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TLC and GC-MS of Alkaloids of *Fumaria vaillantii* and *F. asepala*

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Abstract

Fumaria species belong Fumariaceae family are rich in isoquinolines, protoberberine and quaternary protoberberine alkaloids used in traditional medicine. Fumaria vaillantii were collected from central zone of Saveh. In this research was describes determination and identification of tertiary bases in alkaloid extracts the F. vaillantii by quaternary extract method, spectrophotometry, TLC and GC-MS techniques. Fruits and leaves of F. vaillantii and F. asepala had the highest alkaloids content. Total alkaloid of fraction 2 (quaternary benzophenantridine alkaloids, protopine alkaloids) was higher than other alkaloid fractins. There were 10 and 8 types of alkaloids in all fractions of F. vaillantii and F. asepala in the TLC analysis, respectively. The some new compounds include 6-amino-5-pentenoic-alpha-Lactame, 1H-imidazole and 1,10-dicyanodecane in fraction 1 of F. vaillantii and 4-(2'-hydroxy-3'-iso propyl amino propoxy) phenylacetic acid and 6-amino-5-pentenoic-alpha-Lactame in fraction 4 of F. asepala were determined by GC-MS analysis. F. vaillantii and F.asepala had some quaternary protoberberine alkaloids but they were different from other reported compounds. A number of compounds of α-Lactams (Aziridinones) presented in alkaloids of F. vaillantii and F.asepala. Traditional antibiotics antibiotics penicillins, penems and cephalosporins are Beta-Lactam compounds. Antifungal activity was evaluated using the culture of round disks of Aspergillus on the medium contain different concentrations of the alkaloid fractions. The all alkaloid fractions in varies concentrations exhibited broad spectrum inhibition against Aspergillus flavus and A. niger. The minimum concentration and the maximum fungicide concentration against the test Aspergilluses were 0.04 mg/ml and 0.12 mg/ml of the all fractions, respectively. Our results showed that F. vailantii have four type alkaloids with high anti Aspergillus effects.

Keywords: *A. flavus*; *A. niger*; *Aspegillus*; α-Lactams; Aziridinones; Isoquinolines; Protoberberine

Introduction

Fumaria vaillantii and *F. asepala* from *Fumariaceae* family commonly known as Shahtareh in Iran, are annual herbs, rich in isoquinoline alkaloids and have been used in traditional medicine. The isoquinolines are one of the largest groups of alkaloids as aporphine, protoberberine, protopine and benzophenanthridine [1]. The protoberberines (tetra hydro protoberberines and quaternary protoberberines) are distributed in such plant families as *Papaveraceae*, *Berberidaceae*, *Fumariaceae*. The biological activity of *Fumaria* is mostly associated with the presence of isoquinoline alkaloids in the plant [2]. Apart from regular Thin Layer Chromatography (TLC) and column chromatography Techniques, reverse phase HPLC, GC-MS, and capillary electrophoresis techniques have been reported for the determination of isoquinoline alkaloids from plant extracts [3]. The major chemical constituents of *F. vaillantii* are alkaloid such as protopine, narceimine, tetrahydrocoptisine, narulimidine, methyl fumigate,

