Mycopath (2018) 16(2): 51-55 **Research Article**

Identification of antimicrobial compounds from *n*-hexane stem extract of *Kochia indica* by GC-MS analysis

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Abstract

The aim of this study was to carry out the identification of antimicrobial compounds from the *n*-hexane stem extract of *Kochia indica* Wight by GC-MS analysis. Dried stem of *K. indica* was extracted in methanol and its *n*-hexane fraction was separated by fractionation. The GC-MS analysis of *n*-hexane fraction revealed the presence of 11 compounds among them hexadecanoic acid, methyl ester (24.09%) and di-*n*-octyl phthalate (4.49%) possess antifungal activities whereas, *γ*-sitosterol (13.42%) and stigmasterol (4.00%) showed antibacterial activities, while 9, 12-octadecadienoic acid, methyl ester (3.92%); β-sitosterol (18.60%) and *n*-hexadecanoic acid (4.57%) exhibited antimicrobial activities. In general, *n*-hexane stem extracts appeared to be an effective source of active antifungal and antibacterial compounds.

Keywords: Antimicrobial compounds, *n*-hexane fraction, GC-MS analysis, *Kochia indica*.

Introduction

Plant pathogens play a fundamental role in plant production in terms of quality, quantity and profitability. In order to control the threat caused by diseases, farmers use fungicides to control the damage caused by different pathogens (Shuping and Eloff, 2017). However, there have been many environmental issues as well as health problems regarding the use of synthetic chemical pesticides. Development of resistance and environmental toxicity related with these chemicals has provoked researchers and cultivators to explore other possible ways. To compensate these harmful effects, biological approaches have been used to minimize losses and maximize yield growth and economy (Parka *et al*., 2002; Javaid *et al.*, 2018). To provide effective, cheap and less toxic plant based biological products, continued research is required (Martinez, 2012). Many plant species namely *Cenchrus pennisetiformis*, *Withania somnifera* and *Nigella sativa* have been investigated for this purpose (Akhtar and Javaid, 2018; Khurshid *et al.*, 2018; Aftab *et al.*, 2019). Some plants can produce a variety of antimicrobial compounds such as alkaloids, flavonoids, terpenoids, phenolics, glycosides, tannins and fatty acids in order to protect themselves against various phytopathogens (Ribera and Zuniga, 2012).

Chenopodiaceae is a large family, consisting of approximately 180 genera and 2500 species. Many species of this family are known to exhibit allelopathic effects by producing different phytotoxic compounds (Jefferson and Pennacchio, 2003). Methanolic extracts of different parts of *Chenopodium* spp. were found very effective in inhibiting the growth of *Macrophomina phaseolina* and *Fusarium oxysporum* (Javaid and Amin, 2009; Javaid and Rauf (2015). According to chemical composition of *Chenopodium* album it contains saponins, flavonoids (e.g. phenolic amide), cinnamic acid amide, apocortinoid, alkaloids (e.g. chinoalbicin), phenols, lignans and xyloside (Sherazi *et al*., 2016). *Kochia indica* is a member of this family growing wildly in Pakistan and many researchers revealed that its extract contains abundant flavonoids (Chou and Talalay, 2011) and alkaloids (Youssef, 2013). Therefore, it has many biological properties like, antioxidant (Abou Zid, 2011), heart tonic (Youssef, 2013) and anticancer (Mazzio and Soliman, 2009). Plant extracts normally contains a fusion of bioactive compounds that may work in synergism to restrain growth of fungi (Masoko and Eloff, 2005). In the present study, *n*-hexane fraction of *K. indica* stem extract was investigated to identify possible antimicrobial constituents.

Materials and Methods

K. indica was collected from Lahore, Pakistan, its stem was separated, cut into small pieces and dried. To prepare methanolic extract, 1 kg dried and crushed stem material was dipped in methanol (2 × 5 L) for 2 weeks at room temperature. Thereafter, it was filtered. The crude extract was obtained by evaporating the filtrate on rotary evaporator. The extract was mixed in distilled water (200 mL) and fractionated using *n*-hexane following Javaid *et al.*

(2019). The *n*-hexane fraction was subjected to GC-MS analysis to identify various compounds present in this fraction. Literature survey was carried out to check the antimicrobial properties of different identified compounds.

Results and Discussion

GC-MS chromatogram of *n*-hexane fraction of methanolic stem extract is shown in Fig. 1. A total of 11 compounds were recognized in this fraction which are presented in Table 1 along with their molecular weights, chemical formulae, retention times and peak area percentages. Hexadecanoic acid, methyl ester (24.09%), β-sitosterol (18.60%) and *γ* sitosterol (13.42%) were among the main compounds recognized and constituted 56.11% of the total. 4-Pyrimidinecarboxylic acid (9.49%) and 6- octadecadienoic acid, methyl ester (8.90%) were found up to moderate extent. On the other hand, heptadecanoic acid, 16-methyl-, methyl ester (5.63%), *n*-hexadecanoic acid (4.57%), di-*n*-octyl phthalate (4.49%), stigmasterol (4.00%), 9,12-octadecadienoic acid, methyl ester (3.92%), and 2-propenoic acid, pentadecyl ester (2.89%) were the compounds found in less concentrations.

