IJPSR (2015), Vol. 6, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 March, 2015; received in revised form, 30 April, 2015; accepted, 09 June, 2015; published 01 September, 2015

# *INVITRO* ANTIOXIDANT STUDY AND SEARCH FOR A NOVEL BIOACTIVE COMPOUND FROM LEAVE FRACTIONS OF *CANTHIUM COROMANDELICUM* (BURM.F.) ALSTON

Sanjeeb Kumar Patro and Dinakar Sasmal *

Department of Pharmaceutical Sciences and Technology, Birla Institute Technology, Mesra, Ranchi-835215, India

**Keywords:**

*Canthium coromandelicum* (Cc), Phytochemical, TPC, TFC, DPPH, hydroxyl radical, HPTLC, Spectral analysis.

**Correspondence to Author:**
**Dr. D. Sasmal,**

Professor,

Department of Pharmaceutical Sciences and Technology, BIT, Mesra, Ranchi, Jharkhand, India, 835215

**E-mail** : dsasmal@bitmesra.ac.in

**ABSTRACT: Background:** Plants are enriched with various types of phytoconstituents present in the form of secondary metabolites that ensure its medicinal value. Keeping this in view, the present investigation was carried out focusing on its antioxidant property that may mainstay of exploring other therapeutic potency. Hence, phytochemical investigation followed with antioxidant property was evaluated in the leaf extracts of the selected plant *Canthium coromandelicum* (Burm.f.) Alston, collected from eastern coastal belt of south Odisha. **Objective:** To carry out the phytochemical studies of various leaf extracts and fractions of *Canthium coromandelicum* (Burm.f.) Alston. *In vitro* antioxidant activities were also estimated to justify the leaves being used in traditional medicine as an antioxidant. **Methods:** *In vitro* antioxidant activity was assayed by DPPH scavenging activity and 2-deoxy-D-ribose degradation. Estimation of total phenolic content and total flavonoid content were done. Further HPTLC finger print of active fractions was performed to isolate and purify the active components presence in them. One of the isolated purified compounds was characterized by various spectral methods. **Results:** Preliminary phytochemical analysis of leaf extracts confirmed the presence of various phytoconstituents such as flavonoids, terpenoids, saponins, steroids, tannins, quinones and gums. Ethyl acetate fractions of the extract showed potential antioxidant property compared to all the other extracts and fractions. Further, Methanolic extract was found to be rich in phenolic and flavonoid contents compared to the aqueous extract; hence, ethyl acetate fraction of the methanolic extract was chosen for *In vitro* antioxidant activity. The spectral characterization isolated purified compound was found to be a flavonoid glycoside i.e., Kaempferol 3-O-β-D-glucopyranoside. **Conclusion:** The present preliminary phytochemical analysis coupled with TPC, TFC and *in vitro* antioxidant study evaluated the herbal as a potential source of antioxidants with identification of a novel bioactive compound that opens vista to explore its novel bioactive compound which will help for various pharmacological activities.

**INTRODUCTION:** Oxidative free radicals bear responsibility in situations causing health problems like asthma, cancer, cardiovascular diseases, cataract, diabetes, gastrointestinal inflammatory diseases, liver disease, muscular degeneration and other inflammatory processes [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(9).3841-52

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(9).3841-52

These free radicals found in chemical compounds which contain an unpaired electron spinning on the peripheral orbits around the nucleus. The family of free radicals generated from the oxygen is called Reactive Oxygen Species (ROS) causing damage to other molecules by extracting electrons from them in order to attain stability [2]. In normal conditions of cellular metabolism, ROS (Reactive oxygen species) are formed continuously which are scavenged and converted to nonreactive species by different intracellular enzymatic and/or non enzymatic antioxidant system [3]. Excess production or an ineffective elimination of ROS in biological system induce oxidative stress and cause damage to

all types of bio molecules such as proteins, lipids and nucleic acids [4]. Antioxidants may act as free radical scavengers, reducing agents and chelating agents for transitional metals; quenching of singlet oxygen molecules and/ or activating of antioxidative defense enzyme system to suppress the radical damages in biological systems [5, 6]. Antioxidants thus play an important role in the protection of human system against damage by reactive oxygen species [7, 8].

The body possesses antioxidant defense mechanisms such as enzymatic antioxidant systems (superoxide dismutase (SOD), glutathione peroxidase (GPx), Catalase (CAT) etc. and non enzymatic antioxidant systems like ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, etc). Normal levels of antioxidant system in the body are not sufficient for the eradication of the free radical injury [9]. Therefore, inhibition of free radical- induced oxidative damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of these diseases. Thus the need of the body for antioxidants from external sources becomes mandatory.

Synthetic antioxidants like butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) commonly used have side effects and are also Carcinogenic [10]. Thus, there is a need for more effective, less toxic and cost effective antioxidants. Medicinal plants appear to have desired advantages and hence there occurs a growing interest in natural antioxidants from plant sources [2]. In recent years, it has been investigated that many plant species are serving as source of antioxidants and received therapeutic significance [11-13].

*Canthium coromandelicum* (*Canthium parviflorum* belongs to family*:* Rubiaceae) is a thorny subscandent shrub with spreading branches distributed throughout India in scrub forests and dry plains. It occurs in peninsular India, coramandel coast and in dry plains. Its leaves are simple, small, obovate, opposite with interpetiolar stipules and axillary spines. Its leaves and roots are medicinally important; astringent, sweet, thermogenic, diuretic, febrifuge, constipating, anthelmentic, and as tonic used in vitiated conditions of cough, diarrhea, strangury, fever, leucorrhoea, intestinal worms, and general debility [14]. It is traditionally used against snake bites[15]. Macerated paste of leaf is externally applied twice a day to treat scabies and the ring worm infection[16]. Decoction of leaves is used for wound healing in animals. Significant antioxidant and diuretic activity was exhibited by extracts of leaves[17]. *Canthium* as herbal medicine is used for the treatment of diabetes among major tribal groups in South TamilNadu[18]. Though the ethno medicinal importance of this plant is known but the phytochemical analysis on the basis of such kind of medicinal property has not been known yet. Hence, the present investigation was aimed to find both the qualitative and quantitative phytochemical profile and studying the *in vitro* antioxidant activity in various fractions of the leaf extract.

## MATERIALS AND METHODS:

### Collection of Plant materials:

Leaves of *Canthium coromandelicum* (Cc) were collected from in and around the campus of College of Pharmaceutical sciences (CPS) Mohuda village near Brahmapur, Ganjam (Odisha) during the month of June-August 2012. The plant material had been identified by Prof. S. K. Das, Taxonomist, Department of Biosciences, CPS, Brahmapur, Ganjam, and Odisha and further authenticated from taxonomy section of Botanical Survey of India (BSI), Howrah, Kolkata. The voucher specimen (CNH/76/2012/Tech.II/899) was retained in the Department of Pharm. Sciences & Technology, BIT- Mesra, at Ranchi for future reference.

### Preparation of plant extracts:

1kg of air dried powder was successively extracted with 6 Liters of solvents viz., petroleum ether (40-60 grade), and then with chloroform, methanol and distilled water using Soxhlet apparatus for 72 hours or till colorless state at a temperature not exceeding the boiling point of the respective solvent. The obtained crude extracts were filtered through with Whatman No. 1 filter paper and then concentrated under vacuum at 50ºC by using rotary evaporator. All the extracts were studied with their physical characteristics, % of yield, qualitative and quantitative phytochemical study and potent *in-vitro* antioxidant activities. Methanolic extract and its ethyl acetate fraction were found to be

exhibiting more potent *in-vitro* antioxidant activity than other fractions. Hence, ethyl acetate fraction was stored for further use.

