HEXANE SOLUBLE BIOACTIVE COMPONENTS OF *Chenopodium murale* STEM

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Abstract

Nettle leaf goosefoot (*Chenopodium murale* L.) is a common winter weed of *Chenopodiaceae*. Studies have shown that extracts of this weed possess antioxidant, antimicrobial, larvicidal and nematicidal activities. In the present study, *n*-hexane soluble fraction of methanolic stem extract of this weed was subjected to GC-MS analysis to identify various biologically active constituents. There were 28 compounds in this fraction. Major compounds included oleic acid (16.55%), palmitic acid (11.22%), β-sitosterol (9.63%), hexadecanoic acid (7.71%) and methyl oleate (5.90%). Other prominent compounds were piperine (4.75%), nonacosane (4.69%), monoplalmitin (4.21%), γ-sitosterol (3.91%), methyl linoleate (3.88%), neocurdione (3.86%) and ethanonone (3.25%). The compounds such as stigmasterol (2.92%), 1,4-benzenedicarboxylic acid (2.78%), tetracosanoic acid (1.19%), stearic acid (1.36%), tridecanoic acid (1.35%), tridecanal (1.30%), phytol (1.29%), docosanoic acid, methyl ester (1.23%), octadecanoic acid (1.20%), 4-pyrimidinecarboxylic acid (1.05%), 2-hydroxy-2-phenylbutanamide (0.91%), hexacosanoic acid (0.76%), cholestrol (0.61%), methyl octacosanoate (0.55%) and tetracosanoic acid (0.30%) were present in low concentrations. A thorough literature survey showed that most of the identified compounds possessed antifungal and/or antibacterial properties while very few of them also possessed antioxidant potential. This study concludes that *n*-hexane soluble fraction of methanolic stem extract of *C. murale* is a big storehouse of antimicrobial compounds.

Keywords: Bioactive components, *Chenopodium murale,* GC-MS analysis, Hexane soluble, Stem.

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INTRODUCTION

Most of the plant diseases can be controlled by using synthetic products that are easily available in the market. These could reduce the adverse impact of phytopathogens on plant growth and yield but their field applications are not safe for humans (Mamarabadi *et al.,* 2018). During the last few decades, improper and excessive use of chemical formulations found hazardous to the human's and animal's health, and the environment. Therefore, increased awareness towards their toxic effects has led the foundation of more extensive research for easily biodegradable and environmentally safe plant-based products (Zaker, 2016; Khan and Javaid, 2020). Phytoconstituents are natural, easily convertible into common eco-friendly organic products with minimum or no adverse effects on the plants physiological processes. They exhibited various biological activities against a wide range of plant pathogens and can be used as bio-fungicidal, bio-pesticidal and bio-nematicidal products (Rahman *et al.,* 2016; Sevindik *et al.,* 2017; Mohammed *et al.,* 2018; Tembo *et al.,* 2018). These can easily be adopted by the farmers as an alternative remedy to conventional synthetic chemical formulations with less hazardous impact on the ecosystem, nontoxic for antagonistic microorganisms, have a specific target range, limited field persistence and have a shorter life span with no residual threats (Shuping and Eloff, 2017).

The family *Chenopodiaceae* is widespread, native to tropical and subtropical regions of Asia, Australia, Africa and Central America (Akopian *et al.,* 2020). Some of the species have economic importance and are used as medicinal plants for their bioactive phytoconstituents (Sen *et al.,* 2020). *C. murale,* commonly known as nettle leaf goosefoot, is one of the most important fast-growing members of the family *Chenopodiaceae*. It was introduced from Europe and now grows along roadsides, waste places and deserted lands (Riaz *et al.,* 2018). It is a rich source of secondary metabolites such as flavonoids, terpenoids, diterpenoids, sesquiterpenes, sterols, glycosides, tannins, lignins, carotenoids, phenolic acids, alkaloids, glucosinolates, saponins, coumarins and hydrocarbons (Zhou *et al.,* 2019; Abd-El-Gawad *et al.,* 2020). The modern studies have proved that *C. murale* plant extracts exhibit antioxidant, antibacterial, antifungal, larvicidal and nematicidal activities (Abdel-Aziz *et al.,* 2014). Therefore, the present study was designed to identify the biologically active components through GC-MS analysis present in *n*-hexane stem extract of *C. murale.*

