In -Vitro Antioxidant Activity of *Capsicum Chinense* plant Leaves by Hydro-Methanolic Extraction

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Abstract-Generation of the reactive oxygen species (ROS) or intracellular abilities to elimination of ROS are out of balance, which leads to oxidative stress. It causes excessive damage to all biomolecules, including lipids, proteins, DNA, and RNA. The antioxidant activity of plants is caused by these phytochemicals, which interact with the antioxidants substances that prevents oxidation and oxidatives injury caused by free radical. As a result, it can potentially neutralize reactive oxygen species or free radicals. Capsicum Chinense, a member of the Solanaceae family, is a rumbustious, easily-growing species that is found from the Tezpur DefenseResearch Laboratory, in Indian state of Assam, that the king chilli is the hottest chilli worldwide.This species is utilized as hepatoprotective, antitumor, diuretic, anti-inflammatory, and bactericidal medicines due to its antioxidant activity. Numerous researches carried out in the last few years have shown that many types of chilli consist of phenolic chemicals, carotenoids, and capsaicinoids—all among which have been shown to have biological activity. In this study we identify the anti-oxidant impact of hydro-methanolic extract of leaves parts of Capsicum Chinense or to estimate the Anti-oxidant effects of hydro-methanolic extract and compare to it with Silymarin by DPPH Radical Scavenging Assay methods because there is no scientific data presented to the anti-oxidant activities of this plant. The current investigation also showed that Capsicum Chinense leaves portions contain a variety of secondary metabolites. These phytochemicals may be a significant source of pharmacological compounds, meaning that the plant species have enormous possibility for used in the treat for a range of chronic illnesses. The species' crude extract exhibits encouraging antioxidant

potential as well, supportive the old-style uses of this plant with scientific evidence.

Key Words: - oxidative stress, anti-oxidants, Capsicum Chinense, DPPH, Silymarin

Introduction

The generation of Reactive oxygen species (ROS) or the intracellular abilities to remove ROS are out of balance, which leads to oxidative stress. It causes excessive damage to all biomolecules, including lipids, proteins, DNA, and RNA[1]. This damage can set off the onset of numerous illnesses, including cancer, oxygen toxicity, ageing, atherosclerosis, lipofuscinosis, and liver injury[2, 3]. them as possible antioxidants against a range of diseases caused by free radicals[4]. The antioxidant activity of plants is caused by these phytochemicals, which interact Antioxidants are substances which stop oxidation or oxidatives injury caused by free radical. As a result, it can potentially neutralize reactive oxygen species or free radicals. The presence of these phytochemicals in plant products has also led to recent investigations revealing with other organisms in the environment to prevent the growth of bacteria or fungi. Because these compounds inhibit infections and have little toxicity to host cells, they are thought to provide the foundation for the development of new antimicrobial medications[5].

One of the few horticultural products that is as easily obtainable as chilli peppers is this particular variety. This fruit is used in the cooking of many different cuisines worldwide. The chili pepper is a member of the genera Capsicum, which is a part of the Solanaceae family of plants. This fruit contains a wide range of essential nutrients, such as proteins, fats, carbs, and fiber. There is a higher concentration of chemicals in chilli peppers that might impact biological processes. Numerous research carried out in the last few years have shown that many types of chilli consist of phenolic chemicals, carotenoids, and capsaicinoids—all among which have been shown to have biological activity. Regarding several bioactive substances, polyphenols are the ones that have undergone the most research. Some of the most important bioactivities associated with each one of these substances include the ability of polyphenols to lower blood pressure, lower blood sugar levels, and fight inflammation. This study offers a thorough examination of the both vivo as well as in-vitro bioactivities linked to capsaicinoids and polyphenols present in a range of chilli products. These specifics are useful when preparing meals or food ingredients that have many purposes. The

leaves of the capsicum Chinense plant contains the occurrence of alkaloid, glycosides, saponin, tannin, or other phenolic compound which possess antioxidants activity.

Widely identified by its popular name, King Chilli, sometimes called "Bhut Jolokia," is a highly hot kind of chilli that comes from India's northeast. It has been found from the Tezpur Defense Research Laboratory, in Indian state of Assam, that the king chilli is the hottest chilli worldwide[6].

Figure-1 Leaves, fruits and flowers of Capsicum Chinense

Material and Method

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Methanol, were bought by Shaila enterprise. Analytical grade reagents were utilized in all other cases.

Plant material collection and authentication

Identification and Collection of the Plant: The leaves part of the *Capsicum Chinense* plant has been collected from the local area of Mazbat, Assam and were air dried in the shade.

Authentication of the Plant: The herbal plant that is *Capsicum Chinense* is used in the study was authenticated by Botanical Survey of India, Noida, U.P.

Authentication no- BSI/BGIR-8(3)2021/88

Preparation of plant extract

Plant extraction

Dried leaves of Capsicum Chinese were collected and then grinded into coarse powder via clean mortar or pestle and are store in an air-tight container to protect from moisture. Hydro Methanolic method was used in the study for the extraction of the plants. Plant extracts was ready by the maceration extraction process where

100gm crude drug powder was soaked in 75% methanol in beaker for 72 hrs. at room temperature with occasional stirring. After 72 hrs the liquid phase stained, filtered using filter paper and evaporated to dryness in hot air oven as a result the extract was obtained and weighed[7].

