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Research Article

Characterization and pharmacological screening of flavonoids isolated from *Begonia trichocarpa Dalzell*

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ABSTRACT

This work was designed to characterization and evaluation of in vitro Anti -oxidant, Anti-microbial activity, estimation of cytotoxicity of cells and anti-diabetic activity of fractnation compound from methanol extract of Begonia trichocarpa belong to family Begoniaceae. Isolation and characterization of phytoconstituents from Begonia trichocarpa for the first time. Isolation of phytoconstituents were carried out By means of column chrotography and physiochemical and spectroscopic analysis carried out to identified two compound luteolin and quercetin studied for different pharmacological activity of quercetin. Quercetin showed good anti-microbial activity, MIC of quercetin was found in between 800- 3200µg/ml against different microorganisms. MIC of E.coli, S.mutans and P. aerogenosa, S. aureus and S.typhimurium was found 800 µg/ml, 1600 µg/ml 3200µg/ml respectively. A dose dependent anti-oxidant activity was found in both DPPH free radical scavenging method and Nitric oxide scavenging method. DPPH and Nitric oxide assay of quercetin shows maximum percentage of inhibition 64.51% and 49.43% at the concentration of 400µg/ml, whereas ascorbic acid exhibits 70.63%, 53.34% respectively and IC_{50} values of DPPH assay was 650.1950µg/ml, and ascorbic acid 165.5796 µg/ml respectively. IC_{50} value of Nitric oxide scavenging assay 381.7617µg/ml and ascorbic acid was 338.179. MTT assay showed 43.34892% viability at 100 µg/ml a nd Ic50 values was 71.8009 /ml. In vitro anti diabetic activity by Inhibition assay for alpha - glycosidase showed 88.52% inhibition at 200µg/ml. Anti-oxidant, Anti-microbial activity, estimation of cytotoxicity of cells and anti - diabetic activity of *Begonia trichocarpa dalzell* may be due to the presence of quercetin.

Keywords: Anti- microbial activity; *Begonia trichocarpa dalzell*; Cytotoxicity of cells; DPPH assay; Luteolin.

INTRODUCTION

Herbal medicine or traditional treatment has been jumped over and renewed interest in the treatment against different diseases. Herbal medicines are generally non -toxic and with less or no side effects. WHO has recommended effectiveness and rather than the use of herbal medicine. The use traditional remedies is advantageous but has some limitations the main limitation is lack of its scientific support so researchers have forced to use of modern scientific methodology and technique to provide a new face to herbal medicine through isolation, characterization and estimation of its therapeutic activity. Chromatography is the common method used for fractnation of phytoconstituent, different type of chromatographic method are TLC, Gas chromatography, liquid chromatograpy and column chromatography. Out of this column chromatography is commonly used for fractionation of phytoconstituents. Characterization of compound was carried with spectroscopic analysis. (Agarwal S S., 2007.)

Begonia trichocarpa belong to family Begoniaceae, called kalle puli in Malayalam, which is an endangered species growing in the central part of kerala, Traditionally Pawra Tribe of Satpura Hills, Maharashtra, India, used for Opacity of eyes. 1or 2 drops of begonia leaf juice are dropped in eyes for one time. Khatadya is the local name of Begonia trichocarpa in pawra tribes. (Database from Wikipedia),

The present study aim to characterize column chromatography fractionated compound quercetin and estimate its antimicrobial activity, anti- oxidant activity, cell cytotoxicity and anti-diabetic may be the effect of quercetin.

MATERIALS AND METHOD

Plant collection and preparation of extract

Whole plant of Begonia trichocaropa was collected from the rural areas of Kottayam district Kerala, India. The whole plant material was identified, authenticated by Head of the department, Post Graduate and Research Department of Botany, St Thomas College, Pala,

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Kottayam. Begonia trichocarpa leaf was washed, shade dried, powered and successively extracted with Hexane, petroleum ether, chloroform, ethyl acetate and methanol and water (in the order of increasing polarity).The solvents were removed completely under reduced pressure and a semi solid mass was obtained (Pulok k Mukherjee., 2002)

