antioxidants that are present in herbs are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds.¹⁵ Many researchers are focused on the powerful but non toxic antioxidants from natural sources, such as natural antioxidants which could prevent formation of ROS. Also prevent the use of synthetic antioxidants that are suspected of causing or promoting negative health effects.¹⁴ The phytochemical screening revealed presence of various constituents like alkaloids, glycosides, phenolic acids, flavanoids, steroids and tannins. These chemical constituents may be responsible for antioxidant activity as several such compounds are known to possess potent antioxidant activity.¹⁶ Various chemical constituents have been isolated from plant, and the observed antioxidant activity may be due to presence of any of these constituents. The plant exhibits various pharmacological activities like anticancer¹⁷, antidiabetic^{18,19}, antihyperlipidemic²⁰, wound healing²¹ and other several activities. These properties may be due to its antioxidant activity.

Gallic acid is standard antioxidant; hence it was used as reference standard. Silymarin was used as reference standard in the *in vivo* studies; to compare antioxidant activity of MECS with silymarin it was used. The result showed that MECS (leaves extract) exhibited maximum DPPH scavenging activity of 86.17% at 1000 µg/ml, whereas for silymarin it was 94.22% and for gallic acid it was 98.03% at 1000 µg/ml.

The IC₅₀ values of MECS 380 µg/ml, silymarin 305 µg/ml and gallic acid 280 µg/ml are respectively.

Figure: 1 DPPH scavenging activity of standard gallic acid, silymarin and methanolic plant extract.



4. Conclusion

Searching for the new natural antioxidants has become an urgent demand due to the health hazards accompanying the use of synthetic ones and the strict need of such antioxidants to decline many health disasters caused by liberated free radicals.

The outcome results clearly prove that the methanolic extract of leaves of *Cucumis sativus* has significant antioxidant action *in vitro* and the antioxidant activity could be utilized as a new natural antioxidant in food and therapeutics.

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