Most of the compounds identified in the present study have also been isolated from other plant species and are known to exhibit antimicrobial activities. Compounds namely 9, 12-octadecadienoic acid-methyl ester; hexadecanoic acid, methyl ester; heptadecanoic acid, 16-methyl-, methyl ester and 6-octadecadienoic acid, methyl ester belong to fatty acids methyl esters group. Previous studies show that most of the belonging to this group exhibit antifungal activity (Agoramoorthy *et al*., 2007; Lima *et al*., 2011; Banaras *et al*., 2017). These constituents were also found previously in *Syzygium cumini* and reported to have antifungal activities (Javaid *et al*., 2018) In methanolic extracts of *Nymphaea lotus* and *Spondias mombin*, sterols and fatty acid methyl esters; 9, 12 octadecadienoic acid methyl ester were recognized and have antimicrobial activities (Odumosu *et al*., 2018). Stigmasterol, a phytoconstituent previously identified in methanolic stem extract of *Ficus religiosa* and in the aerial parts of *Fluggea leucopyrus*, has been recognized to possess antimicrobial activity (Manorenjitha *et al*., 2013; Sudha *et al*., 2013). Likewise, Bhawanker *et al*. (2013) isolated hexadecanoic acid and stigmasterol from ethanolic extracts of *Aloe vera* and also identified their antibacterial and antifungal activity against *Streptomyces greseus* and *Candida albicans.* Antimicrobial activity of β-sitosterol has been also proved by Sen *et al*. (2012) against *E. coli*, *Pseudomonas aeruginosa*, *S. aureus* and *Klebsiella pneumonia* using agar disk diffusion method. Likewise, antibacterial activity of *γ*-sitosterol was reported by Jim and Duke (1998). *n*-Hexadecanoic acid, an important antimicrobial compound identified in the present study was also previously isolated from *Brassica nigr*a oil and evaluated against *Staphylococcus aureus*, *Aspergillus niger Pseudomonas aeruginosa* and *Candida albicans* through cup plate agar diffusion assay by Abdel-Karim, (2017). Similarly, di-*n*-octyl phthalate has been isolated from various plant species including *Dracaena cochinchinensis*, *Limonium bicolor*, *Schleichera oleosa*, *Caesalpinia sappan* and *Plantago major*, and is reported as an antifungal compound (Senthilkumar *et al*., 2011; Romeh, 2013). From the present study, it is concluded that *n*-hexane fraction of *K. indica* stem extract possesses potent antimicrobial compounds.

Table 1: Compounds identified in *n*-hexane fraction of methanolic stem extract of *K. indica* through GC-MS.

Sr. No.	Names of compounds	Molecular Formula	Molecular weight	Retention time (min)	Peak area (%)
1	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	18.866	24.09
2	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	19.208	4.57
3	9,12-Octadecadienoic acid,methyl ester	$C_{19}H_{34}O_2$	294	20.439	3.92
4	6-Octadecadienoic acid, methyl ester	$C_{19}H_{36}O_2$	296	20.494	8.90
5	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298	20.710	5.63
6	2-Propenoic acid, pentadecyl ester	$C_{18}H_{34}O_2$	282	22.151	2.89
7	Di-*n*-octyl phathalate	$C_{24}H_{38}O_4$	390	24.008	4.49
8	Stigmasterol	$C_{29}H_{48}O$	412	29.152	4.00
9	β-Sitosterol	$C_{29}H_{50}O$	414	29.649	18.60
10	4-Pyrimidinecarboxylic acid	$C_5H_4N_2O_2$	124	30.017	9.49
11	*γ* -Sitosterol	$C_{29}H_{50}O$	414	30.198	13.42

Table 2: Potential antimicrobial constituents in *n*-hexane fraction of methanolic stem extract of *K. indica*.

Compound No.	Names of compounds	Property	Reference
1	Hexadecanoic acid, methyl ester	Antifungal, antimicrobial	Lima *et al*. (2011) Abdul-Karim *et al*. (2017) Javaid *et al*. (2018)
2	*n*-Hexadecanoic acid	Antimicrobial	Abdul-Karim *et al*. (2017)
3	9,12-Octadecadienoic acid, methyl ester	Antifungal, antimicrobial	Lima *et al*. (2011) Odumosu *et al*. (2018)
4	6-Octadecadienoic acid, methyl ester	Not found	-
5	Heptadecanoic acid, 16-methyl-, methyl ester	Not found	-
6	2-Propenoic acid, pentadecyl ester	Not found	-
7	Di-*n*-octyl phathalate	Antifungal	Senthilkumar *et al*. (2011) Romeh (2013)
8	Stigmasterol	Antifungal, antibacterial	Sudha *et al*. (2013) Manorenjitha *et al*. (2013) Bawankar *et al*. (2013)
9	β-Sitosterol	Antimicrobial	Sen *et al*. (2012)
10	4-Pyrimidinecarboxylic acid	Not found	
11	γ -Sitosterol	Antibacterial, antimicrobial	Jim and Duke (1998)