MATERIALS AND METHODS

Preparation of extract

Fresh plants of *C. murale* were collected from the vicinity of Punjab University, Lahore. The stems were separated from the leaves, washed thoroughly with tap water to remove any debris. Initially the plant material was dried under shade and then completely dried at 40 ºC in an oven. The dried material was grinded to a coarse powder with the help of a mechanical grinder. Dried and coarsely powdered stem material (2 kg) were extracted with methanol (5 L) for 14 days at room temperature followed by filtration through Whatman No. 1 filter papers. Methanol was then evaporated in a rotary evaporator at 45 °C to gain thick paste of stem extract (87 g). The crude methanolic stem extract was then mixed vigorously with distilled water (200 mL) and after that this mixture was partitioned in a separating funnel with 300 mL of *n*-hexane. Solvent was then evaporated in a rotary evaporator and 7.5 g extract of *n*-hexane sub-fraction was collected (Naqvi *et al.,* 2019).

GC-MS analysis

The *n*-hexane sub-fraction was analyzed by GC-MS. It was carried out on GC-MS QP2010 apparatus. Measured volume of the sub-fraction (1 µL) was injected with an auto injector into the gas chromatograph instrument. Capillary column was 30 × 0.25 mm × 0.25 µm. Split ratio of injection samples was 10:0 and flow rate of helium was 1.69 mL min^{-1}. Flow of column was 153.9 mL min^{-1} and the pressure was maintained at 100 kPa. Linear velocity was observed as 47.2 cm s^{-1}. Oven temperature was raised up to 50 °C for 3 min and the temperature was raised at the rate of 11

°C up to 320 °C. Injection temperature was kept at 200 °C (Naqvi *et al.,* 2019).

RESULTS AND DISCUSSION

The GC-MS chromatogram of *n*-hexane fraction is given in Fig. 1 that indicates the presence of 28 constituents. Details of the identified compounds regarding their retention time, peak area percentage, molecular formulae and weights are presented in Table 1. Among the identified compounds, oleic acid **(8)** (16.55%) followed by palmitic acid **(3)** (11.22%), β-sitosterol **(26)** (9.63%), hexadecanoic acid **(2)** (7.71%) and methyl oleate **(5)** (5.90%) were found predominantly. The constituents present in moderate concentrations included piperine **(22)** (4.75%), nonacosane **(20)** (4.69%), monoplalmitin **(12)** (4.21%), γ-sitosterol **(28)** (3.91%), methyl linoleate **(4)** (3.88%), neocurdione **(18)** (3.86%) and ethanonone **(17)** (3.25%). The compounds namely stigmasterol **(25)**, 1,4-benzenedicarboxylic acid **(9)**, tetracosanoic acid **(19)**, stearic acid **(10)**, tridecanoic acid **(11)**, tridecanal **(1)**, phytol **(6)**, dioctyl phthalate **(14)**, docosanoic acid, methyl ester **(13)**, octadecanoic acid **(7)**, 4-pyrimidinecarboxylic acid **(27)**, 2-hydroxy-2-phenylbutanamide **(16)**, hexacosanoic acid **(21)**, cholestrol **(24)**, methyl octacosanoate **(23)** and tetracosanoic acid **(15)** were present in less concentrations with peak areas ranging from 0.30 to 2.92%.

Most of the identified phytoconstituents were found to exhibit biological activities against a wide range of fungal and bacterial pathogens. For instance, compound **8**, previously extracted from seed of *Annona hypoglauca* Mart., was found highly effective against *Candida albicans, Aspergillus flavus, Escherichia coli* and *Staphylococcus aureus* (Dos Santos *et al.,* 2015). Similarly, compound **3, 5** and **20** have been reported to inhibit the growth of many fungal and bacterial species including *C. albicans, Aspergillus parasiticus, A. flavus, E. coli, Pseudomonas aeruginosa, Bacillus subtilis* and *S. aureus* (Mostafa *et al.,* 2015; El-Hawary *et al.,* 2018; Marathe *et al.,* 2020). Recently, Moraru *et al.* (2019) isolated the compound **22** from *Piper nigrum* L. and used it in the synthesis of silver nanoparticles. The tested nanoparticles showed the pronounced inhibitory potential against the *E. coli, S. aureus* and *C. albicans.*

