Journal of Animal & Plant Sciences, 33(3): 2023, Page: 511-517
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2023.3.0643

BIOACTIVE COMPONENTS OF ETHYL ACETATE EXTRACT OF *CASSIA FISTULA* FLOWERS

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ABSTRACT

This study was carried out to identify the phytoconstituents in ethyl acetate fraction of flower extract of Indian laburnum (*Cassia fistula* L.) and to enlist their bioactivities as reported in the literature. For this purpose, dry flowers of the selected plant species were extracted in methanol and the ethyl acetate soluble compounds of this extract were separated using separating funnel technique. The isolated ethyl acetate fraction was analyzed by GC-MS that indicated the presence of 39 phytoconstituents. Among these, 2-butenal, 2-ethyl- (21.12%), decane (9.13%) and 1-decanol, 2-hexyl- (8.09%) were identified as principal constituents. Four compounds namely cyclohexanone, 2,3-dimethyl- (4.45%), benzene, 1-ethyl-3-methyl- (3.18%), phenol, 4-propyl- (3.24%), and heptadecane (3.89%) were ranked as moderately abundant. Less abundant compounds included mesitylene (2.81%), nonane, 4-methyl- (2.79%), nonane, 3-methyl- (2.74%), nonadecane (2.66%), naphthalene (2.13%), tetratetracontane (2.05%), trifluoroacetoxy hexadecane (2.01%), octane, 2,5-dimethyl- (1.88%), nonane, 2-methyl- (1.75%), octane, 2,6-dimethyl- (1.66%), decane, 2,5,6-trimethyl- (1.71%), hexacosane (1.61%), heptadecane, 2,6,10,14-tetramethyl- (1.38%), benzene, 1,2,3-trimethyl- (1.38%), benzofuran, 2,3-dihydro- (1.34%), benzene, nitro- (1.24%), 17-pentatriacontene (1.31%), hexadecane (1.29%), pentadecane (1.46%), 5-octadecene, (E)- (1.13%), tetradecane (1.03%), and oxalic acid, allyl hexadecyl ester (1.01%). The remaining 9 compounds were present as the least abundant compounds with peak areas <1%. Literature showed that among the identified compounds, some possess antimicrobial, antioxidant, anti-inflammatory and anticancer properties.

Keywords: Flower extract, *Cassia fistula,* Ethyl acetate, GC-MS analysis, Phytochemical profile.

Published first online February 10, 2023 Published final June 18, 2023

INTRODUCTION

Plant-derived products have recently become of great interest owing to their versatile applications in nutraceuticals, pharmaceuticals, traditional, modern and folk medicine system (Rahman *et al*., 2020). Around 15 to 29% of higher plant species have been found to be used medicinally and that of 72% of pharmacologically active plant derived components were discovered on the basis of ethnomedicinal use of the plants (Thomford *et al*., 2018). The foremost steps to utilize the biologically active compounds from plant resources are extraction, isolation, characterization and pharmacological screening (Diniz *et al*., 2020; Ergün, 2022; Nawaz *et al.*, 2023). The adverse effects, therapeutic limitations and high cost of synthetic drugs are the key factors that are driving attraction towards ethnopharmacognosy where a thousand of phytochemicals found with no adverse effects (Shukla, 2020; Javaid *et al.*, 2022). Plant extracts are gaining importance because of their unmatched chemical diversity and opportunity towards new drug discoveries that have been investigated by a number of researchers worldwide (Majolo *et al*., 2019; Javaid *et al.*, 2021). The reported biological activities of the phytoconstituents include herbicidal, antioxidant, antimicrobial, anticancer, analgesic, wound healing and antidiarrheal properties (Khan and Javaid, 2020a; Javed *et al.*, 2021; Akbar *et al.*, 2022). In Asian countries, the use of herbal medicines for the treatment of various diseases shows a long history of human interactions with the environment (Sadia *et al*., 2018).