Column chromatographic fractionation of methanol extract of Begonia trichoocarpa Column of (500×10mm)was packed with silica gel G (100-200# Nice) slurry(in chloroform) was used as stationary phase, Gradient elution was performed from nonpolar to polar solvents. Eluted fractions were collected, pooled together, evaporated and carried out TLC on pre-coated aluminum sheets of silica gel GF 254, 0.2mm to find out the presence of component in eluted fractions. Iodine detection was done. Total 390 fractions were eluted, out of this the fractions 11-20,281-291, 306-310 and 371-390 has single banding pattern and was confirmed by TLC study. The fraction 306-310 combined and dried at room temperature, the residue was tested for its purity and named as CCL3.The same was preceded for fractions 381-390 and named as CCL4.The remaining fractions were not taken for further work because of low yield and as well as impure. The structural elucidation of compounds were done with help of FTIR, LC-Q-TOF, 13C NMR and 1 H NMR. (HARBORNE J B., 1998)

Estimation of anti-oxidant activity in vitro methods

Estimation of anti-oxidant activity of CCL3 was performed by the method of DPPH radical scavenging activity and Nitric oxide radical scavenging activity.

DPPH free radical scavenging activity

DPPH solution (60M) was mixed with test solution in DMSO at different concentrations. The sample was kept for 15 minute at room temperature and decrease in absorbance was measured. Ascorbic acid is used as standard and Methanol was used as blank. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula. (Velusamy Kalpanadevi et al., 2012).

$$\%\, Inhibition = \frac{(B - T)}{C} \times 10$$

B = absorption of blank sample (t= 0 min)

C = absorption of test extracts solution (t=15 minutes)
T = absorption of test solution.

Nitric oxide radical scavenging activity

Determination of nitric oxide radical scavenging activity was determined by sodium nitro prusside method (Marcocci et al., 1994.) sodium nitro prusside dissolved in phosphate buffer saline (pH7.4) was prepared and mixed with sample at various concentration (100-400µgm/ml). The mixture was incubated at 29^{0}C, after 150 minute of incubation. Incubated solution was mixed with Griess reagent and incubated at room temperature for 30 minutes, the absorbance at 546nm was measured. Ascorbic acid was used as standard. The amount of nitric oxide radical inhibition was calculated by using equation,

$$\%\, Inhibition\ of\ NO\ radical = \frac{(A0 - A1)}{A0} 100$$

Where A0 is the absorbance before reaction and A1 absorbance after reaction has taken place with Griess reagent (Francis M. Awah et al.,2010)

Evaluation of antimicrobial activity and minimum inhibitory concentration

Minimum inhibitory concentration of isolated against different microorganism was done by Tube dilution method. Double fold serial dilution was done to dilute the initial concentration of the plant extracts in to sterile nutrient broth to obtain 5mg/ml concentration. The sterile plant extract transferred in to sterile nutrient broth to obtain 5mg/ml concentration. The above process was repeated to several times to obtain dilutions of 10mg/ml, 20mg/ml, 40mg/ml, 80mg/ml, 160mg/ml and 320mg/ml, Having obtained the different concentrations of the extracts, each concentration was inoculated with standardized Microorganism cell suspension and incubation was done at 37^{0}C for 24 hours. A single test tube without plant extract is kept as control. The growth of the inoculum in the broth is indicated by turbidity or cloudiness of the broth and the lowest concentration of the extract which inhibited the growth of the test organism were taken as the Minimum Inhibitory Concentration (MIC). The OD of the culture where checked at 600nm. Microorganisms tested for antimicrobial activity and MIC are 1. E coli MTCC-40, 2. Staphylococcus aureus MTCC-6908, 3. Salmonella typhimurium MTCC-3224 4. Streptococcus mutans MTCC- 497 5. Pseudomonas aerogenosa MTCC- 4673 (Andrew JM., 2001)

Determination of in vitro anti proliferative effect on cultured heLa cells

Determination of in vitro anti proliferative effect of compound on cultured heLa cells was determined by the % difference in viability by standard MTT assay method after 24 hours of incubation described by Arung et al in 2000. The cells was washed with 1x PBS and then added MTT solution (3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) to the culture. It was then incubated at 370C for 3 hours. MTT was removed by washing with 1x PBS and DMSO was added to the culture Incubation was done at room temperature for 30 minutes until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a micro plate reader (ELISASCAN, ERBA).

