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Extraction, Isolation and Purification of Kaempferol from *Cassia fistula* L. Flowers

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Abstract

Cassia fistula L., (CFL) (Fabaceae) commonly called the Golden Shower Plant has an abundance of phytopharmaceuticals. It has been used as the oldest natural sweetener. Due to its eminent ethno-medicinal values since ages, it has been used in Ayurveda, Chinese and Unani medicine for innumerable diseases and illnesses like cold, congestion, sore throat, jaundice, wound healing, typhoid, constipation, cancer, etc. Kaempferol (KP) is one such active metabolite present in CFL flowers that displays prominent bioavailability. In recent times, life-threatening diseases have escalated worldwide, which has shifted the focus from synthetic medicines to herbal medicines. In the present work, for the first time, the extraction of KP (a flavonoid, 3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) from CFL flowers was done using methanol as solvent. High-Performance Liquid Chromatography (HPLC) was employed further for the isolation and purification of KP from CFL flowers. Spectral analysis such as Fourier Transformer Infrared Spectroscopy (FTIR), Liquid Chromatography-Mass Spectrometry (LC-MS), and Nuclear Magnetic Resonance (NMR) was conducted for the structural determination of isolated KP. Through Thin Layer Chromatography (TLC) fingerprinting, only 15% purity of KP was achieved, whereas by using HPLC this enrichment reached approximately 85-90%. By performing different spectral characterisations, isolated and purified KP was substantiated as the spectroscopic data obtained agreed well with the literature. Extraction, isolation, and purification of KP from CFL flowers offer a green alternative to explore the therapeutic utility of this important phytoestrogen for the development of several design and real-world applications in pharmaceutical material science for various biological activities.

Keywords: Flavonoids, HPLC, Kaempferol, Polyphenol

1.0 Introduction

Plants display a plethora of bioactive metabolites and are natural reservoirs of herbal medicines. Using natural products in specified formulations for medicaments of innumerable ailments is an age-old belief[1]. Through the perseverance of great pioneers and folk medicines, this therapeutic envisioning has been converted into Ayurvedic, Homeopathy and Unani medicines and is practised worldwide. Consumption of natural products over the ages against diseases has created a positive influence on human civilisation.

Currently, most primary healthcare needs are fulfilled by natural products in most developing countries. This has attracted the attention of developed countries since the medical infrastructure is witnessing a phase of high-cost healthcare expenses with soaring financial austerity[2]. Around 80% of the world population depends on natural products obtained from plant extracts[3]. Therapeutic properties of plant species have made an excellent

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endowment to the evolution of traditional therapies. Hence the phytopharmaceuticals present in these have found a wide range of applications in different fields of medicine, agriculture and pharmaceutical material science.

1.1 Botanical Classification

Kingdom - *Plantae*
Subkingdom - Tracheobinota
Super Division - Spermatophyta
Division - Mangoliophyta
Class – Magnoliopsida
Sub Class - Rosidae
Order - Fabales
Family - *Fabaceae*
Sub Family - Caesalpiniaceae
Genus - *Cassia*
Species - *C. fistula*

Cassia fistula L. (CFL) is one such important ethno-medicinal herbal plant with diverse pharmacological effects[4]. It is commonly known as the golden shower tree and belongs to the *Fabaceae* family. It is a deciduous tree with a beautiful bunch of yellow flowers which are also termed ornamental flowers[5]. It is native to India, Sri Lanka, Amazon and other South Asian countries. CFL is termed as "*Aragvadha*" interpreted as a disease killer.

Natural products derived from these plants' phytochemicals are reported to possess different antimicrobial, antioxidant, anti-inflammatory, anticancer, antidiabetic, antipyretic, antimelasmic, wound healing and many more properties[6]. Upon phytochemical screening, it was reported that CFL has an amalgamation of different phytochemicals such as anthraquinones, Flavanoids, alkaloids, carbohydrates, amino acids and many more[7]. The presence of phytochemicals in abundance in different parts of the plant has made it a potent and viable asset in the pharmacy field.

1.2 Phytochemistry of CFL

Alcoholic extracts of roots have been used as a remedy for cardiac illness, fever and wounds. Leaves are consumed as laxatives and are also applied for eczema and ringworms[8]. The entire plant is consumed for diarrhoea treatment. Also, the fruits and seeds are used for treating dermal infection, abdominal pain and rheumatoid fever[9]. According to previous studies, bioactive amento flavone isolated from leaves has excellent antioxidant properties[10]. Flowers are considered to be effective against cancer activities, gastrointestinal problems and wound healing. It's employed for skin ageing also[11].

