GC-MS analysis of bioactive compounds present in medicinally important *Periploca hydaspidis*

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Abstract: The present study investigates the pharmaceutically bioactive compounds in Methanol, n-hexane and ethyl acetate extracted samples from the root of *Periploca hydaspidis* through Gas Chromatography and Mass Spectroscopy analysis. The mass spectrum obtained was compared with the data base of National Institute of Standards and Technology (NIST) which contains more than 62000 patterns of the mass spectrum. During matching with NIST library the match factor greater than 700 was considered only for better and pure results. The GC-MS analysis revealed the presence of various important compounds in the extracts like Lupeol, Furanol, Decanal, Decanoic acid, Dioxane and Oxirane. Besides these compounds the analysis also revealed the presence of antibiotics, fatty acids and protein.

Keywords: Bioactive compound, GC-MS, *Periploca hyaspidis*, antibiotics, fatty acids

INTRODUCTION

In recent years there has been an intensive search for substances with considerable pharmaceutical properties. Plants are known to produce certain chemicals which are naturally toxic to bacteria and fungi. In bryophytes anatomical barriers are less effective and secondary metabolites with antimicrobial activity are the most effective defense mechanism (Harborne, 1973). Defense substances belong to a wide range of different chemical classes including flavonoids and isoflavonoids. It is reported that intake of antioxidants such as vitamin C reduces the risk of coronary heart diseases and cancer. It is possible to reduce the risk of chronic diseases and to prevent disease progression by either enhancing the body's natural antioxidant defense or by supplementing with proven dietary antioxidants (Stanner *et al*., 2004). Several studies revealed that phenols, mainly flavonoids, from medicinal plants are safe and exert anticarcinogenic, antimutagenic, antitumor, antibacterial, antiviral, and anti-inflammatory effects (Ozgová *et al*., 2003; Khaleeq *et al*., 2018; Maria and Bakht, 2018).

Periploca hydaspidis (P/NYC-02GCmatta) represents the family *Asclepiadaceae*. The plant is a twirling climber, normally has no leaves while its branches are flat, green in color and have diameter of 1.5mm. If the Leaves are present, are linear or lanceolate; nerves in leaves are obscure, petiole 1-2mm long. Flowers are in lax axillary trichotomous cymes. The number of seeds in one coating is 10-50. Flowering is perennial and usually occurs in September-October. Locally the plant is called as *Taroona* and is used as purgative (personal communication with local people). This plant is usually found in Swat, Pakistan at altitude of 850m to 1600m. The present study was carried out to analyze the roots of *Periploca hyaspidis* by GC-MS.

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MATERIALS AND METHODS

Plant material

The present research was conducted at the Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar Pakistan. Different parts of the Plant (stems and roots) were obtained from various sites of District Swat, KP. The plant specimen was identified by plant taxonomist at the Department of Botany, government post-graduate Jehan Zeb College Swat (KPK). For the present study, roots were thoroughly washed with distilled water to remove any dust particles, dried for eight days in dark and grinded with electric grinder.

Crude extract preparation

One thousand gram of powdered root materials was soaked separately in six liters of methanol in extraction flasks and kept at 24ºC in dark for 7 days and shaken three times daily. The samples were filtered through Whatman filter paper No.1. Three liters of fresh methanol was mixed with the remaining residue, filtered and the whole procedures were adopted thrice. The filtered sample was dried below 45ºC under vacuum pressure in Rotary evaporator. The resulting extract was taken and kept in dark for drying in an amber color bottle at room temperature.

Fractionation of crude extract

The dried methanolic extract was taken and 80 g of it was dissolved in 500ml sterile distilled water and mixed with analytical grade n-hexane (300ml) in separatory funnel, shaken gently and allowed to stand for 15minutes to

separate the two phases. The upper n-hexane phase was collected and the lower aqueous phase was re-fractionated three times with n-hexane. All fractions of n-hexane were pooled together and dried at 45°C under vacuum pressure with a rotary evaporator. The same procedures were followed for ethyl acetate. The lower aqueous phase at the end of the process was dried at 45ºC under vacuum rotary evaporator.