naralumidine, bicuculline and fumariline. The isoquinoline alkaloids adlumiceine, adlumidiceine, cortisone, cryptopine, fumaricine, fumariline, fumaritine, fumarophycine, Omethylfumarophycine, parfumine, sinactine, N-methylstylopine. The beta-lactam antibiotics constitute the most important family of antimicrobial agents, both in terms of the large number of compounds available and in terms of prescription volume. Until recently, beta-lactams were not considered strong candidates for Therapeutic Drug Monitoring (TDM), as they are assumed to have a wide therapeutic index and the most frequent adverse effects involve non-dose-related allergic reactions. But concepts are changing, and several studies demonstrate the advantage of TDM for these antibiotics. A Hydrolyzed α-lactams (*Aziridinones*) leads to an α-amino acid and a β-Lactam to a β-amino acid. Lactams can polymerize to polyamides. In addition to the cyclopentenone, cyclohexenone and hexahydroindenone systems, products derived from bicyclic lactams now include optically active piperidines, pyrrolidines, pyrrolidinones and tetrahydroisoquinolines [4]. These favorable properties make the bicyclic lactams highly adaptable tools for asymmetric synthesis. *Aspergillums* species are among the most abundant fungi worldwide. *Aspergillus spp* secrete a wide variety of enzymes that degrade polymers within the substrate into molecules that can be taken up to serve as nutrients. *Aspergillus spp* can form mycotoxins that are toxic for animals and humans. *A. flavus* produces aflatoxin, which is one of the most carcinogenic natural molecules. *A. niger* is one of the most important microorganisms used in biotechnology. It has been in use already for many decades to produce extracellular enzymes and citric acid. *A. niger* strains produce a series of secondary metabolites, but it is only ochratoxin A that can be regarded as a mycotoxin in the strict sense of the word [5]. The present paper was describes determination and identification of tertiary bases in alkaloid extracts the *Fumaria vaillantii* and *F. asepala* by extract method, spectrophotometry, TLC and GC-MS techniques, and antifungal activity of alkaloid extracts of *F. vaillantii* and *F. acepala*.

Material and Methods

Plant materials

Fumaria vaillantii Loisel and *F. asepala* Boiss were collected from their natural habitats in Saveh (Markazi province, Iran) and was determined by Dr. Babak Delnavaz [6]. Herbarium specimens of each sample are deposited with number 8629 and 8630 in the Central Herbarium of University of Saveh (S.IAU). In this research, the alkaloids of both species were extracted in 4 different fractions, the total alkaloid was measured and alkaloids analyzed by Thin Layer Chromatography (TLC) and Gas Chromatography Mass (GC-MS).

Extraction of alkaloids

Separation of the Quaternary Protoberberine Alkaloids (QPA) from the crude plant extract containing various kinds of alkaloids was performed using modified method of Slavikova and Slavic and includes individual steps:

- **Crude extract:** 5 g of individual organs (leaves, stems and roots) were dried (at room temperature and shade), powdered and extracted with 25 ml methanol in a Soxhlet extractor apparatus at below 60°C for 2 h and then evaporated to 0.5 ml in vacuum rotary evaporator.
- **Fraction 1 (lipophilic compounds and non-basic alkaloids):** The methanol residue was taken up in 25 ml sulfuric acid (1%) and 25 ml ether. The ether phase was separated and maintained (F1).
- **Fraction 2 (quaternary benzophenantridine alkaloids, protopine alkaloids, tertiary bases soluble in ether):** The aqueous acid solution brought to pH 8-10 with saturated Sodium Carbonate(Na_2CO_3) solution and it was mixed with 25 ml ether. The ether phase was separated and maintained (F2).
- **Fraction 3 (quaternary protoberberine alkaloids bases soluble in ether):** The aqueous alkali solution brought to pH >13 with sodium hydroxide (NaOH) solution 10 N and it was mixed with 25 ml ether. The ether phase was separated and maintained (F3).
- **Fraction 4 (non-polar compounds and highly polar quaternary alkaloids bases soluble in chloroform):** The aqueous alkali solution brought to pH 6-8 with sulfuric acid (1%) + citric acid (1%) and KI (1%); and it was mixed with 25 ml chloroform [7]. The chloroform phase was separated and maintained (F4).

