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ANTIOXIDANT CAPACITIES OF A FLAVONOID COMPOUND ISOLATED FROM *C SALIGNUS* LEAVES

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ABSTRACT

A flavonoid compound was isolated from the methanolic extract of *C.salignus* leaves and the structure was elucidated as 3′, 4′, 5, 7 Tetra Hydroxy Flavonol by UV, IR, NMR and Mass Spectra. This compound was isolated first time from this plant extract. In present study antioxidant capacities of the isolated compound was determined by two different assays, like DPPH radical scavenging activity method and Hydrogen Peroxide scavenging activity method. For both the models ascorbic acid was used as reference standard. Alterations of the absorbance due to free radicals were tested in control, test (isolated compound from *C salignus* extract), & also standard (stock solution of ascorbic acid) groups. Our findings suggested that, isolated compound of *C salignus* leaves possessed a very good antioxidant activity in dose dependant manner and also comparable with the reference standard one.

KEY WORDS: Isolation, 3′, 4′, 5, 7 Tetra Hydroxy Flavonol, Anti-oxidant.

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INTRODUCTION

Body cells and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or are induced by exogenous damage [1]. Free radicals can attract various inflammatory mediators, contributing to a general inflammatory response and tissue damage. To protect themselves from reactive oxygen species, living organisms have developed several effective mechanisms [2]. The antioxidant-defense mechanisms of the body include enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, but also non-enzymatic counterparts such as glutathione, ascorbic acid, and α-tocopherol. Positive influence could be attributed to the non-nutritive phytochemicals such as carotenoids, alkaloids, vitamins, minerals, flavonoids and other phenolics which also possess antioxidant activity and protect body against free radical damage [3]. The increased production of reactive oxygen species during injury results in consumption and depletion of the endogenous scavenging compounds. Flavonoids may have an additive effect to the endogenous scavenging compounds. Flavonoids can interfere with ≥3 different free radical–producing systems. Flavonoids can prevent injury caused by free radicals in various ways. One way is the direct scavenging of free radicals. Flavonoids are oxidized by radicals, resulting in a more stable, less-reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical.Selected flavonoids can directly scavenge superoxides, whereas other flavonoids can scavenge the highly reactive oxygen-derived radical called peroxynitrite. Epicatechin and rutin are also powerful radical scavengers [4]. The scavenging ability of rutin may be due to its inhibitory activity on the enzyme xanthine oxidase. By scavenging radicals, flavonoids can inhibit LDL oxidation in vitro [5]. Flavonoids (both flavonols and flavanols) are most commonly known for their antioxidant activity in vitro. At high experimental concentrations that would not exist in vivo, the antioxidant abilities of flavonoids in vitro may be stronger than those of vitamin C and E, depending on concentrations tested [6]. Quercetin group of compound is reported to exhibit the highest antiradical property toward hydroxyl and peroxyl radicals and superoxide anions, and this predominance has been well attributed to its structural characteristics [7, 8, 9, 10]. Furthermore, as flavonoid compounds present a strong affinity for iron ions (which are known to catalyze many processes leading to the appearance of free radicals), their antiperoxidative activity could also be ascribed to a concomitant capability of chelating iron [11, 12.] The plant, *Callistemon salignus,* commonly known as bottle brush, is a genus of several species in myrtaceae family. Since this plant belongs to the same myrtaceae family as clove, eucalyptus, it is expected that it might also be a store- house of many chemicals of medicinal and pharmacological interest.In our present study we have investigated the antioxidant activities of the isolated flavonoid compound.

MATERIALS AND METHODS

Plant material: Fresh leaves of *C. salignus* were collected from Kolkata, West Bengal and identified by the Botanical Survey of India, Howrah.

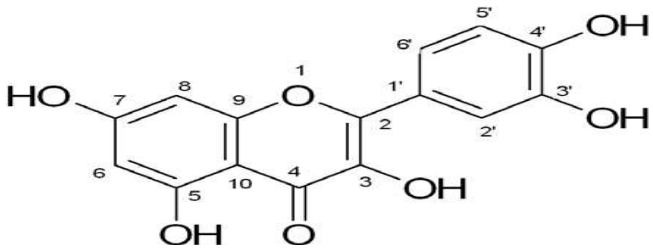
Reagents and Chemicals: All the reagents and chemical were from S.D Fine Chemicals and Loba Chemie Pvt. Ltd. Mumbai, India and were analytical grade.