CFL flowers are reported to be rich in different phytochemicals such as Rhein and anthraquinone, KP, a flavonoid, Leucopelargonidin tetramer, an anthocyanin, fistulin, triterpenes, alkaloids, heptacosanoic acids, hentriacontanoic, triacontanoic, nonacosanoic, gibberellic acid etc[12]. The flowers display impressive anticancer activity. Rhein from the flowers was isolated and studied against colon cancer cells[13].

KP present in CFL flowers is a tetrahydroxyflavone with four hydroxy groups and is a yellow colour compound. Through epidemiological data it was indicated that high consumption of KP has led to a reduction of proliferating different types of cancer such as skin, colon, ovary, liver, stomach etc[14]. Also, it displays a wide array of other biological activities such as anti-inflammatory, antioxidant, cardioprotective, neuroprotective and anti-diabetic activities[15].

Literature is available for the biological activity of commercially purchased KP, but there is a scarcity of data on anticancer, antimicrobial activity and wound healing of isolated KP from CFL flower extract. Few research papers support the biological activities of isolated bio-active components of CFL flowers by combinatorial approach. Some researchers reported the antimicrobial activity of the film laden with CF seed extracts, but rarely any literature is available about the antimicrobial/anticancer activity of isolated and purified single bio-active components from CFL flower extract. Less data is available on the antimicrobial and anticancer activity of film loaded with green synthesised silver nanoparticles using isolated bio-active components of CFL flowers.

This present work focuses on the extraction, isolation and purification of KP from CFL flowers providing a green alternative, a suitable constituent from a bioresource for the development of various applications for therapeutic use.

2.0 Materials and Methodology

2.1 Collection of Plant Materials

CFL flowers were collected in bulk from Varanasi, India.

2.2 Preparation of Extract

Freshly blossomed flowers were collected, washed with distilled water thoroughly and shade-dried at normal

room temperature. After drying the flower petals were ground into a fine powder and then stored in air-tight containers. 1kg of flower powder was extracted with 5 l (1:5 w/v) of 95% of methanol for 48 hours. The extract was filtered using Whatmann grade 1 filter paper and the filtrate was reduced to dryness using a rotary evaporator at 40°C to remove methanol from the extract. The evaporated extract was stored in a vacuum desiccator until further use[16].

2.3 Isolation of Kaempferol from Extract

The yielded extract was subjected to column chromatography over silica gel 30 g (60-120 mesh size). The yielded extract was eluted with hexane along with silica gel and a steady slurry was added into the column. The fraction was eluted with a combination of hexane and ethyl acetate (6:4) and was collected in different flasks. The obtained fractions were scrutinised and checked individually on TLC plates (F1-F7). On examination, similar fractions were combined and were eluted again with a combination of hexane and ethyl acetate (7:3) on TLC plates[16].

2.4 HPLC Isolation and Purification of Kaempferol

HPLC is an advanced instrument used for quantification, separation and identification of compounds in mixtures. Further for complete isolation and purification, fractions 3-7 were employed for HPLC analysis[17].

2.5 Spectroscopic Investigation

FTIR, 13C^{1}H NMR and LCMS were carried out to identify the isolated compound and its purity determination. FTIR (Model: FTIR-8400, Shimadzu) was employed to determine the physicochemical properties of isolated and pure KP. An adequate amount of the sample was placed on the FTIR spectrometer. The scanning range for adsorption was from 4000 to 600cm^{-1} with resolution set at 32cm^{-1} over 32 scans.^{1}HNMR and 13CNMR were recorded on Bruker Avance Neo 500MHz NMR Spectrometer with dimethyl sulfoxide (DMSO) as eluent. The MS identifies molecular weight by converting the molecules into ions and based on their mass and charge, the ions are separated. MS values were achieved by using Thermo-Xcalibur™ data acquisition (Thermo Scientific)[18].

3.0 Results and Discussions

3.1 Identification of Isolated Compounds

TLC analysis displayed the presence of other phytochemicals such as Rutoside and Sennoside which were validated by the standards. Therefore, the fractions which showed similar bands (F3-F7) on TLC profiling were subjected to HPLC. HPLC analysis of fraction 7 for isolation of 90% pure KP was achieved successfully. The retention time of the fraction sample F7 against the standard data was compared which established that the results were in good agreement with reported literature. By using Empower 2 software data analysis, a calibration curve was determined which provides the content of purified KP in the sample. For standard KP the retention time in HPLC analysis was done based on Retention Time (RT). In HPLC RT signifies the time utilised by a solute to run through the chromatographic column representing the compound elution time which is taken to determine the required length of the chromatographic run. Therefore in HPLC graphs, it was revealed that for the isolated sample, KP is 5.665min whereas RT for standard KP is 5.810min, concluding that the retention times were very close to each other, thus after complete assessment, F7 was identified as pure KP[19] (Figure 1).