Alkaloids assay: The existence and content of total alkaloids of plant organs and alkaloid content of fractions of

Fumaria vaillantii and *F. asepala* analyzed by spectrophotometry, TLC and GC- MS. Total alkaloid measurement: Alkaloid of protopine is used to draw the standard calibration curve. 10 mg of protopine (Caspian Tamine Drugs Company of Iran) were dissolved in 10 ml methanol. Six additional calibrations were prepared by 1:2 serial dilutions with methanol-water (50:50). Standard solutions were prepared 0 µg ml-1 -200 µg ml-1. Total alkaloids were measured at 254 nm by spectrophotometer (Uv-visible Shimatzu) [8]. Thin Layer Chromatography (TLC) TLC did according to modified methods as described by Cava and Reed, (1965). 200 µg of alkaloids fraction of plants organs were applied on to TLC plates. TLC solvent systems routinely used was Chloroform: Ether: Ethanol: Water (8:8:3:1) and TLC plates were 0.2 mm thick silica gel aluminum packed plates [9]. Alkaloids were identified using TLC and Dragendorff reagent. The mobility and characters of each of alkaloids analyzed by TLC were compared with Removing Factor (RF). Statistical analysis: Statistical significance of differences was determined by one-way analysis of variance (Minitab) followed by Turkey analysis [10]. The data were presented as the mean ± Standard Error Means (SEM). Difference was considered significant at $p < 0.05$.

GC-MS analysis: GC-MS Analysis performed on a Fisons Trio 1000 mass selective detector with electronic impact ionization (70 eV). The column used was a HP^{-1} [11]. Helium was used as a carrier gas at 1.0 mL min^{-1}. The injection temperature was 280°C. The column temperature was held initially at 200°C for 8 min, increased to 250°C min-1 at 10°C min^{-1} and then held at 250°C for 30 minutes. The MS source temperature was operated at 250°C and the transfer line was maintained at 280°C.The mass range scanned was 125 g-450 g and scan rate was 26 scans/s [12]. Antifungal assay *A. niger* and *A. flavus* prepared from institute of Pasture of Iran. The different concentration of aquatic solution of alkaloid fractions (F1, F2, F3 and F4) added in culture medium of *Aspergillus* [13]. Medium culture of *Aspergillums* were containing potato dextrose agar (39 g/l), different concentrations of the alkaloid fractions (0 mg/ml, 0.04 mg/ml, 0.08 mg/ml and 0.12 mg/ml). The mediums were sterilized by autoclaving at 12°C for 15 min. Three round disk of *Aspergillus* with 0.5 mm diameter of mycelia were placed onto the medium surface of each petri dish, at equal distance. The sealed petri dishes were incubated at 37°C for 1-4 days, in an incubator [14]. Statistical analysis of data Total alkaloid measurement was done with 3 repeat, at least. The SPSS 11.5 statistical package program was used for statistical analysis [15]. Differences among treatments were compared by analysis of variance of the One Way ANOVA test (significance at the $p<0.05$ and 0.01 level) and Duncan test to compare means.

Results

Total alkaloid

The total alkaloid of roots, shoots, leaves and fruits of *F. vaillantii* were 1.94 mgg^{-1}, 1.84 mgg^{-1}, 3.41 mgg^{-1} and 3.45 mgg^{-1}. Fruits and leaves had the highest alkaloid contents [16]. There was significant difference between alkaloid contents of the roots and shoots with fruits and leaves. Roots, shoots, leaves and fruits of *F. asepala* contain sequencing 1.27 mgg^{-1}, 1.45 mgg^{-1}, 3.08 mgg^{-1} and 2.6 mgg^{-1} total alkaloids. Leaves had the highest alkaloid contents. There was significant difference between alkaloid contents of all organs [17]. The level of total alkaloids of *F. asepala* was lower than *F. vaillantii*. There was significant difference between alkaloid contents of two species (Table 1).

Table 1: Total alkaloid (mgg-1) of organs of F.asepala and F. *vaillantii.*

organs	*F. vaillantii*	*F. asepala*
root	1.94 ± 0.21	1.27 ± 0.33
shoot	1.84 ± 0.18	1.45 ± 0.28
leave	3.41 ± 0.20	3.08 ± 0.12
fruit	3.45 ± 0.21	2.6 ± 0.05

Comparison of fractions alkaloids in both species showed that the alkaloid level of fraction 1 in the shoot and root; and fraction 2 in the leaves and fruits were higher [18]. The fractional alkaloid level in the whole plant of both species was significantly higher than the rest of the fractions (Table 2).