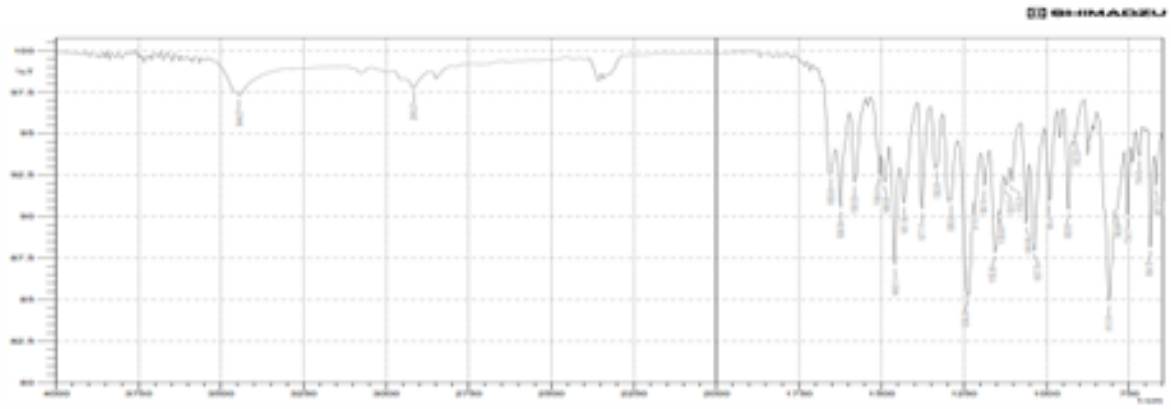


Figure 1: FTIR spectral data of quercetin

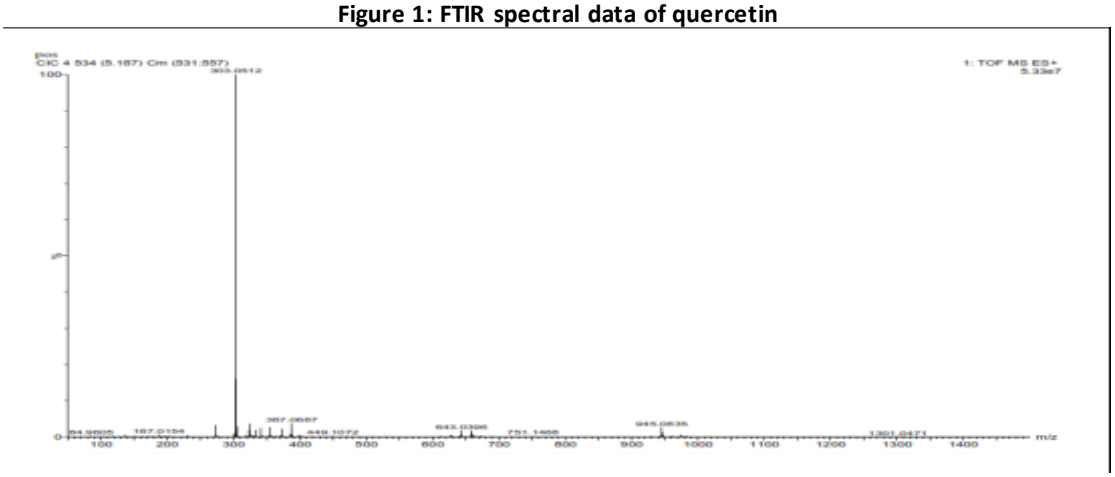


Figure 2: LC-Q-TOF data of quercetin

Table 1: MIC of the compound quercetin

concentration of extract (µg/ml)	E. col		Staphylococcus aureus		S.typhimurium	
	% of inhibition	MIC (µg/ml	% of inhibition	MIC (µg/ml	% of inhibition	MIC (µg/ml)
12.5	41.63%	800	36.29		37.26	3200
25	57.44%		46.12		48.68	
50	65.48%		53.75		54.92	
100	72.37%		57.54		61.79	
200	87.31%		66.47		72.22	
400	95.12		75.97		82.97	
800	99.71%		85.19		85.81	
1600	99.15%		88.63		96.42	
3200	99.71%		98.59		99.71	

