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ANALYSIS OF *N*-BUTANOL FLOWER EXTRACT OF *CASSIA FISTULA* THROUGH GC-MS AND IDENTIFICATION OF ANTIMICROBIAL COMPOUNDS

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A B S T R A C T

This study was carried out to identify antimicrobial phytoconstituents present in *n*-butanol soluble part of methanolic flower extract of *Cassia fistula* L. The flowers were dipped in methanol for two weeks, filtered and the solvent was evaporated on a rotary evaporator. The *n*-butanol fraction was separated in a separating funnel and analyzed by GC-MS. There were 18 phytoconstituents in this fraction. The most abundant compound was 1H-indene, octahydro-, *cis*- with 35.13% peak area followed by cyclohexene, 1-butyl- (21.88%) and cyclohexane, butyl- (12.87%). Other compounds included 9-heptadecanol (3.70%), behenic alcohol (1.63%), decane, 3-methyl- (2.90%), cyclohexane, 1-methyl-3-(1-methylethenyl)-, *cis*- (1.25%), 3-hexanol, 5-methyl- (1.67%), acetaldehyde isopentyl propyl acetal (1.69%), undecane (2.12%), 1,3-dioxane, 2-ethyl-5-methyl- (1.13%), acetaldehyde butyl pentyl acetal (3.04%), acetaldehyde dipentyl acetal (3.24%), cycloheptasiloxane, tetradecamethyl- (1.11%), Cyclooctasiloxane, hexadecamethyl- (1.77%), cyclononasiloxane, octadecamethyl- (1.48%), *n*-hexadecanoic acid (2.01%), and tetracosamethyl-cyclododecasiloxane (1.46%). A thorough literature survey showed that *n*-hexadecanoic acid; cyclohexane, butyl-; cyclooctasiloxane, hexadecamethyl-; cyclononasiloxane, octadecamethyl- and behenic alcohol in *n*-butanol fraction possess antifungal, antibacterial and/or antiviral properties.

Keywords: *Cassia fistula,* Flower extract; GC-MS analysis, *n*-Butanol.

INTRODUCTION

Nature is blessed with a variety of plants having economic importance. *Cassia fistula* L. is one of those blessed natural plants. It is an ornamental plant commonly known as Amaltas and is found in many countries like South America, China, Srilanka, Brazil, India and Pakistan. It is also known as golden shower due to the presence of beautiful yellow flower bunches. Beside its ornamental characteristics, it holds a significant medicinal importance. It has been reported as a storehouse of phytochemicals with a wide range of antibacterial, antifungal, antioxidant, anticancer, insecticidal and antidiabetic properties (Rajkumar and Jebanesan, 2004; Duraipandiyan and Ignacimuthu, 2007; Chaerunisaa *et al.*, 2020). Extracts made from seeds of *C. fistula* have been reported as growth regulators for larval stages of many insects (Raja *et al.,* 2000). In previous studies, methanolic extracts of *C. fistula* leaves were also tested on larvae of mosquito and they showed larvicidal potential as well (Govindarajan *et al.,* 2008). Each plant part of *C. fistula* such as roots, leaves and flowers has been reported as an excellent source of phenolics, alkaloids and teriterpenes (Asseleih *et al.,* 1990). It also contains a good purgative quality which is beneficial for pregnant women and kids (Satyavati and Sharma, 1989).

As per WHO, 70% of the world's population rely on traditional medicinal practices for their health needs. In developed countries, many people rely on medicinal plants in order to combat their health-related problems (Kunle *et al.,* 2012). Drugs obtained from these plants are considered less toxic with little or no side-effects as

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compared to synthetic medicines. There are several techniques used to isolate organic compounds from plant extracts. Both qualitative and quantitative analyses can be performed with the help of HPLC and mass spectrometry. Moreover, recent studies revealed that GC-MS is a good technique for the identification of compounds present in different plant parts (Khan and Javaid, 2019, 2020a, b; Banaras *et al.,* 2020, 2021). The presence of phytochemicals in *C. fistula* is admitted and discovered as well but still there may be many compounds which are yet to be discovered. In addition, little work is done on phytochemical analysis of *C. fistula* plants growing in the agro-ecological conditions of Lahore, Pakistan. Therefore, the main focus of the present study was to identify the phytochemicals found in *n*-butanol flower extract of *C. fistula* through GC-MS analysis, and to find out their antimicrobial properties through literature survey.

