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ISOLATION AND CHEMICAL CHARACTERIZATION OF POTENTIAL BIOACTIVE COMPOUNDS FROM *CASSIA UNIFLORA*

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Keywords:

Cassia uniflora, Bioactive compounds, Spectral analysis, Flavonoid

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ABSTRACT: Objective: The present study was designed for Isolation of phytoconstituents from pharmacologically potent extracts of leaves of *Cassia uniflora* based on *in-vitro* pharmacological screening and their subsequent characterization. **Methods:** Crude extracts of leaves, stems, and fruits of *cassia uniflora* were prepared using various solvents such as water, methanol and hydro alcohol. These extracts were screened for *in-vitro* pharmacological activities like antioxidant, anti-inflammatory, and anti-diabetic activities. These active extracts were subjected to column chromatography by means of different mobile phases followed by TLC. The isolated compounds were subjected to IR, ^{1}H NMR, ^{13}C NMR, and LC-MS spectral analysis for chemical characterization. **Results:** The methanol extract of leaf of *Cassia uniflora* was found to be potent when compared with other extracts. This extract was subjected to column chromatography to get fractions and eluted fractions were run in TLC mobile phase with different solvent ratios. The fractions with similar R_f values to standard were united and crystallized. The spectral analysis confirmed that the isolated compounds were found to be Methyl inositol, Luteolin, pentacosane, and Triacontan-1-ol. **Conclusion:** various extracts from different parts of the plant *of Cassia uniflora* were prepared. Methyl inositol, Luteolin, pentacosane, and Triacontan-1-ol were isolated from the methanol extract of leaves and characterized.

INTRODUCTION: The high cost of contemporary medicines (mostly imported) and their unavailability in remote areas, and most prominently, the severe side effects of certain drugs, have resulted in a considerable return to traditional medicine.

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Since huge portions of pharmaceutical drugs are derived from medicinal plants, the demand for these raw materials has been progressively increasing. The evaluation of the effectiveness of the plant has been recommended by the World Health Organization (1980) in conditions where there is a lack of safe synthetic drugs. The chemicals obtained from the medicinal plants act as, imminent sources of new therapeutic agents, precursors for the synthesis of helpful drugs, and used as therapeutic purposes, Such as artemisinin for the cure of multidrug-resistant malaria, silymarin for hepatic protection; Vincristine and Vinblastine for various types of cancers.

Although herbs have been praised for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance for a while. However, a blind dependence on synthetics is ended, and the people are returning to the naturals with hope of safety and security. Plants, particularly used in Ayurveda offer biologically active molecules and lead structures for the progress of modified derivatives with improved activity and reduced toxicity. Hence, research to stumble on the scientific proof for claims of plants used for Indian Ayurvedic system of medicine has been intensified. Furthermore, these preparations are systematically evaluated and disseminated properly; our indigenous population can be given better access to effective drug treatment and improved health status. Thorough research on the chemistry and pharmacology of products of plant origin is greatly necessary, and this may ultimately lead to the discovery of new medications that can be used in the treatment of several diseases [1, 2].

Cassia (Senna) is a large genus belonging to the family Caesalpinaceae consisting of approximately 500 species as herbs, shrubs, and trees and are largely scattered all over Asia, including India, Mauritius, China, East Africa, South Africa, America, Mexico, West Indies, and Brazil. *Cassia uniflora* is an invasive weed, found in farming fields, wastelands, and open forests. *C. uniflora* is an annual, erect herb, 15-20 cm high, young appressed hairy branches, branches, and branchlets 4-5 angled. Leaf rachis is 6-12 cm long; leaflets are 3-5 pairs, ovate-lanceolate to oblanceolate, fulvous hairy on the lower side, white pubescent to glabrescent on upper side and is reported from Mahabubnagar and Kurnool districts.

C. uniflora possesses enormous medicinal value and has been utilized by tribal people in wound remedy, eczema and Combat dropsy [3]. This plant was selected from the books "Flora of the presidency of Madras". Vol.1 Adlard & Sons Ltd., London. Rep.Ed.1997, Dehradun [4]. Most of the *Cassia* species are rich in alkaloids, anthraquinones, anthocyanosides, phenolic flavonoids, saponins, tannins, terpenoids, steroids and cardiac glycosides [5-17]. Microbiologists, natural product chemists, and ethnobotanists are in a search of novel bioactive compounds have antioxidant, antimicrobial and anti-inflammatory properties from medicinal plants to treat transmittable diseases. *Cassia* species have been reported to possess Antioxidant [18-20], Antimicrobial [21-25], Antibacterial, Antifungal [26-31], Anti-inflammatory, Anthelmintic, Anti-arthritic [32-35], Antidiabetic [36-38], Hepatoprotectant [39-41] and Cytotoxic activities [42-47].

C. uniflora leaves are found to have larvicidal activity, a phytotoxic effect due to the existence of allele chemicals, and were analyzed by HPTLC [48, 49]. Anti-inflammatory, Analgesic, and Anti-arthritic, Antioxidant, Antimicrobial and Antibacterial activity of *C. uniflora* leaves have also been reported, which exposed that the leaves have been contained terpenoids, steroids, flavonoids and pungent and bitter essential oils [50-55]. The search for naturally occurring Luteolin has an immense concern in industries as well as in scientific researches as it has potent antioxidant and anti-inflammatory activity. Qualitative and quantitative information has not been given on effective isolation of the Luteolin. It is useful to explore their content in the *C. uniflora* leaves. Thus, in light of vast potentiality of medicinal plants as source of antimicrobial, anti-inflammatory and antioxidant compounds, an attempt was made to investigate phytochemicals from the leaves of *C. uniflora*. Moreover, there is very few literature information was established on the isolation of bioactive compounds. Four potential bioactive compounds are successfully isolated and characterized from the methanol extract of *C. uniflora* leaves; those are Methyl Inositol, Luteolin, n-pentacosane, and Triacontan-1-ol. This is the first report to isolate these bioactive compounds from *Cassia uniflora* leaves.

FIG. 1: PICTURE OF *CASSIA UNIFLORA*

MATERIALS AND METHODS:

Plant Collection and Authentication: During the vegetative stage, Stem, fruits, and leaves of *Cassia uniflora* were collected from plants grown without pesticides in the fields from Rajahmundry, East Godavari district. The herbarium specimen was identified and authenticated by Dr. K. N. Reddy, Department of taxonomy, Laila Impex R&D Centre, Vijayawada. They were collected in the month of January 2011. The plant materials were deposited in a raw drug museum. The voucher no. of *Cassia uniflora* leaf material was 3317. The Stem, fruits, and leaves were cut down and separated from other parts of the plants, cleaned and air-dried under the shade and powdered mechanically to a fine powder for further experimental use.

Preparation of Plant Extract: The extracts of various parts of the plant were prepared in a sequential procedure. At first by soaking each 100g of dried leaf powder in 600 ml of methanol, hydro-alcohol (60% methanol in water), and water, next by soaking each 45g of dried stem powder in 300ml of methanol, hydro- alcohol (60% methanol in water) and water, finally by soaking each 35g of dried fruits powder in 250ml of methanol, hydro-alcohol (60% methanol in water) and water for about 48 h to obtain methanol, hydroalcoholic and aqueous extracts of leaves, stem, and fruits of *C. uniflora*. At the last, the plant extracts were filtered using Whatman filter paper. The filtrates were then concentrated under reduced pressure in vacuum at 40 °C for 25 min using a rotary evaporator. Codes have been given for the above extracts as shown below.

CULM: *Cassia uniflora* leaf methanolic extract.

CULH: *Cassia uniflora* leaf hydro alcoholic extract.

CULW: *Cassia uniflora* leaf aqueous extract.

CUSM: *Cassia uniflora* stem methanol extract.

CUSH: *Cassia uniflora* stem hydroalcoholic extract.

CUSW: *Cassia uniflora* stem aqueous extract.

CUFM: *Cassia uniflora* fruit methanolic extract.

CUFH: *Cassia uniflora* fruit hydroalcoholic extract.

CUFW: *Cassia uniflora* fruit aqueous extract. These codes were used for the extracts in further discussion.

Pharmacological Screening: The obtained various extracts of different parts of *C. uniflora* were screened for *in-vitro* antioxidant activity by superoxide radical scavenging, DPPH radical scavenging, and ABTS radical scavenging activity, anti-inflammatory activity by 5-Lipoxygenase inhibitory activity, ant diabetic activity with alpha-amylase and alpha-glycosidase assay. Among all the extracts, the methanol extract of leaves of *C. uniflora* was found to be highly potent. So, it was forwarded to fractionation by column chromatography.

Column Chromatography: 30 g of methanol leaf extract was adsorbed on 90 gm of silica gel and finally 120 gm of silica gel adsorbed extract was packed over 360 gm of silica gel (100-200 mesh) (Merck) and then eluted with chloroform, mixtures of chloroform and acetone, acetone and methanol of increasing polarity to obtain fractions. The bed volume of solvents for the elution was 750 ml. Two-bed volumes were used. Elution first starts with 100% chloroform and then using a mixture of chloroform and acetone in the ratio of 98:2, 94:6, 90:10, 80:20, 50:50, and 100% acetone, finally 100% methanol. All the collected fractions were dart for TLC. Based on the TLC profile, fractions with similar R_f values were pooled into fraction 1-8 and filtered through Whatman number-1filter paper. The filtrates were concentrated under reduced pressure in a rotary vacuum evaporator and air-dried to a constant weight at room temperature.

The collected fractions (F1-8) were screened for *in-vitro* antioxidant activity. Fractions-1, 6 and 8 have been found good ABTS radical scavenging activity compared to the remaining fraction, and hence these fractions were isolated by column chromatography.

