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Isolation and Characterization of 4', 5, 7,-trihydroxy isoflavone from *Crinum defixum* Ker-Gawler plant leaves and their Biological activities

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A B S T R A C T

The present study was carried out to isolation and characterization of Flavone compounds present in the *Crinum defixum* Ker-Gawler leaves, which is an important medicinal plant. Worldwide trends towards the utilization of natural plant remedies have created an enormous need for the use of medicinal plants. Different pharmacological properties of *C.defixum* Ker-Gawler have already been reported. The plant was extracted for various solvents in increasing order of polarity from using n-hexane, chloroform, ethyl acetate, acetone, ethanol, butanol and methanol. Thus, the present study was performed to investigate the preliminary phytochemical screening, isolation and characterization of flavone compounds present in the *C.defixum* Ker-Gawler leaves using FT-IR, GC-MS, NMR and MASS Spectral techniques and their biological activities.

Keywords: *Crinum defixum* Ker-Gawler, Phytochemical screening, Isolation, Characterization, FT-IR, GC-MS, NMR, MASS Spectra, 4', 5, 7-Trihydroxy isoflavone.

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1. Introduction

Medicinal plants play important role in human health; they produce a definite physiological action to the human body. Phytochemicals are bioactive chemicals of plant origin. These bioactive constituents are called secondary

metabolites such as alkaloids, tannins, flavonoids, glycosides, carbohydrates and phenolic compounds. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruit, seeds etc. i.e., the whole plant contains active components[1].The plant extracts and bioactive compound which isolated from medicinal plants are used for antibacterial, antifungal and antiviral therapy[2]. The presence of phytochemicals in the plant parts may differ from one part to another. The plant derivative drugs have been in practice for a very long time [3]. The plant derivative drugs may be of importance to the pharmaceutical industry, researchers and folk healers in the natural areas[4].The biologically active compounds present in plant material are separated from various suitable solvents are used in the extraction procedure [5]. Therefore the aim is ethno-medical use and subsequently the isolation and characterization of compound which will be added to the potential list of drugs.

C.defixum Ker-Gawler (Amaryllidaceae) has abundantly growing on rivers, canals in dry and wet conditions. The *C.defixum* have commercial, economical and medicinal importance. The *C.defixum* Ker-Gawler is one of the *Crinum* genus [6]. It is commonly known as Bon-naharu (Meaning wild garlic) this plant having number of medicinal activities. The leave extracts are used to treat pimples, itching, body-ache, leprosy, paronychia and otitis. The crushed bulbs are used to treating nausea, emetic, emollient, diaphoretic, burns, whitlow and carbuncle [7].The bulbs of this plant is fusiform, flowers are sessile, fragment at night and tinged with red [8]. The *C.defixum* is reported to contain the active constituents such as caranine, crinamine, crinine, galanthamine, hippestrine, galanthine, haemanthamine. In recent years the new alkaloid 5 - hydroxyhomolycorine has also been reported [9]. The ethanol and methanol extracts of the *C.defixum* Ker-Gawler have been reported to possess free radical scavenging activity, antianalgesic activity antigenotoxic properties [10]. The main focus of this study was isolation and characterization of flavone compounds present in the *C.defixum* Ker-Gawler leaves and their biological activities.

2. Materials and method

2.1. Collection of plant materials

The leaves of *C.defixum* Ker - Gawler were collected from Poondi village, Thanjavur District, Tamilnadu. The botanical identity (Voucher No: A.A.R 003 on 04-02-2013) of the plant of was confirmed by Dr. S.John Britto, Rapinat Herbarium, St. Joseph's College, Tiruchirappalli.

2.2. Preparation of Extracts

The fine powder (5 kg) was extracted with 95% ethanol at room temperature for ten days. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator and extracted for various solvents in increasing order of polarity from using n-hexane, chloroform, ethyl acetate, acetone, ethanol, butanol and methanol. After that the extract was taken in a beaker and kept in a water bath and heated at 30-40 ºC till all the solvent got evaporated. All the extracts were tested for the presence bioactive compounds by using standard methods. The dried extract was subjected to preliminary phytochemicals, isolation and characterization of flavone compounds present in the *C.defixum* Ker-Gawler leaves.