Table 1a: MIC of the compound quercetin

concentration of extract (µg/ml)	Streptococcus mutans		Pseudomonas aerogenosa	
	% of inhibition	MIC (µg/ml)	%of inhibition	MIC(µg/ml)
12.5	39.08	1600	36.63	1600
25	46.30		46.67	
50	53.43		54.50	
100	60.20		62.23	
200	75.48		71.34	
400	90.46		83.17	
800	98.31		89.54	
1600	99.71		99.15	
3200	99.43		99.71	

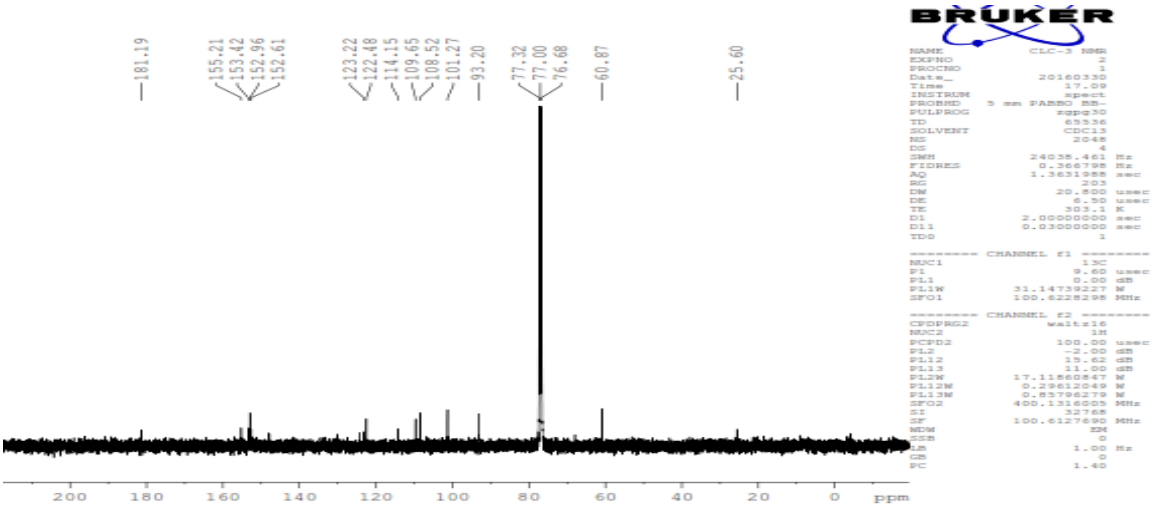


Figure 3: 1H NMR data of quercetin

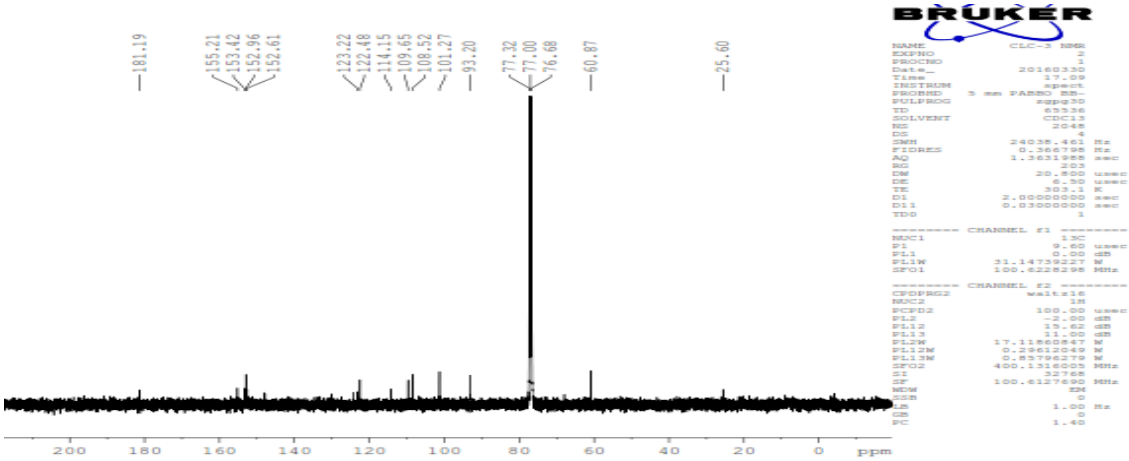


Figure 4: 13CNMR data of quercetin

Table 2: DPPH radical scavenging activity of compound quercetin

Concentration (µg/ml)	Percentage Inhibition of Compound CCL3	Percentage Inhibition of Ascorbic Acid
50µg/ml	36.29	33.59
100 µg/ml	44.08	40.79
200 µg/ml	53.22	64.28
400 µg/ml	64.51	70.63

Table 3: Nitric oxide radical scavenging activity of compound quercetin

Concentration (µg/ml)	Percentage Inhibition of compound CCL3	Percentage Inhibition of Ascorbic acid
50µg/ml	13.11	17.40
100 µg/ml	24.08	29.25
200 µg/ml	37.07	42.11
400 µg/ml	49.43	53.34

Table 4: Percentage Viability of compound quercetin

Sample volume (µg/ml)	Average Absorbance 540nm	Percentage Viability
6.25 µg/ml	0.6888	89.47779
12.5 µg/ml	0.5394	70.07015
25 µg/ml	0.4446	57.75526
50 µg/ml	0.4165	54.10496
100 µg/ml	0.3337	43.34892

