ETHYL ACETATE EXTRACT OF *Chenopodium murale* ROOT, A SOURCE OF BIOACTIVE COMPOUNDS

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Abstract

Chenopodium murale L. is a winter weed of family Chenopodiaceae. In this study, bioactive compounds present in ethyl acetate fraction of root extract of *C. murale* were identified. The weed plants were collected from Jehlem, Pakistan. Its roots were dried, powdered and extracted in methanol. After evaporation of the solvent, the remaining extract was mixed in water and partitioned with *n*-hexane, chloroform and finally with ethyl acetate. The last fraction was analyzed through GC-MS that indicated the presence of 15 compounds. These included the three major compounds namely *o*-xylene (15.03%), cyclopentanol (13.42%) and 2-hexanol (13.99%). The moderately and less abundant compounds were ethylbenzene (5.47%); methyl acetate (6.00%); cholestrol (4.33%); 2-phenanthrenol (3.01%); cyclohexanone (5.32%); *p*-xylene (5.12%); furostan-3,26-diyl dibenzoate (3.29%); dihexyl phthalate (4.99%); tricosanoic acid (2.74%); dioctyl phthalate (4.99%), hexanal (3.05%) and ergostane (1.29%). Literature survey showed that 10 of the identified compounds exhibited various biological activities including antifungal, antibacterial, antioxidant, anticancer and antipsoriatic. However, this study concludes that most of the compounds were antimicrobial in nature.

Keywords: Antimicrobial, Bioactive compounds, *Chenopodium murale,* Ethyl acetate extract, Natural compounds, Root.

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INTRODUCTION

Phytochemicals are natural non-nutritious compounds necessary for normal physiological processes in the human body (Rezaeian *et al.,* 2015). Various type of phytochemicals including glycosides, flavonoids, acids, tocopherols, tannins, steroids, phenolic, saponins and ascorbic acid, are known to show antioxidant behavior. These compounds can be used to treat various chronic diseases namely atherosclerosis, cancer, diabetes and hepatitis (Granda and De Pascual-Teresa, 2018). Plants are also an excellent source for potential antifungal agents. Alkaloids, fatty acids and their methyl esters, flavonoides, essential oils, saponins, tannins and many other classes of natural compounds present in plants are known for their antifungal activities and can be utilized for synthesis of new compounds (Kanwal *et al.,* 2010; Scorzoni *et al.,* 2016; Ali *et al.,* 2017). Many antifungal drugs which are being used nowadays such as amphotericin B, micafungin and caspofungin, were originally isolated from natural sources (Scorzoni *et al.,* 2016). Likewise, many antibacterial drugs are either natural compounds themselves or are derivatives of a natural product scaffold (Brown *et al.,* 2014). Natural compounds belonging to various classes such as alkaloides, coumarins, lipoids, macrolides, macrolactams, phenolics, quinones, steroids and terpenoids, showed potent antibacterial activities and are very important in the development of antibacterial drugs (Dai *et al.,* 2020).

Members of family Chenopodicaea are a rich source of many bioactive compounds (Khan *et al.,* 2018, 2020; Khan and Javaid, 2019, 2020a,b,c). *C. murale* is a winter weed of this family that generally grows along the road-sides and cultivated fields. This plant species possesses a number of bioactivities such as antifungal, antibacterial and anti-inflammatory. It also has many medicinal applications like hepatoprotective, analgesics and hypotensive effects (Javaid and Amin, 2009; Saleem *et al.,* 2014). It contains a number of terpenoides, flavanoids and saponins and show antioxidant properties (Abbas *et al.,* 2012). In addition, it contains acid phosphatases that are highly important biological enzymes used in various clinical and forensic studies (Zaman *et al.,* 2020). Recently, Naqvi *et al.* (2020) reported bioactive substances present in *n*-hexane stem extract of *C. murale.* The present study was undertaken to explore bioactive compounds present in ethyl acetate fraction of methanolic root extract of *C. murale.*