GC-MS analysis

The GC-MS analysis of the plant extracted with different solvents was carried out through Agilent Mass Hunter work station Gas chromatograph interfaced to mass spectrometer (GC-MS). Triple Quadrupole Acquisition method was followed during the process. The machine was equipped with a non polar column packed with film made of 95% Dimethylepolysiloxane and 5% phenyl (Agilent USB393752HHP-5MS-30m length × 250µm diameter × 0.25µm film thickness). For the detection of the compounds an electron ionization source with 70eV energy was used. Ultra pure Helium gas (99.99%) was used as a carrier gas for mobile phase with split mode at septum purge flow rate of 3ml/min. The employed injection volume was 1µL with split ratio of 20:1. The temperature of the injector was 250°C.The temperature strategy in the oven was programmed as, 50°C for 5 min, then 10°C/min to 180°C for 15 min, then 10°C/min to 280°C for 20 min and lastly 20°C/min to 300°C for 1 min. The pressure was 9.7131 psi and the constant flow rate was 1.1929ml/min with average velocity of 39.805cm/sec. The total run time was 65min. The solutions were prepared by taking 1gm of each extract and making it soluble in 20ml of their respective solvents. The solutions were filtered through Whatman no. 1 filter paper to remove any solid particles. All the solutions used were clean and transparent. All the chemicals used were highly pure analytical grade. The mole percent peak area was calculated according to the following formula (Hossain *et al*., 2011)

Mole % component (Peak area) = area under peak/total area of all peaks×100

STATISTICAL ANALYSIS

Data are shown as mean values of three replications. MSTATC computer software was used for statistical analysis (Russel and Eisensmith, 1983).

RESULTS

The phytochemical components of crude methonalic roots extracts are represented in table 1 (fig. 1). The data revealed that crude methanolic extract contained butanoic acid with peak area of 13.62% with a high match factor of 852. 1,3-dioxane with peak area of 1.09% and match factor of 770 was also detected in the tested extract. This compound is medicinally important showing antibacterial, anti-inflammatory and antioxidant activities. Tetrahydro-2-methyl-2-furanol (peak area 6.59%), representing the alcoholic group of compounds was also detected in the subject extract. Compounds representing the –one functional group like, 5-hydroxy-2-methyl hexanone (2.63%), 3,4-dimethyl-3-hydroxy-2-pyrrolidinone (50.96%), 4-sec-butoxy-2-butanone (5.93%) were also detected during the GC-MS analysis.