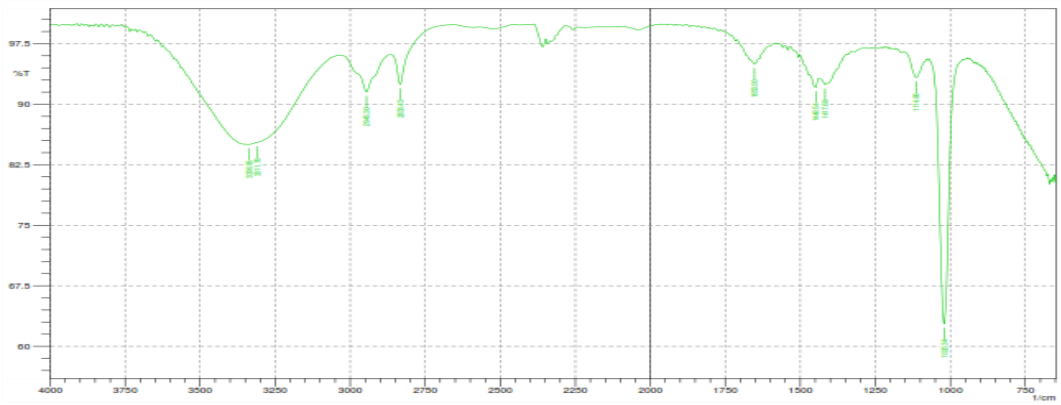


Figure 5: FTIR spectral data of luteolin

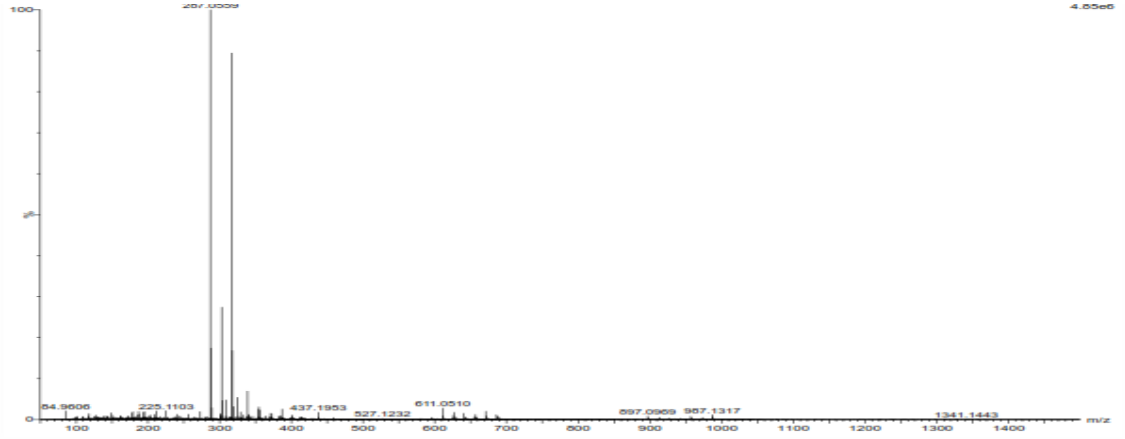


Figure 6: LC-Q-TOF data of luteolin

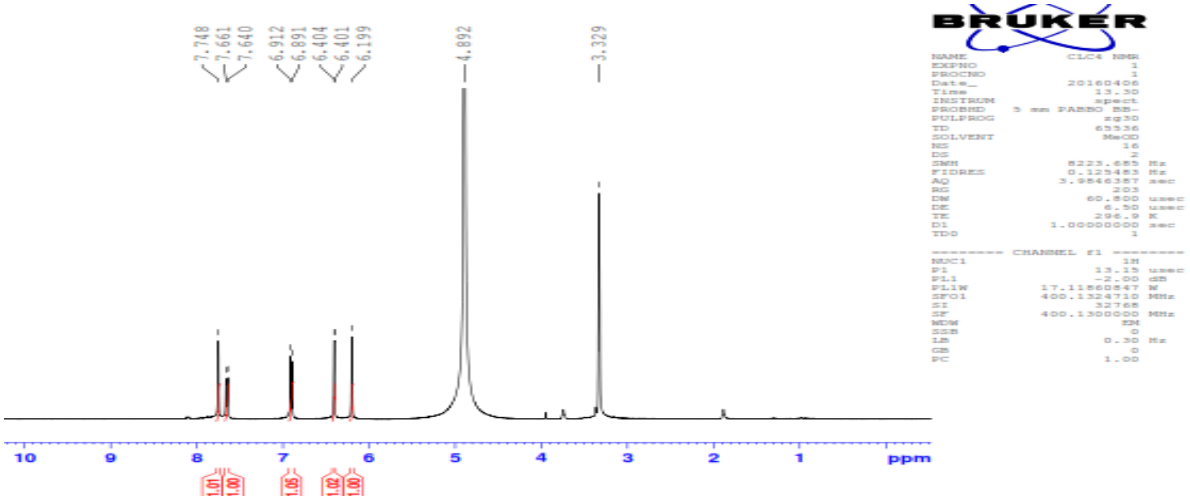


Figure 7: 1H NMR data of luteolin

$$\%Viability = \left(\frac{OD\ of\ Test}{OD\ of\ Control}\right) \times 100$$

Cytotoxicity assay by Direct Microscopic observation was also carried out. (Rathinam prema et al., 2012)

In-vitro evaluation of Anti diabetic activity by Inhibition assay for alpha-glycosidase

Alpha glycosidase activity was measured by in vitro by determination of reducing sugar arising from hydrolysis of sucrose by alpha glycosidase enzyme. The effect of isolated compound quercetin on α glycosidase activity was assayed according to the method Matsui et al., with slight modifications (Ankita bachhawat J et al., 2011)

RESULT AND DISCUSSION

Interpretations and observations of fractionation compound

The compound CCL3 in its IR (KBr) cm-1 spectra exhibited absorption band at 3500-3250cm-13444.87 for OH stretching and 2916.37 for CH aromatic Stretching, band at 1750-1500 cm-1 for 1653 and 1624.06 C=C