Extraction Method

Maceration Method

Because of its ease of usage, maceration is a particularly often used technique for extracting polysaccharides. This method involves placing the medication, in powder form, in a container with the solvent and letting it sit at room temperature for three days while stirring constantly[8].

The combination is strained, the residual solid (marc) is pressed, and the collected liquids are filtered after that. This extraction method's primary goals are to remove unwanted components and retrieve desirable compounds with therapeutic relevance. The general maceration mechanism is leaching [9].

In-vitro Antioxidant activities

Antioxidant Assay

The antioxidant activities of the plants extraction were determined by 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay methods**.** Entirely the assays were carry out in triplicate or average value were considering.

DPPH Radical Scavenging Assay

The free radical scavenging capacity of the hydro-methanolic extract of Capsicum Chinense leaves parts, determine by using DPPH. DPPH solutions (0.004% w/v) was prepare in 95% ethanol. Methanolic extracts given plants were mix with the ninety five percent ethanol and water respectively to prepared stock solutions (10 milligram /100ml). From this stock solution 1ml, 2ml, and 3ml, of solutions were taken in 3 test tube respectively and by sequential dilutions with the similar solvents, the final volume of each test tube was made up to 10 ml whose concentration was then 10 µg/ml, 20 µg/ml, 30 µg/ml respectively for all extracts. Fresh prepared of DPPH solutions (0.004% w/v) was add in each of these test tube or later 10 min, absorbance was taken at 517 nm using in a spectrophotometer (Double beam UV–visible spectrophotometer). Silymarin were uses as a reference standard drug or dissolve in a distill water to make the stocks solution with the similar concentrations. A control samples was preparing contains the same volumes without any extraction or references standard. % scavenging of the DPPH free radicals was measure uses the following equations [328-330].

% DPPH radicals-scavenging = (Absorbance of control - Absorbance of test Sample) X 100
(Absorbance of control)

Results

Table-I Result of phytochemical test Preliminary phytochemical investigation of Capsicum Chinense

TESTS	HAE
Tests for Alkaloid	
1. Mayer's Tests	+
2. Wagner's Tests	+
3. Hager's Tests	+
4. Dragendroff's tests	+
Tests for Saponins	
1. Foam test	++
Test for Flavonoids	
1. Alkaline reagent tests	+
2. Lead Acetate tests	+
Tests for Tannins	
1. Gelatine + extract	++
Tests for Phenolic compound	
1. Ferric chloride solution	++
Tests for Terpenoids	
1. Salkowski test	++

+ represents presence; ++ represents present in more concentrations; - represents absence.

The phytochemical testing of Hydro-Methanolic extraction of leave of Capsicum Chinese shows the presence of alkaloid, flavonoid, saponin, tannin, phenol, terpenoid and carbohydrates.

Phytochemical screening

Preliminary phytochemicals screening of the *Capsicum Chinense* leaves extraction shows that the plants is riches in various actives ingredient (2^{nd}ry plant metabolite). The results of the phytochemicals screening revealed strongest to moderate presence of alkaloid, flavonoid, saponins and carbohydrates (Table-1).

In-Vitro Antioxidant Activity

DPPH radical scavenging activity

DPPH radical scavenging potential of Capsicum Chinense leaves extract at different concentrations investigated in the present study was determined together with standard antioxidant (Silymarin) at the same concentrations. Capsicum Chinense leaves extracts (hydro-methanolic extracts) showed significant scavenging effect on DPPH free radical in concentration dependent manner. When compared with standard antioxidants used in the experiment, the extract showed relatively lower DPPH free radical scavenging potentials.

Table-2 Antioxidant activities of hydro-methanolic extracts of leaves parts of Capsicum Chinense by DPPH method.

S.NO.	Hydro methanolic Extraction of Capsicum Chinense		Silymarin	
	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition
01	10	13.61	10	21.45
02	20	21.12	20	23.83
03	30	26.44	30	36.87

Graphical representation I shows the % inhibition of DPPH radicals by extraction of Capsicum Chinense