Compound **4** and **26** have previously been reported from medicinal plants *Vitex kwangsiensis,* C. Pei and *Schimpera arabica* Hochst. & Steud. with strong antioxidant properties (Hidayathulla *et al.,* 2018; Tian and Liu, 2018). Likewise, compound **9, 14, 18, 21** and **23** exhibited significant growth inhibitory effects against *C. albicans, Aspergillus niger, S. aureus, Pseudomonas fluorescens* and *E. coli* (Jusoh *et al.,* 2019; Anzian *et al.,* 2020; Chetia and Saikia, 2020). Tao *et al.* (2018) reported the compound **2** in higher concentrations in leaf extract of *Phyllostachys heterocycle* with strong antimicrobial activities. Previously, compound **10** was also tested against five strains of *E. coli*, where it showed remarkable growth inhibition (Zhang *et al.,* 2017). Similarly, compound **1, 6, 7, 11, 15, 19, 24** and **28** exhibited strong antimicrobial properties against a number of susceptible microorganisms (Sribalan *et al.,* 2016; Fernandes *et al.,* 2017; Saha and Bandyopadhyay, 2020).

Conclusion

The present study provides insight into the phytochemical composition of *n*-hexane stem extract of *C. murale* and demonstrated the bioactive potential of the identified compounds. It concludes that the non-polar *n*-hexane fraction of *C. murale* stem extract is a rich store-house of antimicrobial compounds.

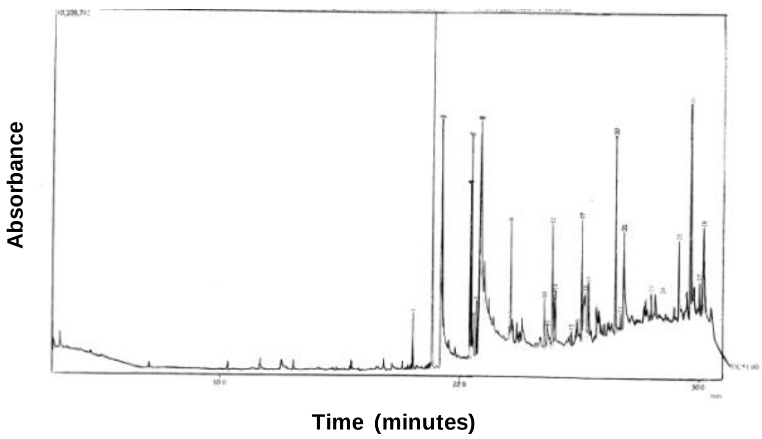

Fig. 1: GC-MS chromatogram of *n*-hexane sub-fraction of methanolic stem extract of *Chenopodium murale.*