3.2 Spectral Characterisation

The structural confirmation was done by spectral characterisations – FTIR, LCMS, 13C and ^{1}H NMR.

3.3 FTIR

FTIR spectra of purified KP depicted characteristic adsorption peaks, a wide band at 3308cm^{-1} displaying O-H stretching of four hydroxyls. Adsorption peaks at 1658cm^{-1} represented a carbonyl bond (C=O). Sharp adsorptive peaks at 1609cm^{-1} show the presence of

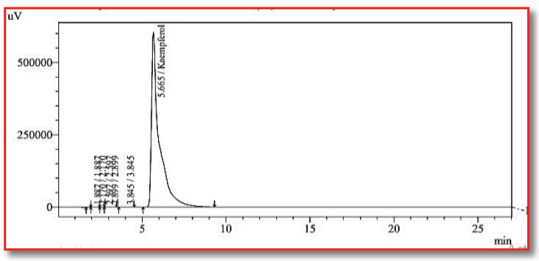


Figure 1. HPLC chromatogram of isolated Kaempferol.

aromatic compounds (C=C). The peaks at 1568cm^{-1} and 1506cm^{-1} are due to vibrations of the benzene ring. The peak at 1374 and 1304 was attributed to the carboxylic group (C=OH). The peak at 1169cm^{-1} corresponds to stretching vibrations of the carbonyl group (C=O). These obtained significant peaks are in good agreement with the previous literature[20] (Figure 2).

3.4 NMR

The ^{1}H NMR spectra of isolated KP was recorded at 500MHz frequency on (δ) scale (ppm; d_6 DMSO), eluents hexane: ethyl acetate (50:50): 12.48 (s, 1H), 10.22 (s, 1H) 8.04 (d, J =8.5 Hz, 2H) 6.92 (dt, J = 8.8 Hz, 2.4 Hz, 2H), 6.40–6.19 (s, J= 8.8 HZ, 1H)[21] (Figure 3).

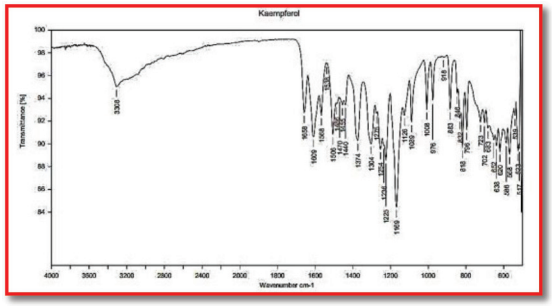


Figure 2. FTIR spectra of isolated Kaempferol.

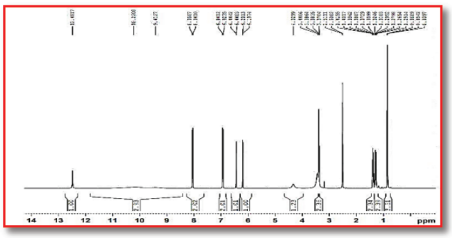


Figure 3. ^{1}H NMR spectra of isolated Kaempferol.

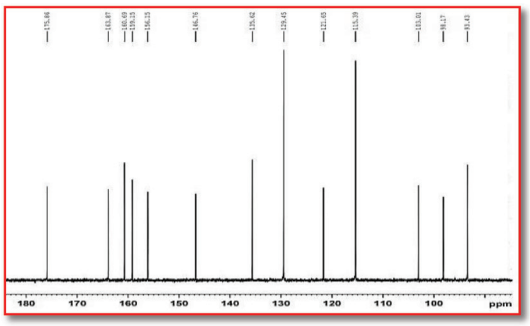


Figure 4. ^{13}C NMR of isolated Kaempferol.

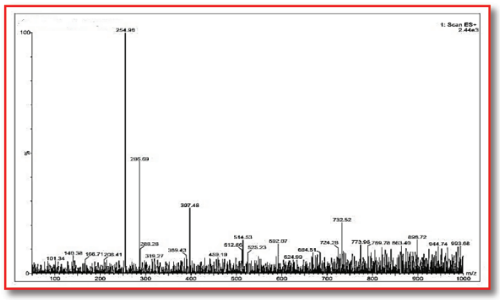


Figure 5. LCMS spectra of isolated Kaempferol.