2.3. Phytochemical screening

The preliminary phytochemical analysis of various extracts of *C.defixum* Ker-Gawler plant leaves revealed the following phytochemicals (Table.1).

2.4 Isolation of Phytocomponent:

The 95% ethanolic extract was subjected to column chromatographic separation eluted with chloroform/ methanol system (4.75:0.25, 4.85:0.15, and 4.95:0.05). The fraction was crystallized with methanol to obtain crystalline yellow needles. The isolate compounds were identified by spectral studies.

3. Results and Discussion

3. Characterization of 4', 5, 7,-trihydroxy isoflavone

3.1. GC-MS Analysis

The GC- MS analysis of methanolic extract of *Crinum defixum* Ker-Gawler leaves shows the RT value is 17.13 and Peak area is 100% composition. The data was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The results were clearly indicating the 4', 5, 7-Trihydroxy isoflavone. Similarly the 4',5,7-Trihydroxy isoflavone was identified chloroform and butanol fractions of *Crinum defixum* Ker-Gawler shows same value of RT(chloroform = 17.32, butanol = 17.27) and 100% compositions. So 4',5,7-Trihydroxy isoflavone is a majaor compound of *Crinum defixum* Ker-Gawler leaves(Fig.1).

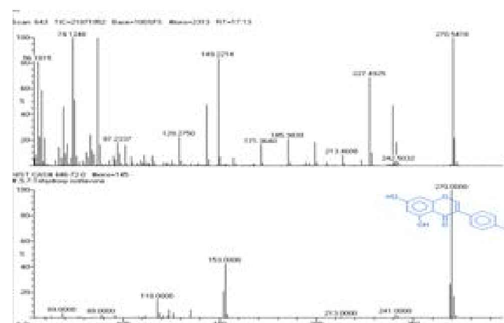

Figure 1: GC-MS- Spectrum of 4', 5, 7,-trihydroxy isoflavone

3.2. FT-IR Analysis

IR (KBr) -The absorption appeared at 3230 cm^{-1} indicating the OH vibrations of hydrogen bonds, the absorptions 1462 and 1444 cm^{-1} indicating that C-H vibrations of two CH_3 protons, the absorption 1383 cm^{-1} indicate that CH_3 vibration of CH_3 proton, the absorptions 1326, 1264 cm^{-1} indicate that OH vibration of phenol OH groups and the absorption 965 cm^{-1} indicate that CH vibration of C=C groups are present in the molecule.

3.3. ^{1}H- NMR Analysis

^{1}H NMR ($CDCl_3$, TMS) 500MHz - The peaks appeared at ppm: 6.28 (1H, t, H-2), 6.844 (2H, d, H-3), 7.045 (1H, d, H-6), 6.906 (1H, d, H-8), 7.429 (2H, d, H2' & 6') and 6.562 (2H, d, H3' & 5'). The peaks at 7.045, H-6 (J =16.5 Hz), 6.906, H-8 (J = 16.0) showing doublets resonated to two

aromatic protons present in the phenol hydroxyl group in the ring A. The peaks 7.429, H-2' & 6' (J = 6.5) and 6.562, H-3' & 5' (J = 2.5) showing two *m*-coupled doublets appeared in the ring B. The one singlet appeared at 6.28, H-2 (J = 2.5), and one doublet appeared at 6.844, H-3, (J = 8.5) in the ring A.