MATERIALS AND METHODS

Collection of plant material: The fresh, healthy looking flowers (whole inflorescence) of *C. fistula* were collected during May 2020 from different areas of University of the Punjab Lahore, during early hours of the day. The flowers then shifted to the lab for further scientific studies. After that petal were separated from sepals, placed on plain papers and shade dried.

Preparation of extract: One hundred grams of dried petals of C. *fistula* were soaked in 500 ml of methanol. After 15 days, the whole material was double filtered. The methanol was evaporated by using rotary evaporator and the resultant biomass was mixed in 100 ml of water. This mixture was partitioned with 100 ml of *n*-butanol with the help of separating funnel. After 3 hours, two distinct layers were observed, the *n*-butanollayer was then separated, filtered and placed in glass vials for compounds identification through GC-MS analysis.

GC-MS analysis: Gas Chromatography machine model 7890B (Agilent) and Mass spectroscopy was 5977 (Agilent) used for the identification of antimicrobial compounds from extracted 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 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 m/z, 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.

Literature survey: A thorough survey of literature was done in order to collect information regarding antimicrobial activities of each identified compound. Structures of the potential antimicrobial compounds were drawn using software ChemDraw.

RESULTS AND DISCUSSION

There were 18 compounds in the *n*-butanol fraction of methanolic flower extract of *C. fistula* as indicated in GC-MS chromatogram shown in Figure 1. Information regarding retention time, peak area percentages, and molecular formulae and weights of the compounds are given in Table 1. Structures of the antimicrobial compounds are presented in Figure 2. The principal compound in this fraction was 1Hindene, octahydro-, *cis*-with 35.13% peak area. Two other compounds namely cyclohexene, 1-butyl- and cyclohexane, butyl- were also ranked as highly abundant with 21.88% and 12.87% peak areas, respectively. Among these, the last compound is a derivative of cyclohexane. Many previous studies have shown a variety of biological activities such as analgesic, anticancer, antioxidant, anti-inflammatory, cytotoxic and antithrombin, by various derivatives of cyclohexane (Shoaib *et al.,* 2019).

Moderately abundant compounds included 9-heptadecanol (3.70%), acetaldehyde dipentyl acetal (3.24%), acetaldehyde butyl pentyl acetal (3.04%), decane, 3-methyl- (2.90%), undecane (2.12%) and *n*-hexadecanoic acid (2.01%) (Table 1). *n*-Hexadecanoic acid or palmitic acid has been reported in a number of plant species including *Albizia adianthifolia* (Abubakar and Majinda, 2016), *Mesembryanthemum edule* (Omoruyi *et al.,* 2014) and *Buchholzia coriacea* (Ojinnaka*et al.,* 2015). This compound is known for its antimicrobial activity against a number of bacterial species namely *Streptococcus mutans*, *S. sanguis, S. gordonii, Fusobacterium nucleatum, Candida albicans* and *Porphyromonas gingivalis* (Huang *et al.,* 2011). It also possesses a number of other biological activates such as mosquito larvicide (Rahuman *et al.,* 2000), hypocholesterolemic, antioxidant (Kumar *et al.,* 2010), and anti-inflammatory (Aparna *et al.,* 2012).