3.5 ^{13}C NMR

The ^{13}C NMR spectra of isolated KP was recorded at 500 MHz frequency on (δ) scale (ppm; d_6 DMSO), eluents hexane: ethyl acetate (50:50): 175.86 (C-4, carbonyl, C=O), 163.87 (C-7, carbonyl, C=O),156.15 (C-5, carbonyl, C=O),146.76 (C-2, aromatic), 135.62 (C-3, aromatic), 129.45 (C-2', C-6', aromatic), 115.39 (C-3', C-5', alkenes C=C), 103.01 (C-10, alkenes C=C), 98.17 (C-6, alkenes C=C). The peaks exhibited by the isolated KP were similar to standard spectral data of commercially available KP[22] (Figure 4).

3.6 LCMS

LCMS analysis for isolated KP was calculated for Electrospray Ionisation (ESI) for positive mode and was found at *m/z* 286.9 which is consistent with the mass of gross empirical formula $C_{15}H_{10}O_6$ (Figure 5).

This formula corresponds to the reported literature thus validating isolation of KP from CFL methanol extract[22].

4.0 Conclusion

In conclusion, for the first time, the present work reports the isolation and purification of KP from the methanolic extract of CFL flowers. The yielded isolated compound KP (3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) was pale yellow. Through TLC analysis enrichment of KP was obtained only to 15-20%. While through HPLC fingerprinting isolated KP was further enriched and its purification was accomplished at around 90%. The characterisation of the isolated KP was carried out by using multispectroscopic techniques such as FTIR, LCMS 1C and ^{1}H NMR. FTIR spectrum showed characteristic adsorbent peaks similar to reported standard KP data. NMR and LCMS results espouse the isolation of pure KP from methanolic extract.