3.4. ^{13}C- NMR Analysis

iii) ^{13}C NMR($CDCl_3$, TMS) 500MHz - The peaks appeared at ppm: 128.21(C-2), 101.78 (C-3), 205.90 (C=O), 158.69 (C-5), 125.94 (C-6), 158.00 (C-7), 125.94 (C-8), 139.00 (C-9), 127.82 (C-10), 129.02 (C-1'), 104.75 (C-2',C-6'), 115.52 (C-3', C-5') and 157.29 (C-4'). The ^{13}C NMR spectrum showed the presence of 13 carbon signals including the overlapping peaks of C-3' & C-5' and C-2'& C-6' thus indicating the presence of 15-carbon skeleton. The ^{1}H and ^{13}C NMR values for all the carbons were assigned on the basis of HMQC and HMBC correlations. The carbonyl carbon is bonded at C-4 position (C 205.90 (C=O)) when the carbonyl is not hydrogen bonded. But Hydrogen bonded with neighboring 5-Hydroxy group in the downfield. Carbon bonded to the hydroxyl group C-5, C-7 and C-4' appeared at 158.69, 158.00 and 157.29. The ^{13}C NMR resonance at C 125.94 which showed HMBC correlations with ^{1}H NMR resonance at H-6 & H-8 was attributed to C-7. The ^{1}H NMR peaks at 7.429, H-2' & 6' (J = 6.5) and 6.562, H-3' & 5' (J = 2.5) is coupled (HMQC correlations) with ^{13}C NMR data of 7.429, d, (6.5) and 6.562, d, (2.5) respectively in the ring B. In the ^{1}H NMR spectrum of the compound shows the aromatic proton signals of two *m*-coupled doublets of 6.28, t and 6.562, d, (each J = 2.5 Hz) showing HMQC correlations to the carbon resonances at C-2 128.21and C-3' and 5' 115.52. Based on their above mentioned data and comparisons of ^{1}H and ^{13}C NMR data with those given in the literature and corresponding to the molecular formula $C_{15}H_{12}O_5$, it was concluded that 4', 5, 7,-trihydroxy flavone i.e., Genistein extracted from leaves of *Crinum defixum* Ker-Gawler. Even though a lot of work has been done based on the ^{1}H and ^{13}C-NMR parameters of flavonoids [11,12,13, 14].