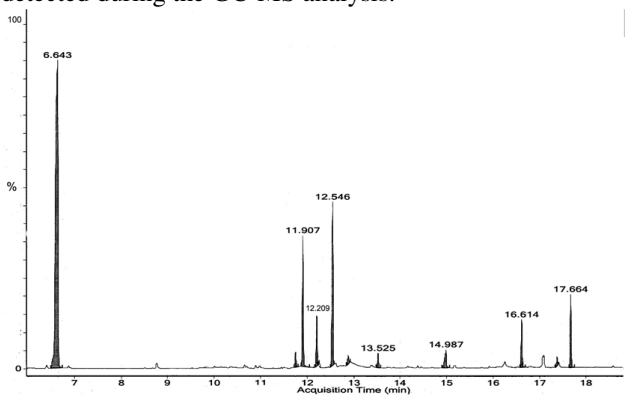

Fig. 1: Chromatogram of root crude methanol extracted sample

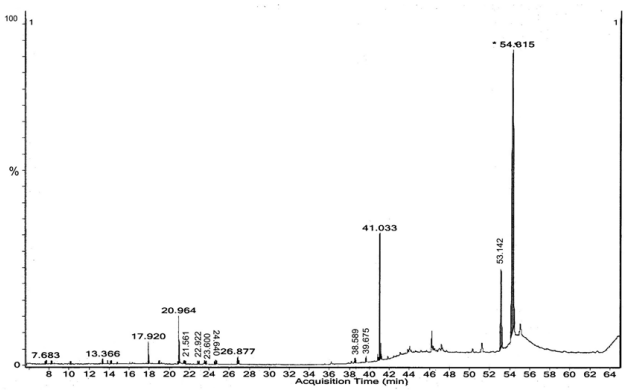

Fig. 2: Chromatogram of root n-hexane extracted sample

Analysis of the data indicated that aldehyde group containing of compounds were predominant in the tested extract (table 2; fig. 2). The group is represented by 2-decenal, tetra decanal hexadecanal and octadecanal with peak area of 0.1%, 0.3%, 0.6% and 0.19% respectively. Some of these compounds are pharmacologically important. Lipids representing compound 1-monolinoleoylglycerol trimethylsilyl ether with peak area of 0.46% were also detected in the extract. 2-(9-octadecenyloxy)-, (Z)- ethanol with peak area of 0.1% represented the alcoholic functional group. Some other important phytocehmicals identified were Lup-20(29)-en-3-ol, acetate, (3β)-, 12-oleanen-3-yl acetate, (3α)-, 1-monolinoleoylglycerol trimethylsilyl ether and 4,8,12,16-tetramethylheptadecan-4-olide 5.97%, 1.2%, 0.46% and 0.1% were also detected in the tested extract. n-hexadecanoic acid with peak area of 0.1% was also present in the extract.

Table 1: Phytochemical constituents of root crude methanol extracted sample

Compound name	Molecular formula	Molecular weight	Retention time	Match factor	NIST number	ID number	Peak area %
3,4-dimethyl-3-hydroxy-2-Pyrrolidinone	$C_6H_{11}NO_2$	129	6.643	707	196873	918	50.96
tetrahydro-2-methyl-2-Furanol	$C_5H_{10}O_2$	102	12.209	846	1210	33101	6.59
Butanoic acid	$C_9H_{16}O_3$	172	12.546	852	282446	33325	13.62
1,3-Dioxane	$C_4H_8O_2$	88	13.525	770	118505	10920	1.09
5-hydroxy-2-methyl-Hexanone	$C_7H_{14}O_2$	130	14.987	681	151941	6447	2.63
4-sec-Butoxy-2-butanone	$C_8H_{16}O_2$	144	16.614	605	236316	8169	5.93
2,2'-[1,4-butanediylbis(oxymethylene)]bis-Oxirane	$C_{10}H_{18}O_4$	202	17.664	616	69644	8114	9.49

Table 2: Phytochemical constituents of root n-hexane extracted sample

Compound name	Molecular formula	Molecular weight	Retention time	Match factor	NIST number	ID number	Peak area %
2-Decenal, (E)-	$C_{10}H_{18}O$	154	13.366	768	53573	816	0.1
Tetradecanal	$C_{14}H_{28}O$	212	17.920	922	36005	5397	0.3
Hexadecanal	$C_{16}H_{32}O$	240	20.964	907	158514	5691	0.6
2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	21.561	805	337240	2225	0.1
2-Heptadecanone	$C_{17}H_{34}O$	254	22.922	756	12339	2220	0.1
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	23.600	786	42975	9049	0.1
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	24.640	841	335494	6723	0.1
Octadecanal	$C_{18}H_{36}O$	268	26.874	870	36188	5716	0.19
4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	38.589	728	110183	61522	0.1
2-(9-octadecenyloxy)-, (Z)- Ethanol	$C_{20}H_{40}O_2$	312	39.675	756	36235	18654	0.1
1-Monolinoleoy lglyceroltrimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	41.033	674	18126	37079	0.46
12-Oleanen-3-yl acetate, (3α)-	$C_{32}H_{52}O_2$	468	53.142	862	244056	153829	1.2
Lup-20(29)-en-3-ol, acetate, (3β)-	$C_{32}H_{52}O_2$	468	54.315	879	194307	12142	5.97

The phytochemical constituents of ethyl acetate extracted samples are shown in table 3 (fig. 3). The data indicated that the sample contain Ethyl iso-allocholate with peak area of 0.098% which is a good antibiotic. The sharpness of the peak represented the pureness of the compound in the extract. A flavoring agent 4-hydroxy-3-methoxy-benzoic acid with peak area of 3.27% was also detected in the extract having vinillic acid as its synonym. A bioactive fatty acid, n-hexadecanoic acid with a high peak area of 11.45%, which is also called as palmitic acid was also detected in the extract. 1,2-benzenedicarboxylic acid, diisooctyl ester with the highest peak area of 73.64% was also present in the extract.