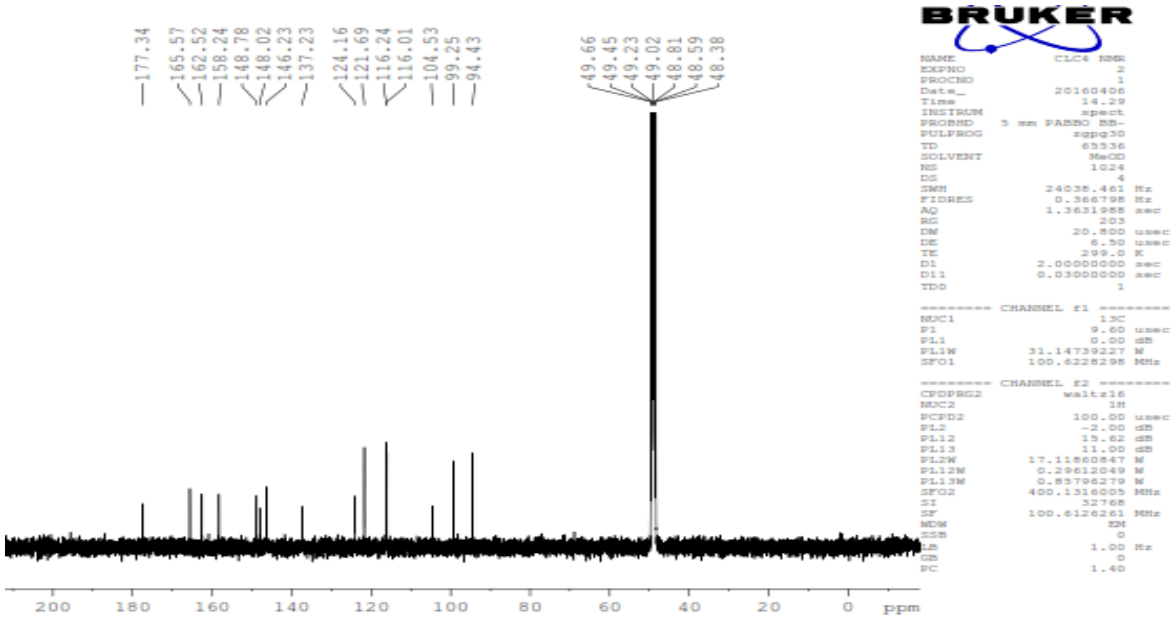
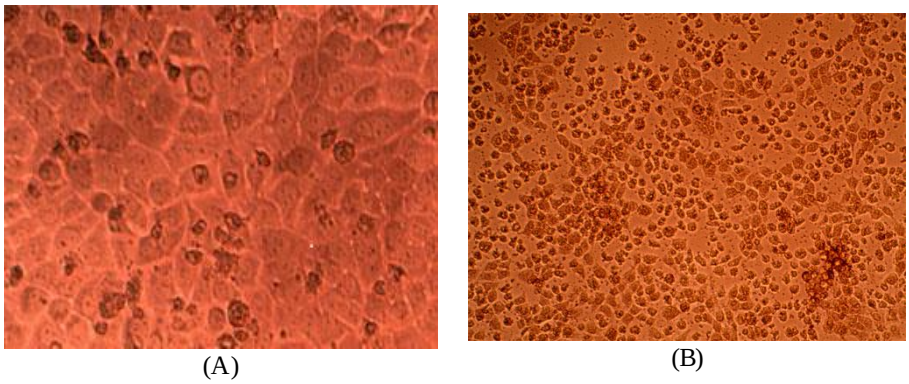


Figure 8: 13C NMR spectra of luteolin



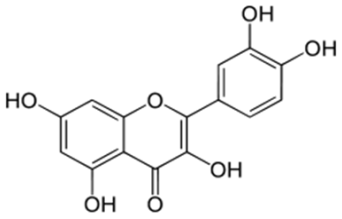
(A) (B)

Figure 9: Cytotoxicity assay by direct microscopic observation A. Microscopic observation of control, B. Isolated quarcetin

Stretching. The LC-MS data showed a peak at retention time 5.1and molecular weight of 303 in positive mode. H NMR showed the presence of 5 OH groups at the position 3, 6, 8, 3 and 4'.The band at 13.146, 13.094, 4.058, 3.868 and 3.770 indicate the phenolic and alcoholic OH respectively. Aromatic ring proton(s) at 7th, 9th, 2'nd 5' 6' indicate the band at 6.023, 6.54, 6.91, 6.97 and 7.88 respectively. The Band at 1.602, 1.876 and 1.278 indicate the presence of glucose at aromatic ring. The glycoside part which contain aromatic group that indicate the band between 6 -8, it shows presence of 10 protons.13CNMR shows band at 181.19 indicate c=o group, aromatic carbon indicate the band at101.27-155.21 and 25.60, 60.87, 93.20 are aliphatic carbon band. (J B Harborne., 1988, Schlor research library., 2012).