MATERIALS AND METHODS

Extraction procedure

C. murale plants growing as wild in Jehlem, Pakistan were collected during March 2018. Originally 2 kg fresh plant material of the weed was collected. The roots were separated and after thorough washing with tap water, cut into small pieces and dried under shade. The root pieces were completely dried in an electric oven at 40 ºC. The dried roots were grinded to a coarse powder and 50 g were extracted in 500 mL of methanol for two weeks at room temperature. Thereafter, the material was filtered and the methanol was evaporated in a rotary evaporator getting a thick biomass of root extract. This extract was mixed with 100 mL distilled water and shacked well. It was successively partitioned with 4 × 200 mL of *n*-hexane, 200 mL of chloroform and finally with 200 mL of ethyl acetate. The last fraction of methanolic extract was used for GC-MS analysis (Naqvi *et al.,* 2020).

GC-MS analysis

This ethyl acetate fraction was subjected to GC-MS analysis. The analysis was done on a GC-MS QP2010 machine. A volume of 1 µL of the fraction was injected with an auto injector into the gas chromatograph instrument. GC-MS procedure described by Naqvi *et al.*

(2019) was followed in this study. A detailed survey of the literature was carried out in order to collect information regarding biological activities of the compounds identified in this study.

RESULTS AND DISCUSSION

GC-MS chromatogram of ethyl acetate fraction is shown in Fig. 1, which revealed the presence of 15 compounds. Details of the identified compounds are shown in Table 1 and their structures are illustrated in Fig. 2. The major compounds were *o*-xylene (**6**); 2-hexanol (**2**) and cyclopentanol (**1**) with peak areas of 15.03%, 13.99% and 13.42%, respectively. The compounds namely methyl acetate (**3**); ethylbenzene (**5**); cyclohexanone (**8**); *p*-xylene (**7**); dihexyl phthalate (**9**); dioctyl phthalate (**12**) and cholestrol (**10**) showing peak areas of 6.00%, 5.47%, 5.32%, 5.12%, 4.99%, 4.99% and 4.33% were categorized as moderately abundant ones. Less abundant compounds were furostan-3,26-diyl dibenzoate (**14**); hexanal (**4**); 2-phenanthrenol (**13**); tricosanoic acid (**11**) and ergostane (**15**) with peak areas ranging from 1.29 to 3.29%.

The most abundant compound **6** was previously identified in the ethyl acetate extract of *Bacillus atrophaeus* and is known to possess strong antimicrobial activities against a wide range of phytopathogens namely *Alternaria solani*, *Fusarium oxysporum*, *Verticillium dahlia*, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus* (Mohamad *et al.,* 2018). Similarly, Compound **13** was previously isolated from the extracts of *Coleus forskohlii* with strong antimicrobial potential against *Candida albicans* and *Klebsiella pneumonia* (Rajkumar and Malathi, 2015). GC-MS analysis of ethanolic aqueous extracts of *Tamarindus indica* revealed the presence of compound **2** in higher concentrations with potent antimicrobial and anti-inflammatory properties (Mehdi *et al.,* 2021). Likewise, compound **1** was identified from the seed extracts of *Trigonella foenum-graecum* (Rao, 2017), and compound **7** was found abundantly in a medicinal plant *Abutilon indicum* (Tiwari *et al.,* 2016), with strong antimicrobial and antioxidant properties. Compound **8** was isolated from the ethyl acetate extract of *Amphirosellinia nigrospora* with antibacterial activity against *Ralstonia solanacearum* (Nguyen *et al.,* 2019). Likewise, Yu *et al.* (2018) worked on *Nectria* sp. bioactive metabolites and reported the presence of compound **8** with effective antibacterial potential against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichisa coli*. Jash *et al.* (2018) also reported the antimicrobial and food preservation properties of compound **4**. Compound **9** and **12** were previously identified from the extracts of *Avicennia officinalis* with strong anticancer and medicinal properties, respectively (Zhang *et al.,* 2018).