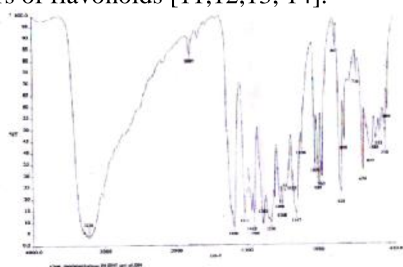

Figure 2: IR-Spectrum of 4', 5, 7,-trihydroxy isoflavone

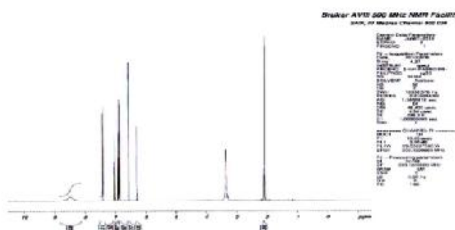

Figure 3: ^{1}H-NMR - Spectrum of 4', 5, 7,-trihydroxy isoflavone

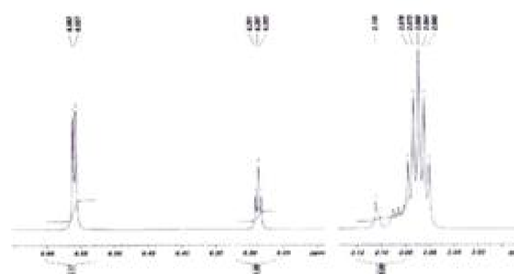

Figure 3: a. Enlarged ^{1}H-NMR - Spectrum of 4', 5, 7,-trihydroxy isoflavone

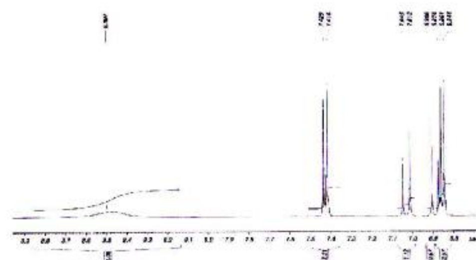

Figure 3: b. Enlarged ^{1}H-NMR - Spectrum of 4', 5, 7,-trihydroxy isoflavone

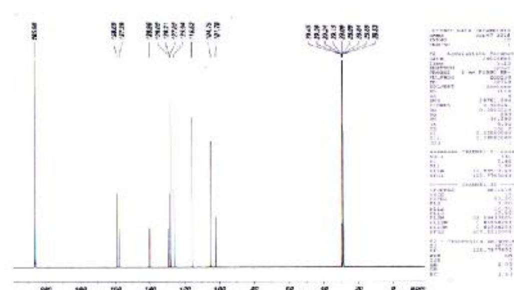

Figure 4: ^{13}C- NMR - Spectrum of 4', 5, 7,-trihydroxy isoflavone

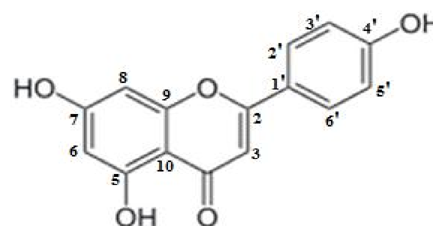

Figure 5: Structure of 4', 5, 7,-trihydroxy isoflavone (Genistein)

4. Biological activities of of 4', 5, 7,-trihydroxyisoflavone

4. 1. Antioxidant activity (DPPH Method)

1,1-Diphenyl-2-Picryl-hydrazyl (DPPH) is free radical but stable[15, 16]. DPPH is violet in colour which donates a hydrogen ion in to the solution [17]. The colour change is maintained by spectrophotometer and free radical scavenging activity is calculated. The method described by Shimada *et al*., 1992;[18] was used. Stock solution of 25 µg/mL of DPPH in methanol was made. Different concentrations of isolated compound (methanol fraction)

(20, 40, 60 and 80 µg/mL) were chosen for *in-vitro* antioxidant activity. L-Ascorbic acid was used as the standard. Briefly, a 2 mL aliquot of DPPH methanol solution (25µg/mL) was added to 0.5 mL sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a (UV 1800-SHIMADZU) spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity and % scavenging was calculated by the following equation.

$$\text{Radical scavenging activity (\%)} = 100 - \left[\frac{A_C - A_S}{A_C}\right] \times 100$$

Where $A_{C=}$ control is the absorbance and $A_{S=}$ sample is the absorbance of reaction mixture (in the presence of sample).

Statistical Analysis

Tests were carried out in triplicate for 3-5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a non-linear regression algorithm.

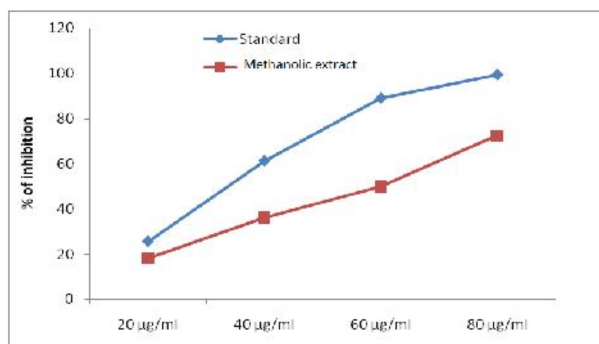

Figure 6: Comparison of % scavenging of DPPH by ascorbic acid *vs* 4',5,7,-trihydroxy isoflavone

4. 2. Antimicrobial activity (disc diffusion method)

The Antimicrobial activity was evaluated by isolated (methanol fraction) compound of *C.defixum* Ker-Gawler leaves using disc diffusion method.

4.2.1. Preparation of Media

Nutrient Agar (NA-Himedia) Media for Bacteria

Composition of Media

Animal's tissue	:	5.00 g
Sodium chloride	:	5.00g
Beef extract	:	1.50g
Yeast extract	:	1.50g
Agar	:	15.0g

4.2.2. Preparation of medium:

Suspend 28.0 grams in 1000 mL distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121˚c) for 15 minutes. Mix well and pour into sterile Petri plates.

4.2.3. Potato Dextrose Agar (PDA-Himedia) Media for Fungi

Composition of Media.