Isolation of Bioactive Compounds from Fractions: 2 g of fraction-1 (F-1) was adsorbed on 6 g of silica gel, and finally 8 g of silica gel adsorbed extract was packed over 50 g of the silica gel column and chromatographed. The bed volume of solvents for elution was 70 ml, three-bed

volumes were used. The extract was first eluted with a mixture of hexane and chloroform in the ratio of 98:2, and then the ratio was changed to 95:5.

1 g of fraction-6 (F-6) was adsorbed on 3 g of silica gel, and finally, 4 g of silica gel adsorbed extract was packed over 40g of the silica gel column and chromatographed. The bed volume of solvents for the elution was 100 ml, two-bed volumes were used. The extract was first eluted with 100% ethyl acetate, then a mixture of ethyl acetate and acetone in the ratio of 99:1, 96:4, and 90:10.

10 g of fraction-8 (F-8) was adsorbed on 30 g of silica gel, and finally, 40 g of silica gel adsorbed extract was packed over 120 g of silica gel column and subjected to column chromatography. The bed volume of solvents for the elution was 250 ml, two-bed volumes were used. The extract was first eluted with 100% ethyl acetate, then a mixture of ethyl acetate and methanol in the ratio of 95:5, 90:10, 85:15, 80:20, and 50:50.

Thin Layer Chromatography (TLC) Procedure: The TLC development was set as twin through chamber were examined in various solvent systems such as chloroform, methanol, hexane, and ethyl acetate. The best possible solvent system for the identification of compounds was determined by altering the ratios of solvents. These fractions were run on silica gel 60 F254 pre-coated aluminum plate of 0.2 mm thickness for the observation of spots after collection. Visualization was carried out by UV chamber. The retardation factor (R_f) was designed using the following formula.

R_f = Distance moved by the solute/Distance moved by the solvent

Chemical Characterization of Bioactive Compounds: Isolated bioactive fractions were subjected to spectral analysis using FT-IR, NMR, and LC-MS for chemical characterization.

FT-IR spectra were recorded on Bruker Alpha TKBR and ATR spectrophotometer operating at 500-4000 cm^{-1} using KBr pellet technique that shows peaks at different wavenumber ranges.

^{1}H and ^{13}C-NMR spectra were run on a Bruker AV NMR instrument equipped with 5 mm ^{1}H and ^{13}C operating at 400 MHZ and 100MHZ, respectively with tetramethylsilane (TMS) as an internal standard.

An Agilent 6400 series Triple, Quad (QQQ) LC-MS (Agilent, Beijing, China) instrument equipped with an electrospray ionization source (ESI) was employed to analyze the isolated compounds. The optimized detection parameters were as follows: Chromatographic conditions: Wavelength set as 190 to 400 nm, the Flow rate was 0.4 ml/min, the sampling volume was 5µl, and the column temperature was 30 °C.

Mass spectrometry conditions: negative ion mode, atomization gas pressure-40 psi, dry gas, velocity-9 l/min, drying temperature- 350 °C, ionization voltage- 3,000V, electron spray ionization (ESI), detection of anion way-auto MSN, scanning range-200~800 m/z.

RESULTS AND DISCUSSION:

Total Yield Extracts: The leaves stem and fruits of *Cassia uniflora* plant materials were collected dried and were extracted with different solvents. The final yield of various extracts in different solvents was calculated and listed in **Table 1**.

TABLE 1: FINAL YIELD OF VARIOUS EXTRACTS IN DIFFERENT SOLVENTS

Extract code	Quantity (g)	Solvents used	Volume (ml)	Yield (g)
CULM	100	Methanol	600	20.39
CULH	100	Hydro-alcohol	600	25.71
CULW	100	Water	600	30.89
CUSM	45	Methanol	300	4.0
CUSH	45	Hydro-alcohol	300	5.55
CUSW	45	Water	300	6.86
CUFM	35	Methanol	250	l4
CUFH	35	Hydro-alcohol	250	6.0
CUFW	35	Water	250	4.43

All the extracts were screened for *in-vitro* antioxidant, anti-inflammatory, and anti-diabetic activity. Amongst, the methanol extract of leaf was found to be a potent antioxidant, anti-inflammatory, and anti-diabetic activity, hence it was subjected to column chromatography for fractionation.

Fractionation: About 16 fractions were eluted from methanol extract using various solvents of increasing polarity. Based on the TLC profile, column fractions with similar R_f values were combined into various fractions (F-1 to 8) and crystallized. This process was repeated several times to get desired quantities. The weight of various fractions in different solvents (%) and mixing patterns is given in **Table 2**. The isolated fractions were screened for *in-vitro* antioxidant activity. Fractions-1, 6, and 8 have been found good ABTS radical scavenging activity compared to the remaining fraction; hence these fractions were isolated by column chromatography.

TABLE 2: MIXING PATTERN OF CULM COLUMN

Name of the solvent	Fractions	Weight (g)
Chloroform	F-1	2
2% Acetone in Chloroform	F-2	0.5
6% Acetone in Chloroform	F-3	0.6
10% Acetone in Chloroform	F-4	0.3
20% Acetone in Chloroform	F-5	0.540
50% Acetone in Chloroform	F-6	1.33
Acetone	F-7	3.85
Methanol	F-8	14

Isolation of Bioactive Compounds: About four subfractions were isolated (SF-1 to 4) from F-1 based on the TLC profile. The SF-1 and SF-2 were isolated with hexane and chloroform (98:2) and the SF-1 spot was identified in the TLC mobile phase solvent ratio of hexane: ethyl acetate (8:2) showed an R_f value of 0.87 equal to that of standard n-Pentacosane and SF-2 spot was identified in the solvent ratio of Hexane: chloroform (8:2) showed an R_f value of 0.37 equal to that of standard Triacontan-1-ol. The SF-1 and 2 were crystallized and were named as Compound - 3 and 4. Mixing patterns for all subfractions of fraction 1 is shown in **Table 3**.

TABLE 3: MIXING PATTERNS FOR SUB FRACTIONS OF F-1

Name of the solvent	Volume (ml)	Sub Fractions	Yield (g)
2% Chloroform in Hexane	70	SF-1	0.170
2% Chloroform in Hexane	2 × 70	SF-2	0.08
5% Chloroform in Hexane	70	SF-3	0.02
5% Chloroform in Hexane	2 × 70	SF-4	0.40

About three subfractions (SF-1 -3) from F-6 were isolated based on the TLC profile. The SF-1 was isolated with 100% ethyl acetate and the spot was identified in the TLC mobile phase solvent ratio of chloroform: methanol (8:2) showed an R_f value of 0.75 **Fig. 2** similarities that of standard Luteolin. The SF-1 pure compound crystals were formed, filtered from the mother liquor and washed with acetone. The crystals were soluble in methanol and named as Compound-2. Mixing patterns for all subfractions of a fraction 6 is shown in **Table 4**.

TABLE 4: MIXING PATTERNS FOR SUB FRACTIONS OFF-6

Name of the solvent	Volume (ml)	Sub Fractions	Yield (g)
Ethyl acetate	100	SF-1	0.6
Ethyl acetate	100	SF-2	0.01
1% Acetone in ethyl acetate	2 × 100		
4% Acetone in ethyl acetate	2 × 100	SF-3	0.10
10% Acetone in ethyl acetate	2 × 100		

Five subfractions (SF-1-5) from F-8 were isolated based on the TLC profile. The SF-3 was isolated with a mixture of ethyl acetate and methanol (90:10) and SF-4 was isolated with a mixture of ethyl acetate and methanol (85:15 and 80:20) and both the spots were Identical and Identified in the TLC mobile phase solvent ratio of chloroform: methanol (6:4) showed an R_f value of 0.5 **Fig. 2** similarities that of standard Methyl Inositol. These two pure crystalline compounds were filtered from the mother liquor and washed with hexane. The crystalline compound was soluble in methanol and named as Compound-1. Mixing patterns for all subfractions of a fraction 8 is shown in **Table 5**.

TABLE 5: MIXING PATTERN FOR SUB FRACTIONS OF F-8

Name of the solvent	Volume (ml)	Sub Fractions	Yield (g)
Ethyl acetate	250	SF-1	0.04
Ethyl acetate	250	SF-2	0.02
5% Methanol in Ethyl acetate	2 × 250		
10% Methanol in Ethyl acetate	2 × 250	SF-3	0.50
15%Methanol in Ethyl acetate	2 × 250	SF-4	3.31
20% Methanol in Ethyl acetate	2 × 250		
50% Methanol in Ethyl acetate	2 × 250	SF-5	2.90

Chemical Characterization of Purified Compounds: The purified bioactive compounds were characterized by spectral studies, *i.e.*, FT-IR, NMR, and LC-MS. The pure compound-1 was obtained as colorless, white crystalline solid having MP 180-182 °C. Fourier transmission Infra-red **(**FT-IR) spectrum of compound-1 was presented in **Fig. 2**. The characteristic peaks were observed at 3403.65, 3318.09 cm^{-1} (O-H stretch), 2951.77 and 2907.38 cm^{-1} (C-H stretch), 1452.43-1341.83 cm^{-1}

(CH_3 group of methoxy), 1452.43 cm^{-1} (Asymmetric bending deformation of CH_3 group), 1381.44 cm^{-1} (Bending vibration of CH_3), 1280.73-1192.87 cm^{-1} (H-O bend), 1129 cm^{-1} (C-O stretch), 1072.47, 1002 and 961 cm^{-1} (C-O-C stretch), 901-750 cm^{-1} (C-C bend), 661-437 cm^{-1} (C-H bend) respectively.