Table 2: Alkaloid content of Fractions 1, 2, 3, 4 of *F. asepala* and *F. vaillantii* organs.

species	organs	F.1	F.2	F.3	F.4
	root	0.41 ± 0.21	0.91 ± 0.4	0.52 ± 0.38	0.10 ± 0.1
	shoot	0.45 ± 011	0.73 ± 0.19	0.37 ± 0.27	0.28 ± 0.12
	leave	1.21 ± 0.27	1.17 ± 0.33	0.68 ± 0.12	0.34 ± 0.09
	fruit	1.49 ± 0.16	1.02 ± 0.32	0.59 ± 0.32	0.33 ± 0.15
F. vaillantii	total	3.56	3.83	2.16	1.05
	root	0.21 ± 0.18	0.51 ± 0.22	0.31 ± 0.08	0.24 ± 0.06
	shoot	0.49 ± 0.14	0.43 ± 0.12	0.30 ± 0.15	0.22 ± 0.16
	leave	1.02 ± 0.14	1.11 ± 0.35	0.60 ± 0.18	0.36 ± 0.23
	fruit	0.85 ± 0.31	0.97 ± 0.28	0.56 ± 0.34	0.22 ± 0.05
F. asepala	total	2.57	3.02	1.71	1.04

TLC analysis

Alkaloids kind of plants organs, were compared by TLC analysis and Dragendorff reagent.

Roots: Only one alkaloid was observed in all fractions of roots of *F. vaillantii* and *F. asepala* and removing factors of these alkaloids were similar but in fraction 4 wasn't any alkaloid (Figure 1).

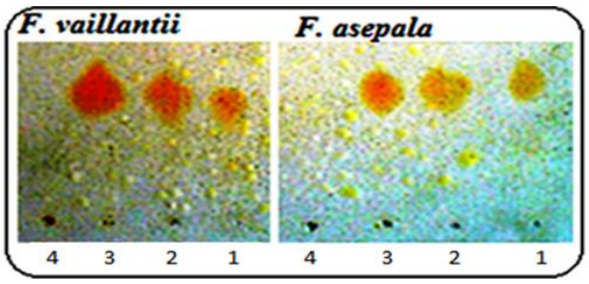

Figure 1: TLC analysis of alkaloid fractions of roots of *F. vaillantii* and *F. asepala*.

Stems: In fractions 2, 3 and 4 stems of *F. vaillantii* was observed one alkaloid common and Removing Factor (RF) of these alkaloids were 0.36 but fraction 4 contain another alkaloids in RF 0.25to. In fractions 2, 3 and 4 stems of *F. asepala* was observed one alkaloid common and Removing Factor (RF) of these alkaloids were 0.36, but fraction1 contain one alkaloid in RF 0.25 (Table 3).

Table 3: Removing Factor (RF) of TLC analysis of alkaloid fractions of organs of *F. vaillantii* and *F. asepala.*

F.4	F.3	F.2	F.1	N.	organs	species
0.31	0.31	0 .31	0.31	1	root	*F.*

-	-	-	0.25	1	stem	*vaillantii*
0.36	0.36	0.36	-	2		
-	-	0.07	-	1	leave	
-	0.2	-	-	2		
-	-	0.28	-	3		
-	-	0.42	-	4		
0.92	0.92	0.92	0.92	5		
-	-	0.28	-	1	fruit	
-	-	0.45	-	2		
-	-	0.55	-	3		
0.31	0.31	0.31	0.31	1	root	*F. asepala*
-	-	-	0.25	1	stem	
0.36	0.36	0.36	-	2		
-	-	0.07		1	leave	
-	-	-	0.11	2		
-	-	0.35	-	3		
-	-	0.5	-	4		
0.92	0.92	0.92	-	5		
-	0.25	0.25	-	1	fruit	
0.33	-	-	-	2		

Leaves: In leaves of *F. vaillantii*, alkaloid with RF 0.92 was common in all fractions but in fractions 2 and 3 were observed 3 and 1 other different alkaloids subsequently [19, 20]. In leaves of *F. asepala*, alkaloid with RF 0.92 was common in fractions 2, 3 and 4 but in fractions 2 were observed other different alkaloids and in fraction 1 only alkaloid with RF 0.11.