Potatoes infusion from	:	200.00g
Dextrose	:	20.00 g- allignment
Agar	:	15.00g

4.2.4. Preparation of medium:

Suspend 39.0 grams in 1000 mL distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121˚c) for 15 minutes. Mix well before dispensing in specific work, when pH 3.5 is required; acidify the medium with sterile 10 % tartaric acid. The amount of acid required for 100 mL of sterile cooled medium is approximately 1 mL. do not heat the medium after addition of acid.

4.2.5. Microorganisms

Pure culture of *Escherichia coli* specie of bacteria and *Candida albicans* specie of fungi were procured from *Rontgen Laboratory,* Thanjavur. These microorganisms were identified and confirmed by Microbiologists, Department of Microbiology, Thanjavur Medical College, Thanjavur.

4.2.6. Preparation of 24 hours pure culture

A loop full of each of the microorganisms was suspended in about 10mL of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37ºC for 24 hours except for fungal which was incubated at 25ºC for 24-48 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8˚C until use.

4.2.7. Preparation of plant extracts solutions for the experiment

The dried isolated (methanol fraction) compound of *C.defixum* Ker-Gawler were weighed (10mg/mL) and dissolved in sterile distilled to prepare appropriate dilution to get required concentrations of about 50µL (50 mg), 100µL (100 mg) and 150µL (150 mg). Control used as respective solvent (Aqueous). They were kept under refrigerated condition unless they were used for the experiment. Standard solution as chloramphenicol (25mg/mL) for bacteria and fluconazole 30µL (30 mg) for fungi used to compare the test solution. They were kept under refrigerated condition unless they were used for the experiment.

4.2.8. Preparation of dried filter paper discs

Whattman filter paper (No:1) was used to prepare discs approximately 6 mm in diameter, which are placed in hot air for sterilization. After sterilization, the discs were loaded with different concentrations of prepared plant extract solutions and again kept under refrigeration for 24 hrs.

4.2.9. Application of discs to inoculated agar plates

Previously prepared paper discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down firmly to ensure complete contact with the agar surface. The discs were placed on the medium suitably apart and the plates were incubated at 5°C for 1 hr to permit good diffusion and then transferred to incubator at 37°C for 24 hrs. After completion of 24hrs, the plates were inverted and placed in an incubator set to respective temperature for 24 hrs.

4.2.10. Antimicrobial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka et al., 2007)[19] using plant extracts. Petri plates were prepared by pouring 30 mL of NA/PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of

micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Escherichia coli* were spread on Nutrient agar plates for bacteria and *Candida albican was* spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µL, 100 µL and 150 µL) were laid down on the surface of inoculated agar plate. The plates were incubated at 37 C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate.

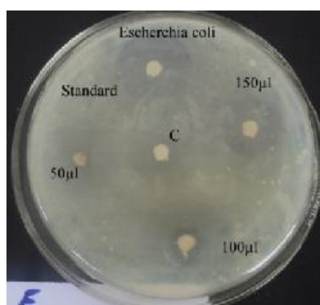

Figure 7: Zone inhibitions of Antibacterial activity of 4', 5, 7,-trihydroxy isoflavone against *Escherchia coli* bacteria at different concentrations.

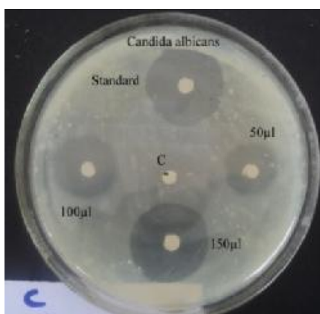

Figure 8: Zone inhibitions of Antifungal activity of 4', 5, 7,-trihydroxy isoflavone against *Candida albicans* fungi at different concentrations

