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ANTIOXIDANT POTENTIAL OF PHLOROGLUCINOL; AN *IN-VITRO* APPROACH

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Keywords:

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ABSTRACT: Phloroglucinol is a polyphenol and a component of phlorotannins belonging to the Laminariaceae family. Phloroglucinol has many positive effects on health. The present study was aimed to evaluate the antioxidant potential of phloroglucinol by comparing it with Standard antioxidant *viz*, ascorbic acid. The antioxidant potential of phloroglucinol was estimated using free radicals such as 1, 1-diphenyl-2-picrylhydrazyl (DPPH), superoxide anion (O_2^-), nitric oxide (NO^-), hydroxyl radical (OH), hydrogen peroxide (H_2O_2) by standard methods. The study shows that phloroglucinol is more powerful against DPPH and hydrogen peroxide radicals, when compared to other radicals. The IC_{50} value of Phloroglucinol is 42 ± 1.00 µg/ml for DPPH, 53.66 ± 1.52 µg/ml for Nitric oxide, 102 ± 2.00 µg/ml for Superoxide, 180 ± 3.60 µg/ml for Hydroxyl and 52.3 ± 1.52 µg/ml for Hydrogen peroxide radicals. *In-vitro* methods suggest that phloroglucinol is effective against DPPH and hydrogen peroxide radicals. Thus our study showed that the phloroglucinol exhibited antioxidant activity against all the free radicals and could be considered as a source of natural antioxidant.

INTRODUCTION: A free radical is any species capable of independent existence containing one or more unpaired electrons [1]. The unpaired electron alters the chemical reactivity of the molecule/atom, making it more reactive than the corresponding non-radical form. Reactive Oxygen Species (ROS) are an inevitable and highly toxic consequence of metabolism in an atmosphere with 21% oxygen. ROS are forms of Oxygen that are more reactive than molecular oxygen (O_2), and they include the superoxide anion (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2). ROS plays an important role in the pathogenesis of various serious diseases, such as neurodegenerative disorders, cancer, cardiovascular disease, atherosclerosis, cataracts and inflammation [2]. The oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems.

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Substances that inhibit oxidation, and are capable of counteracting the damaging effects of oxidation in body tissue are termed antioxidants. They prevent damage caused by free radicals. They create a barrier from free radical damage that results in decaying process. Oxidation causes aging in the skin so antioxidants like Vitamin C, Vitamin E, goji berry, pomegranate, ellagic acid and green tea can reduce the process of aging. Antioxidants are intimately involved in the prevention of cellular damage [3]. Trace metals like selenium, iron, copper, zinc and manganese are required for proper function of antioxidant enzyme systems in the body.

Some natural and synthetic antioxidant like Vitamin E is a fat-soluble Vitamin present in nuts, seeds, vegetable, fish oils, whole grains (wheat, germ) and fortified cereals. Vitamin C is a water-soluble Vitamin present in citrus fruits, juices, green peppers, cabbage, spinach, broccoli, kiwi and strawberries. Beta-carotene is a precursor to Vitamin A (retinol) that is present in liver, egg yolk, milk, butter, carrots, yams, tomato, peaches and grains. Butylated Hydroxyl Toluene (BHT), Butylated Hydroxyl Anisole (BHA) and gallic acid are synthetic antioxidants [4].

Phloroglucinol is a compound from *Ecklonia cava*, a species of brown algae. It is a naturally occurring secondary plant metabolite used for gastrointestinal disorder worldwide. Recently, this biomolecule has attracted attention for drug synthesis because of its anti-inflammatory [5], anti-microbial, anti-allergic [6] and Human Immunodeficiency Virus (HIV)-1 reverse transcriptase and protease inhibitor activities [7]. So, it was planned to investigate on the antioxidant potential of phloroglucinol.

MATERIALS AND METHODS:

Chemicals: Phloroglucinol were purchased from Sigma - Aldrich. Ascorbic acid and all other chemicals procured were of highest grade available commercially.

DPPH Free Radical Scavenging Activity: The free radical scavenging activities of phloroglucinol were measured *in-vitro* by 1, 1-diphenyl-2-picryl hydrazyl (DPPH) assay [8]. 1 ml of sample at different concentrations (10, 25, 50, 100 and 200 µg/ml) was added to 1 ml of ethanolic solution of DPPH. The mixture was shaken and allowed to stands at room temperature for 20 mins and the absorbance was measured at 517 nm using a spectrophotometer. The scavenging activity of the sample was calculated using the formula.

$$\% \text{ Inhibition} = [Abs_{Control} - Abs_{Sample} / Abs_{Control}] \times 100$$

