- Short communication

Bangladesh J. Bot. **52**(2): 393-399, 2022 (June) DOI: https://doi.org/10.3329/bjb.v51i2.60438

PHYTOCHEMICAL PROFILE OF *N*-HEXANE FLOWER EXTRACT OF *CASSIA FISTULA* L.

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Keywords: Cassia fistula, Flower extract, GC-MS analysis, ***n***-hexane

Abstract

Flowers of *Cassia fistula* L. were collected from Lahore, Pakistan and extracted in methanol followed by separation of *n*-hexane soluble compounds in a separating funnel. The separated *n*-hexane phase was analyzed by GC-MS for identification of various possible phytochemicals. Among the 31 identified compounds, the predominant compound in this fraction was butanoic acid, methyl ester with 17.81% peak area followed by 3-eicosene, (E)- (6.83%), 2-ethylformanilide (6.59%), naphthalene (6.10%), trifluoroacetoxy hexadecane (5.88%), 5-octadecene, (E)- (5.58%), phenylethanolamine (5.09%), 1-hexene-3,5-dione (4.68%) tetratetracontane (3.42%), which were ranked as moderately abundant compounds. Eleven compounds including benzenebutanamine (3.80%), heptadecyl trifluoroacetate (3.65%), heptadecane (3.08%), octadecyl trifluoroacetate (2.30%), 2-pyrrolidinone, 1-methyl- (1.78%), 1-undecanol (1.64%), ethylene glycol, O,O-di(pivaloyl)- (1.48%), benzyl alcohol (1.38%), hexadecanoic acid, 15-methyl-, methyl ester (1.32%), 1-docosene (1.27%) and heneicosane (1.25%), with their peak areas between 1 and 4% were categorized as less abundant compounds. The remaining 9 compounds with peak areas less than 1% were categorized as least abundant or minor compounds.

Pakistan is rich in a variety of medicinal plants possibly because of diverse climatic zones ranging from arid and semi-arid to temperate and tropical. Around 2000 plant species from Pakistan are known to be medicinally important (Ullah 2017). Need for medicinal plants is increasing all over the world due to recognition of natural products. These plants and their products are gaining popularity because of low toxicity, availability, low cost and pharmacologically effectiveness as compared to synthetic drugs (Saet *et al*. 2007). About 40% of drugs, including those to cure cancer, approved during the last 20 years are of natural origin. At present China, France, Italy Japan, UK and USA are the major international markets of medicinal plants trade. Keeping in view the present increase in medicinal plants trade, it is estimated that this trade will reach to $5 trillion by 2050 (Ullah 2017). Many recent studies have shown that plants of Pakistan namely *Datura metel* L. (Jabeen *et al.* 2022), *Melia azedarach* L. (Akbar *et al.* 2022), *Sonchus oleraceous* L. (Banaras *et al*. 2020), *Chenopodium quinoa* Willd. (Khan and Javaid 2020) and *Chenopodium murale* L. (Naqvi *et al*. 2020) contain a variety of potent biologically active compounds.

Golden shower (*Cassia fistula* L.) is a slow growing tree species belonging to Fabaceae. It is native to Indian Subcontinent. Now it has been distributed in various countries like Bangladesh, Brazil, China, East Africa, Mauritius, Mexico, Nepal, South Africa and West Indies and is being cultivated as an ornamental tree (Bhalodia *et al*. 2012). It is a very useful plant from medicinal and pharmacological point of view as all parts of this plant have shown various biological activities (Sharma *et al.* 2020; Ferdosi *et al*. 2021, 2022). All parts of this plant are known to be used to cure diarrhea and intestinal disorders (Biswas and Ghosh 1973). It also has therapeutic value and exerts

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analgesic and antipyretic effects (Patel *et al.* 1965). Bark extract possesses antioxidant, antibacterial and anti-inflammatory activities (Ilavarasan *et al.* 2005, Chaerunisaa *et al.* 2020). Leaf extract of *C. fistula* exhibits hepatoprotective, antimicrobial, hypoglycemic and wound healing properties (Bhakta *et al.* 1998, 1999). Fruits, flowers and seeds have been used to treat various ailments such as abdominal pain, fever, and skin diseases (Bahorun *et al.* 2005). Duraipandiyan *et al*. (2011) isolated a compound rhein from flowers of *C. fistula* that exhibited significant larvicidal and antifeedant potential against *Helicoverpa armigera*. Studies regarding phytochemical analysis and bioactivities of *C. fistula* from Pakistan are very few. Therefore, the present study was undertaken to explore phytochemical profile of *n*-hexane fraction of methanolic leaf extract of *C. fistula* through GC-MS analysis.