General Instrument details for isolation: Melting points were determined with capillary melting point apparatus. UV spectophotometer Shimazdu Pharmaspec –

1700, made in Japan; IR: The infrared spectra were recorded on IR spectrophotometer (Shimadzu 8201 PC) in KBr phase ;1H and 13C NMR spectra were recorded in DMSO d 6 as internal standard on a Bruker 300MHz and600MHzDPX spectrometer and the chemical shifts are reported in δ units ; Mass spectra (positive mode) were obtained using LC-ESI-Q-TOF micro mass spectrometer.

Extraction and isolation: The leaves were shade dried and powdered. Then defatted with n hexane and then macerated in methanol in a beaker for 48hrs. Occasional shaking and stirring was done. Then it was filtered through muslin cloth for the methanol extract. Then filtrate was concentrated to dryness under the vacuum. The percentage yield extractive was calculated withreferences to the air-dried drug.

Several chemical tests like Alkaloids, Carbohydrates ,Glycosides; Fixed oils and Fats, Gums and Mucilage, Phenolic compounds and Tannins, Proteins and free Amino acids; Saponin, Sterols; Volatile oils etc were done to detect several and different group of compounds present in the extractions [4]. To get the isolated product , the dried methanol extract was subjected to column chromatography over silica gel (60 – 120 mesh) and graded elutions were carried out with eluents $CHCl_3$ followed by different $CHCl_3$-Ethyl acetate mixtures (90:10, 65:35, 35:65, 25:75). Structure was elucidated as 3′, 4′, 5, 7 Tetra Hydroxy Flavonol by UV, IR, NMR and Mass Spectra [13]. Melting point of the compound was found 316 ^{o} C. Structure of the compound is below



3′, 4′, 5, 7 Tetra Hydroxy Flavonol

Antioxidant assay **:**The evaluation of antioxidant activity of the isolated flavonoid compound (ICCS) of methanolic extracts of *C salignus* was done through two different *in vitro* assays. Both the assays were carried out in triplicate and average values were considered. Statistical Analysis was done by t –Test: paired Two Samples for Mean.

DPPH radical scavenging activity method [14] ***:*** The free radical scavenging activity of the extracts was determined using DPPH. DPPH solution (0.1 mM) was prepared in 95% methanol. Isolated compound of *C salignus* (ICCS) was mixed with 95% methanol to prepare the stock solution (0.5 mg/mL). Freshly prepared 1 mL of DPPH solution (0.1 mM) was added to 3 mL of various concentrations (10-100 µg/mL) of ICCS. After 30 min, absorbance was measured at 517 nm. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Ascorbic acid was used as a reference compound. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with the isolated compound) using the following formula - absorbance of the control minus absorbance of the test sample divided by absorbance of the control multiplied by 100 (Hundred) [15] .

Hydrogen Peroxide Scavenging Activity [16] The ability of the isolated flavonoid compound (ICCS) of *C salignus* extracts to scavenge hydrogen peroxide was determined according

to the method of Ruch et al.(1989).A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4).Hydrogen peroxide concentration was determined spectrophotometrically. ICCS (10,25,50,100µg/ml) in distilled water was added to hydrogen peroxide solution(0.6 ml,40mM).Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against blanksolution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both ICCS and standard compound was calculated as described previously.

RESULT AND DISCUSSION

It is extremely important to point out there is a correlation between antioxidant activity and phyto-chemical screened. Flavonoid compounds are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Phenolic compounds like tannin, flavonoid possess ideal structural chemistry for free radical scavenging activity and are the ideal antioxidants and act by different mechanisms [17]. Two methods (DPPH Scavenging Method and Hydrogen Peroxide Scavenging Method) were chosen for the antioxidant activity of the isolated compound (ICCS) as these two methods gave good results for crude extract evaluation. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolourize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in Absorbance [18].Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects [19].For both the methods it has been found that it is also same or comparable with the ascorbic acid in dose dependent and significant manner. Comparison of the antioxidant activity of different concentrations of ICCS and ascorbic acid is shown result Table 1 and also in the Figure 1for DPPH scavenging activity. This activity was increased by increasing the concentration of the sample ICCS. It exhibited a significant antioxidant activity in dose dependent manner. For hydrogen peroxide scavenging activity also result is almost same (Tabulated in table 2, and graphical representation shown in Figure 2). After comparing these two methods, the second method gave somewhat better result than the first one. This significant activity may be due to the presence of flavonol group in the structure of ICCS. The antioxidant activity of the quercetin group of compound is attributed to the presence of catechol group in the B ring and OH group at the position 3 of the AC ring within the molecule [20].