This study concludes that most of compounds present in ethyl acetate fraction of root extract are antifungal and antibacterial in nature.

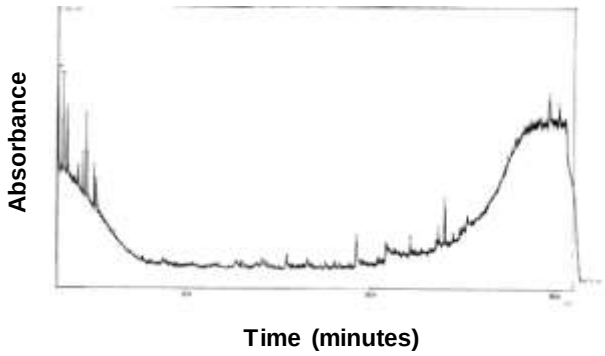

Fig. 1: GC-MS chromatograms of ethyl acetate fraction.

Table 1: Compounds identified in ethyl acetate fraction.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Cyclopentanol	$C_5H_{10}O$	86	3.050	13.42
2	2-Hexanol	$C_6H_{14}O$	102	3.317	13.99
3	Methyl acetate	$C_3H_6O_2$	74	3.53	6.00
4	Hexanal	$C_6H_{12}O$	100	4.142	3.05
5	Ethylbenzene	C_8H_{10}	106	4.400	5.47
6	*o*-Xylene	C_8H_{10}	106	4.575	15.03
7	*p*-Xylene	C_8H_{10}	106	5.008	5.12
8	Cyclohexanone	$C_6H_{10}O$	98	5.125	5.32
9	Dihexyl phthalate	$C_{20}H_{30}O_4$	334	19.225	4.99
10	Cholestrol	$C_{28}H_{49}NO$	415	22.167	4.33
11	Tricosanoic acid	$C_{23}H_{46}O_2$	354	23.675	2.74
12	Dioctyl phthalate	$C_{24}H_{38}O_4$	390	24.008	4.99
13	2-Phenanthrenol	$C_{14}H_{10}O$	194	27.267	3.01
14	Furostan-3,26-diyl dibenzoate	$C_{41}H_{54}O_5$	626	29.675	3.29
15	Ergostane	$C_{28}H_{50}$	386	30.200	1.29

Table 2: Bioactivity of components of ethyl acetate fraction.

Sr. No.	Names of compounds	Bioactivity	Reference
1	Cyclopentanol	Antimicrobial and antioxidant	Rao (2017)
2	2-Hexanol	Antimicrobial and anti-inflammatory	Mehdi *et al.* (2021)
3	Methyl acetate	No activity reported	-
4	Hexanal	Antimicrobial and food preservation properties	Jash *et al.* (2018)

5	Ethylbenzene	No activity reported	-
6	*o*-Xylene	Antifungal, antibacterial and antioxidant	Tiwari *et al.* (2016); Mohamad *et al.* (2018)
7	*p*-Xylene	Antimicrobial, antioxidant and antipsoriatic	Tiwari *et al.* (2016)
8	Cyclohexanone	Antifungal and antibacterial	Yu *et al.* (2018); Nguyen *et al.* (2019)
9	Dihexyl phthalate	Anticancer	Zhang *et al.* (2018)
10	Cholestrol	No activity reported	-
11	Tricosanoic acid	No activity reported	-
12	Dioctyl phthalate	Medicinal properties	Zhang *et al.* (2018)
13	2-Phenanthrenol	Antifungal and antibacterial	Rajkumar and Malathi (2015)
14	Furostan-3,26-diyl dibenzoate	No activity reported	-
15	Ergostane	Medicinal properties	Wang *et al.* (2015)