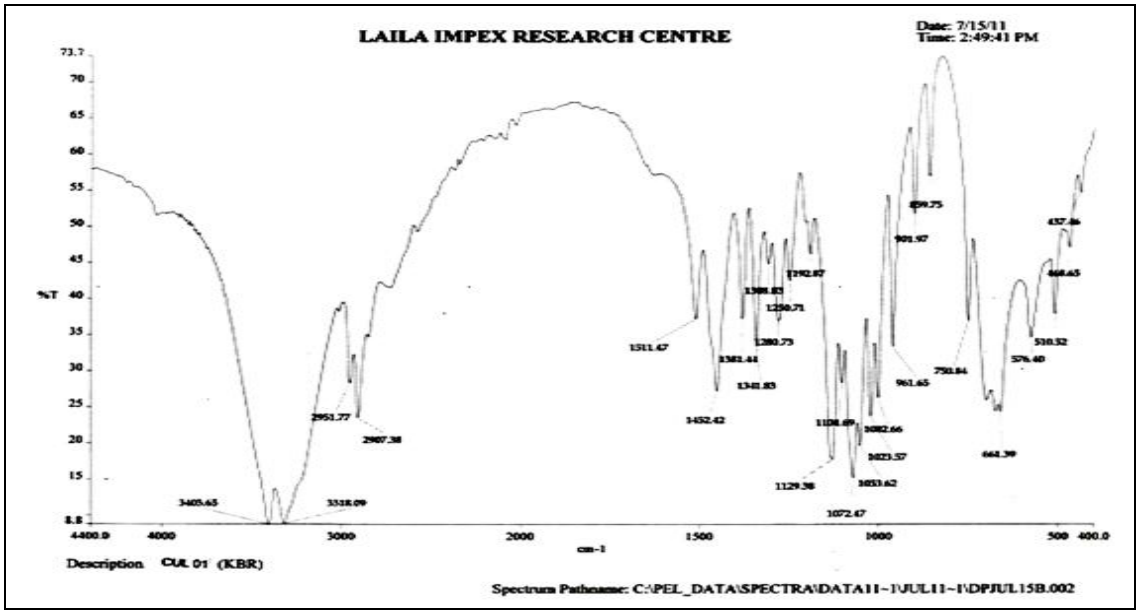

FIG. 2: IR SPECTRUM OF THE ISOLATED COMPOUND-1

^1H and ^{13}C nuclear magnetic resonance (NMR spectra of compound-1 were recorded in deuterated water (D_2O). Corresponding chemical shift (δ) values are expressed in ppm, and coupling constants (J) values are expressed in Hertz. ^1H NMR spectrum of compound-1 with resonance was shown in **Fig. 3**. The spectrum showed the presence of different δ values at

δ = 3.536 (S, 3H, OCH_3)

δ = 3.276 (t J=10.0Hz, 1H, CH)
δ = 3.574 (t J=9.6Hz, 1HCH)
δ = 3.670 (d d J=10.0, 2.8Hz, 1HCH)
δ = 3.756 (d d J=10.0, 2.8Hz, 1 H, CH) δ =3.974 (m, 2H, CH,)

The ^1H NMR spectrum of the compound revealed the presence of different types of neighboring H atoms and their splitting.

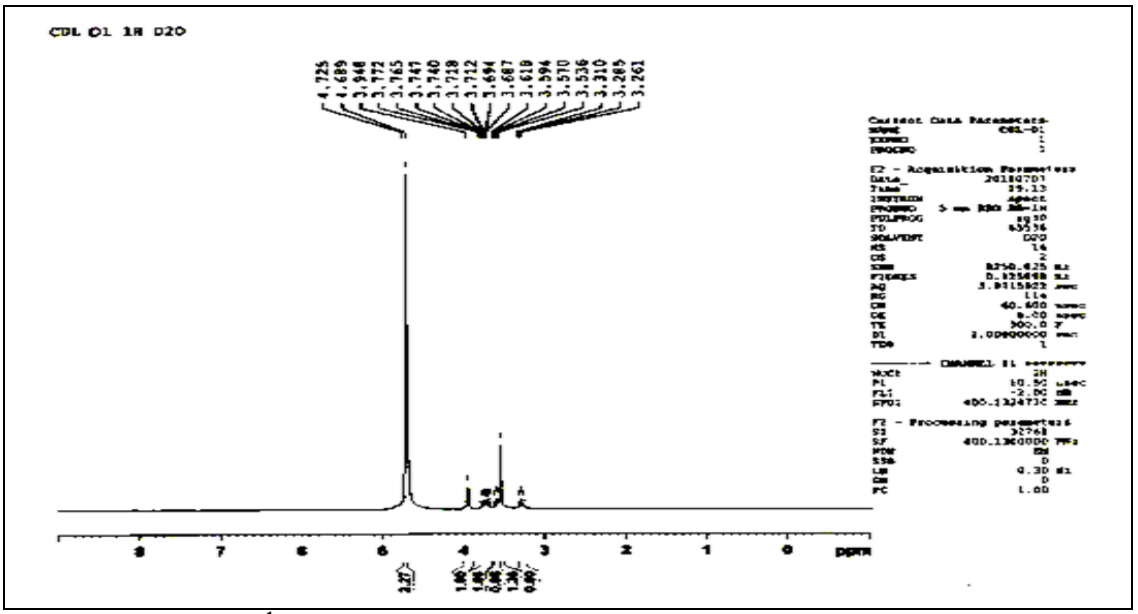

FIG. 3: ^1H NMR SPECTRUM OF THE ISOLATED COMPOUND-1

The ^{13}C NMR spectrum of compound-1 in **Fig. 4** showed different peaks at δ=59.70 (C, OCH_3), six oxygenated methane carbons resonating at δ=69.91 (C, CH), δ=70.66 (C, CH), δ=71.55 (C, CH), δ=71.77 (C, CH), δ= 72.18 (C, CH), δ=82.81 (C, CH) respectively. The ^{13}CNMR spectrum of the compound showed the presence of different C atoms in the compound.

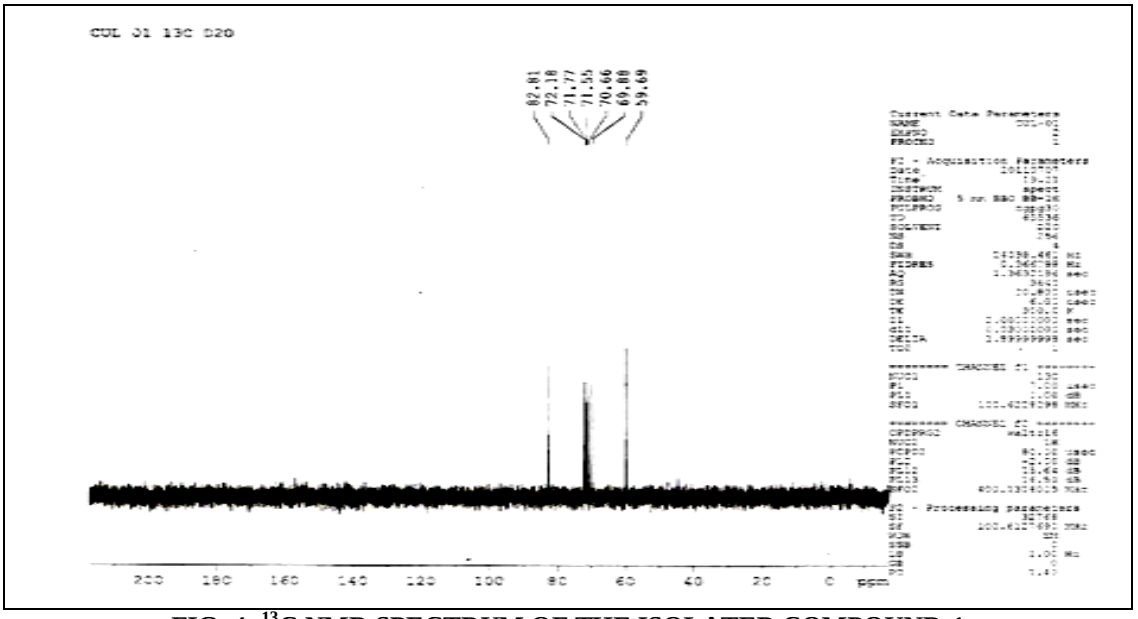

FIG. 4: ^{13}C NMR SPECTRUM OF THE ISOLATED COMPOUND-1

The molecular weight of pure compound-1 was established by LC-MS analysis. Based on the existence of negative ion [*m/z* 193 (M-H) $^-$] confirmed that the molecular weight is 194 as in the mass spectrum **Fig. 5**. The molecular formula was shown to be $C_7H_{14}O_6$ with different functional groups.