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

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak Area(%)
1	1H-Indene, octahydro-, *cis*-	C_9H_{16}	124.22	3.667	35.13
2	9-Heptadecanol	$C_{17}H_{36}O$	256.46	3.770	3.70
3	Behenic alcohol	$C_{22}H_{46}O$	326.6	3.930	1.63
4	Cyclohexane, butyl-	$C_{10}H_{20}$	140.27	4.027	12.87
5	Cyclohexene, 1-butyl-	$C_{10}H_{18}$	138.25	4.188	21.88
6	Decane, 3-methyl-	$C_{11}H_{24}$	156.31	4.354	2.90
7	Cyclohexane, 1-methyl-3-(1-methylethenyl)-, *cis*-	$C_{10}H_{18}$	138.25	4.405	1.25
8	3-Hexanol, 5-methyl-	$C_7H_{16}O$	116.2	4.502	1.67
9	Acetaldehyde isopentyl propyl acetal	$C_{10}H_{22}O_2$	174.28	4.583	1.69
10	Undecane	$C_{11}H_{24}$	156.31	4.686	2.12
11	1,3-Dioxane, 2-ethyl-5-methyl-	$C_7H_{14}O_2$	130.18	5.521	1.13
12	Acetaldehyde butyl pentyl acetal	$C_{11}H_{24}O_2$	188.31	5.704	3.04
13	Acetaldehyde dipentyl acetal			6.911	3.24
14	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	519.07	9.223	1.11
15	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	593.2	11.174	1.77
16	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	667.4	12.868	1.48
17	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	14.819	2.01
18	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	889.8	18.161	1.46

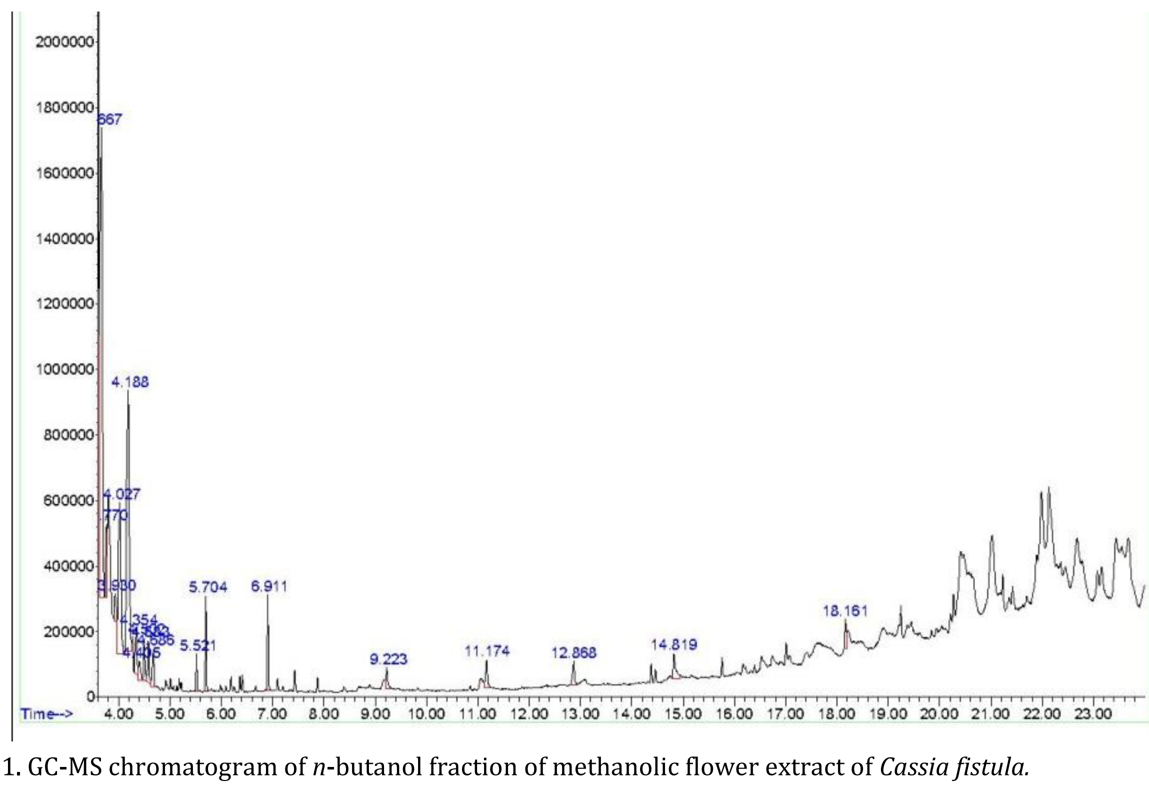
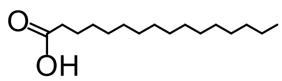
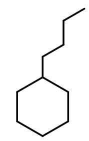