**Preliminary phytochemical studies:**

The different extracts of *C. coromandelicum* were tested for the presence of major class of secondary metabolites like steroids, alkaloids, glycosides, saponins, flavonoids, phenolic compounds, tannins, triterpenoids, carbohydrates, proteins and fats etc. Phytochemical screening of all the extracts was carried out according to the standard methods prescribed (Harborne, 1998) with some modifications[19].

**Quantitative phytochemical screening:**

**Estimation of total Phenolic content:**

0.1 ml of the sample (10 µg/ ml) was mixed with 0.5 ml of folin-Ciocalteau reagent (diluted with 1:10 ratio with distilled water) and 1.5 ml of sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml with distilled water. It was allowed to stand for 2 h. and the absorbance was measured at 750 nm Using UV-Visible spectrophotometer (Shimadzu UV 1700). Gallic acid was used as standard and results were expresses as mg/g Gallic acid equivalent [20].

**Estimation of total flavonoid content:**

0.5 ml of the sample (100 µg/ ml) was mixed with 1.5 ml of methanol (75% v/v), 0.1ml of Aluminium chloride (10% w/v), and 0.1 ml of potassium acetate (1M) and 2.8 ml of distilled water. The mixture was allowed to stand for 30 minutes in room temperature and the absorbance was measured at 435 nm using spectrophotometer (Shimadzu UV 1700). Quercetin was used as standard and results were expressed as mg/g Quercetin equivalent[20].

**Estimation of *in vitro* Antioxidant activity:**

**Free radical scavenging activity using DPPH (2, 2-Diphenyl Picryl Hydrazide):**

The ability of the samples to scavenge the free radicals was estimated by *in vitro* method using a stable nitrogen centered radical viz. DPPH. Scavenging of DPPH free radical determines the free radical scavenging capacity or antioxidant potential of the test sample, which shows its effectiveness, prevention, interception and repair mechanism against injury in a biological system. The free radical scavenging activity of extracts and fractions were measured using DPPH by the method of Hallowell, 1989. 0.25 ml of the test solution was added to a methanolic solution of DPPH (100 µM, 2.95 ml) at different concentrations (200-1000 µg/ ml). The mixture was shaken vigorously and incubated in a room temperature for 20 min in dark condition. The absorbance of resulting solutions was recorded at 517 nm by UV-Visible spectrophotometer (Shimadzu UV 1700). All the assays were carried out in triplicates against ascorbic acid as a standard. Blank was prepared without the addition of DPPH and for control 0.2 ml of methanol (without test sample) was added. Decreased absorbance values indicated a higher free radical scavenging activity. The percentage of DPPH scavenging activity was determined from the following equation as:

$$\text{DPPH scavenging activity (\%)} = [(AC - AS)/AC] \times 100$$

Where AC is the absorbance value of the control & AS is the absorbance value of the added test samples solution.

**Hydroxyl radical scavenging activity:**

The deoxyribose method was used for determining the scavenging effect on OH as prescribed (Hallowell, 1989; Arti *et al*., 2010). The reaction mixture contained ascorbic acid (50 µM), $FeCl_3$ (20 µM), EDTA (2 µM), $H_2O_2$ (1.42 mM), deoxyribose (2.8 mM), with different concentrations of samples in a final volume of 1 ml in potassium phosphate buffer (10 mM, pH 7.4). It was incubated at $37^0$C for 1h and then 1 ml of 2.8% TCA and 1 ml of 1% TBA were added. The mixture was heated in a boiling water bath for 15 min. It was cooled and absorbance was measured at 532 nm with the spectrophotometer (Shimadzu UV 1700) [21-22].

**Fractionation of active extract:**

The extract which contains highest amount of Phytochemical constituents was subjected to further fractionation by separating funnel method using different solvents like water, n- Butanol, and ethyl acetate. In this method, weighed amount of selected solvent extract was suspended in water (5 times) to made slurry. Some amount of slurry and the extracted solvent (100 ml) was added carefully in the stoppered separating funnel. It was then

shaken continuously for 15 minutes intermittently monitored by the Stopper to release excess pressure. It was kept for sometime undisturbed until the layers were clearly separated. The Stopper was then opened and the lower layer containing extracted slurry was drained off and the upper layer containing solvent extract was collected by pouring it out from the top of the separating funnel. Solvent extract obtained were filtered and concentrated in a rotary evaporator. All the fractions were kept in desiccators.

**Column chromatographic isolation:**

About 1gm of dried ethyl acetate fraction of methanolic extract (EA-MECC) was than column chromatographed over silica gel G (60-120) using methanol: chloroform as eluent in increasing polarity index up to 15%. 25 fractions of 25 ml each were collected. They were tabulated (**Table 7**). The dried isolated Column fraction of powdered Cc was further made flash chromatographed by using same solvent system with increased order of polarity index up to 10%. The collected eluent from flash chromatography was evaporated by using Rota evaporator to get pure fine yellow crystalline powder.

**HPTLC analysis:**

A number of solvent systems were tried, and the satisfactory resolution was obtained in solvent system, chloroform: methanol (8:2) for leaf ethyl acetate fraction of methanolic extracts of *Canthium coromandelicum* (EA-MECC).

Chromatographic separation of ethyl acetate fraction of methanolic extract of *Canthium coromandelicum* was performed on 5 cm × 10 cm aluminum-backed HPTLC plates coated with 200 µg layers of silica gel 60GF254 (Merck, Darmstadt, Germany). Before use, the plates were prewashed with methanol and activated at 110°C for 5 min. Crystalline powder of EA-MECC column sub fraction designated by SKP-LF (5 µl each) was applied on to HPTLC plate 13 mm apart from middle of bands by spray-on technique along with nitrogen gas supply for simultaneous drying of bands, by means of Camag (Switzer-land) Linomat V sample applicator fitted with a 100 µl syringe (Hamilton, Bonaduz, Switzerland). A constant spot application rate of 0.15 µl/ s was used. Plates were developed with appropriate solvent system. Before development the chamber was saturated with mobile phase for 20 min at room temperature (32 ± 2°C). Chromatography was performed in Camag's twin-trough chamber. Wavelength for detection was evaluated from complete UV spectrum. Scanning was performed with a Camag TLC scanner 3 under control of Camag winCATS planar chromatography manager software (version 1.4.2). The slit dimensions were 6 mm × 0.45 mm and the scanning speed was 20 mm/s.

**Characterization of isolated compound:**

The flash chromatographic pure fine yellow phytoconstituent was characterized by using various spectroscopic analyses like UV, LC-MS, IR, and NMR ($^{1}H$ &$^{13}C$).

**SEM (Scanning Electron Microscope) of (SKP-LF/ ICF-PCc):**

The particles observed SEM (Jeol instrument, Japan). Scanning electron photographs were taken at an accelerating voltage of 20 Kv, chamber pressure of 0.6 mm Hg. The test sample was observed under scanning electron microscope. The sample was mounted directly on to the SEM sample stub using double slide sticking tape and coated with platinum film(thickness of 200 nm) under reduced pressure (0.001 mm of Hg). Elemental analysis of SKP-LF/ (ICF-PCc) performed through SEM.