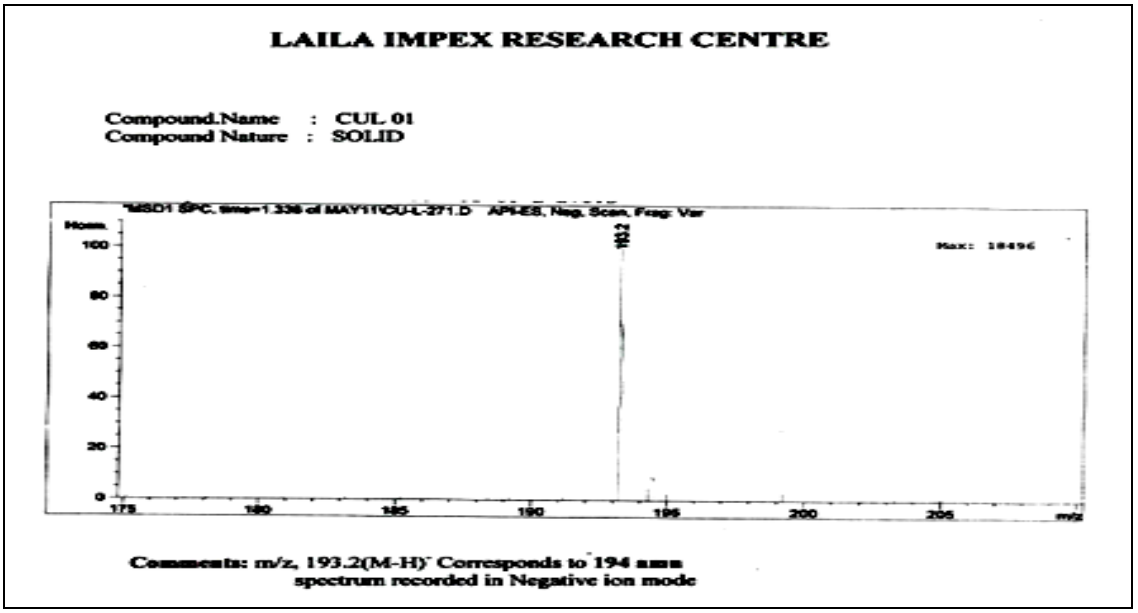

FIG. 5: MASS SPECTRUM OF THE ISOLATED COMPOUND-1

The chemical characteristics and spectral data are resemblances with the 3-O methyl D-chiro inositol [56]. Thus, it can be confirmed that the isolated compound is methyl inositol, which is not previously reported in the title plant. Chemically, it is (1S, 2S, 4S, 5R) -6- methoxy Cyclohexane-1, 2, 3, 4, 5- pentol.

Compound 2 was obtained as a pale yellow powder having MP 328-330 °C. Fourier transmission Infra-

red **(**FT-IR) spectrum of pure compound-2 was given away in **Fig. 6**. The characteristic peaks were observed at 3419 cm^{-1} (O-H stretch), 2923 cm^{-1} 2855.94 cm^{-1} (C-H stretch), 1655.23 cm^{-1} (C=O stretch), 1608.83 cm^{-1} (C=C alkene stretch), 1569.89 cm^{-1}, 1511.78 cm^{1} and 1445 cm^{1} (C=C aromatic stretch), 1371 cm^{-1} (O-H bend), 1265 cm^{-1} and 1166 cm^{-1} (C-O stretch), 1025 cm^{-1} and 520 cm^{-1} (C-H bend), 1124.41 cm^{-1} (C-CO-C bend) respectively. These interatomic bonds confirm that the isolated compound is flavonoid.

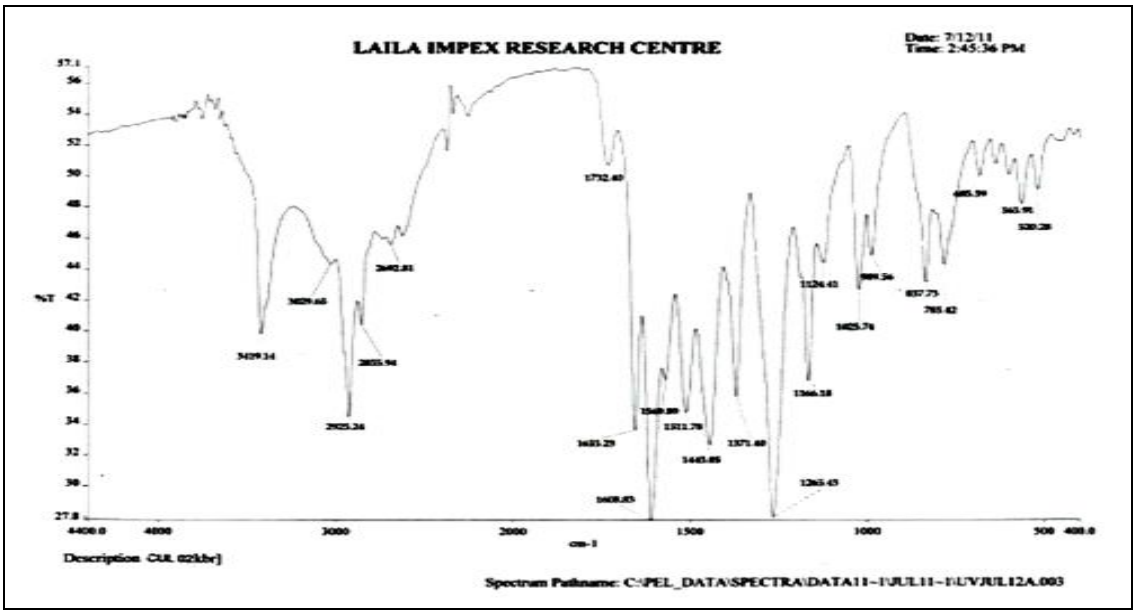

FIG. 6: IR SPECTRUM OF THE ISOLATED COMPOUND-2

The ^{1}H NMR spectra of compound-2 were recorded in d_6-DMSO **Fig. 7**. The spectrum showed the presence of various δ values at

δ =6.19 (1H, d J=2Hz CH), δ =6.49 (1H, d J=2.0Hz, CH) are for H-6 and H-8 of ring A, δ =6.910 (1H, d *J*=8.1 Hz, CH), δ =7.47(1H, d *J*=1.6 Hz, CH), δ=7.427 (1H, dd, *J*=8.5, 1.6 Hz, CH) are for 3, 4–disubstituted system ring B, δ = 6.665 (S, CH) for isolated proton attached at 3-position adjacent to a carbonyl group in ring B of flavones.

Further information to establish the structure was accomplished based on the carbon-13 data of the compound.

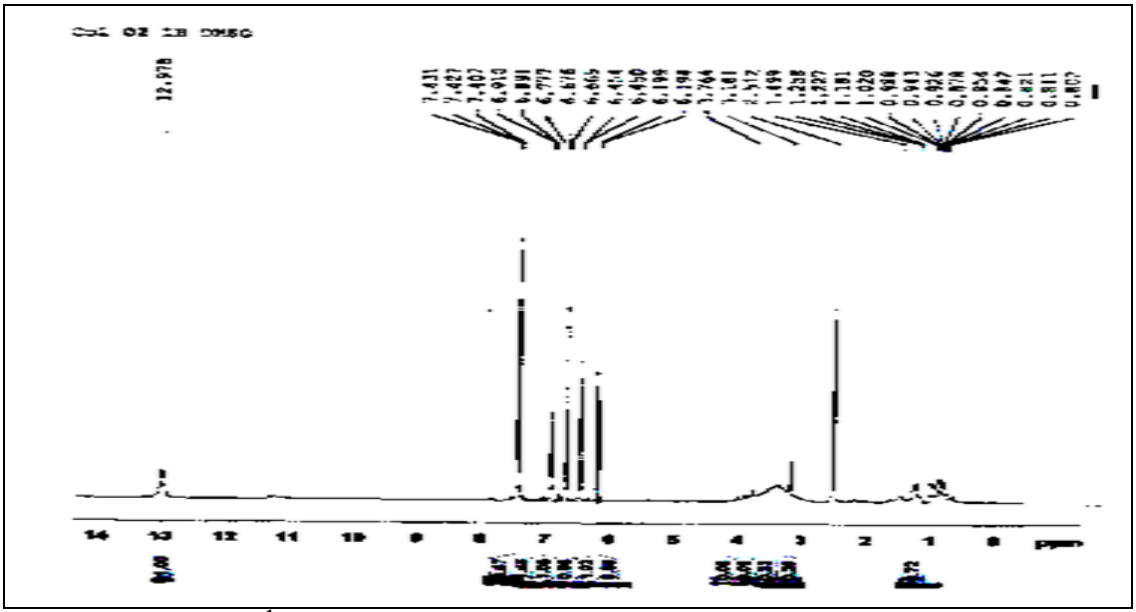

FIG. 7: ^{1}H NMR SPECTRUM OF THE ISOLATED COMPOUND-2

The ^{13}C NMR spectrum of the compound-2 was taken in D_2O. The spectrum in **Fig. 8** showed different peaks at

δ=164.20 (C, C=C)
δ=102.85 (C, C=C)
δ=181.61 (C, C=O)