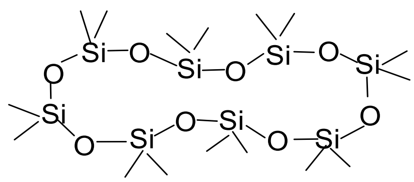
Figure 1. GC-MS chromatogram of *n*-butanol fraction of methanolic flower extract of *Cassia fistula.*



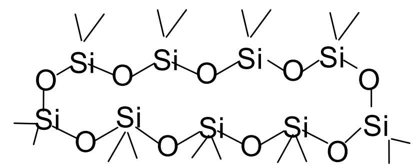
n-Hexadecanoic acid



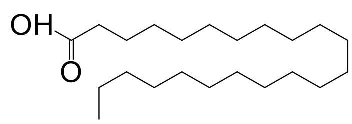
Cyclohexane, butyl-



Cyclooctasiloxane, hexadecamethyl-



Cyclononasiloxane, octadecamethyl-



Behenic alcohol

Figure 2. Structures of antimicrobial compounds identified in *n*-butanol fraction of methanolic flower extract of *Cassia fistula.*

Compounds namely cyclooctasiloxane, hexadecamethyl- (177%), acetaldehyde isopentyl propyl acetal (1.69%), 3-hexanol, 5-methyl- (1.67%), behenic alcohol (1.63%), cyclononasiloxane, octadecamethyl- (1.48%), tetracosamethyl-cyclododecasiloxane (1.46%), cyclohexane, 1-methyl-3-(1-methylethenyl)-, *cis*- (1.25%), 1,3-dioxane, 2-ethyl-5-methyl- (1.13%), and cycloheptasiloxane, tetradecamethyl- (1.11%) were the less abundant ones (Table 1). Among these, cyclooctasiloxane, hexadecamethyl- and cyclononasiloxane, octadecamethyl- have shown antibacterial and antifungal properties, respectively (Al Bratty *et al.,* 2020). Behenic alcohol also known as docosanol, is a saturated 22-carbon aliphatic fatty alcohol, generally used as an emulsifier, emollient and thickener in cosmetics. It is also known for its antimicrobial properties (Ranganathan, 2014). Food and Drug Administration (FDA) has approved it as a pharmaceutical antiviral agent (Katz *et al.,* 1991). This compound is especially effective against a wide variety of lipid-enveloped viruses such as HSV-1 and HSV-2. At the time of early in replication, this compound prevents fusion of virus with host cell membrane (Aoki, 2015).

This study concludes that *n*-butanol fraction of flower extract of *C. fistula* contains *n*-hexadecanoic acid; cyclohexane, butyl-; cyclooctasiloxane, hexadecamethyl-; cyclononasiloxane, octadecamethyl- and behenic alcohol, which possess antimicrobial properties.

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Malik F. H. Ferdosi	:	Collection of plant materials and GC-MS analysis
Arshad Javaid	:	Supervised the work and finalized the paper.
Iqra H. Khan	:	Prepared structures of compounds and contributed in writing and reviewing the manuscript
Shahbaz Ahmed	:	Contributed in writing
Nadeem Shad	:	Literature survey