5.0 Acknowledgements

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6.0 References

1. Bationo RK, Dabiré CM, Hema A, Ch RH, Palé E, Nacro M. HPTLC/HPLC-mass spectrometry identification and NMR characterization of major flavonoids of wild lemongrass (*Cymbopogon giganteus*) collected in Burkina Faso. Heliyon. 2022; 8(8):e10103. https://doi.org/10.1016/j.heliyon.2022.e10103 PMid:35991980 PMCid: PMC9382276
2. Mohan SC, Socrates SH. Phytochemical analysis of flower extracts of different *Cassia s*pecies by using gas chromatography-mass spectrometry. J Biol Chem. 2019; 13(1):1-11. https://doi.org/10.3923/ijbc.2019.1.11
3. Jameel M, Ali A, Ali M. Extraction and isolation of new compounds from traditional herbal medicine *Clerodendrum phlomidis* Linn. Future J Pharm Sci. 2017; 3(2):118-23. https://doi.org/10.1016/j.fjps.2017.04.003
4. Sharma A, Kumar A, Jaitak V. Pharmacological and chemical potential of *Cassia fistula* L- A critical review. Herb Med J. 2020; 100407. https://doi.org/10.1016/j.hermed.2020.100407
5. Ashraf Ali Md. *Cassia fistula* Linn: A review of phytochemical and pharmacological studies. Int J Biol Pharm Allied Sci. 2019; 8(6). https://doi.org/10.31032/IJBPAS/2019/8.6.4734
6. Chaerunisaa AY, Susilawati Y, Muhaimin M, Milanda T, Hendriani R, Subarnas A. Antibacterial activity and subchronic toxicity of *Cassia fistula* L. barks in rats. Toxicol Rep. 2020; 7:649-57. https://doi.org/10.1016/j.toxrep.2020.04.013 PMid:32461915 PMCid: PMC7240208.
7. Meena AK, Ilavarasan R, Perumal A, Singh R, Ojha V, Srikanth N, *et al*. Evaluation for substitution of stem bark with small branches of *Cassia fistula* Linn for traditional medicinal uses: A comparative chemical profiling studies by HPLC, LC-MS, GC-MS. Heliyon. 2022; 8(8):e10251. https://doi.org/10.1016/j.heliyon.2022.e10251 PMid:36033318 PMCid: PMC9404363
8. Ali Khan B. Investigation of the effects of extraction solvent/technique on the antioxidant activity of *Cassia fistula* L. J Med Plant Res. 2012; 6(3). https://doi.org/10.5897/JMPR11.1289
9. Sanoria S, Qadrie Zl, Gautam Sp, Barwal A. *Cassia fistula*: Botany, phytochemistry and pharmacological leverages - A review. Int J Pharm Sci. 2020; 1-7. https://doi.org/10.22159/ijpps.2020v12i6.37310
10. Srividhya M, Hridya H, Shanthi V, Ramanathan K. Bioactive amento flavone isolated from *Cassia fistula* L. leaves exhibits therapeutic efficacy. 3 Biotech. 2017; 7(1). https://doi.org/10.1007/s13205-017-0599-7 PMid:28401469 PMCid: PMC5388655
11. Rahman FB, Ahmed S, Noor P, Rahman MMdM, Huq SMA, Akib MdTE, *et al*. A comprehensive multi-directional exploration of phytochemicals and bioactivities of flower extracts from *Delonix regia* (Bojer ex Hook.) Raf., *Cassia fistula* L. and *Lagerstroemia speciosa* L. Biochem Biophys Rep. 2020; 24:100805. https://doi.org/10.1016/j.bbrep.2020.100805 PMCid: PMC7495018.
12. Singh R, Khanam H, Pandey J. The biological properties and medical importance of *Cassia fistula*: A mini-review. Chem Proceedings. 2023; 14(95). https://doi.org/10.3390/ecsoc-27-16149
13. Duraipandiyan V, Baskar AA, Ignacimuthu S, Muthukumar C, Al-Harbi N. Anticancer activity of Rhein isolated from *Cassia fistula* L. flower. Asian Pac J Trop Dis. 2012; 2:S517-23. https://doi.org/10.1016/S2222-1808(12)60213-8
14. Pham DQ, Pham HT, Han JW, Nguyen TH, Nguyen HT, Nguyen TD, *et al*. Extracts and metabolites derived from the leaves of *Cassia alata* L. exhibit *in vitro* and *in vivo* antimicrobial activities against fungal and bacterial plant pathogens. Ind Crops Prod. 2021; 166:113465. https://doi.org/10.1016/j.indcrop.2021.113465
15. Alam M, Irshad M, Hassadi GE, Younis S. Bioefficacies of *Cassia fistula*: An Indian labrum. Afr J Pharm Pharmacol. 2009; 3(6):287-92. https://doi.org/10.5897/ajpp.9000068
16. Antonisamy P, Agastian P, Kang C-W, Kim NS, Kim J-H. Anti-inflammatory activity of rhein isolated from the flowers of *Cassia fistula* L. and possible underlying mechanisms. Saudi J Biol Sci. 2019; 26(1):96-104. https://doi.org/10.1016/j.sjbs.2017.04.011 PMCid: PMC6319193.
17. Zahara K, Bibi Y, Masood S, Nisa S, Qayyum A, Ishaque M, *et al*. Using HPLC-DAD and GC-MS analysis isolation and identification of anticandida Compounds from *Gui Zhen Cao* Herbs (Genus *Bidens*): An important Chinese medicinal formulation. Molecules. 2021; 26(19):5820. https://doi.org/10.3390/molecules26195820 PMid:34641363 PMCid: PMC8510080.
18. Fang X, Szołtysik R, Tang J, Bajkacz S. Efficient extraction and sensitive HPLC-MS/MS quantification of selected ecdysteroids in plants. J Food Compos Anal. 2022; 110:104580. https://doi.org/10.1016/j.jfca.2022.104580
19. S. Qian Y, Ramamurthy S, Candasamy M, Shadab Md, Kumar RH, Meka VS. Production, characterization and evaluation of Kaempferol nanosuspension for improving oral bioavailability. Curr Pharm Biotechnol. 2016; 17(6):549-55. https://doi.org/10.2174/1389201017666160127110609 PMid:26813303
20. Gabriela, Weiss Angeli V, Colombo M, Scherer Koester L. Investigation of the compatibility between kaempferol and excipients by thermal, spectroscopic and chemometric methods. Journal of Thermal Analysis and Calorimetry. 2019; 142(3):1249-60. https://doi.org/10.1007/s10973-019-09092-w

21. Telange DR, Patil AT, Pethe AM, Tatode AA, Anand S, Dave VS. Kaempferol-Phospholipid complex: formulation and evaluation of improved solubility, *In Vivo* bioavailability and antioxidant potential of Kaempferol. J Excip Food Chem. 2016; 7(4):89.

22. Raćkowska E, Bobrowska-Korczak B, Giebułtowicz J. Development and validation of a rapid LC-MS/MS method for determination of methylated nucleosides and nucleobases in urine. J Chromatogr B. 2019; 1128:121775. https://doi.org/10.1016/j.jchromb.2019.121775 PMid:31493699.