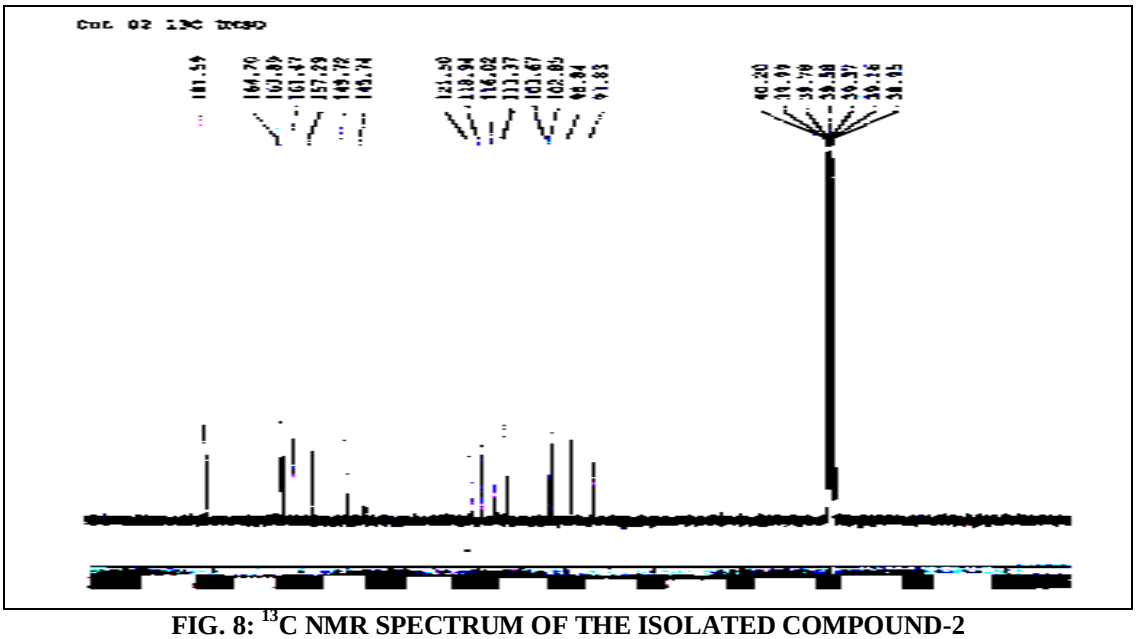

FIG. 8: ^{13}C NMR SPECTRUM OF THE ISOLATED COMPOUND-2

The molecular weight of pure compound-2 was recognized by LC-MS analysis. Based on Negative molecular ion $(M-H)^-$ found at m/z 285.2 $(M-H)^-$ confirmed that molecular weight is 286 as in the mass spectrum **Fig. 9.** The Molecular formula was shown to be $C_{15}H_{10}O_6$.

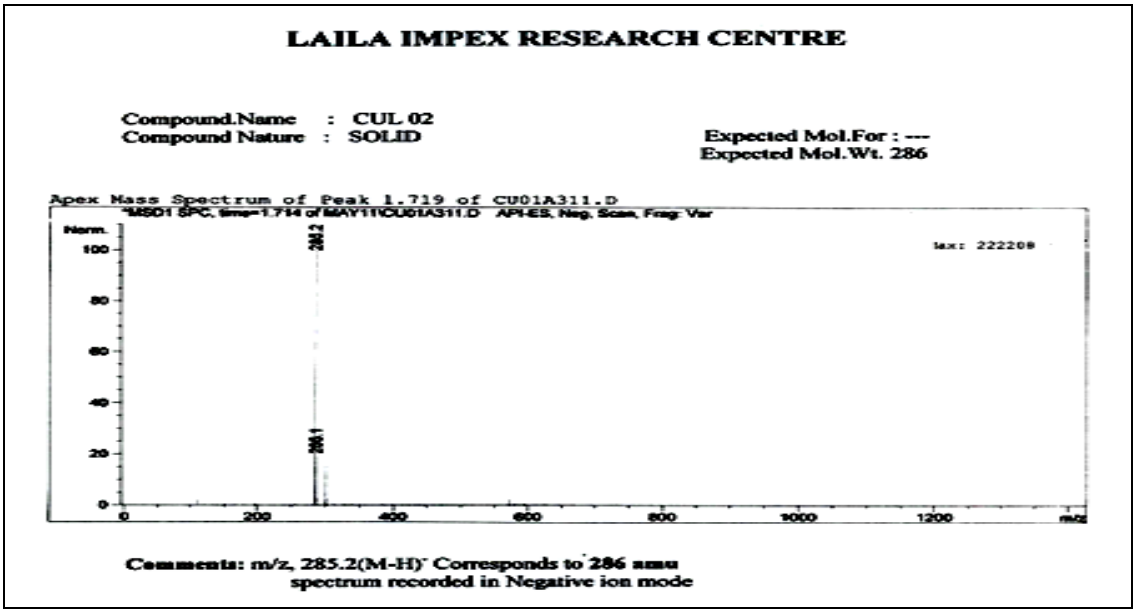

FIG. 9: MASS SPECTRUM OF THE ISOLATED COMPOUND-2

The spectral data and chemical characteristics are in good agreement with the molecular structure of Luteolin [57-59]. Thus, it can be confirmed that the isolated compound is found to be Luteolin, which is not reported earlier in the patrician plant. Chemically, it is 2-(3, 4-Dihydroxyphenyl) -5, 7-dihydroxy-4-Cromenone.

Compound 3 was obtained as a solid; from methanol having MP 52-55 °C. The characteristic peaks of the Fourier transmission Infra-red **(**FT-IR) spectrum of pure compound-3 were observed at 2914 cm^{-1} and 2846 cm^{-1} (C-H stretch), 1469 cm^{-1} and 1407 cm^{-1} (C-H bend), 725-720 cm^{-1} (Methyl of long-chain alkane) respectively.

The ^{1}H and ^{13}CNMR spectra of compound-3 were taken in $CDCl_3$. ^{1}H NMR spectrum of compound-3 with resonance was shown in **Fig. 10**. The spectrum showed the presence of different δ values at

δ = 0.880 (t J=6.8 Hz CH_3,)

δ =1.256 (CH_2)

δ =1.540 (CH_2)

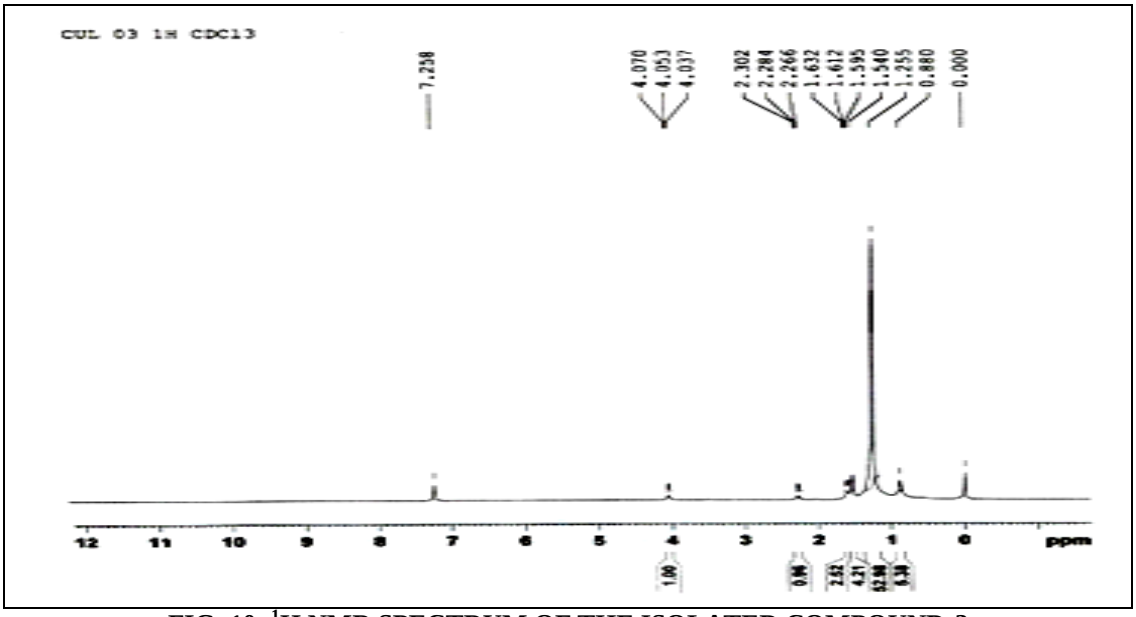

FIG. 10: ^{1}H NMR SPECTRUM OF THE ISOLATED COMPOUND-3

The ^{13}C NMR spectrum of compound-3 in **Fig. 11** Showed peaks at

δ =22.70-37.56 (C, CH_2)

δ =14.09 (C, CH_3)

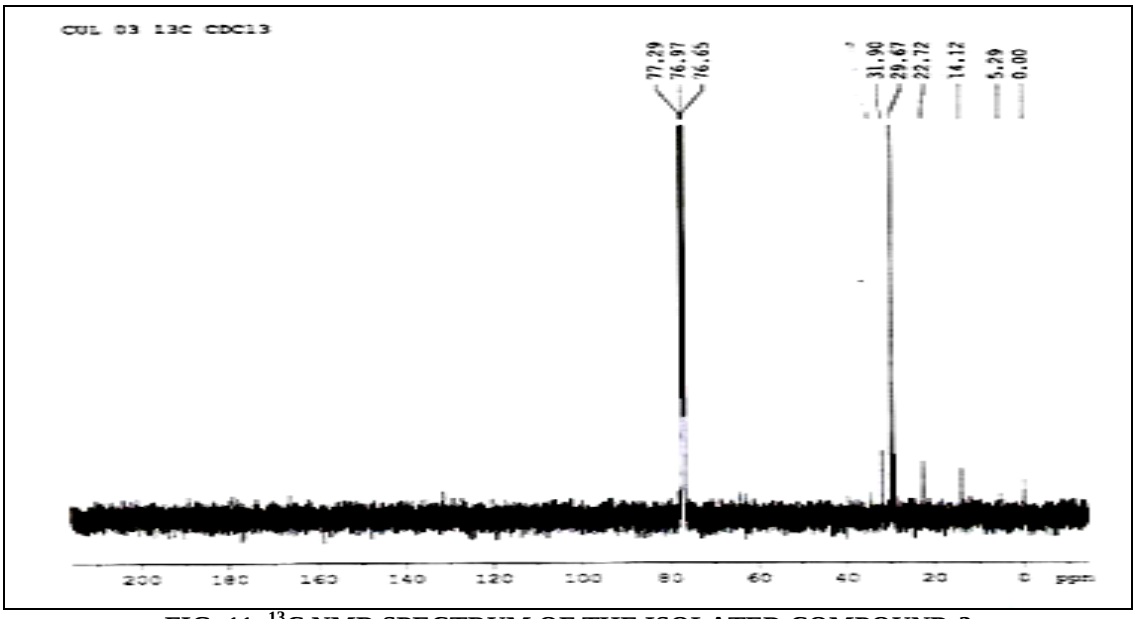

FIG. 11: ^{13}C NMR SPECTRUM OF THE ISOLATED COMPOUND-3

The molecular weight of pure compound-3 was confirmed by LC-MS analysis. Based on positive molecular ion $(M+H)^{+}$ observed at m/z, 353.4 corresponds to molecular weight 352 as in the mass spectrum **Fig. 12.** The Molecular formula was shown to be $C_{25}H_{52}$.