Cassia fistula L. commonly known as Amulthus or golden shower tree belongs to the family Caesalpiniaceae (Siddiqua *et al*., 2018). It is native to Pakistan, Sri Lanka, Amazon, India, South Africa, China, West Indies, Mexico and Brazil (Tanveer *et al*., 2019). It is a deciduous semi-wild tree known for the beauty of its yellow color flower bunches. It has greenish grey bark, compound leaves and leaflets each of 6–13 cm long (Rahman *et al*., 2020). It has long cylindrical pendulous

pods which are nearly straight, smooth and brown-black in color which bear ovate black seeds (Sharma, 2017). Its flowers contain tannins, anthraquinone, oxyanthraquinone, phlobaphenes, rhein, fistulic acid, oxyanthraquinone and volatile oils (Sharma *et al*., 2021). It has been used extensively in Ayurvedic medicine system for the procurement of many intestinal disorders like healing ulcers (Zhao *et al.,* 2013). The plant has a high therapeutic value and exerts analgesic and antipyretic effects due to its mucilage and fiber contents (Nagpal *et al*., 2011). Flower extracts are used extensively against liver troubles, skin disorders, prurtius, diabetes, leucoderm, haematemesis, tuberculous glands and rheumatism (Sharma *et al*., 2020). There are reports indicating its hepatoprotective, anti-inflammatory, antifertility, antioxidant and antibacterial properties (Ferdosi *et al.,* 2022). Besides its pharmacological uses, the plant extracts are also recommended as pest and disease control agents in agriculture (Sony *et al*., 2018; Chaerunisaa *et al*., 2020). Thus, *C. fistula* is well anchored in its traditional uses. Therefore, the present study was carried out to evaluate the chemical profile of *C. fistula* flowers ethyl acetate extract through GC-MS analysis.

MATERIALS AND METHODS

Preparation of extract: During the month of June 2019, flowers of *C. fistula* were collected from Lahore Pakistan and dried under the shade. One hundred grams of the dried flowers were soaked in methanol and filtered after two weeks. Thereafter, the solvent was evaporated on a rotary evaporator and the resultant gummy biomass was suspended in 100 ml of distilled water. Same volume of *n*-hexane was added to this mixture and shacked well for five minutes. The whole mixture was transferred to a separating funnel and left for two hours to separate the *n*-hexane and aqueous phases. *n*-Hexane layer was separated and to the aqueous phases, 100 ml *n*-hexane was added again and the process was repeated thrice to separate all the *n*-hexane soluble compounds. Then 100 ml of chloroform was added to the aqueous phase. After separating chloroform layer, finally 100 ml of ethyl acetate were mixed in the aqueous phases and after separating the layer, ethyl acetate fraction was subjected to GC-MS analysis for compounds identification (Khan and Javaid, 2020b).

GC-MS analysis: The gas chromatograph (GC) machine model 7890B and that of mass spectroscopy (MS) 5977A were used for the identification of different compounds from the sample while both were branded by Agilent technologies. 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 80 ºC and then raised 10 ºC per minute up to 300 ºC. Inlet temperature was 280 ºC. MS conditions were as mode: scan 50…500, the source temperature was 230 ºC and quadrupole temperature was 150 ºC. 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.

RESULTS AND DISCUSSION

A total of 39 compounds were identified in the ethyl acetate fraction as shown in Fig. 1 and Table 1. On the basis of peak area percentages, the compounds were categorized into four groups namely the highly abundant or principal constituents (>8%), moderately abundant (3 to 7.99%), less abundant (1 to 2.99%) and the least abundant or minor compounds (<1%). The highly abundant compounds were 2-butenal, 2-ethyl- (21.12%), 1-decanol, 2-hexyl- (8.09 %) and decane (9.13%). The most abundant compound 2-butenal, 2-ethyl- has been reported in various *Allium* spp. namely *A. tuberosum*, *A. chinense* and *A. fistulosum* (Azadi *et al.,* 2009), and belongs to unsaturated aliphatic aldehydes. Compounds of this class are known for their antifungal activity against *Microsporum canis* and *Tricophyton mentagrophytes* (Battinelli *et al.,* 2006). Antimicrobial activity of olive oil against a variety of food-borne bacteria and fungi was primarily because of some aldehydes (Kubo *et al.,* 1995). 1-Decanol, 2-hexyl- was found in methanolic extract of *Solena amplexicaulis* and is known to possess antimicrobial activity (Krishnamoorthy and Subramaniam, 2014).