**Statistical analysis:**

All data on antioxidant activity tests were the average of triplicate analysis. Linear regression analysis was used to calculate the $IC_{50}$ values.

**RESULTS:**

The findings from the experimental work have been summarized under different sub headings.

**Phytochemical screening:**

The physical characteristics and the percentage of yield of all the extracts of *C. coromandelicum* were demonstrated in **Table 1** and **Table 2**. Preliminary phytochemical screening of different metabolites (steroids, alkaloids, phenolic groups, tannins, coumarins and anthraquinones etc.) were done in four different extracts of *C. coromandelicum* and the results were recorded in **Table 3.** In *C.*

*coromandelicum*, tannins showed their presence in all the three extracts followed by saponins, flavonoids, phenols; terpenoids in two different extracts except pet ether and chloroform; steroids illustrated their occurrence in chloroform extract, alkaloids and glycosides confirmed their existence only in methanolic extracts.

**Total Phenolic content:**

Total Phenolic content (TPC) was estimated by using folin-Ciocalteau reagent and expressed as mg/ g Gallic acid equivalent (GAE). In leaves, methanolic extract showed significant value of TPC 43mg of gallic acid equivalent. Whereas, the total phenolic content was found to be insignificant of gallic acid equivalent per 1gm of aqueous extract (Tab. 4 & Fig. 1b).

**Total flavonoid content:**

Total flavonoid content (TFC) was estimated and expressed as mg/g Quercetin equivalent. In leaves, methanolic extract of *C. coromandelicum* showed highest value of TFC 116.6 mg/g of Quercetin equivalent. Whereas, the total flavonoid content of aqueous extract was found to be 96.6 mg/g of Quercetin equivalent (**Table 4** and **Fig. 1a**).

**Free radical scavenging activity using DPPH:**

The free radical scavenging activity of extracts was studied by its ability to reduce the DPPH. In *C. coromandelicum* leaves, the extract MECC scavenged the DPPH radical with $IC_{50}$ value of 141.13 µg/ ml. Amongst all extracts, the methanolic extract showed higher activity with low $IC_{50}$ value of 141.13 µg/ ml followed by, chloroform extract having scavenging activity with $IC_{50}$ value of 908.78 µg/ ml (**Table 5**). Similarly, in various fractions of methanolic extract, ethyl acetate fraction showed higher activity with low $IC_{50}$ value of 117.03 µg/ ml followed by n-Butanol fraction with $IC_{50}$ value of 510.23 µg/ ml as comparing with Ascorbic acid as standard (**Table 6**).

**Hydroxyl radical scavenging activity:**

Kept in view, the hydroxyl radical scavenging activity was being carried out. It was found that, there was a considerable difference in the scavenging activity. In leaves of *C. coromandelicum*, $IC_{50}$ values of OH radical scavenging activity ranges from 250.87 in methanolic extract followed by 899.72 in chloroform extract to >1000 µg/ ml in aqueous extract as compared with Quercetin being standard (**Table 5**). Similarly, in various fractions of methanolic extract, ethyl acetate fraction showed higher activity with low $IC_{50}$ value i.e. 241.80 µg/ ml followed by n-Butanol fraction with $IC_{50}$ value of 795.38 µg/ ml (**Table 6**).

**HPTLC analysis:**

Based on the above results, HPTLC fingerprinting of purified SKP-LF isolated from flash chromatography was taken. In HPTLC fingerprint observations, SKP-LF exhibited four peaks at 425 nm **($\lambda_{max}$)** with $R_f$ values in the range of 0.02 to 0.96 in the optimized solvent system of chloroform: methanol (8:2). The peak number 2 further showed higher percentage of area of 71.77% (**Table 8; Fig. 2)** and contains more quantity of bioactive component than other peaks. Hence, this major component was further purified with flash chromatography and characterized with the several spectroscopic analysis to get structural moiety of organic compound present in it.

**TABLE 1: PHYSICAL CHARACTERISTIC OF DIFFERENT CRUDE LEAVE EXTRACTS OF *C. COROMANDELICUM***

| Plant extract | Consistency | Colour | Odour |
|---|---|---|---|
| Pet. ether extract | Sticky solid | Dark greenish | Agreeable |
| Chloroform extract | Semi solid | Dark greenish | Agreeable |
| Methanolic extract | Sticky solid | Dark brownish | Agreeable |
| Aqueous extract | Semi solid | Dark greenish | Characteristics organic |

**TABLE 2: PERCENT YIELD OF DIFFERENT CRUDE LEAVE EXTRACTS OF *C. COROMANDELICUM***

| Plant extracts | Weight of dry powder | Weight of dry extract | % yield |
|---|---|---|---|
| Pet. ether extract | 500 gm. | 30 gm. | 6 |
| Chloroform extract | 500 gm. | 20 gm. | 4 |
| Methanolic extract | 500 gm. | 25 gm. | 5 |
| Aqueous extract | 500 gm. | 50 gm. | 10 |

**TABLE 3: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF DIFFERENT EXTRACTS OF *C. COROMANDELICUM***

| | Pet. Ether | Chloroform | Methanol | Aqueous |
|---|---|---|---|---|
| Alkaloids | -ve | -ve | ++ve | -ve |
| Glycosides | -ve | -ve | ++ve | -ve |
| Tannins | -ve | +ve | ++ve | +ve |
| Saponins | -ve | -ve | +++ve | ++ve |
| Flavonoids | -ve | -ve | +++ve | +ve |
| Phytosterols | +ve | -ve | +ve | -ve |
| Terpenoids | -ve | -ve | ++ve | ++ve |
| Phenols | -ve | -ve | ++ve | +ve |
| Steroids | -ve | +ve | -ve | -ve |
| Carbohydrates | -ve | -ve | +ve | -ve |
| Proteins | -ve | -ve | -ve | -ve |
| Fats | +ve | -ve | -ve | -ve |

+++ Present significantly; ++ Present moderately; + present slightly; - Absent

**TABLE 4: TOTAL PHENOLIC AND FLAVONOID CONTENT ANALYSIS OF METHANOLIC AND AQUEOUS EXTRACTS OF *C. COROMANDELICUM***

| Sl. No | Extract/ Fraction | Total Phenolic content (mg/g Gallic acid equivalent) | Total flavonoid content (mg/g Quercetin equivalent) |
|---|---|---|---|
| 1 | ME-CC | 46 | 116.6 |
| 2 | AQ-CC | - | 96.6 |

**TABLE 5: *IN VITRO* ANTIOXIDANT ACTIVITY OF VARIOUS LEAF EXTRACTS OF *C. COROMANDELICUM***

| Test | $IC_{50}$ value (µg/ml) | | | | % of inhibition | |
|---|---|---|---|---|---|---|
| | PECC | CFCC | MECC | AQCC | Ascorbic acid | Quercetin |
| DPPH | >1000 | 908.43 | 141.13 | >1000 | 92.13 | |
| OH radical | >1000 | 899.72 | 250.87 | >1000 | | 83.71 |

**TABLE 6: *IN VITRO* ANTIOXIDANT ACTIVITY OF VARIOUS FRACTIONS METHANOLIC EXTRACT OF LEAVES OF *C. COROMANDELICUM*.**

| Test | $IC_{50}$ value (µg/ml) | | | % of inhibition | |
|---|---|---|---|---|---|
| | EA-MECC | BU-MECC | AQ-MECC | Ascorbic acid (10µg) | Quercetin (10µg) |
| **DPPH** | 117.03 | 510.23 | >1000 | 92.13 | |
| **OH radical** | 241.80 | 795.38 | >1000 | | 83.71 |