DISCUSSION

Plants with medicinal value are the reservoirs of new drugs. A substantial number of modern medicines are created either directly or indirectly from the medicinal plants. The root extracted with Methanol, n-hexane and ethyl acetate solvents was subjected to GC-MS analysis to find out the active chemical constituents. The GC-MS analysis of the phytochemical components of roots samples extracted with methanol indicated the presence of butanoic acid with a high match factor of 852 and 1, 3-dioxane with peak area of 1.09% and match factor of 770with NIST library having similarity with *Periploca*

Table 3: Phytochemical constituents of root ethyl acetate extracted sample

Compound name	Molecular formula	Molecular weight	Retention time	Match factor	NIST number	ID number	Peak area %
4-hydroxy-3-methoxy- Benzoic acid	$C_8H_8O_4$	168	17.408	719	6514	21968	3.27
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	24.794	898	335494	6723	11.45
1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	41.091	882	113206	20061	73.64
9,19-Cyclochloestene-3,7-diol, 4,14-dimethyl-, 3-acetate	$C_{31}H_{52}O_3$	472	46.246	749	186587	138012	0.06
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	46.535	767	43053	6556	0.098
Lup-20(29)-en-3-ol, acetate, (3β)-	$C_{32}H_{52}O_2$	468	54.313	840	194307	12142	11.15

laevigata (Hajji *et al.,* 2010a). These compounds are medicinally important possessing antibacterial, anti-inflammatory and antioxidant activities (Shah *et al*., 2014). Tetrahydro-2-methyl-2-Furanol (peak area 6.59%), representing the alcoholic group of compounds were also present in the extract. Compounds possessing the '–one' functional group like, 5-hydroxy-2-methyl hexanone (2.63%), 3,4-dimethyl-3-hydroxy-2-pyrrolidinone (50.96%), 4-sec-butoxy-2-butanone (5.93%) were also detected during the GC-MS analysis having similarity with Hajji *et al*. (2010b) who reported the same compounds in *Mirabils jalaba*.

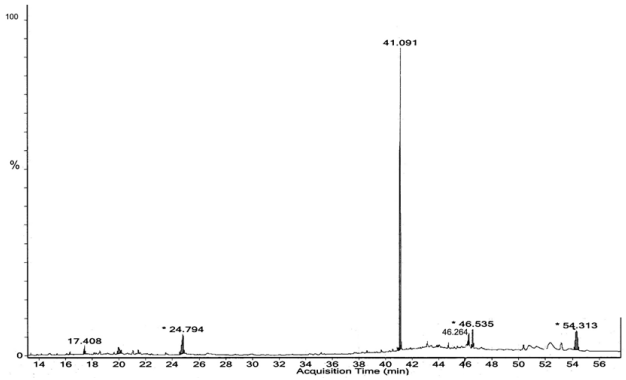

Fig. 3: Chromatogram of root ethyl acetate extracted sample

The results of GC-MS analysis of root n-hexane extracted samples revealed that aldehyde group containing compounds were predominant in the extract. The group was represented by 2-decenal, tetra decanal hexadecanal and octadecanal having pharmacological significance as indicated previously by Laila *et al.* (2013). Lipids, represented by the 1-monolinoleoylglycerol trimethylsilyl ether with peak area of 0.46% were also detected in the extract; anyhow Laila *et al*. (2013) reported a bigger peak area of 1.46% for the same compound. 2-(9-octadecenyloxy)-, (Z)- ethanol with peak area of 0.1% represented the alcoholic functional group present in the roots of plant. Some other pharmacologically important compounds like Lup-20(29)-en-3-ol, acetate, (3β)-, 12-Oleanen-3-yl acetate, (3α)-, 1-monolinoleoylglycerol trimethylsilyl ether and 4,8,12,16-tetramethylheptadecan-4-olide were also detected in the extract, which showed similarity with the results of Senthamarai *et al*. (2012), who reported the same compounds in *Sauropus androgynous* except 12-Oleanen-3-yl acetate.

The phytochemical constituents of ethyl acetate extracted samples from the root tissues indicated the presence of ethyl iso-allocholate possessing antibiotic activity (Kalaiselvan *et al*., 2012; Shah and Hossain, 2014). The sharpness of the peak represented the pureness of the compound in the extract (Laila *et al*., 2013). A flavoring agent 4-hydroxy-3-methoxy- benzoic acid (Vinillic acid) with peak area of 3.27% was also detected in the extract (Hajji *et al*., 2009; 2010 a, b). A bioactive fatty acid, n-hexadecanoic acid (palmitic acid) with a high peak area of 11.45% was detected in the extract. 1, 2-benzenedicarboxylic acid, diisooctyl ester with the highest peak area of 73.64% was also present in the extract. Similar results are also reported by Daferera *et al.* (2000).

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