Moderately abundant compounds included cyclohexanone, 2,3-dimethyl- (4.45%), benzene, 1-ethyl-3-methyl- (3.18%), phenol, 4-propyl- (3.24%), and heptadecane (3.89%) (Table 1). Among these, heptadecane was previously identified from two species of *Scutellaria* namely *S. multicaulis* and *S. bornmuelleri* from Iran (Zahra *et al.,* 2019), and benzene, 1-ethyl-3-methyl- from *Pulicaria undulata* from Saudi Arabia (Alshehri and Ghobashy, 2020). There is not any report of biological activities of these moderately abundant compounds.

Less abundant compounds included octane, 2,5-dimethyl- (1.88%), heptadecane, 2,6,10,14-tetramethyl- (1.38%), octane, 2,6-dimethyl- (1.66%), decane, 2,5,6-trimethyl- (1.71%), nonane, 4-methyl- (2.79%), nonane, 2-methyl- (1.75%), nonane, 3-methyl- (2.74%), mesitylene (2.81%), benzene, 1-ethyl-2-methyl- (1.31%), benzene, 1,2,3-trimethyl- (1.38%), benzene, nitro- (1.24%), tetradecane (1.03%), naphthalene (2.13%), benzofuran, 2,3-dihydro- (1.34%), hexadecane (1.29%), pentadecane (1.46%), 5-octadecene, (E)- (1.13%), nonadecane (2.66%), hexacosane (1.61%), trifluoroacetoxy hexadecane (2.01%), tetratetracontane (2.05%), 17-pentatriacontene (1.31%), and oxalic acid,

allyl hexadecyl ester (1.01%) (Table 1). Some of these compounds have biological activities. Naphthalene, trifluoroacetoxy hexadecane and tetradecane possess antifungal, antibacterial and anti-inflammatory properties (Ibrahim *et al.,* 2017), tetratetracontane is an antioxidant agent (Mallick and Dighe, 2014), while hexadecane is an antibacterial agent (Gideon, 2015). Oxalic acid, allyl hexadecyl ester possesses acaricide properties and can be used to control ticks and mites (Zayed and Samling, 2016). 17-Pentatriacontene is known for its antiarthritic, anticancer, anti-inflammatory and antibacterial activities (Dinesh *et al.,* 2018).

Compound namely nonane, 5-methyl- (0.68%), benzyl alcohol (0.95%), benzene, 1-methyl-3-propyl- (0.76%), benzene, 1-methyl-4-propyl- (0.66%), resorcinol (0.94%), cetene (0.63%), isopropyl 2-ethylhexanoate (0.75%), sulfurous acid, butyl heptadecyl ester (0.98%), and 1-heptanol, 2,4-diethyl- (0.89%) were ranked as the least abundant ones (Table 1). Among these, benzyl alcohol possesses antimicrobial properties (Lucchini *et al.,* 1990), while resorcinol is known for its antifungal activity against human pathogenic fungus *Candida albicans* (Ansari *et al.,* 2015).