**TABLE 7: COLUMN SUB FRACTION OF EA-MECC BASED ON POTENT *IN VITRO* ANTIOXIDANT ACTIVITY.**

| Fraction | Solvent ratio | Eluent | Marked as |
|---|---|---|---|
| 1-5 | Chloroform (100) | Grayish Mass (50mg) | ----- |
| 6-15 | Methanol :Chloroform (20:80) | Yellowish powder Mass (100 mg) | (ICF-PCc)/SKP-LF |
| 16-25 | Methanol :Chloroform (25:75) | Yellowish brown Mass (50mg) | |

**TABLE 8: HPTLC FINGER PRINT PROFILE OF SKP-LF/ (ICF-PCc) SCANNED AT 425 nm ($\lambda_{max}$)**

| Fraction | Peak | $R_f$ | Max. height | Area | Area % |
|---|---|---|---|---|---|
| SKP-LF | 1 | 0.02 | 49.2 | 837.6 | 11.55 |
| | 2 | 0.27 | 148.6 | 5204.8 | 71.77 |
| | 3 | 0.42 | 21.6 | 583.1 | 8.04 |
| | 4 | 0.96 | 21.5 | 626.3 | 8.64 |

**TABLE 9: ELEMENTAL ANALYSIS OF SKP-LF THROUGH SEM:**

| Element | App Conc. | Intensity Corrn. | Weight% | Weight % Sigma | Atomic % |
|---|---|---|---|---|---|
| C K | 76.00 | 1.3562 | 66.11 | 1.33 | 73.30 |
| O K | 11.29 | 0.4172 | 31.92 | 1.33 | 26.57 |
| Pt M | 1.39 | 0.8319 | 1.97 | 0.47 | 0.13 |
| Totals | | | 100.00 | | |

**SEM & EDX (Energy Dispersive Spectroscopy) spectroscopy of SKP-LF/ (ICF-PCc):** In this study, the compound isolated from flash chromatography (*i.e.*, SKP-LF) was pure as compared to compound isolated by column chromatography with respect to no. of elements present in both the compounds can be shown in **Table 9** and **Fig. 3.**

**Characterization of SKP-LF/ (ICF-PCc):**
From the preliminary phytochemical chemical investigations, the isolated bioactive component was found to be more positive test only towards flavonoids and glycosides.

Yellowish crystalline powder mass, m.p: 220 -240 $^{\circ}$C [23] ; UV ($\lambda_{max}$ nm): 210, 266, 339; IR (KBr)-cm$^{-1}$: 2956.01, 2923.22, 2853.78, 1734.06, 1653.05, 1606.05, 1559.50, 1507.42, 1466.91, 1362.75, 1282.71, 1208.44, 1182.40, 1078.24, 1016.52; $^{1}$H-NMR (400 MHz, $CD_3OD$): δ (ppm) 8.03 (d, 2H, *J* = 8 Hz, 2', 6',), 6.89 (d, 3',5' *J* = 8Hz), 6.4 (s, 1H , 8), 6.2 (s,1H, 6) 5.27 (d,2H 6"), 4.63 (d, 2H 6", 1"), 4.44 (s, 1H 6" OH), 3.70 (s, 1H 2"), 3.53 (s, 1H 4") , 3.42 (d, 2H 2"and 3"), 3.20 (d, 2H 3" and 4") [Kazuaki *et al.*, 2011]; $^{13}$C-NMR (400 MHz, $CD_3OD$): δ (ppm) 178.079 (C-4), 164.534 (C-7), 161.62 (C-5), 160.14 (C-4'), 157.62 (C-2, 9), 133.98 (C-3), 130.856 (C-2',6'), 121.317 (C-1'), 114.639 (C-3',5'), 104.288 (C-10), 102.565 (C-1"), 98.437 (C-6), 93.307 (C-8), 76.18 (C-5"), 74.3 (C-3"), 72.3 (C-2"), 69.859 (C-4"), 61.139 (C-6")[24].

Based on all the above descriptions of spectral analysis data like UV, IR, $^{1}$H NMR, $^{13}$C NMR and LC-MS, the given compound SKP-LF was identified as: 5, 7, 4'-trihydroxy – 3- O- β- D-galactopyranosyl flavon Or Kaempferol 3-O-β-D-glucopyranoside (**Fig. 4,5, 6, 7, 8 & 9**).