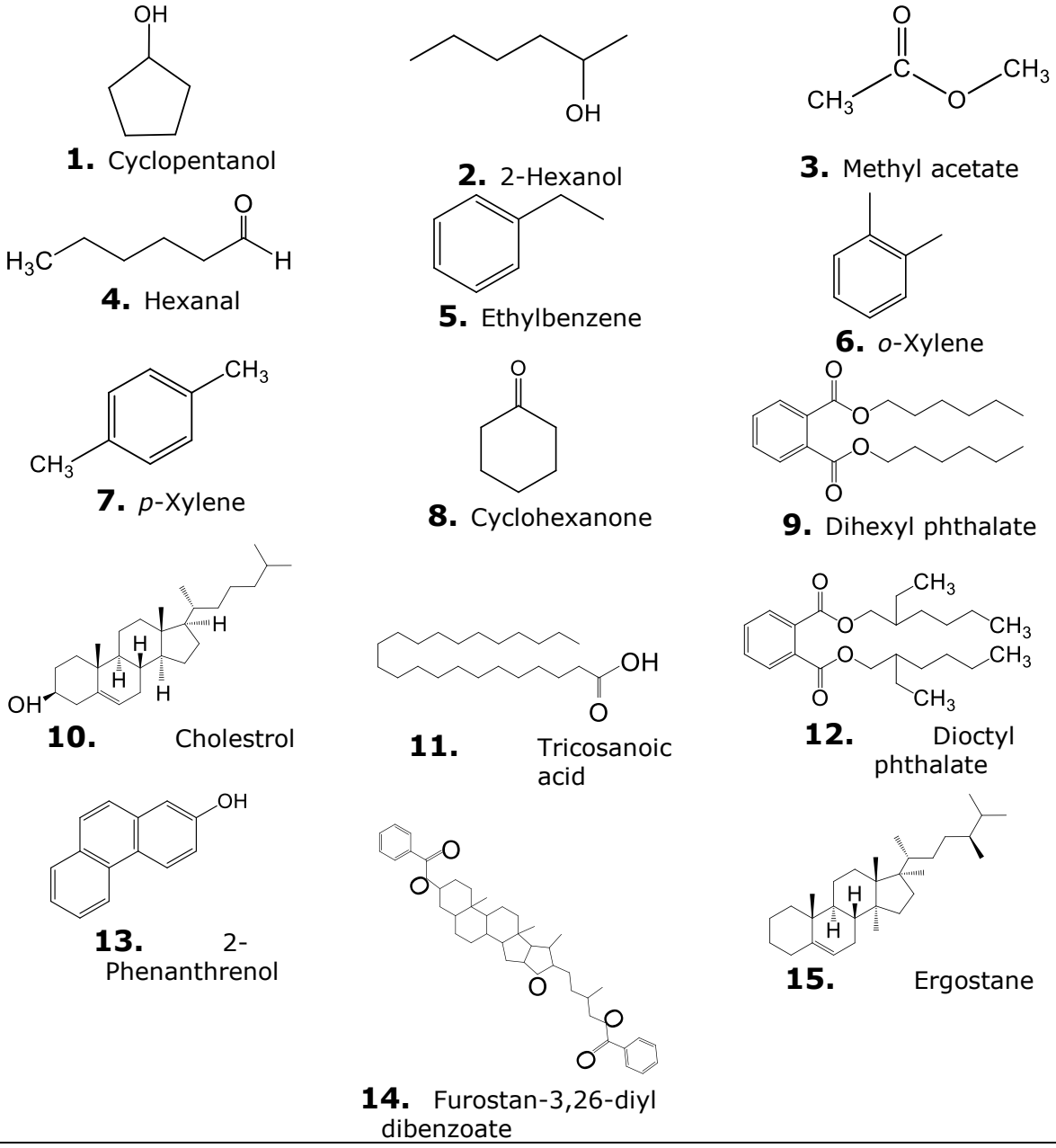

Fig. 2: Structures of compounds in ethyl acetate fraction of root extract.

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