Table 1: Compounds identified in ethyl acetate fraction of methanolic extract of *Cassia fistula* flowers through GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Octane, 2,5-dimethyl-	$C_{10}H_{22}$	142.28	4.129	1.88
2	Heptadecane, 2,6,10,14-tetramethyl-	$C_{21}H_{44}$	296.57	4.194	1.38
3	Octane, 2,6-dimethyl-	$C_{10}H_{22}$	142.28	4.242	1.66
4	Cyclohexanone, 2,3-dimethyl-	$C_8H_{14}O$	126.19	4.298	4.45
5	Decane, 2,5,6-trimethyl-	$C_{13}H_{28}$	184.36	4.475	1.71
6	Nonane, 5-methyl-	$C_{10}H_{22}$	142.28	4.518	0.68
7	Nonane, 4-methyl-	$C_{10}H_{22}$	142.28	4.544	2.79
8	Nonane, 2-methyl-	$C_{10}H_{22}$	142.28	4.586	1.75
9	Benzene, 1-ethyl-3-methyl-	C_9H_{12}	120.19	4.625	3.18
10	Nonane, 3-methyl-	$C_{10}H_{22}$	142.28	4.665	2.74
11	Mesitylene	C_9H_{12}	120.19	4.723	2.81
12	Benzene, 1-ethyl-2-methyl-	C_9H_{12}	120.19	4.843	1.31
13	Decane	$C_{10}H_{22}$	142.28	5.022	9.23
14	Benzene, 1,2,3-trimethyl-	C_9H_{12}	120.19	5.392	1.38
15	Benzyl alcohol	C_7H_8O	108.14	5.521	0.95
16	Benzene, 1-methyl-3-propyl-	$C_{10}H_{14}$	134.21	5.744	0.76
17	Benzene, 1-methyl-4-propyl-	$C_{10}H_{14}$	134.21	5.951	0.66
18	Benzene, nitro-	$C_6H_5NO_2$	123.10	6.281	1.24
19	Tetradecane	$C_{14}H_{30}$	198.39	6.344	1.03
20	Naphthalene	$C_{10}H_8$	128.17	7.713	2.13
21	Benzofuran, 2,3-dihydro-	C_8H_8O	120.15	8.014	1.34
22	Resorcinol	$C_6H_6O_2$	110.11	8.926	0.94
23	Hexadecane	$C_{16}H_{34}$	226.44	9.146	1.29
24	Phenol, 4-propyl-	$C_9H_{12}O$	136.19	10.141	3.24
25	Cetene	$C_{16}H_{32}$	224.42	10.387	0.63
26	Pentadecane	$C_{15}H_{32}$	212.41	10.492	1.46
27	Isopropyl 2-ethylhexanoate	$C_{11}H_{22}O_2$	186.29	11.861	0.75
28	5-Octadecene, (E)-	$C_{18}H_{36}$	252.47	12.895	1.13
29	Nonadecane	$C_{19}H_{40}$	268.51	12.984	2.66
30	Hexacosane	$C_{26}H_{54}$	366.70	14.133	1.61
31	Trifluoroacetoxy hexadecane	$C_{18}H_{33}F_3O_2$	338.44	15.158	2.01
32	Heptadecane	$C_{17}H_{36}$	240.46	15.233	3.89
33	Tetratetracontane	$C_{44}H_{90}$	619.18	17.270	2.05
34	17-Pentatriacontene	$C_{35}H_{70}$	490.93	19.964	1.31
35	Sulfurous acid, butyl heptadecyl ester	$C_{21}H_{44}O_3S$	376.6	20.836	0.98
36	Oxalic acid, allyl hexadecyl ester	$C_{21}H_{38}O_4$	354.52	21.221	1.01
37	2-Butenal, 2-ethyl-	$C_6H_{10}O$	98.14	21.812	21.12
38	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242.44	23.356	8.09
39	1-Heptanol, 2,4-diethyl-	$C_{11}H_{24}O$	172.31	23.985	0.89

Table 2: Properties of compounds identified in ethyl acetate fraction of methanolic extract of *Cassia fistula* flowers as reported in the literature.