Where,

$Abs_{control}$ = Absorbance of control
Abs_{sample} = Absorbance of sample

Nitric Oxide Scavenging Activity: The effect of phloroglucinol on NO radical scavenging activity was measured using ascorbic acid as standard. NO radical scavenging activity was measured by the method [9]. Sodium nitro prusside (5 Mm) in PBS (Phosphate Buffer Solution) was mixed with different concentrations of phloroglucinol (10, 25, 50, 100 and 200 µg/ml) at 25 ºC for 15 min. Then, Griess reagent (1% Sulphanilamide, 2% ortho phosphoric acid, 0.1% Naphthyl ethylenediamide) was added to the mixture. The absorbance was measured at 546 nm. The scavenging activity of the sample was calculated using the formula.

$$\% \text{ Inhibition} = [Abs_{Control} - Abs_{Sample} / Abs_{Control}] \times 100$$

Super Oxide Scavenging Activity: Super oxide scavenging activity was measured following by the method [10]. Super oxide anions were generated in a non-enzymatic phenazine methosulphate-nicotin amide adenine dinucleotide (PMS-NADH) System by oxidation of NADH and assayed by reduction of NBT (Nitro Blue Tetrazolium). In this study, 3 ml of tris-HCl buffer containing 1ml of NBT 50 µM, 1 ml of NADH (78 µM) and phloroglucinol in the range of 10 - 50 µg were added. The reaction was started by adding 1 ml of PMS solution (10 µM) to the mixture and incubated at 560 nm. The scavenging activity of the sample was calculated using the formula. The IC_{50} values of phloroglucinol were calculated and that of ascorbic acid.

$$\% \text{ Inhibition} = [Abs_{Control} - Abs_{Sample} / Abs_{Control}] \times 100$$

Hydroxyl Radical Scavenging Activity: Scavenging of the hydroxyl free radical was measured by the method [11]. The reaction mixture contained sample (10, 25, 50, 100 and 200 µg/ml). Deoxyribose (3.75 mM), Hydrogen peroxide (1 mM), Potassium Phosphate buffer (20 mM, pH = 7.4), $FeCl_3$ (0.1 mM), EDTA (0.1 mM) and Ascorbic acid (0.1 mM) was incubated in a water bath at 100 ºC for 20 mins. The absorbance of the resulting solution was measured in UV-visible spectrophotometer at 532 nm. The scavenging activity of the sample was calculated using the formula.

$$\% \text{ Inhibition} = [Abs_{Control} - Abs_{Sample} / Abs_{Control}] \times 100$$

Hydrogen Peroxide Radical Scavenging Activity: The ability of the phloroglucinol to scavenge hydrogen peroxide radical was determined by the method [12]. The reaction mixture containing 500 µl of phosphate buffer and 400 µl of 2 mM hydrogen peroxide was mixed with various concentrations of phloroglucinol and incubated at room temperature for 5 mins. Then 2 ml of dichromate reagent was added and the decrease in colour intensity was measured at 570 nm. 2 ml of dichromate acetic acid reagent alone served as blank where as the reaction mixture without compound served as control. The scavenging activity was calculated according to the following formula.

$$\% \text{ Inhibition} = [Abs_{Control} - Abs_{Sample} / Abs_{Control}] \times 100$$

RESULTS AND DISCUSSION: The DPPH assay method is derived from the reduction of DPPH, a stable free radical [13]. The free radical DPPH with an odd electron gives a maximum absorption at 517

nm (purple colour). When antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (*e.g.*, a free radical scavenging antioxidant) and is reduced to the DPPHH and as consequence the absorbance is decreased resulting in decolourization (yellow colour) with respect to the number of electrons gained. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug [14]. The DPPH radical scavenging activity was detected and compared to ascorbic acid. **Fig. 1** shows that the % inhibition of phloroglucinol and ascorbic acid. The IC_{50} value of phloroglucinol was found to be 42 ± 1.00 µg/ml for ascorbic acid 32.53 ± 2.25 µg/ml respectively.

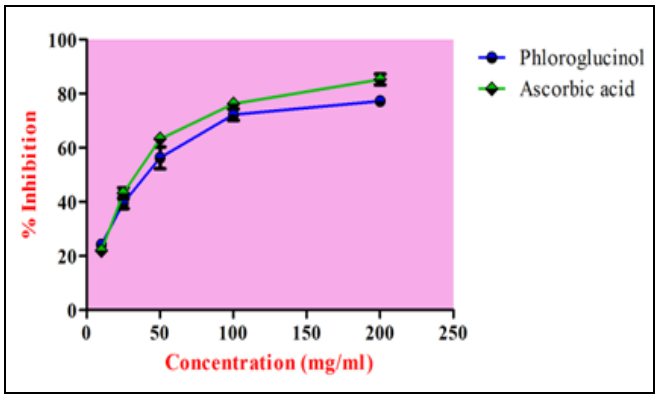

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY OF PHLOROGLUCINOL

Nitric Oxide is an essential bioregulatory molecule required for several physiological processes like neural signal transmission, muscle diseases, inflammatory bowel disease, sepsis and septic shock, immune response, control vasodialation and control of blood pressure [15, 16]. However, the elevation of the nitric oxide results in several pathological conditions, including cancer. Additionally, increasing evidence shows that NO modulates neurotoxin induced cell damage and is involved in neuronal cell death in Parkinson's disease (PD) and other neurodegenerative disorders such as Alzheimer disease [17].