The spectral data and spectrum are similar to the long-chain Alkane pentacosane [60]. Thus, it can be incorrigible that the isolated compound is Pentacosane. Chemically, it is n-Pentacosane.

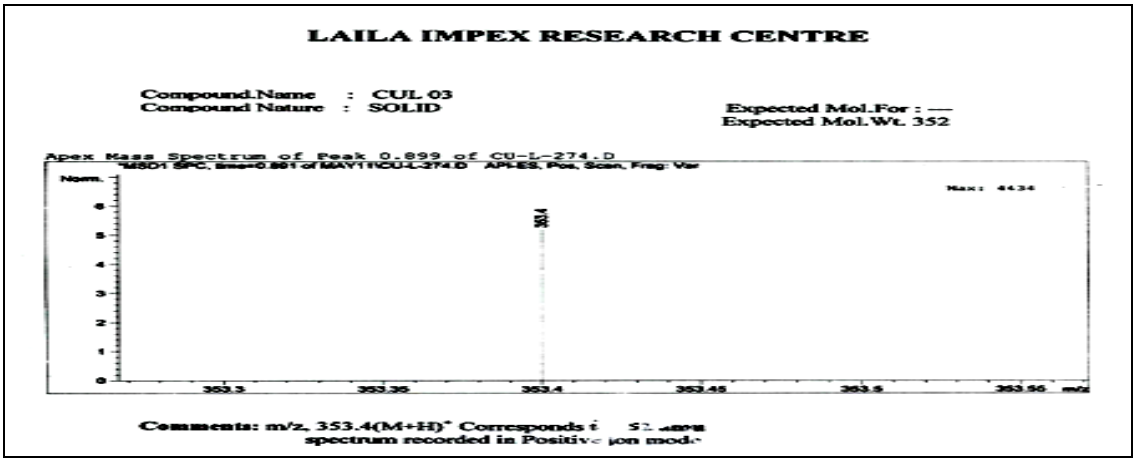

FIG. 12: MASS SPECTRUM OF THE ISOLATED COMPOUND- 3

Compound 4 was obtained as a solid having MP 82-84 °C. Fourier transmission Infra-red (FT-IR) spectrum of pure compound-4 was shown in **Fig. 13**. The characteristic peaks were observed at 3398 cm^{-1} (O-H stretch), 2914 cm^{-1} and 2847 cm^{-1} (C-H stretch), 1436 cm^{-1} and 1359 cm^{-1} (CH_2 and CH_3 bend), 1219 cm^{-1}, and 1120 cm^{-1} (C-O stretch), 772 cm^{-1} (O-H bend) respectively.

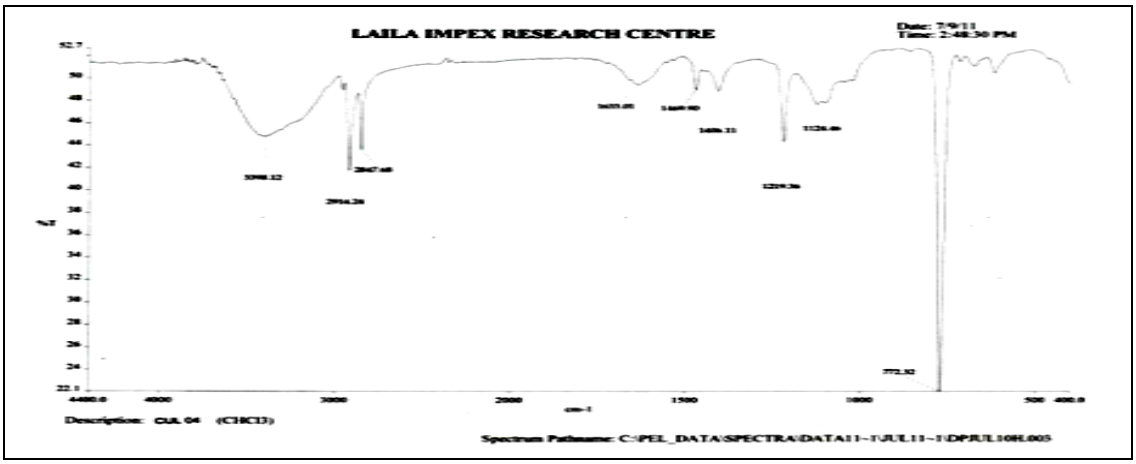

FIG. 13: IR SPECTRUM OF THE ISOLATED COMPOUND-4

The ^{1}H and ^{13}C NMR spectra of compound-4 were taken in $CDCl_3$. The ^{1}H NMR spectrum was publicized in **Fig. 14**. The spectrum showed the presence of different δ values at

δ=0.87 (t J=5.79Hz, CH_3)
δ=3.64 (t J=5.79 Hz, CH_2)
δ=1.25, brs (CH_2)

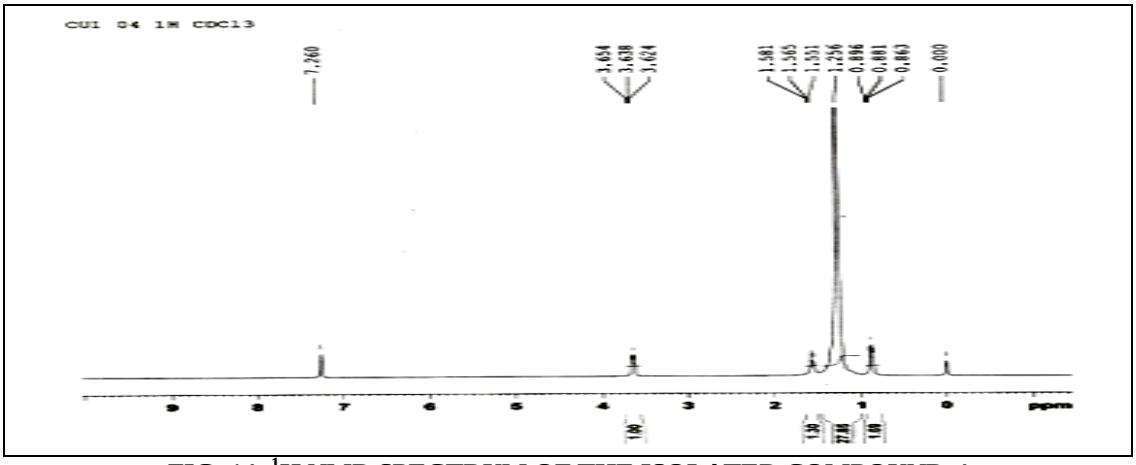

FIG. 14: ^{1}H NMR SPECTRUM OF THE ISOLATED COMPOUND-4

The ^{13}C NMR spectrum of compound-4 in **Fig. 15** showed resonance at δ=62.30 (C, CH_2), δ=22.0-32.9 (C, CH_2), δ= 14.3 (C, CH_3).

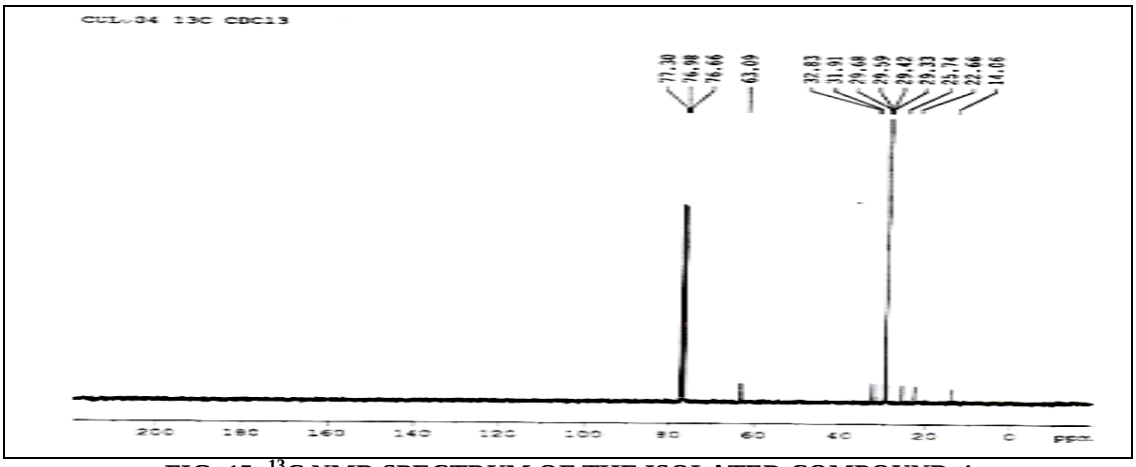

FIG. 15: ^{13}C NMR SPECTRUM OF THE ISOLATED COMPOUND-4

The molecular weight of pure compound-4 was confirmed by LC-MS analysis. Based on positive molecular ion $(M+Na)^+$ found at *m/z* 461.6 confirmed the molecular weight is 483 as in the mass spectrum **Fig. 16.** The molecular formula was given away as $C_{30}H_{62}O$.