Fruits: In fruits of *F. vaillantii* only in fraction 2 was observed 3 alkaloid with RF 0.27, 0.45 and 0.55. In fruits of *F.asepala* alkaloid RF 0.25 was common in fraction 2 and 3; and alkaloid fraction 0.33 was observed in fraction 4 only.

GC MS analysis of alkaloid fractions of *F. vaillantii*

F1: Analysis showed 14 compounds include ammonia (CAS); ethanol (Jayson); phenol (Phenol, 2,6-Bis(1,1-Dimethylethyl)-4-Methyl); cyclohexane(2-Hexylthiophene); 1-ethyl-1-methyl neophytadiene (2,6,10-Trimethyl 4-Ethylene-14-Pentadecne); 2-hexadecen-1-ol (2,6,10-Trimethyl14-Ethylene-14-Pentadecne Neophytadiene); palmitic acid (Emerson 143); linoleic acid (Telfairic Acid); linoleic acid (9,12,15-Octadecatrienoic Acid); 6- amino-5-pentenoic-alpha.-lactame (Abhexone); 1h-imidazole 2-nitro-(Azomycin) and 1,10-dicyanodecane (1H,5H-Pyrrolo but only 6-amino-5-pentenoic-alpha-lactame; 1h-imidazole; 2-nitro-(CAS) and 1,10-dicyanodecane were alkaloids [21].

F2: Analysis showed 5 compounds include ammonia (CAS), ethanol (Jayson), ethane (Pronarcol), phenol (Phenol, 2,6-Bis(1,1-Dimethylethyl) 4-Methyl), 1, 1'-Oxybis- (Ally dimethyl (Prop-1-Ynyl) Silane but never each weren't alkaloids [22].

F3: Analysis showed 5 compounds include ethanol (Jaysol), 1, 1'-oxybis- (Pronarcol), ethyl acetate (Acetidin), Pyridine, 2-butyl-3,4,5,6-tetrahydro- (2-Butyl-3,4,5,6-tetrahydro pyridine) and Pentadecanecarboxylic acid (Emerson= palmitinic acid) but never each weren't alkaloids [23].

F4: Analysis showed 33 compounds include ammonia (CAS), ethanol, 1,1'-oxybis-(CAS), dichloromethane (Solaesthin), freon 20 (Trichloromethane), 1-butanol (Gamma Valerol acetone) , ethanol (Emkanol), 2-ethoxy, methyl benzene (Phenyl methane), ethanoic acid (Acetic Acid Butyl Ester), 7-oxabicyclo[2.2.1] heptane (Socineole), benzene (Benzene, 1-Methyl-2-(1-Methylethyl), 1,8-cineole (2-Oxabicyclo[2.2.2]), tetradecane, phenol (2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one), di isopropyl methyl phosphate (Thiophene, 2-Propyl), butylated hydroxytoluene (4-Methyl-2,6-DI-Tert-Butylphenol), pentanamide (Le Vulinanilide), 4-oxo-N-phenyl, 6-n-butyl-2,3,4,5-tetrahydropyridine (Cyclohexane, 1-Ethyl-1-Methyl), octadecane, methyl palmitate (Uniphat A60), palmitinic acid (Emersol), eicosane, octa-sulfur (Cyclooctasulfur), methyl stearate (Octadecanoic Acid, Methyl Ester), Ethyl Acetamidoacetate (Glycine, N-Acetyl-, Ethyl Ester), levulinanilide, hexadecane (Cetane), n-tricosane (Phenol, 4-(2-Aminopropyl), phenol, 4-(2-aminopropyl), nonacosane, N-acetyl glycine ethyl ester and 1,2-benzenedicarboxylic acid (Phthalate= Bisoflex 81) but only levulinanilide, phenol, 4-(2-aminopropyl)-, 6-n-butyl-2,3,4,5-tetrahydropyridine and glycine, n-Acetyl-, Ethyl Ester were alkaloids (Tables 4-6).