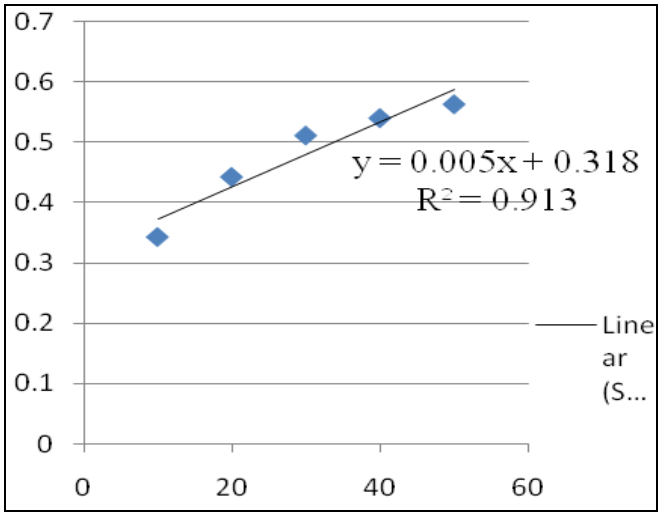


**FIG. 1(A) STANDARD CURVE OF QUERCETIN;**

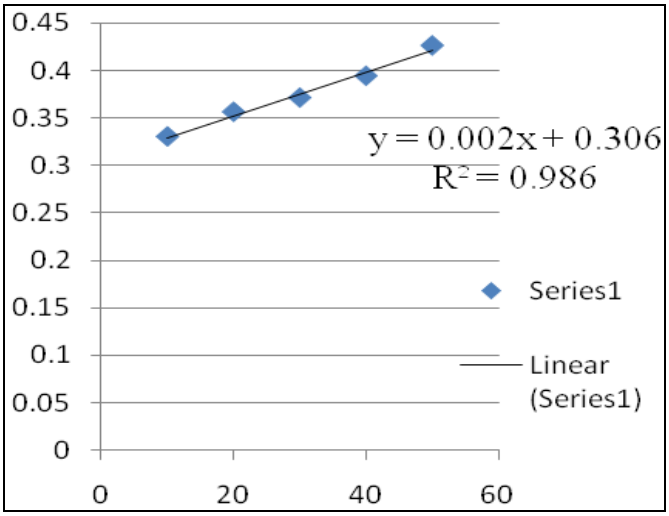


**FIG. 1(B) STANDARD CURVE OF GALLIC ACID**

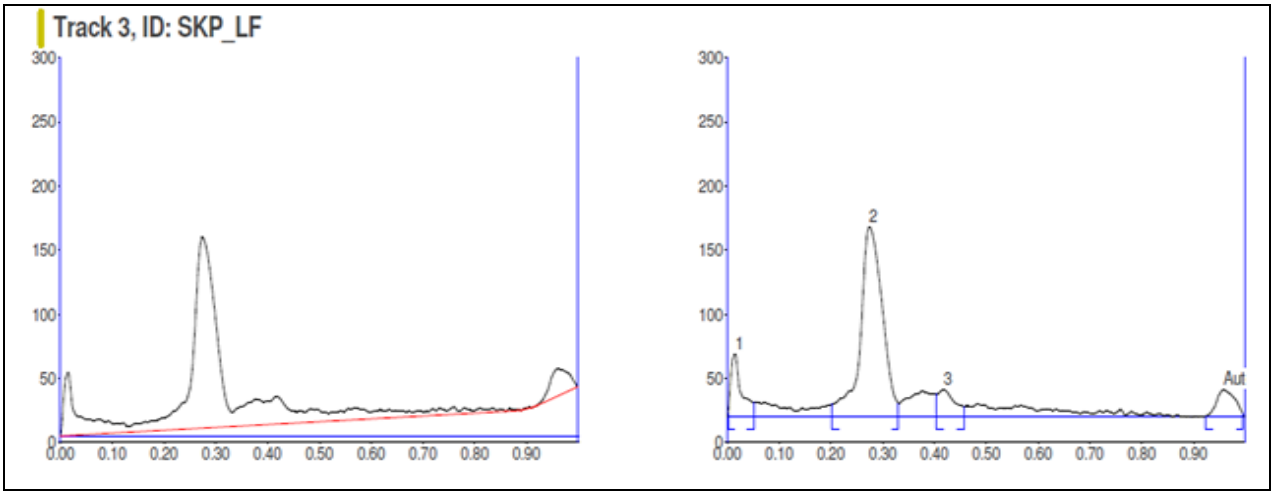


**FIG. 2: HPTLC CHROMATOGRAM OF PEAK NUMBER 2 SCANNED AT 425nm ($\lambda_{max}$) OF COLUMN FRACTION OF SKP-LF OF *C COROMANDELICUM.***