Table 1
DPPH Scavenging Activity of isolate flavonoid (ICCS)

Treatment	Concentration (µg/ ml)	Absorbance (Mean ± S.E)	% Antioxidant Activity
Control	-	0.073 ± 0.005	-
Ascorbic Acid	10	0.041 ± 0.0028	43.83
Ascorbic Acid	25	0.031 ± 0.0005	57.53
Ascorbic Acid	50	0.027 ± 0.0002	63.01
Ascorbic Acid	100	0.025 ± 0.005	65.75
ICCS	10	0.046 ± 0.006	36.98
ICCS	25	0.033 ± 0.003	54.79
ICCS	50	0.028 ± 0.004	61.64
ICCS	100	0.026 ± 0.007	64.38

Here calculated t- value (2.37) was less than the table values at 2.5 % level of significance. So it can be concluded that there is no statistical significant difference exists between ICCS and ascorbic acid at that level. P value = 0.048.

Table 2
Hydrogen Peroxide Scavenging Activity of isolated flavonoid (ICCS)

Treatment	Concentration (µg/ ml)	Absorbance (Mean ± S.E)	% Antioxidant Activity
Control	-	0.030 ± 0.0012	-
Ascorbic Acid	10	0.079 ± 0.0005	62.02
Ascorbic Acid	25	0.085 ± 0.007	64.70
Ascorbic Acid	50	0.093 ± 0.0091	67.74
Ascorbic Acid	100	0.096 ± 0.002	70.96
ICCS	10	0.067 ± 0.002	55.22
ICCS	25	0.081 ± 0.003	62.96
ICCS	50	0.089 ± 0.007	66.29
ICCS	100	0.094 ± 0.005	68.08

Here calculated t- value (2.4) was less than the table values at 2.5 % level of significance. So it can be concluded that there is no statistical significant difference exists between ICCS and ascorbic acid. P value = 0.044.

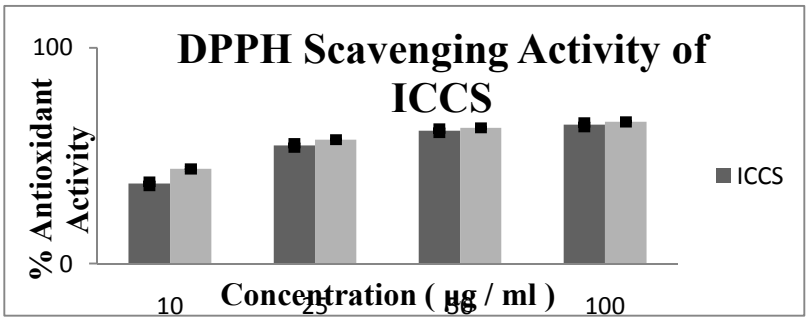


Figure 1
DPPH Scavenging Activity of isolated flavonoid compound (ICCS). Values represented as mean ± standard error

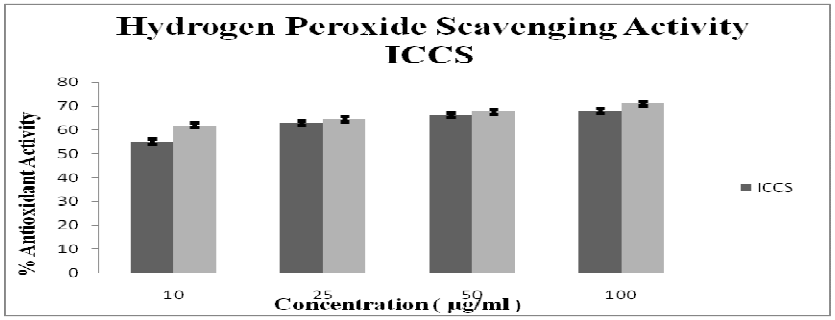


Figure 2
Hydrogen peroxide activity of isolated flavonoid (ICCS) . Values represented as mean ± standard error

CONCLUSION

Our investigations clearly demonstrate that isolated flavonoid compound (ICCS - 3′, 4′, 5, 7 Tetra Hydroxy Flavonol) possesses a significant antioxidant activity.

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