N.Thi Ngoc Tram *et al* (2002) [20] reported that (-) Galanthamine, (+) Haemanthamine (3-Epicrinamine, Hemanthamine), Hipeastrine, (-) Lycorine (Narcissine, Galanthidine) types of alkaloids are reported that the after 1985. Recently a new alkaloid 5 -hydroxy homolycorine has also been isolated from *Crinum defixum* Ker-Gawler bulbs and M.Bordoloi *et al*,(2009) reported that (E)-N' -[(E)-2-butenoylhydrazide2 has been isolated from *Crinum defixum* Ker– Gawler root bulb. This Hydrazide compound imparted a clear dose dependent protective effect against the genotoxic effect of H_2O_2 with those reported in the literature. For the first time 4', 5, 7,-trihydroxy isoflavone (Genistein) has been isolated successfully from the medicinal plant *Crinum defixum* Ker-Gawler leaf under present study. Antioxidant activity of *Crinum defixum* Ker-Gawler leaves clearly indicates that the higher in antioxidant potential in DPPH assay method (table-3) and Antimicrobial activity of *Crinum defixum* Ker-Gawler plant extract were effective against both antibacterial and anti-fungal activities (table-4 and 5).

4. Conclusion

The preliminary phytochemical analysis of various extracts of *Crinum defixum* Ker-Gawler leaves contains many bioactive chemicals like alkaloids, flavonoids, saponins, terpenoids, amino acids and phenolic compounds (table-1). The GC- MS studies of *Crinum defixum* Ker-Gawler leaves clearly indicate that the 4', 5, 7-Trihydroxy isoflavone (Genistein) is a major compound (Identified from chloroform, butanol and methanol fractions). It shows very good antioxidant and antimicrobial properties. The isolation of the characterized flavanoids would be useful to prepare plant based pharmaceutical preparation to treat various complications linked with human diseases.

5. Acknowledgement

I wish to express my deep sense of gratitude and most sincere thanks to Honorable Resource Person Dr. G. Chandramohan, Principal, Jairams Arts and Science College, Karur-3, Tamilnadu, India for providing support to finish my research work.

Discussion

Table 1: Preliminary phytochemical constituents of *C.defixum* Ker-Gawler leaves

S.No	Phytochemicals	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Acetone Extract	Ethanol Extract	Butanol Extract	Methanol Extract
1.	Alkaloids	-	Present	Present	Present	Present	-	-
2.	Flavonoids	-	Present	-	-	-	Present	Present
3.	Terpenoids	Present	Present	Present	-	-	-	-
4.	Glycosides	-	-	-	-	-	-	-
5.	Saponins	-	Present	Present	Present	Present	-	-
6.	Steroids	Present	-	-	Present	-	-	-
7.	Carbohydrates	-	-	-	-	-	-	-
8.	Phenolic Compounds	Present	Present	Present	Present	Present	Present	-
9.	Tannins	-	-	-	-	-	-	-
10	Amino acids	Present	-	-	Present	Present	Present	Present

Table 2: ^{1}H and ^{13}C- NMR data of compound-(CD-1) in acetone-*d*6

S.NO	Position	H (*J*, Hz)	c
1.	2	6.28, t, (2.5)	128.21
2.	3	6.844, d, (8.5)	101.78
3.	5		158.69
4.	6	7.045, d, (16.5)	125.94
5.	7		158.00
6.	8	6.906, d, (16.0)	125.94
7.	9		139.00
8.	10		127.82
9.	1'		129.02
10.	2' and 6'	7.429, d, (6.5)	104.75
11.	3' and 5'	6.562, d, (2.5)	115.52
12.	4'		157.29
13.	C=O		205.90

Table 3: DPPH radical scavenging activity of 4’, 5, 7,-trihydroxy isoflavone

S.NO	Concentrations (µg/mL)	4’, 5, 7,-trihydroxy isoflavone	Ascorbic acid (Standard)
1	20	18.18±1.27	25.6±2.04
2	40	36.31±2.54	61.26±4.90
3	60	49.95±3.49	88.98±7.11
4	80	72.5±5.07	99.34±7.94
	IC_{50}	56.52	35.03

Table 4: Antibacterial activity of 4’, 5, 7,-trihydroxy isoflavone against *Escherichia coli* bacteria at different concentrations

Sample	50 µL (mm)	100 µL (mm)	150 µL (mm)	Standard (Chloromphenical for bacteria) (mm)	Control (solvent)
4’, 5, 7,-trihydroxy isoflavone	6±0.42	9±0.63	10±0.70	13±0.91	0

Values are expressed as Mean ± SD for triplicate.