NO is a short-lived (half-life 3-30 s) colourless gas that is moderately soluble in water (up to 2 mmol/ L) but highly soluble in organic solvents [18]. It is lipophilic in nature and can diffuse between cells very easily. NO is generated from the terminal guanido nitrogen atom L-arginine by NADPH-dependent enzymes called Nitric oxide synthases (NOS). NO has an unpaired electron, hence is a free radical. NO becomes nitrosonium cation (NO^+) or nitroxyl anion (NO^-) by donating or accepting an electron, respectively [19]. **Fig. 2** demonstrated the nitric oxide radical scavenging activity of different concentrations of phloroglucinol. The IC_{50} value of nitric oxide radical scavenging activity was found to be 53.66 ± 1.52 µg/ml for ascorbic acid 34.73 ± 1.67 µg/ml respectively.

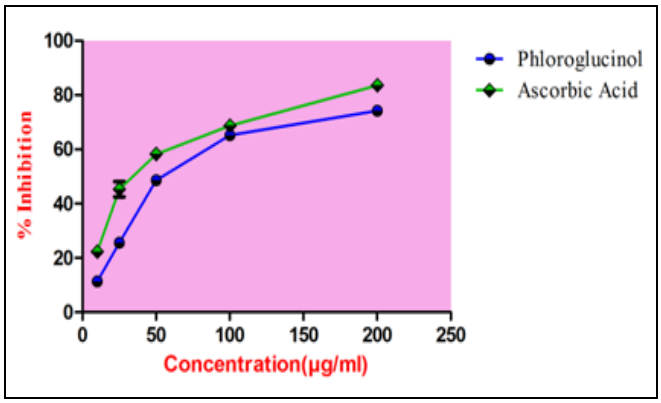

FIG. 2: NITRIC OXIDE RADICAL SCAVENGING ACTIVITY OF PHLOROGLUCINOL

Superoxide anion radical (O_2^-) is generated by four-electron reduction of molecular oxygen into water. This radical is formed in aerobic cells due to electron leakage from the electron transport chain. Superoxide radicals (O_2^-) are also formed by activated phagocytes such as monocytes, macrophages, eosinophils and neutrophils and the production of O_2^- is a crucial factor to kill bacteria through phagocytes. In living organisms, O_2^- is removed by the enzymes called superoxide dismutases (SOD).

Superoxide anion is a week oxidant produced during various biological reactions and is highly toxic [20]. It is known as an initial radical and plays an important role in the formation of other reactive oxygen-species, such as hydrogen peroxide or singlet oxygen. **Fig. 3** shows the superoxide anion radical scavenging activity of various concentrations of phloroglucinol. The IC_{50} value of superoxide anion radical scavenging activity was found to be 102 ± 2.00 µg/ml for ascorbic acid 25.33 ± 2.08 µg/ml respectively.

Hydroxyl radical is the most deleterious and reactive among the ROS and it bears the shortest half-life compared with other free radicals. The oxygen derived hydroxyl radicals along with the

added transition metal ion (Fe^{2+}) causes the degradation of deoxyribose into malondialdehyde which produces a pink chromogen with thio-barbituric acid [21]. Hydroxyl radical can be formed by the Fenton reaction in the presence of reduced transition metals (Fe^{2+}) and H_2O_2, which is known to be the most reactive of all the reduced forms of dioxygen and is thought to initiate cell damage *in-vivo* [22].

Scavenging of hydroxyl radical is an important antioxidant activity because of very high reactivity of the OH radical, enabling it to react with a wide range of molecules found in living cells such as sugars, amino acids, lipids and nucleotides [23]. Thus, removing OH is very important for the protection of living systems. **Fig. 4** shows that the hydroxyl radical scavenging activity of different concentrations of phloroglucinol. The IC_{50} value of hydroxyl radical scavenging activity was found to be 180 ± 3.60 µg/ml for ascorbic acid 30.6 ± 1.50 µg/ml respectively.