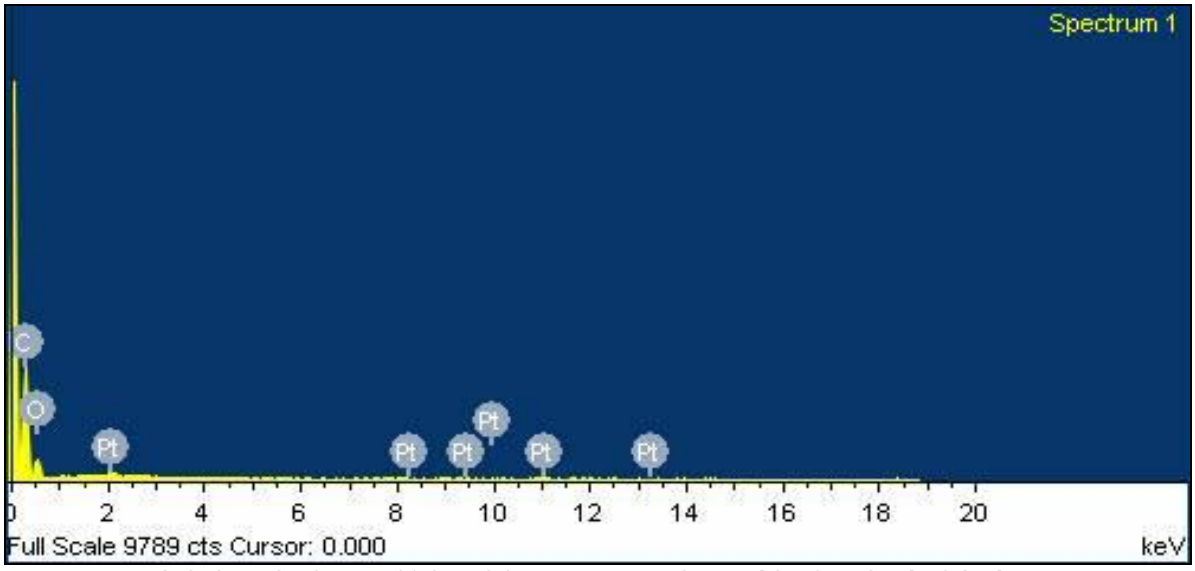


**FIG. 3: SEM OF SKP-LF/ (ICF-PCc) PURIFIED FROM FLASH CHROMATOGRAPHY.**

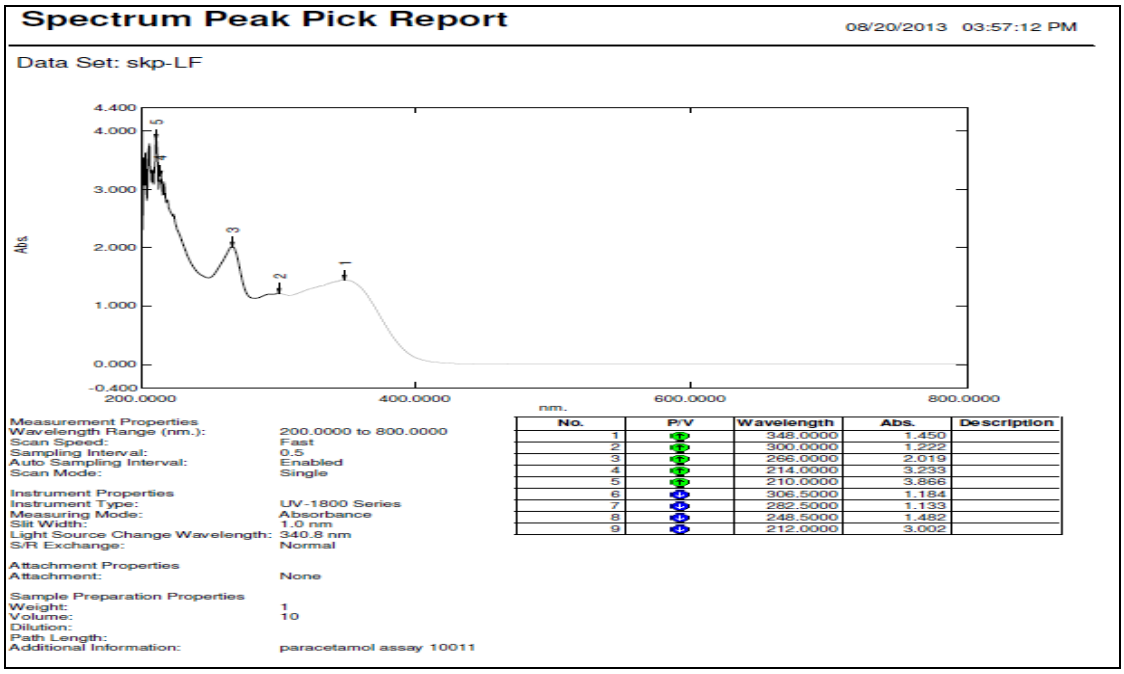


**FIG. 4: UV SPECTRA OF SKP-LF/ (ICF-PCc)**

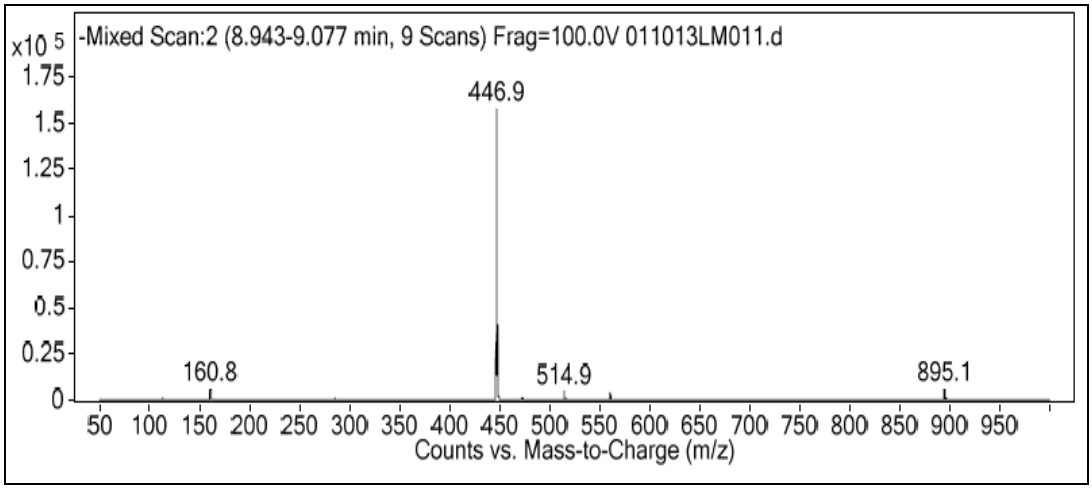


**FIG. 5: MASS SPECTRA OF SKP-LF/ (ICF-PCc)**

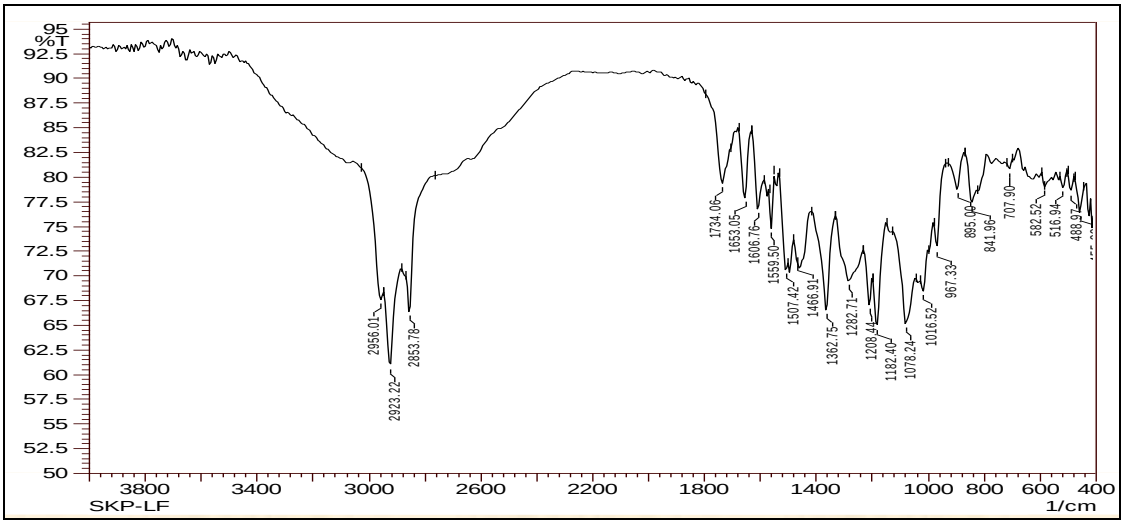


**FIG. 6: IR SPECTRA OF SKP-LF/ (ICF-PCc)**

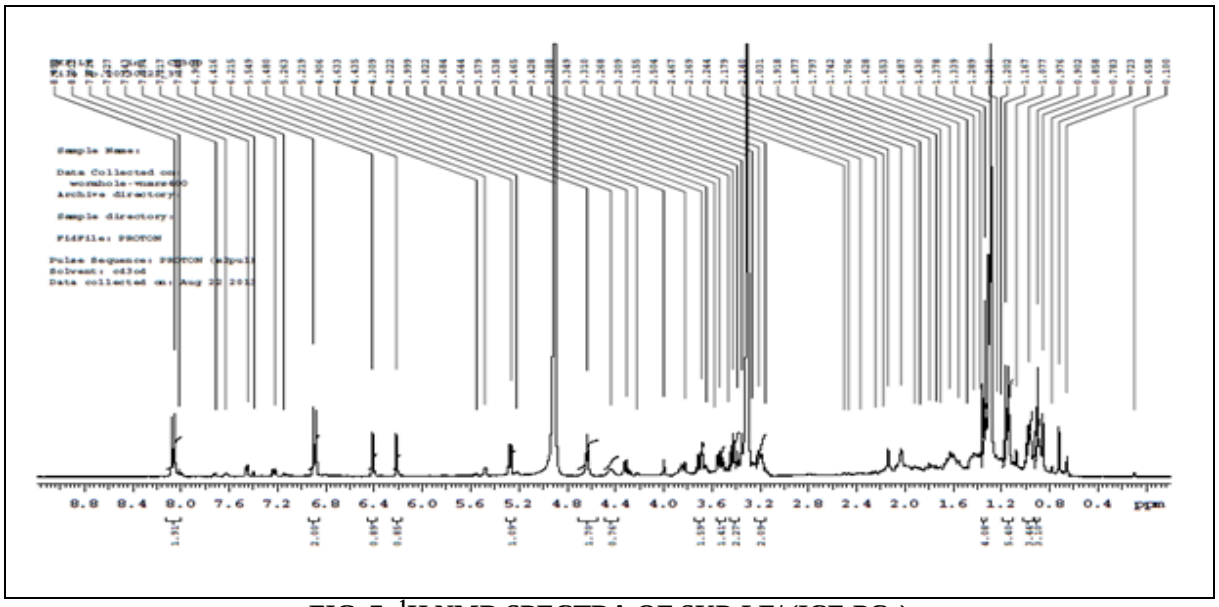


**FIG. 7: $^{1}$H NMR SPECTRA OF SKP-LF/ (ICF-PCc)**

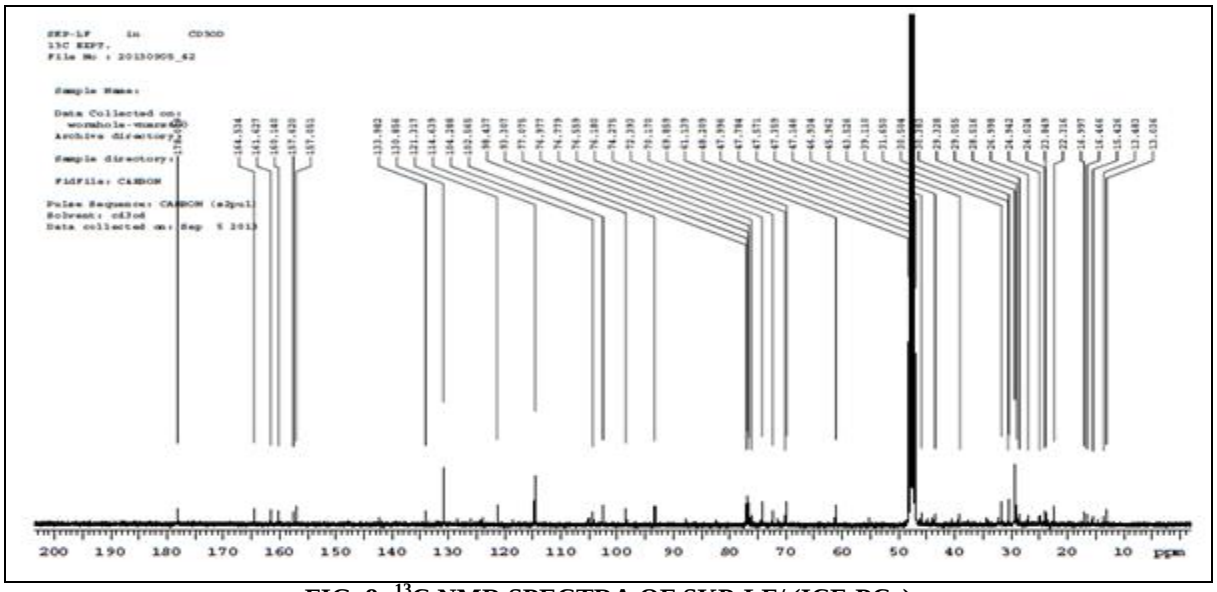


**FIG. 8: $^{13}$C NMR SPECTRA OF SKP-LF/ (ICF-PCc)**

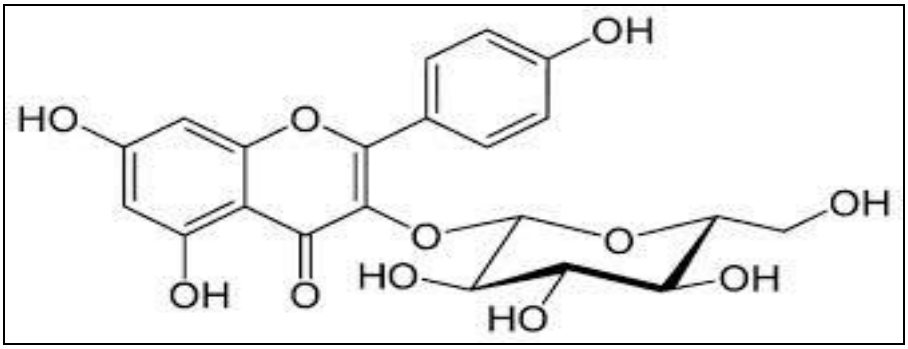


**FIG. 9: STRUCTURE OF POSSIBLE ISOLATED COMPOUND i.e., SKP-LF/ (ICF-PCc)**

**IUPAC name:**

5,7-dihydroxy-2-(4-hydroxyphenyl) – 3 -[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy - 6 - (hydroxymethyl) oxan-2-yl]oxychromen-4-one

**Other names:**

Astragaline/Asragalin/Kaempferol-3-glucoside/Kaempferol-3-O-glucoside/Kaempferol-3-D-glucoside/Kaempferol-3-beta-monoglucoside/Kaempferol 3-O-β-D-glucopyranoside

**DISCUSSION:** The various adverse factors such as environmental, physiochemical, pathophysiological and nutritional etc are antagonistic to the antioxidant defense mechanisms. These factors result in oxidative stress due to failure of the suppressing mechanisms in regulating the reactive oxygen species or the pro-oxidants. Plant derived components are now used as a safe source of antioxidants and chemo protective agents against oxidative damage. Plant derived components such as phenolics, flavonoids and other phytochemicals play significant role in giving protection against oxidative damage.

The phytochemical screening of the extract revealed the presence of steroids, alkaloids, phenolic groups, flavonoids, tannins, coumarins and anthraquinones etc (**Table 3**) among which the flavonoids and polyphenolics are known to have antioxidant properties [22-25]. The total phenolic and flavonoid content were estimated to be 43mg of GAE and 96.6 mg/g QE which were significant in contributing antioxidant potency to the extract concerned. This is supported by the report that polyphenolic and flavonoid content provides the enhanced antioxidant property in rats [8, 11-12].

Phenolic compounds are known to contribute the quality and nutritional values of foods in terms of colour, aroma and flavor and also providing health-beneficial effects [26, 27]. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and molecular damage and damage by microorganisms, insects and herbeveros [28]. The phenolic compounds have been recognized as free radical terminator [26]. The significant differences in the phenolic content of the extracts may be attributed to different solvents used. Like phenolics, the flavonoids serve as potential antioxidant or free radical scavenger by hydrogen donating activity [29]. This is primary attribute to high reactivity of hydroxyl substituent that participates in the reaction [30].