Flowers of *C. fistula* were collected from Lahore, Pakistan during May 2020. The collected flowers were shade dried and 50 g dry material was soaked in 200 ml of methanol for two weeks. After filtration, methanol was evaporated on a rotary evaporator and the obtained biomass was mixed in 100 ml water. This mixture was partitioned with 100 ml *n*-hexane in a separating funnel. Once the two layers were completely differentiated, the *n*-hexane layer was separated, filtered and preserved for identification of the compounds through GC-MS analysis.

GC-MS analysis was carried out on a gas chromatograph (GC) machine model 7890B, Agilent Technologies (USA), assembled with mass spectroscopy (MS) machine model 5977, Agilent Technologies (USA), for the identification of different compounds from the sample. The column used was DB 5 MS (30 m × 0.25 µm × 0.25 µm). Injection volume was 1 µl and carrier gas was helium. Oven ramping; initial temperature was 80ºC and then raised 10ºC per min up to 300ºC. Inlet temperature was 280ºC. MS conditions were as mode: scan 50…500, the source temperature was 230ºC and quadrupole temperature was 150ºC. Chemical compounds were identified by comparison of their spectra with library and arranged in the ascending order of their retention times and retention indices. The relative abundance was reported by using their peak areas.

GC-MS chromatogram of *n*-hexane fraction of methanolic flower extract of *C. fistula* exhibited the presence of 31 compounds (Fig. 1). Details of these compounds such as retention time, peak area percentages, molecular formulae and molecular weights are presented in Table 1. The predominant compound in this fraction was butanoic acid, methyl ester with 17.81% peak area. This compound belongs to fatty acids methyl ester group. Mostly members of this group possess antifungal, antibacterial (Suresh *et al.* 2014, Ali *et al.* 2017), and insecticidal activities (de Meloa *et al.* 2018). Fatty acids methyl ester namely methyl oleate, methyl linoleate and methyl palmitate of vegetable oils reduced growth of *Paracoccidioides* spp. (Pinto *et al.* 2017).

Eight compounds namely phenylethanolamine (5.09%), 5-octadecene, (E)- (5.58%), naphthalene (6.10%), 3-eicosene, (E)- (6.83%), trifluoroacetoxy hexadecane (5.88%), tetratetracontane (3.42%), 2-ethylformanilide (6.59%) and 1-hexene-3,5-dione (4.68%) were ranked as moderately abundant compounds with their peak areas from 4 to 7% (Table 1). Naragani *et al*. (2016) identified 5-octadecene, (E)- from secondary metabolites of *Streptomyces cheonanensis* with strong antimicrobial potential against *Escherichia coli*, *Staphylococcus epidermis*, *S. aureus*, *Penicillium citrinum* and *Aspergillus flavus*. Similarly, naphthalene was isolated from a medicinal plant *Mussaenda frondosa* with potent insecticidal properties (Vadivel and Gopalakrishnan 2011). Balachandar *et al*. (2018) reported that 3-eicosene, (E)- found to be very effective against *E. coli, P. aeruginosa, B. subtilis, B. circulans* and *S. aureus*. Likewise, 1-hexene-3,5-dione was identified from the extracts of *Quercus crassifolia* and tested against bacterial pathogens where it showed the best antibacterial potential against *Streptococcus thermophilus, Lactobacillus bulgaricus* and *E. coli* (Valencia-Avilés *et al.* 2019).

Table 1. Compounds identified in *n*-hexane fraction of methanolic flower extract of *C. fistula* through GC-MS analysis.