Sr. No.	Names of compounds	Bioactivity	Reference
1	Octane, 2,5-dimethyl-	-	-
2	Heptadecane, 2,6,10,14-tetramethyl-	-	-
3	Octane, 2,6-dimethyl-	-	-
4	Cyclohexanone, 2,3-dimethyl-	-	-
5	Decane, 2,5,6-trimethyl-	-	-
6	Nonane, 5-methyl-	-	-
7	Nonane, 4-methyl-	-	-
8	Nonane, 2-methyl-	-	-
9	Benzene, 1-ethyl-3-methyl-	-	-
10	Nonane, 3-methyl-	-	-
11	Mesitylene	-	-
12	Benzene, 1-ethyl-2-methyl-	-	-
13	Decane	-	-
14	Benzene, 1,2,3-trimethyl-	-	-
15	Benzyl alcohol	Antimicrobial	Lucchini *et al.* (1990)
16	Benzene, 1-methyl-3-propyl-	-	-
17	Benzene, 1-methyl-4-propyl-	-	-
18	Benzene, nitro-	-	-
19	Tetradecane	Antifungal, Antibacterial	Ibrahim *et al.* (2017)
20	Naphthalene	Anti-inflammatory, Antimicrobial	Ibrahim *et al.* (2017)
21	Benzofuran, 2,3-dihydro-	-	-
22	Resorcinol	Antifungal	Ansari *et al.* (2015)
23	Hexadecane	Antibacterial	Gideon (2015)
24	Phenol, 4-propyl-	-	-
25	Cetene	-	-
26	Pentadecane	-	-
27	Isopropyl 2-ethylhexanoate	-	-
28	5-Octadecene, (E)-	-	-
29	Nonadecane	-	-
30	Hexacosane	-	-
31	Trifluoroacetoxy hexadecane	Antifungal	Ibrahim *et al.* (2017)
32	Heptadecane	-	-
33	Tetratetracontane	Antioxidant	Mallick and Dighe (2014)
34	17-Pentatriacontene	Antiarthritic, Anticancer, Anti-inflammatory, Antibacterial	Dinesh *et al.* (2018)
35	Sulfurous acid, butyl heptadecyl ester	-	-
36	Oxalic acid, allyl hexadecyl ester	Acaricide, Antiseptic, Pesticide	Zayed and Samling (2016)
37	2-Butenal, 2-ethyl-	Antimicrobial	Battinelli *et al.* (2006)
38	1-Decanol, 2-hexyl-	Antimicrobial	Krishnamoorthy and Subramaniam (2014)
39	1-Heptanol, 2,4-diethyl-	-	-