Physical properties isolated Quercetin

Amorphous yellow powder with melting point 317^{0}c, TLC shows single spot with R*f* value 0.94.



Quarectin

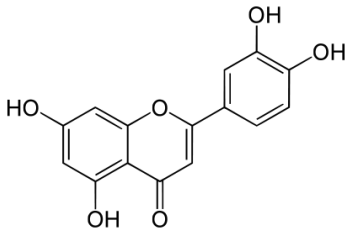
Interpretation and observation of Luteolin

The IR of the compound CCL4 shows absorption band at 3500-3200 cm^{-1} 3336.85 and 3311.78 for phenolic OH, Band exhibited at 3000-2750cm^{-1} 2945.30 and 2833.43 aromatic CH stretching, 1750-1500cm^{-1} 1653 for cyclic ketone, 1500-750cm^{-1} for aromaticity, 1500-1250 cm^{-1} for alkyl group. The LC- MS data exhibited an M+ peak at retention time 5.6 and molecular weight 287.6559 in positive mode. 1H NMR spectra shows 5-aromatic protons of bands at 6.19, 7.7, 6.9, are single let and 6.40, 7.6 which are double let protons and pro-

tons of -OH group merged with solvent peak exhibited at 3.329, intense peak at 4.892 for phenolic –OH. 13C NMR show the presence of 12 aromatic carbon indicating the band between 104 to 165. So, presences of two aromatic ring. Band at 177.34 for C=O. A peak at 99.25, 94.43 for alkyl group. (Vera Francisco et al, 2014, Hao Liuet.et al.2010).

Physical properties of luteolin

Dull crystalline yellow powder with melting point 268^0C. Rf value by TLC: 0.77.



Luteolin

Pharmacological screening

The observations of pharmacological screening of isolated quercetin from Begonia trichocarpa exhibited antimicrobial activity, antioxidant activity, cell cytotoxicity and anti-diabetic activity. MIC of isolated quercetin found in between 800-3200µg/ml against different microorganisms. Anti-microbial activity of plant quercetin was compared with synthetic antibiotic (Itaru Hirai et al., 2010), anti-cancer activity of quercetin was studied (Lara Gibellini et al.,2011),isolated quercetin also exhibited good cytotoxicity, 43.34892 % viability at 100 µg/ml and IC_{50} values was 71.8009 µg/ml. Flavonoid compound quercetin and its derivative exhibits different pharmacological activity (Aneela Maalik et al., 2014) DPPH and Nitric oxide assay of quercetin shows maximum % inhibition 64.51% and 49.43% at the concentration of 400µg/ml and the In vitro anti-diabetic study performed by testing % Inhibition of alpha- glycosidase exhibited by quercetin. Fractnated flavonoid quercetin of Begonia trichocarpa shows antimicrobial activity, anti-oxidant activity, anti-cancer activity and anti-diabetic activity, provide evidence for the different pharmacological activity of naturally occurring flavonoid quercetin.

CONCLUSION

Begonia trichocarpa contain poly phenolic compound quercetin and luteolin, the antioxidant activity, antimicrobial activity (sindhu jose et al., 2016) cytotoxicity and anti-diabetic activity of Begonia trichocarpa may be due to its quercetin content. Flavonoids are polyphenolic compounds possessing 15 carbon atoms represented as C_6-C_3-C_6. Flavonoids have an oxygen bridge between ortho position of benzene ring (a) and the benzylic carbon atom adjacent ring B. Besides the oxygen bridge flavonoids have the typical oxygen pattern in their ring the substitution are –OH,-OCH_3,-O-CH2-O- or glycosides. Flavonoid stabilize the reactive oxygen species by reacting with the reactive compound of the radical. Because of the high reactivity of the hydroxyl group of the flavonoid results its antimicrobial activity, anti-oxidant activity, anti-cancer activity and anti-diabetic activity.

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