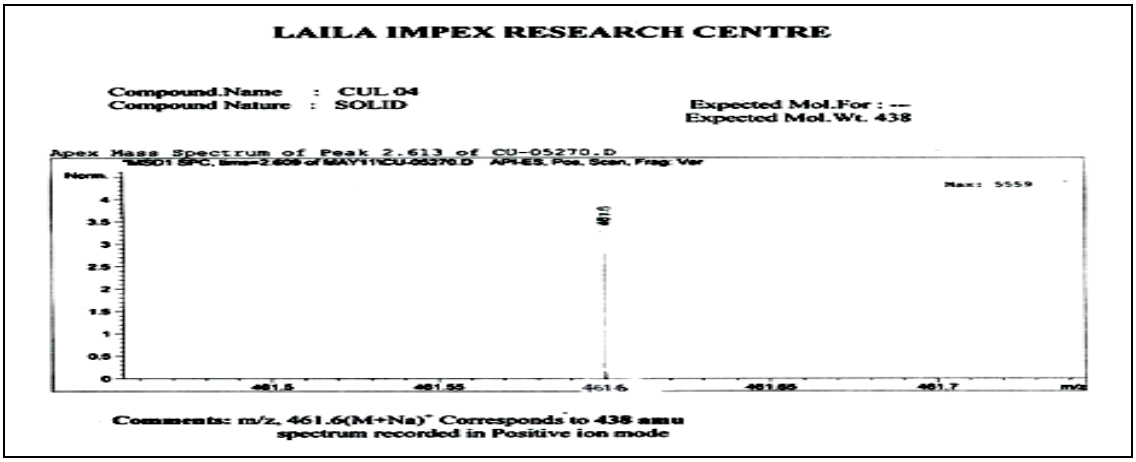

FIG. 16: MASS SPECTRUM OF THE ISOLATED COMPOUND-4

The results are in good accordance with the structure of 1-Tricontanol [61]. Thus, it can be confirmed that the isolated compound is Myricyl alcohol, which has not been previously isolated from this plant. Chemically, it is Triacontan-1-ol.

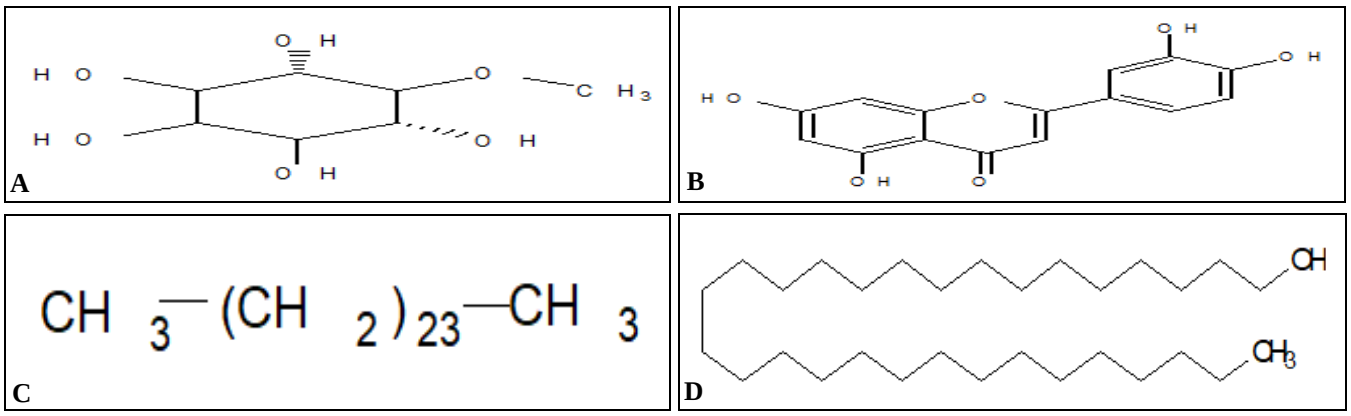

FIG. 17: CHEMICAL STRUCTURES OF ISOLATED COMPOUNDS (A) METHYLINOSITOL; (B) LUTEOLIN; (C) PENTACOSANE; (D)-TRIACONTANOL

CONCLUSION: From the present work, the methanol extract of leaves of *C. uniflora* showed increased antioxidant and anti-inflammatory activity, hence forwarded to fractionalize and column chromatography for isolation of phytoconstituents. Methyl Inositol, Luteolin, n-Pentacosane, and 1-Tricontanol compounds have been isolated successfully using various solvents. It is found that the solvent plays the main role in the extraction of plant constituents. The solvents of 10, 15, and 20% of methanol in ethyl acetate showed the presence of Inositol compared to the 5 and 50% methanol in ethyl acetate and 100% ethyl acetate. Ethyl acetate solvent showed the presence of flavonoid compared to 1, 4 and 10% acetone in ethyl acetate. The solvent of 2% chloroform in hexane showed the presence of long-chain alkane, n-pentacosane and alcohol compared to the 5% chloroform in hexane. The identification of Methyl inositol, Luteolin, n-pentacosane, and 1-Tricontanol was attempted by direct comparison with its retardation factor. The isolated compounds were recognized through FT-IR, NMR and mass spectroscopy. This method is simple, rapid and highly efficient extraction method for extracting the potent bioactive components from *Cassia uniflora* plant.

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REFERENCES:

1. Mazumder PM, Percha V, Farswan M and Upaganlawar A: A wonder gift to medicinal science. Int J Community Pharm 2008; 1: 16-38.
2. Samy RP, Pushparaj PN and Kone PGK: A complication of bioactive compounds from Ayurveda. Bioinformation by Biomedical Informatics Publishing Group 2008: 100-10.
3. www.plants-pedia.com/.../What-is-the-common-name-for-the-Senna... Cached - Block all www.plants-pedia.com results.
4. Reddy CS, Reddy KN, Vatsavaya and Raju S: Suplement to Flora of Andhra Pradesh, India, Deep Publication, New Delhi 2008; 15.
5. Wu QP, Wang ZJ, Fu MH, Tang LY, He Y, Fang J and Gong QF: Chemical constituents from the leaves of *Cassia angustifolia*. Journal of Chinese Medicinal Materials 2007; 30(10): 1250-52.
6. León JAM, García AF, Saavedra CMA, Torres DCGM and Rodríguez ET: Phytochemical screening of *Cassia uniflora* Mill. Revista Cubana de Plantas Medicinales 2011; 16(4): 331-36.
7. Dave H and Ledwani L: A review on anthraquinones isolated from Cassia species and their applications, Indian Journal of Natural Products and Resources 2012; 3(3): 291-19.
8. Veerachari U and Bopaiah AK: Phytochemical investigation of the ethanol, methanol and ethyl acetate leaf extracts of six Cassia species. International Journal of Pharma and Bio Sciences 2012; 3(2): 260-70.
9. Singanaboina K, Chinna V, Ratnampally SK and Damera V: Phytochemical screening in leaf extracts of *Cassia angustifolia* (vahl) grown in different soil treatments. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(7): 571-76.
10. Agnihotri A and Singh V: Phytoconstituents Isolated from stem bark of *C. fistula* Linn. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(7): 1849-58.
11. Christi VE, Mohan S, Poorani TU, Kumari S and Banupriya M, Bhakta T, Deb PK, Bhaumik KN and Saha J: Pharmacognostic investigation, acute toxicity studies and isolation of steroidal compound from the leaves of *Cassia fistula* Linn. Int J Pharm Sci Rev Res 2014; 25(1): 210-14.
12. Mitra P, Ghosh T, Gupta S, Basu B and Mitra PK: Isolation and characterization of a compound from the leaves of *Cassia alata* Linn. EC Chemistry 2016; 2(2): 138-44.
13. Kabila B, Sidhu MC and Ahluwalia AS: Phytochemical profiling of different cassia species: a review. International Journal of Pharmaceutical & Biological Archives 2017; 8(2): 12-20.
14. Christi VE, Mohan S, Poorani TU, Kumari S and Banupriya M: Comparative Phytochemical Study on Some Cassia Species. Asian Journal of Science and Technology 2018; 9(09): 8550-56.
15. Zhao Y, Zhao K, Jiang K, Tao S, Li Y, Chen W, Shumeng Kou S, Chenrong G, Zongrui L, Lusheng G, White WL and Zhan KX: A review of flavonoids from Cassia species and their biological activity. Current Pharmaceutical Biotechnology 2016; 17(13): 1134-46.
16. Srividhya M, Hridya H, Shanthi V and Ramanathan K: Bioactive amento flavone isolated from *Cassia fistula* L. leaves exhibits therapeutic efficacy. 3Biotech 2017; 7(1): 33.