Table 5: Anti-fungal activity of 4’, 5, 7,-trihydroxy isoflavone against *Candida albicans* fungus at different concentrations

Sample	50 µL (mm)	100 µL (mm)	150 µL (mm)	Standard (Nystatin for fungi) (mm)	Control (solvent)
4’, 5, 7,-trihydroxy isoflavone	5±0.35	7±0.49	8±0.56	11±0.77	0

Values are expressed as Mean ± SD for triplicate.

6. References

[1] P. Tiwari, B. Kumar, M. Kaur, G. Kaur, H. Kaur,*int. pharm. Sciencia*, 2011, 1, 98-106.

[2] Anjali Ruikar, RasikaTorane, Amruta Tambe, Vedavati Puranik. Nirmala deshpande. *Int. J. Chemtech. Res*. 2009. 1 (2)

[3] M. Lahlou, *Phytother. Res.,* 2004, 18, 435-445.

[4] K. Das, R. K. S. Tiwari, D. K. Shrivastava, *J.Med. Plant Res.,* 2010, 4 (2), 104-111.

[5] Fennell CW and Van Staden J: *Crinum* species in traditional and modern medicine. Journal of Ethnopharmacology 2001; 78(1):15–26.

[6] Madhava Chetty k, Sivaji k, Tulasi RK, Flowering plants of Chittoor district. 1st ed. Tirupati (india): Students offset Printers; 2008.

[7] K.R. Kirtikar, B,D. Basu, Indian Medicinal Plants,vol. IV (1975) Published by M/S Bishen Singh Mohendra Pal Sing, New Connaught Place,Dehradun, PP. 2473-2474.

[8] Hooker JD, Flora of British India, Published underthe authority of the Secretary of state for India in Council, 1954

[9] Nguyen TNT, Titorenkovab TV, Bankovab V, Handjievab NV, Popovb SS. *Crinum* L. *Amaryllidaceae*. Fitoterpia, 2002; 73: 183-208.

[10] Jeffs PW, Abou-Donia A, CampauD, Staiger D.Structures of 9-*O*-dimethyl-homolycorine and 5 -hydroxyhomolycorine alkaloids of *crinum defixum*, C. *latifolium* Assignment of aromatic substitution patterns from ^{1}H-coupled ^{13}C spectra, J Org Chem. 1985; 50: 1732-1737.

[11] Harborne, J.B. *The Flavonoids: Advances in Research Since1986*; Chapman and Hall: London, UK, 1994; pp. 280-290.

[12] J.B. Harborne and T.J. Mabry, \The Flavonoids: Advances in Research", Chapman and Hall Ltd., London (1982).

[13] E Tayfen; H Sebnem; S Iclal; C Ihsan; O Yukio, *Turk. J. Chem*., 2002, 26, 581-588.

[14] M Fairouz; Z Amar; S Narimane; T Ahmed; R Salah; *Rec. Nat. Prod*., 2010, 4, 91-95.

[15] Sharma OP & Bhat TK, DPPH antioxidant assay revisited. *Food Chemistry*, 113 (2009) 1202.

[16] Barros L, Ferreira MJ, Queiros B, Ferreira ICFR & Baptista P, Total phenols, ascorbic acid, b-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry*, 103 (2007) 413.

[17] Szabo MR, Idtou C, Chambre D &Lupea AX, Improved DPPH determination for antioxidant activity spectrophotometric assay. *Chemistry and Materials Science*, 61(3) (2007) 214.

[18] Shimada K, Fujikawa K, Yahara K, & Nakamura T (1992). Antioxidative properties of *xanthum* on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry, 40:945-948.

[19] Awoyinka *et al*., 2007. Antibiogram was done by disc diffusion method using plant extracts (NCCLS, 1993)

[20] Nguyen TNT, Titorenkovab TV, Bankovab V,Handjievab NV, Popovb SS. *Crinum* L.*Amaryllidaceae*. Fitoterpia, 2002; 73: 183-208.