Sl. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Decane	$C_{10}H_{22}$	142.28	4.983	0.62
2	Benzyl alcohol	C_7H_8O	108.14	5.492	1.38
3	2-Pyrrolidinone, 1-methyl-	C_5H_9NO	99.13	5.568	1.78
4	Benzene, 1,2-diethyl-	$C_{10}H_{14}$	134.22	5.812	0.90
5	Aniline, N-methyl-	C_7H_9N	107.15	5.941	0.71
6	Benzene, 4-ethyl-1,2-dimethyl-	$C_{10}H_{14}$	134.22	6.208	0.63
7	Benzene, 1,2,4,5-tetramethyl-	$C_{10}H_{14}$	134.22	6.715	0.62
8	1-Undecanol	$C_{11}H_{24}O$	172.31	7.642	1.64
9	Naphthalene	$C_{10}H_8$	128.17	7.729	6.10
10	1-Hexadecanol	$C_{16}H_{34}O$	242.44	10.391	3.42
11	Hexadecane	$C_{16}H_{34}$	226.44	10.492	0.72
12	Pentadecane	$C_{15}H_{32}$	212.41	11.768	0.90
13	Phenylethanolamine	$C_8H_{11}NO$	137.08	11.868	5.09
14	5-Octadecene, (E)-	$C_{18}H_{36}$	252.5	12.90	5.58
15	Heneicosane	$C_{21}H_{44}$	296.6	14.129	1.25
16	3-Eicosene, (E)-	$C_{20}H_{40}$	280.5	15.160	6.83
17	Heptacosane	$C_{27}H_{56}$	380.7	16.265	0.77
18	Ethylene glycol, O,O-di(pivaloyl)-	$C_{12}H_{22}O_4$	230.3	16.375	1.48
19	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284.47	16.512	1.32
20	Trifluoroacetoxy hexadecane	$C_{18}H_{33}F_3O_2$	338.4	17.210	5.88
21	Cyclooctene, 1,2-dimethyl-	$C_{10}H_{18}$	138.5	18.152	0.67
22	Heptadecyl trifluoroacetate	$C_{19}H_{35}F_3O_2$	352.47	19.077	3.65
23	Heptadecane	$C_{17}H_{36}$	240.46	19.127	3.08
24	Hexacosane	$C_{26}H_{54}$	366.7	19.99	4.05
25	Octadecyl trifluoroacetate	$C_{20}H_{37}F_3O_2$	366.50	20.796	2.30
26	Tetratetracontane	$C_{44}H_{90}$	619.2	20.838	4.32
27	Benzenebutanamine	$C_8H_{11}N$	121.17	20.962	3.80
28	Butanoic acid, methyl ester	$C_5H_{10}O_2$	102.13	21.788	17.81
29	2-Ethylformanilide	$C_9H_{11}NO$	149.19	21.907	6.59
30	1-Docosene	$C_{22}H_{44}$	308.58	22.379	1.27
31	1-Hexene-3,5-dione	$C_6H_8O_2$	112.12	23.340	4.68

Table 2. Properties of compounds identified in *n*-hexane fraction of methanolic flower extract of *C. fistula* as reported in the literature.

Sl. No.	Names of compounds	Bioactivity	Reference
1	Decane	Antimicrobial	Ubaid *et al.* (2016)
2	Benzyl alcohol	Anti-inflammatory, antimicrobial and antioxidant	Shaaban *et al.* (2012)
3	2-Pyrrolidinone, 1-methyl-	Pesticidal	Deb and Kumar (2019)
4	Benzene, 1,2-diethyl-	Unknown	
5	Aniline, N-methyl-	Unknown	
6	Benzene, 4-ethyl-1,2-dimethyl-	Larvicidal	Ojewumi *et al.* (2017)
7	Benzene, 1,2,4,5-tetramethyl-	Unknown	
8	1-Undecanol	Antioxidant	Qader *et al.* (2012)
9	Naphthalene	Insecticidal	Vadivel and Gopalakrishnan (2011)
10	1-Hexadecanol	Antioxidant	Amudha *et al.* (2018)
11	Hexadecane	Antioxidant, antibacterial and antifungal	Arora *et al.* (2017)
12	Pentadecane	Antibacterial	Arora *et al.* (2017)
13	Phenylethanolamine	Unknown	
14	5-Octadecene, (E)-	Antimicrobial	Naragani *et al.* (2016)
15	Heneicosane	Antimicrobial	Vanitha *et al.* (2020)
16	3-Eicosene, (E)-	Antibacterial	Balachandar *et al.* (2018)
17	Heptacosane	Antibacterial	Konovalova *et al.* (2013)
18	Ethylene glycol, O,O-di(pivaloyl)-	Unknown	
19	Hexadecanoic acid, 15-methyl-, methyl ester	Antioxidant	Prabhu *et al.* (2018)
20	Trifluoroacetoxy hexadecane	Antifungal	Ibrahim *et al.* (2017)
21	Cyclooctene, 1,2-dimethyl-	Unknown	
22	Heptadecyl trifluoroacetate	Antimicrobial	Thekkangil and Suchithra, (2020)
23	Heptadecane	Antifungal	Amudha *et al.* (2018)
24	Hexacosane	Antibacterial and antifungal	Devender and Ramakrishna, (2017)
25	Octadecyl trifluoroacetate	Unknown	
26	Tetratetracontane	Antioxidant and cytoprotective activities	Amudha *et al.* (2018)
27	Benzenebutanamine	Anticancer	Gromek *et al.* (2016)
28	Butanoic acid, methyl ester	Antimicrobial	Abdelillah *et al.* (2013)
29	2-Ethylformanilide	Unknown	
30	1-Docosene	Antibacterial	Kumar *et al.* (2011)
31	1-Hexene-3,5-dione	Antibacterial	Valencia-Avilés *et al.* (2019)