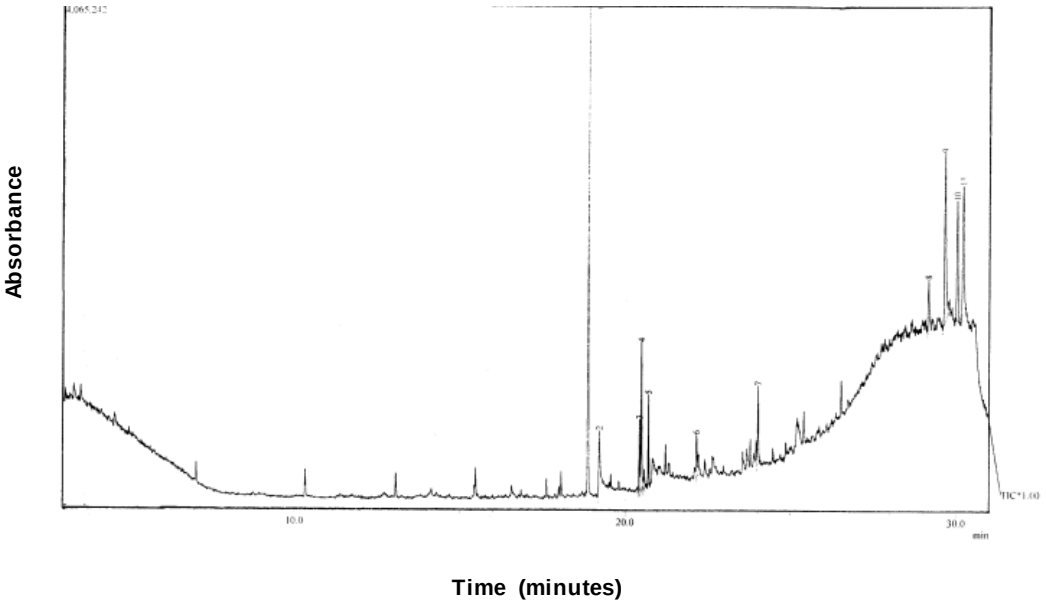
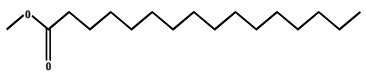
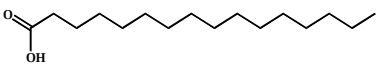


Fig. 1: GC-MS chromatogram of *n*-hexane fraction of methanolic stem extract of *Kochia indica.*

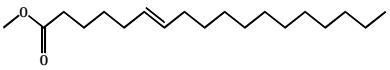


1. Hexadecanoic acid, methyl ester

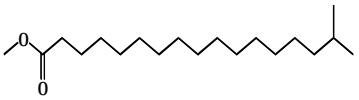


2. *n*-hexadecanoic acid

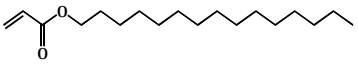
3. 9,12-octadecadienoic acid,methyl ester



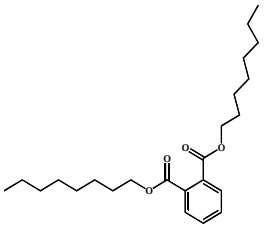
4. 6- Octadecadienoic acid, methyl ester



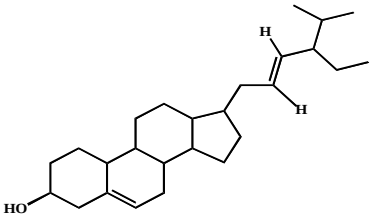
5. Heptadecanoic acid, 16-methyl-, methyl ester



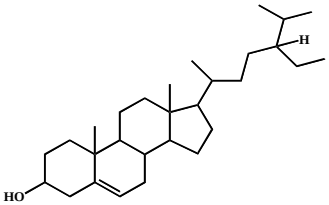
6. 2-Propenoic acid, pentadecyl ester



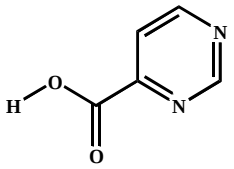
7. Di-*n*-octyl phathalate



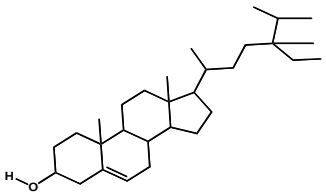
8. Stigmasterol



9. β-sitosterol



10. 4-Pyrimidinecarboxylic acid



11. γ -sitosterol

Fig. 2: Structures of compounds identified in n-hexane fraction of methanolic stem extract of *Kochia indica* through GC-MS analysis.

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