Table 1: Compounds identified from *n*-hexane sub-fraction of methanolic stem extract of *Chenopodium murale* through GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retentio n time (min)	Peak area (%)
1	Tridecanal	$C_{13}H_{26}O$	198	18.050	1.30
2	Hexadecanoic acid	$C_{17}H_{34}O_2$	270	18.858	7.71
3	Palmitic acid	$C_{16}H_{32}O_2$	256	19.233	11.22
4	Methyl linoleate	$C_{19}H_{34}O_2$	294	20.433	3.88
5	Methyl oleate	$C_{19}H_{36}O_2$	296	20.492	5.90
6	Phytol	$C_{20}H_{40}O$	296	20.575	1.29
7	Octadecanoic acid	$C_{19}H_{38}O_2$	298	20.708	1.20
8	Oleic acid	$C_{18}H_{34}O_2$	282	20.867	16.55
9	1,4-Benzenedicarboxylic acid	$C_8H_6O_4$	166	22.142	2.78
10	Stearic acid	$C_{18}H_{36}O_2$	284	23.542	1.36
11	Tridecanoic acid	$C_{15}H_{30}O_3$	258	23.675	1.35
12	Monoplalmitin	$C_{19}H_{38}O_4$	330	23.867	4.21
13	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354	23.950	1.23
14	Dioctyl phthalate	$C_{24}H_{38}O_4$	390	24.000	1.28
15	Tetracosanoic acid	$C_{24}H_{48}O_2$	368	24.683	0.30
16	2-Hydroxy-2-phenylbutanamide	$C_{10}H_{13}NO_2$	179	24.908	0.91
17	Ethanonone	$C_{16}H_{16}O_4$	272	25.100	3.25
18	Neocurdione	$C_{15}H_{24}O_2$	236	25.242	3.86
19	Tetracosanoic acid	$C_{25}H_{50}O_2$	382	25.392	1.56
20	Nonacosane	$C_{29}H_{60}$	408	26.617	4.69
21	Hexacosanoic acid	$C_{27}H_{54}O_2$	410	26.725	0.76
22	Piperine	$C_{17}H_{19}NO_3$	285	26.858	4.75
23	Methyl octacosanoate	$C_{29}H_{58}O_2$	438	28.008	0.55
24	Cholestrol	$C_{27}H_{46}O$	386	28.175	0.61
25	Stigmasterol	$C_{29}H_{48}O$	412	29.150	2.92
26	β-Sitosterol	$C_{29}H_{50}O$	414	29.650	9.63
27	4-Pyrimidinecarboxylic acid	$C_5H_4N_2O_2$	124	30.017	1.05
28	γ-Sitosterol	$C_{29}H_{50}O$	414	30.192	3.91

Table 2: Bioactivity of components of *n*-hexane sub-fraction of methanolic stem extract of *Chenopodium murale*.

Sr. No.	Names of compounds	Bioactivity	Reference
1	Tridecanal	Antimicrobial	Yucel *et al.* (2017)
2	Hexadecanoic acid	Antimicrobial	Tao *et al.* (2018)
3	Palmitic acid	Antifungal, antibacterial	Marathe *et al.* (2020)
4	Methyl linoleate	Antioxidant	Tian and Liu (2018)
5	Methyl oleate	Antifungal, antibacterial	El-Hawary *et al.* (2018)
6	Phytol	Antimicrobial	Saha and Bandyopadhyay (2020)
7	Octadecanoic acid	Antimicrobial	Jasim *et al.* (2015)
8	Oleic acid	Antifungal and antibacterial	Dos Santos *et al.* (2015)
9	1,4-Benzenedicarboxylic acid	Antifungal, antibacterial	Rampilla and Khasim (2020)
10	Stearic acid	Antimicrobial	Zhang *et al.* (2017)
11	Tridecanoic acid	Antimicrobial	Kim *et al.* (2016)
12	Monoplalmitin	No activity reported	-
13	Docosanoic acid, methyl ester	No activity reported	-
14	Dioctyl phthalate	Antifungal, antibacterial	Yusuf-Babatunde *et al.* (2019)
15	Tetracosanoic acid	Antimicrobial	Fernandes *et al.* (2017)
16	2-Hydroxy-2-phenylbutanamide	No activity reported	-
17	Ethanonone	No activity reported	-
18	Neocurdione	Antifungal and antibacterial	Chetia and Saikia (2020)
19	Tetracosanoic acid	Antimicrobial	Fernandes *et al.* (2017)
20	Nonacosane	Antifungal and antibacterial	Mostafa *et al.* (2015)
21	Hexacosanoic acid	Antibacterial and antimicrobial	Anzian *et al.* (2020)
22	Piperine	Antibacterial and antimicrobial	Prabakaran *et al.* (2018), Moraru *et al.* (2019)
23	Methyl octacosanoate	Antifungal	Jusoh *et al.* (2019)
24	Cholestrol	Antimicrobial	Sribalan *et al.* (2016)
25	Stigmasterol	Antifungal and antibacterial	Yusuf *et al.* (2018)
26	β -Sitosterol	Antioxidant and antimicrobial	Hidayathulla *et al.* (2018)
27	4-Pyrimidinecarboxylic acid	No activity reported	-
28	γ -Sitosterol	Antimicrobial	Canli *et al.* (2017)

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