17. Monisha M, Sowmiya M, Ragunathan R and Johney J: Extraction of bio-active compounds from *Cassia auriculata* Pods and leaves and its medicinal uses. Int J Curr Microbiol App Sci 2017; 6(8): 425-34.
18. Kamath BR and Kizhedath S: *In-vitro* study on antioxidant activity of methanolic leaf extract of *Cassia fistula* Linn. International Journal of Basic & Clinical Pharmacology 2018; 7(5): 849-53.
19. Colar FR, Gogi CL, Khudav MM, Chodary MS and Patil SB: Phytochemical and antioxidant properties of some Cassia species. Natural Product Research 2018; 32(11): 1324-28.
20. Gupta V, Rathore DS, Kansara NP and Badiger AM: *In-vivo* antioxidant activity of topical cream of *Cassia tora* L. leaves extract. Dataset Papers in Pharmacology 2013, 1-5.
21. Yadav A, Bhardwaj R and Sharma RA: Phytochemical screening and antimicrobial activity of anthraquinones isolated from different parts of *Cassia nodosa*. Research Journal of Medicinal Plants 2013; 7(3): 150-57.
22. Deshpande SR and Naik BS: Evaluation of *in-vitro* antimicrobial activity of extracts from Cassia obtusifolia L. and *Senna sophera* (L.) Roxb against pathogenic organisms. Journal of Applied Pharmaceutical Science 2016; 6(01): 083-085.
23. Rana R, Saklani K and Gaurav N: Phytochemical analysis and antimicrobial activity of leaf and seed extract of *Cassia fistula*. International Refereed Multidisciplinary Journal of Contemporary Research 2017; 5(4): 24-30.
24. Tapwal A, Pandey P, Chandra S and Rashmi: Antimicrobial aactivity and phytochemical screening of endophytic fungi associated with *Cassia fistula*. International Journal of Clinical and Biological Sciences 2015; 2(7): 15-21.
25. Mehta JP, Parmar PH, Kukadiya NB and Godhani DR: Antimicrobial assay of extracts of *Cassia siamea* (Lam.) and *Cassia Javanica* (Linn.). Journal of Pharmaceutical, Chemical and Biological Sciences 2018; 5(4): 386-95.
26. Robert BD, Ameo S and Onegi B: Assessment of antibacterial activity of crude leaf and root extracts of *Cassia alata* against Neisseria gonorrhea. Afr Health Sci 2014; 14(4): 840-48.
27. Vivekanandan KE and Ajeesh PR: Phytochemical screening and antibacterial evaluation of the leaf and root extracts of *C. alata*. International Journal for Research in Applied Science & Engineering Techn 2018; 6(3): 820-26.
28. Bereksi MS, Hassaïne H, Bekhechi C and Abdelouahid DE: Evaluation of the antibacterial activity of some medicinal plants extracts commonly used in Algerian traditional medicine against some pathogenic bacteria. Pharmacogn J 2018; 10(3): 507-12.
29. Sharma DK: Enumerations on phytochemical, pharmacological and ethnobotanical properties of *Cassia fistula* Linn: yellow shower. The Journal of Phytopharmacology 2017; 6(5): 300-06.
30. Vijayakumari A and A Sinthiya: Biosynthesis of phytochemicals coated silver nanoparticles using the aqueous extract of leaves of *Cassia alata* – characterization, antibacterial and antioxidant activities. International Journal of Pharmaceutical and Clinical Research 2018; 10(5): 138-49.
31. Sony P, Kalyani M, Jeyakumari D, Kannan I and Sukumar RG: *In-vitro* antifungal activity of *Cassia fistula* Extracts against fluconazole resistant strains of Candida species from HIV patients. J Mycol Med 2018; 28(1): 193-00.
32. Anitha J and Miruthula S: Anti-inflammatory and phytochemicals analysis of *Cassia fistula* Linn. Fruit pulp extracts. Int J Pharmacognosy 2014; 1(3): 207-15.
33. Chaudhary S and Kumar A: Phytochemical analysis and assessment of *in-vitro* anthelmintic activity of *Cassia auriculata* Linn. leaves. American Journal of Phyto-medicine and Clinical Therapeutics 2014; 2(2); 161-67.
34. Satpute SM, Bhamburdekar SB and Gaikwad DK: anthelmintic potential of pods and stem bark extracts of *Cassia fistula* L. International Research Journal of Pharmacy 2017; 8(5): 70-72.
35. Choudhary M, Kumar V, Malhotra H and Singh S: Medicinal plants with potential antiarthritic activity. Journal of Intercultural Ethnopharmacology 2015; 4(2): 147-79.
36. Akhila S and Aleykutty NA: Antidiabetic activity studies on *Cassia fistula* fruits. Adv J Pharm Life Sci Res 2015; 3(3): 1-8.
37. Jangir RN and Jain GC: Evaluation of antidiabetic activity of hydroalcoholic extract of *Cassia fistula* Linn. pod in streptozotocin-induced diabetic Rats. Pharmacogn J 2017; 9(5): 599-06.
38. Kumar V, Ranjanasingh, Mahdi F, Mahdi AA and Singh R: Experimental validation of antidiabetic and antioxidant potential of *Cassia tora* (L.): An Indigenous Medicinal Plant. Indian J of Clin Biochem 2017; 32(3): 323-28.
39. Vijayalakshmi S, Ranjitha J, Devirajeswari V and Bhagiyalakshmi M: Pharmacological profile of *Cassia occidentalis* L – a review. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(3): 29-33.
40. Singh VV, Jain J and Mishra AK: Pharmacological and phytochemical profile of *Cassia occidentalis* L: a review. Journal of Drug Delivery & Therapeutics 2016; 6(5): 91-96.
41. Sobeh M, Mahmoud MF, Hasan RA, Cheng H, El-Shazly AM and Wink M: *Senna singueana*: Antioxidant, hepatoprotective, antiapoptotic properties and phytochemical profiling of a methanol bark extract. Molecules 2017; 1502: 1-15.
42. El-Sayed MM, Abdel-Aziz MM, Abdel-Gawad MM, Abdel-Hameed ES, Ahmed WS and Abdel-Lateef EE: Chemical constituents and cytotoxic activity of *Cassia glauca* Lan. leaves. Life Science Journal 2013; 10 (3): 1617-25.
43. Asba A and Meeta B: Evaluation of phytochemicals of *Cassia tora* Linn. and it's cytotoxicity assay using brine shrimp. International Journal of Pharmacognosy and Phytochemical Research 2017; 9(4): 587-95.
44. Ali MI, Aboul-Enein AM, Mohamed SM, Abou elella FM, Mohammed MMD and Hamed AR: Phytochemical, cytotoxicity and antioxidant investigation of *Cassia alata* leaves growing in Egypt. Journal of Innovations in Pharmaceutical and Biological Sciences 2017; 4(4): 97-105.
45. Pawar AV, Patil SJ and Killedar SG: Uses of *Cassia fistula* Linn. as a medicinal plant. International Journal of Advance Research and Development 2017; 2(3): 85-91.
46. Ahmed SI, Hayat MQ, Tahir M, Mansoor Q, Ismail M, Keck K and Bates RB: Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of *Cassia angustifolia* Vahl. BMC Complementary and Alternative Medicine 2016; 16: 460-469.
47. Kumar RS, Narasingappa RB, Joshi CG, Girish TK, Rao UJP and Danagoudar A: Evaluation of *Cassia tora* Linn. against Oxidative Stress-induced DNA and cell membrane damage. J Pharm Bioallied Sci 2017; 9(1): 33-43.
48. Ghayal N, Padhye A and Dhumal K: Larvicidal activity of invasive weeds *Cassia uniflora* and *Synedrella nodiflora*. Int J Pharm Bio Sci 2010; 1(3): 1-10.

49. Vitonde S, Thengane RJ and Ghole VS: Allelopathic effects of *Cassia tora* and *Cassia uniflora* on *Parthenium hysterophorous* L. Journal of Medicinal Plants Research 2014; 8(4): 194-96.
50. Vijay D, Ahmed ABA, Kumar RA, Gnanasekaran D, Mani P, Sebastian C and Rajasekaran: Comparative evaluation of *Senna uniflora* for anti-inflammatory activity. Int J Pharm Tech 2010; 2(2): 325-32.
51. Sheetal, Chaudhari S, Chaudhari MR and Chavan J: Analgesic, anti-inflammatory and anti-arthritic activity of *Cassia uniflora* Mill. Asian Pacific Journal of Tropical Biomedicine 2012; 1(2): 181-86.
52. Khyade MS, Kamble SP and Kurhe AR: Comparative FTIR analysis and free radical quenching properties of three Cassia species. Asian Journal of Pharmaceutical and Clinical Research 2015; 8(5): 119-25.
53. Kothapalli LP, Papule PP, Thomas AB and Bhujbal SS: Evaluation of *Senna uniflora* (mill.) leaf extract and its topical gel formulation for biological activity. International J of Pharmaceutics & Drug Analysis 2017; 5(8): 354-65.
54. Jothirathinam T and Victor VD: Phytochemical screening and antimicrobial activity of *Senna uniflora* (Mill.) H.S Irwinand Barneby. World Journal of pharmaceutical Research 2016; 5(6): 1608-19.
55. León JAM, Cedeño CQA, Rodríguez CET, Zayas CAA and Tamayo ORS: Antibacterial activity of fractions obtained from *Cassia uniflora* leaves against methicillin-resistant *Staphylococcus aureus*. Rev Cubana Quím 2017; 29(3): 444-55.
56. Sridhar C, Krishnaraju AV and Subbaraju GV: Anti-inflammatory constituents of *Teramus labialis*. Ind J Pharma Sci 2006; 68(1): 111-14.
57. Harborne JB and Mabry TJ: The Flavonoids. Advan Res, 1981.
58. Markham KR: Techniques of Flavonoid Identification. Academic Press, New York, 1982.
59. Ramli I, Hamzah AS, Aimi N and Lajis NH: Chemical constituents of *Vitex ovata* (Verbenaceae) Pertanika. J Sci Tech 1997; 5(1): 105-09.
60. Buckingham J and Chapman: Dictionary of a organic Compounds, Chapman and Hall, New York 1982; 5.
61. Nogueira MM, Ollvelra JSD and Ferraz S: Nematicidal hydrocarbons from *Mucuna aterrima*. Phytochem 1996; 42(4): 997-98.

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