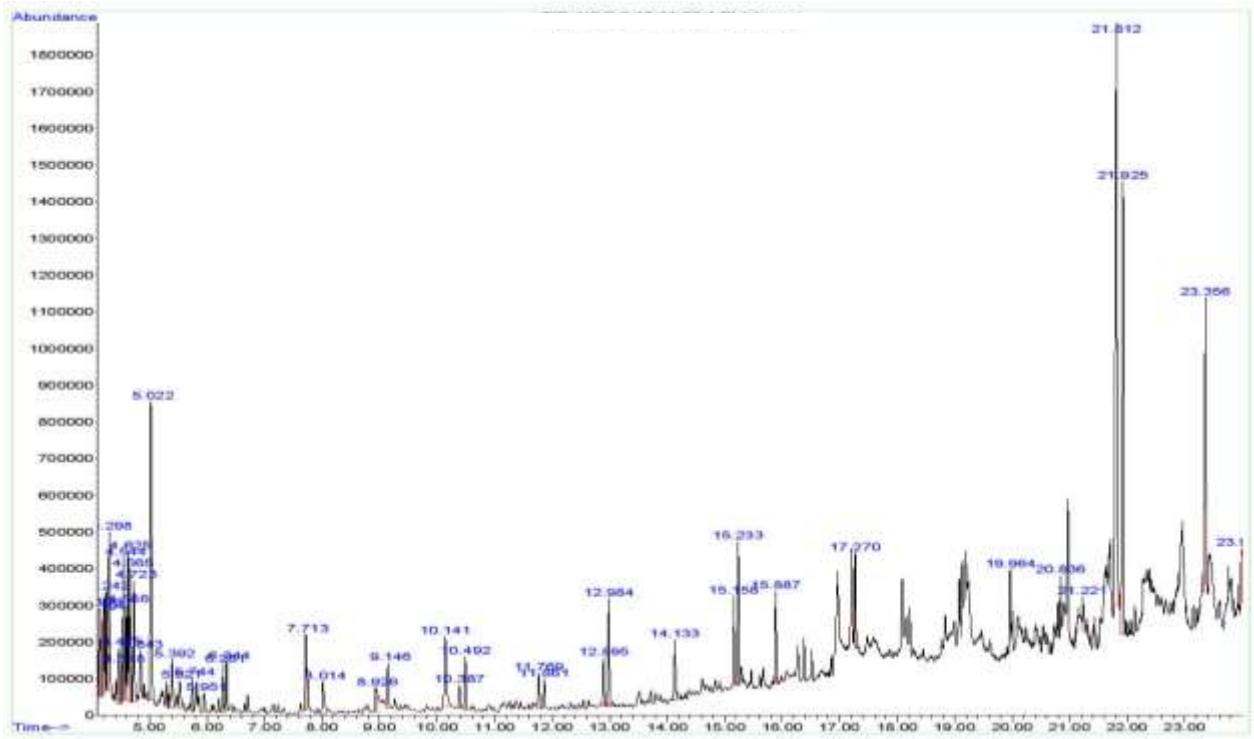

Fig. 1. GC-MS chromatogram of ethyl acetate fraction of methanolic extract of *Cassia fistula* flowers.

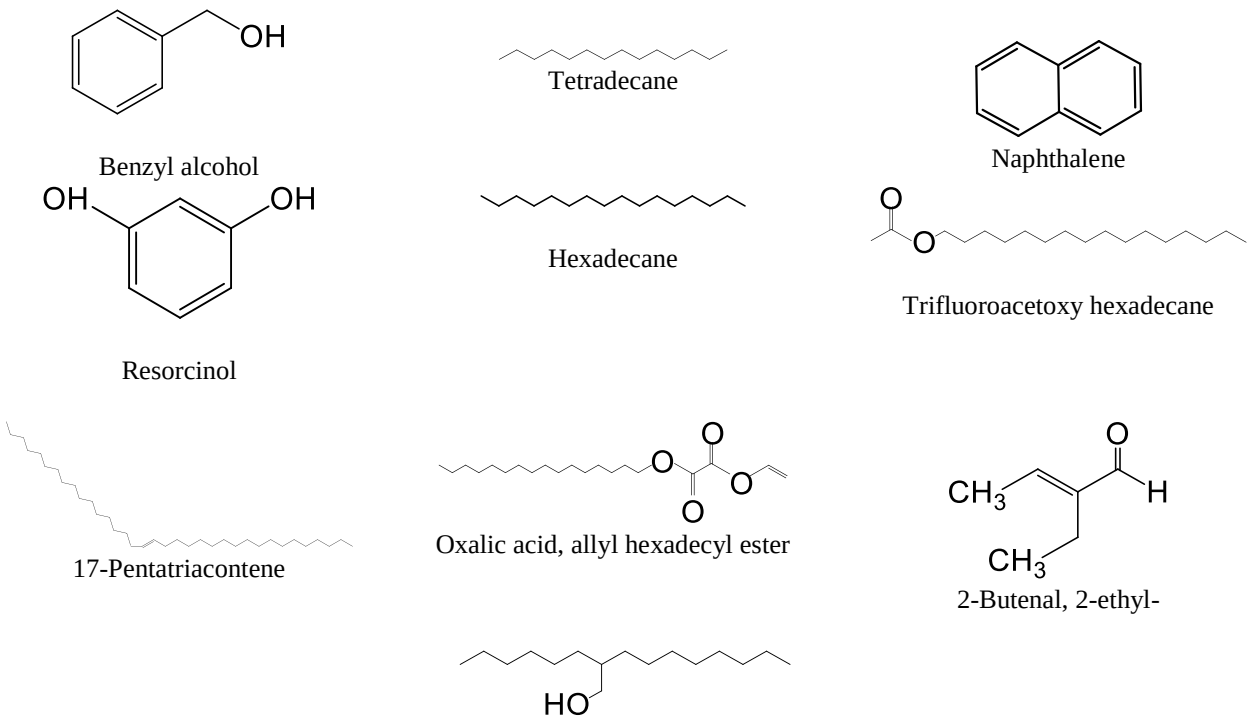

Fig. 2. Structures of biologically active compounds identified in ethyl acetate fraction of methanolic extract of *Cassia fistula* flowers.

Conclusion: This study concludes that 2-butenal, 2-ethyl-; 1-decanol, 2-hexyl- and decane are the major compounds in ethyl acetate fraction of methanolic flower extract of *C. fistula* in addition to a number of other compounds with least to moderate abundance. However, literature shows that only few of them have biological activities.

Conflict of interest disclosure: The authors declare no conflict of interest.

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