The DPPH scavenging activity of the ethyl acetate fraction of the methanolic extract showed higher activity with low $IC_{50}$ value of 117.03µg/ml was recorded. A lower $IC_{50}$ value implicated a batter antioxidant of the fractionated extract. This reactivity validated the use of the extract in free radical scavenging. Similarly the extract showed higher hydroxyl scavenging activity measured 241.80µg/ml compared with the other fractions of the extract. The hydroxyl scavenging activity seem to be the most important one as it can damage almost every molecule found inside the living cell and become the initiator of lipid peroxidation[3].

The correlation studies revealed a negative correlation between the $IC_{50}$ value and TPC and TFC (**Table 4**). $IC_{50}$ value showed a stronger negative relation with TFC as compared to the TPC there by suggesting a pronounced influence of the flavonoids in the antioxidant activity in terms of

DPPH and hydroxyl radical scavenging activity. Our findings are in agreement with the results of various researchers [31-35] suggesting correlation between antioxidant activity and phenolics and flavonoid content. Hence, it may be said that the phenolics and flavonoids are not only the metabolites but also influencing the antioxidant activities of the plant extracts. Wide variety of other phytochemicals may also have an influence on the antioxidant potentials of different plants.

To identify the concerned antioxidant compounds, HPTLC analysis was done with the chromatogram med EA-MECC with $R_f$ values ranging from 0.59 to 0.87. The peak number 2 showed higher percentage of area of about 66.96 may exhibiting flavonoid derivative. Further, the UV, IR, $^{13}C$ NMR, $^{1}H$ NMR and LC-MS data identified the compound as : 5,7,4'-trihydroxy – 3- O- β- D-galactopyranosyl flavon Or Kaempferol 3-O-β-D-glucopyranoside, which was a flavonoid compound that strongly suggesting antioxidant activity of the extract.

**CONCLUSION:** The present preliminary phytochemical analysis coupled with TPC, TFC and *in vitro* antioxidant study evaluated the herbal as a potential source of antioxidants along with identification of flavonoid derivative that opens vista to explore its various pharmacological activities. Plants are considered to be the gift of nature to human beings especially to the naturologists who read their nature and activities, the scientific manipulation of which may make the humankind healthy safe and sound. Day by day data from the plant world is accumulating from folklores and many plants are yet to be scientifically validated. The paper in this regard has made a subject attempt to establish the scientific validity of a plant named *C. coromandelicum* (Rubiaceae) reported for coramandel costal region of India produced to be various ethnomedicinal uses.

**ACKNOWLEDGEMENTS:** The authors would like to acknowledge AICTE (QIP), Govt. of India for financial support, Botanical Survey of India, Kolkata for authentication of plant specimen & Department of Pharmaceutical Science & Technology, Birla Institute of Technology, Mesra, Ranchi, India for providing the research facilities to carry out the study.