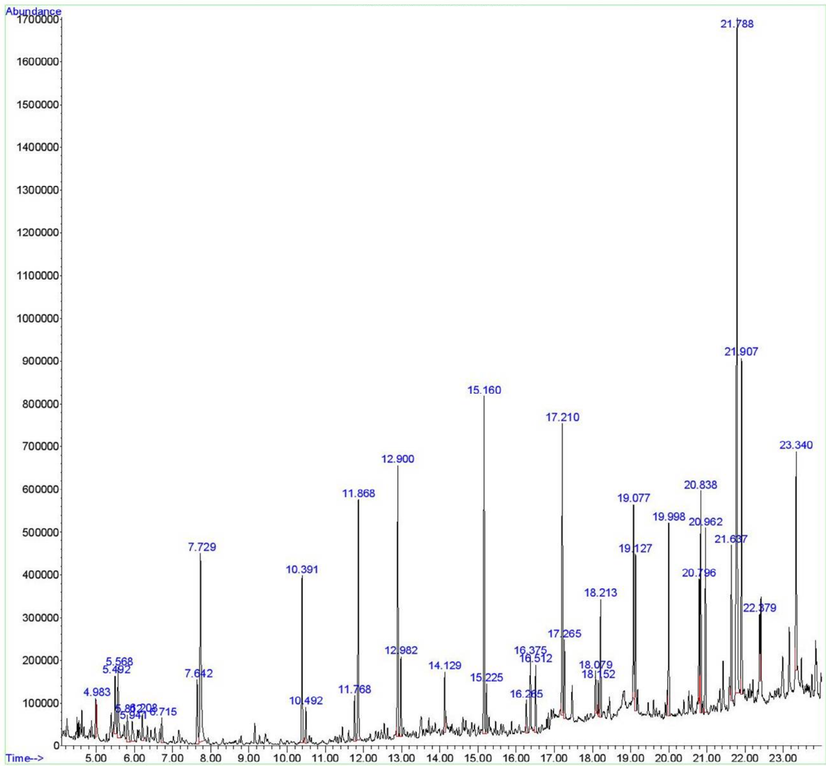

Fig. 1. GC-MS chromatogram of *n*-hexane fraction of methanolic flower extract of *C. fistula.*

Compounds with their peak areas between 1 and 4% were categorized as less abundant compounds. These included benzyl alcohol (1.38%), 2-pyrrolidinone, 1-methyl- (1.78%), 1-undecanol (1.64%), heneicosane (1.25%), ethylene glycol, O,O-di(pivaloyl)- (1.48%), hexadecanoic acid, 15-methyl-, methyl ester (1.32%), heptadecyl trifluoroacetate (3.65%), heptadecane (3.08%), octadecyl trifluoroacetate (2.30%), benzenebutanamine (3.80%) and 1-docosene (1.27%) (Table1). Recently, 2-pyrrolidinone, 1-methyl- was isolated from the essential oils of *Artemisia annua* with excellent pesticidal activity against a stored grain pest *Tribolium casteneum* (Deb and Kumar 2019). Similarly, Vanitha *et al*. (2020) identified the heneicosane from the leaf extracts of *Plumbago zeylanica* and tested it against a wide range of fungal and bacterial pathogens. The findings revealed the highest inhibitory potential against *A. fumigatus* and *S. pneumoniae*.

The least abundant compounds with peak areas less than 1% included decane (0.62%), benzene, 1,2-diethyl- (0.90%), aniline, N-methyl- (0.71%), benzene, 4-ethyl-1,2-dimethyl- (0.63%), benzene, 1,2,4,5-tetramethyl- (0.62%), hexadecane (0.72%), pentadecane (0.9%), heptacosane (0.77%) and Cyclooctene, 1,2-dimethyl- (0.67%) (Table 1). Decane was isolated from the extracts of *Camponotus fellah* (Ubaid *et al*. 2016) whereas, hexadecane and pentadecane were identified from the extracts of *Cenchrus setigerus* (Arora *et al*. 2017) where they exhibited antimicrobial activities against many pathogens. Similarly, Ojewumi *et al*. (2017) worked on lemon grass and reported the presence of benzene, 4-ethyl-1,2-dimethyl- which is used on commercial scale in the formulation of mosquito repellent creams.

It may be concluded from the present study that *n*-hexane fraction of flower extract of *C. fistula* is a rich source of bioactive compounds. Most of the compounds in this fraction namely Hexadecane; 5-Octadecene, (E)-; butanoic acid, methyl ester; 1-Hexene-3,5-dione and heptadecyl trifluoroacetate, possess antimicrobial properties. However, some compounds are also antioxidant (hexadecanoic acid, 15-methyl-, methyl ester), anticancer (benzenebutanamine), anti-inflamatory (benzyl alcohol), pesticidal (2-pyrrolidinone, 1-methyl-) and larvicidal (benzene, 4-ethyl-1,2-dimethyl-) in nature.

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(Manuscript received on 03 December 2021; revised on 17 May 2022)