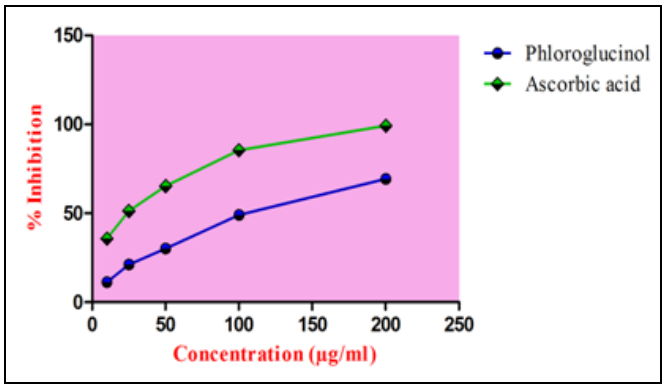

FIG. 3: SUPEROXIDE ANION RADICAL SCAVENGING ACTIVITY OF PHLOROGLUCINOL

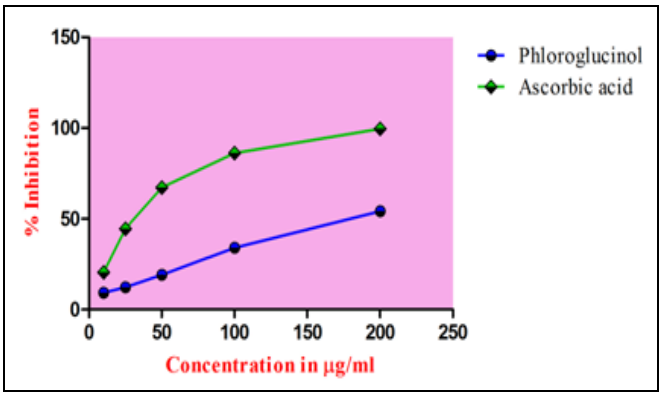

FIG. 4: HYDROXYL RADICAL SCAVENGING ACTIVITY OF PHLOROGLUCINOL

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to the cell because it may give rise to hydroxyl radical in the cells. Thus, removal of H_2O_2 is significant for protection of food systems. H_2O_2 is an intermediate during endogenous oxidative metabolism and mediates radical oxygen formation such as OH, which may be used to predict the scavenging capability of antioxidants in biological systems [24].

Hydrogen peroxide has only a weak activity to initiate lipid peroxidation, but its activity as an active oxygen species comes from its potential to produce the highly reactive hydroxyl radical through the Fenton reaction. **Fig. 5** shows the hydrogen peroxide radical scavenging activity of various concentration of phloroglucinol. The IC_{50} value of hydrogen peroxide radical scavenging activity was found to be 52.33 ± 1.52 µg/ml for ascorbic acid 21.5 ± 2.12 µg/ml respectively.

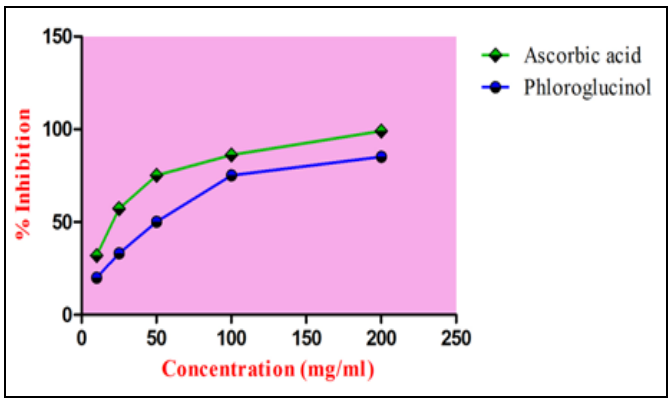

FIG. 5: HYDROGEN PEROXIDE RADICAL SCAVENGING ACTIVITY OF PHLOROGLUCINOL

TABLE 1: COMPARISON OF ANTIOXIDANT ACTIVITY OF PHLOROGLUCINOL AND ASCORBIC ACID

S. no	Free Radical Scavenging methods	IC_{50} Values (µg/ml)	
		Phloroglucinol	Ascorbic acid
1	DPPH	42 ± 1.00	32.53 ± 2.25
2	Nitric oxide	53.66 ± 1.52	34.73 ± 1.67
3	Superoxide anion	102 ± 2.00	25.33 ± 2.08
4	Hydroxyl radical	180 ± 3.60	30.6 ± 1.50
5	Hydrogen peroxide	52.33 ± 1.52	21.5 ± 2.12

CONCLUSION: Free radicals are often generated as by products of biological reactions or from exogenous factors. The involvement of free radicals in the pathogenesis of a large number of diseases is well documented. The results of the present study revealed that phloroglucinol possessed potent free radical scavenging ability with DPPH and hydrogen peroxide radical, when compared to that of other radicals. We conclude that phloroglucinol has high amount of phenolic hydroxyl group's that plays an important role in its antioxidant activities. Therefore, phloroglucinol

has good radical scavenging activity and may be used as a source of natural antioxidant.

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CONFLICT OF INTEREST: Nil

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