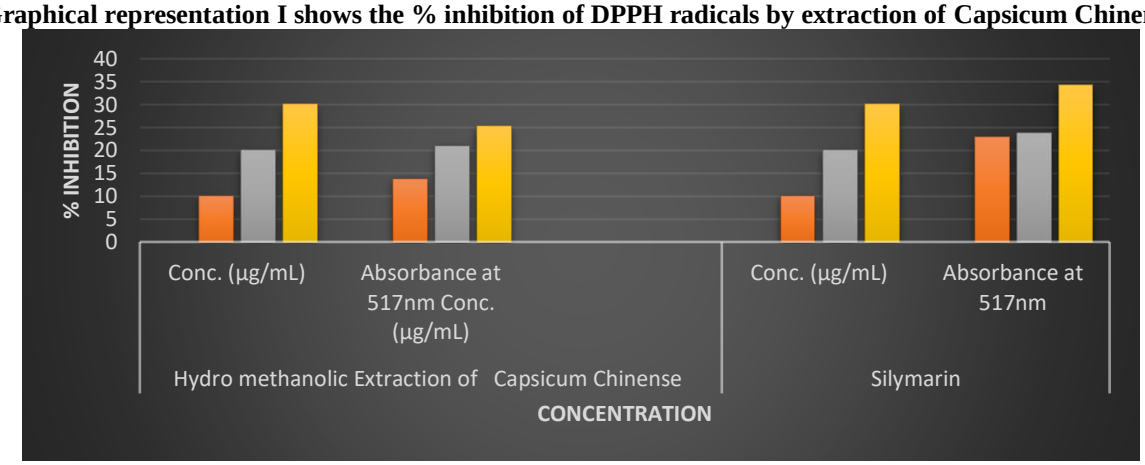
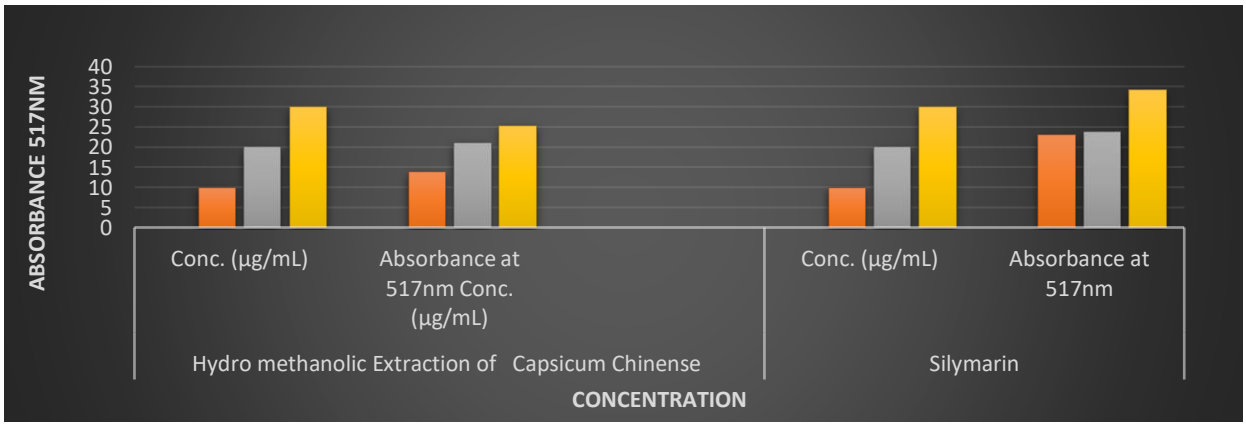


Table-3 Antioxidant activities of hydro methanolic extraction of leaves part of Capsicum Chinense by DPPH method

S.NO.	Hydro-methanolic Extraction of Capsicum Chinense		Silymarin	
	Conc. (µg/mL)	Absorbance at 517nm	Conc. (µg/mL)	Absorbance at 517nm
01	10	13.87	10	22.89
02	20	21.12	20	23.73
03	30	25.34	30	34.21

Graphical representation II showing the absorbance of DPPH radicals by extracts of Capsicum Chinense



Discussion

At normal temperature, DPPH is a purple stable free radical with a distinctive absorbance at 517 nm. An antioxidant called 1,1-diphenyl-2-picrylhydrazine readily stifles nitrogen free radical of DPPH. Purple color decolorization is stoichiometric and depends on the number of electrons acquired[10]. Leaves extracts from Capsicum Chinense demonstrated a substantial, concentration-dependent scavenging activity on the free radical DPPH. In contrast to the conventional antioxidants employed in the study, the extract exhibited comparatively reduced ability to scavenge free radical's DPPH. As a result, in vulnerable biological and food systems, Capsicum Chinense leaf extracts may be able to stop reactive radical species from causing damage to biomolecules like DNA, proteins, polyunsaturated fatty acids (PUFA), and carbohydrates. The high reactive species recognized as hydroxyl radical (HO•) is produced in biologicals system and targets DNA nucleotides, breaking DNA strands and causing cancer and mutagenesis. By removes the hydrogen atom from membrane lipids' polyunsaturated fatty acid, it starts the lipid peroxidation process. It has the ability to harm practically all of the molecules in living cell[11]. The leaves parts extract of Capsicum Chinense demonstrated the capacity to neutralize free radicals produced, and it also demonstrated concentration-dependent hydroxyl radical scavenging that was equivalent to that of the reference standard (silymarin) at the same dosages[12].

Conclusion

The current investigation also showed that Capsicum Chinense leaves portions contain a variety of secondary metabolites. These phytochemicals may be a significant source of pharmacological compounds, meaning that the plant species may having enormous potential uses as a treatments for a range of chronic illnesses. The species' crude extract exhibits encouraging antioxidant potential as well, supporting the traditional use of this plant with scientific evidence. More research is required to produce innovative antioxidant medications.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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