**DECLARATION OF INTEREST:** The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

## REFERENCES:

1. Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. Antioxidant activity in vitro of the selenium-contained protein from the Se-enriched Bifidobacterium animalis 01. Anaerobe 2010; 16(4): 380-386.
2. Havkin – Frankel. D., Dorn. R. and Leustek. T. Plant tissue culture for production of secondary metabolites. Food Technol. 1997; 51(11): 56–61.
3. Shao HB, Chu LY, Lu ZH, Kang CM. primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. Int. J Biol. Sci. 2008; 82: 47-95.
4. Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002; 82: 47-95.
5. Murphy MP, Holmgren A, Goran Larsson N, Halliwell B. unraveling the biological roles of reactive oxygen species. Cell Metab 2011; 13(4): 361-366.
6. Venkatesh S, Deecaraman M, Kumar R, Shamsi MB, Dada R. Role of reactive oxygen species in the pathogenesis of mitochondrial DNA (mt DNA) mutations in male infertility. Indian J Med Res 2009; 129: 127-137.
7. Peng KT, Hsu WH, Shih HN, Hsieh CW, Huang TW, Hsu RWW. The role of reactive oxygen species scavenging enzymes in the development of septic loosening after total hip replacement. J Bone Joint Surg 2011; 93-B: 1201-1209.
8. Ling LU, Tan KB, Lin H, Chiu GNC. The role of reactive oxygen species and autophagy in safingol-induced cell death. Cell death & Disease 2011; 2: 1-12.
9. Paramaguru R, Silambujanaki P, Bavanieswaran M, Rao ChV, Rawat AKS, Vijayakumar M. Free radical scavenging and lipid peroxidation inhibition potential of various fractions of Murraya panniculata. Pharmacologia 2012; 3(5): 138-143.
10. Ali SS, Naresh K, Abhinav L, Angad S, Hallihosur S, Abhishek S, et al. Indian medicinal herbs as sources of antioxidants. Food Res Int 2008; 41: 1–15.
11. Pari L, Amudha K. Antioxidant effect of naringin on nickel induced toxicity in rats: an in vivo and in vitro study. Int J Pharm Sci Res 2011; 2(1): 151-158.
12. Battu GR, Ethadi SR, Veda Priya G, Swathi Priya K, Chandrika K, Venkateswara Rao A. Evaluation of antioxidant and anti-inflammatory activity of Euphorbia heyneana Spreng. Asian Pac J Trop Biomed 2011; 1(2): 191-194.
13. Lawrence R, Kapil L. Antioxidant activity of garlic essential oil (Allium sativum) grown in north Indian plains. Asian Pac J Trop. Biomed. 2011; 1(1): 1-3.
14. Warrier P.K, Nambiar. V.P.K, Ramankutty C, and Vasudevan Nair R. Indian medicinal plants. Orient Blackswan publishers, New Delhi. 1996: Pp-366.
15. Mahishi Parinitha, Srinivasa B.H., and Shivanna, M.B. Medicinal plant wealth of local communities in some villages in Shimoga District of Karnataka. India Journal Ethno pharmacology. 2005; 98 (3): 307-312.

16. Anitha. B., Mohan.V.R, Athiperumalsami.T and Sudha, S. Ethno medicinal plants used by the kanikkars of Tirunelveli District, Tamil Nadu, India to treat skin diseases. Ethno botanical Leaflets. 2008; 12: 171-180.
17. Mohideen. S., Ilavarasan. R., Hemalatha. S., Anitha, N., and Sasikala, E. Wound healing and diuretic activities of Canthium parviflorum Lam. Natural Product Sciences. 2003; 9 (2): 102-104.
18. Ayyanar. M., Sankarasivaraman K., and Ignacimuthu S. Traditional Herbal Medicines used or the treatment of Diabetes among two major tribal groups in south Tamil Nadu, India. Ethnobotanical Leaflets. 2008; 12: 276-280.
19. Harborne JB. Phytochemical Methods, A guide to modern techniques of plant analysis, 3rd Edn. Chapman and Hall, New York, 1998; pp. 1-150.
20. Senthil kumar R, Rajkapoor P, Perumal P. Antioxidant activities of Indigofera cassioides Rottl. Ex. DC. Using various in vitro assay models. Asian Pac J Trop. Biomed 2012; 256-261.
21. Halliwell B, Gutteridge JMC. Free radicals, aging and diseases, in free radicals biology and medicine. Oxford: Oxford University Press; 1989.p. 419-422.
22. Arti RV, Vijayakumar M Rao ChV, Mathela CS. In vitro and in vivo antioxidant properties and DNA damage protective activity of green fruit of Ficus glomerata. Food chem Toxicol 2010; 48: 704-709.
23. Victorio CP, Kuster RM, Lage CLS. Detection of flavonoids in Alpinia purpurata (Vieill.) K. Schum leaves using high performance liquid chromatography. Rev. Bras. Pl. Med. Botucatu 2009; 11(2): 147-153.
24. Kazuaki Yamasaki, Ryogo Hishiki, Eisuke Kato, Jun Kawabata. Study of Kaempferol Glycoside as an Insulin Mimic Reveals Glycon to be the key structure. ACS Medical Chemistry Letters 2011; 2(1): 17-21.
25. Sen S, Chakraborty R, Sridhar C, Reddy YSR. Free radicals, antioxidants, diseases and Phytomedicine: current status and future prospect. Int. J Pharmaceutical Sci. Rev Res 2010; 3(1): 91-100.
26. Atnassova M, Georgieva S, Ivancheva K. Total phenolic and total flavonoid contents, Antioxidant capacity and Biological contaminants in Medicinal Herbs. Journal of University of Chemical Technology and Metallurgy 2011; 46(1): 81-88.
27. Wagh KBM, Wagh VD, Toshniwal SS, Sonawane, BR. Phytochemical, antimicrobial evaluation and determination of total phenolic and flavonoid contents of Sesbania grandiflora flower extract. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(4): 229-232.
28. Vaya J, Belinky PA, Aviram M. Antioxidant constituents from Licorice root: Isolation, Structure elucidation and antioxidant capacity towards LDL oxidation. Free Radical Biol. Med 1997; 23(2): 303-313.
29. Pal RS, Ariharasivakumar G, Girhepunjhe K, Upadhay A. In vitro antioxidant activity of phenolic and flavonoid compounds extracted from seeds of Abrus precatorius. International journal of Pharmacy and Pharmaceutical Sciences 2009; 1:136-143.
30. Heim KE, Tagliaferro AR, Boblia DJ. Flavonoids antioxidants: Chemistry, metabolism and structure-activity relationships. The Journal of Natural Biochemistry 2002; 13: 572-584.
31. Yang JH, Lin HC, Mau JL. Antioxidant properties of several commercial mushrooms. Food Chem 2002; 77: 229-235.
32. Kalikonen MP, Hopia AI, Vuorela HJ, Rauha JP, Philaja K, Kujala TS. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricaltural Food Chem 1999; 47: 3954-3962.
33. Startil P, Klejdus B, Kuban V. Determination of total content of phenolic compounds and their antioxidant activity in vegetative evaluation of spectrophotometric methods. Journal of Agricaltural Food Chem 2006; 54: 607-616.
34. Kratchanova M, Deher P, Ciz M, Lozek A, Mihailrv A. Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction systems. Acta Biochemica Polonica 2010; 57(2): 229-234.
35. Chanu KV, Ali MA, Kataria M. Antioxidant activities of two medicinal vegetables: Parkia javanica and Phlogacanthus thyrsiflorus. Journal of Pharmacy and Pharmaceutical Sciences 2010; 4(1): 102-106.

**How to cite this article:**
Patro SK and Sasmal D: *In vitro* Antioxidant Study And Search For A Novel Bioactive Compound From Leave Fractions of *Canthium Coromandelicum* (Burm.F.) Alston. Int J Pharm Sci Res 2015; 6(9): 3841-52. doi: 10.13040/IJPSR.0975-8232.6(9).3841-52.