Table 4: GC MS analysis of alkaloid fraction F1 of *F. vaillantii*

N.	F1 Compounds	RT	%
1	Ammonia	1.33	7.48
2	Ethanol	1.4	52.43
3	Phenol		
4	Cyclohexane	25.39	1.33
5	Neophytadiene	28.22	1.09
6	2-Hexadece n-1-Ol	29.1	1.28
7	Palmitic Acid	30.85	6.43
8	Linoleic Acid	35.59	6.96
9	Linoleic Acid	35.83	9.3
10	6-Amino-5-Pentenoic-Alpha.-Lactame	49.59	5.73
11	1h-Imidazole	51.07	2.86
12	1,10-Dicyanodecane	53.75	4.09

Table 5: GC MS analysis of alkaloid fraction F2 and F3 of *F. vaillantii*.

N.	F2 Compounds	RT	%	N.	F3 Compounds	RT	%
1	Ammonia	1.33	3.1	1	Ethanol	1.39	46.01
2	Ethanol	1.4	19.16	2	1,1'-Oxybis (CAS)	1.44	50.96
3	Ethane, 1,1'-Oxybis-(CAS)	1.46	70.61	3	Ethyl Acetate	1.69	0.66
4	Phenol	21.08	3.78	4	Pyridine, 2-Butyl-3,4,5,6-Te Trahydro	25.39	0.96
5	Ally dimethyl (Prop-1-Ynyl) Silane	25.39	3.35	5	Pentadecanecarboxylic Acidl	30.78	1.4

Table 6: GC MS analysis of alkaloid fraction F4 of *F. vaillantii*.

N.	F4 Compounds	RT	%	N.	F4 Compounds	RT	%
1	Ammonia	1.34	0.27	16	Butylated-Hydroxy toluene phenol	21.08	0.85
2	Ethanol= Jayson	1.42	0.2	17	Levulin anilide	21.5	0.26
3	1,1'-Oxybis-	1.46	2.62	18	Cetane	23.03	1.38
4	Dichloro methane	1.52	8.27	19	Cyclohexane	25.39	2.91
5	Freon 20	1.75	28.45	20	6-N-Butyl-2,3,4,5-Tetrahydropyridine	25.39	2.91
6	Gamma,Valero lactone	1.92	0.32	21	Octadecane	27.41	1.67
7	Emkanol	2.28	12.35	22	Methyl palmitate	30.02	9.11
8	Methyl benzene	2.78	0.75	23	Palmitinic acid	30.88	3.31
9	Ethanoic Acid	3.33	1.2	24	Eicosane	31.55	1.41
10	7-Oxabicyclo[2.2.1] Heptane	7.72	0.37	25	Cyclooctasulfur	32.46	0.72
11	Benzene,	8	0.42	26	Methyl stearate	35.22	6.89
12	1,8-Cineole	8.15	0.32	27	4-(2-Aminopropyl)	39.15	1
13	Tetrad cane	18.18	0.49	28	Nonacosane	42.1	0.84

14	2,6-Di(T-Butyl)-4-Hydroxy-4-Methyl-2,5-Cyclohexadien-1-one phenol	20.08	0.21	29	Ethyl acetamidoacetate	43.49	2.73
15	Thiophene, 2-propyl-	20.53	0.24	30	Phthalate=Bisoflex 81	44.99	2.7

GC MS analysis of alkaloid fractions of F asepala

F1 analysis showed 45 compounds include

Dihydrazide (Oxamide Bis Hydrazone), Ethanol (Jaysol); Ethane (Ethane, 1,1'-Oxybis); Ethyloxy, Ethyl Acetate (Acetic Acid, Ethyl Ester), Thiophene, 3-Methyl (2-Octylthiophene), Bicyclo [3.2.0] Hept-2,6-Diene-1, 2, 3, 4, 5, 6-D(7) (3-Decen-5-One, 2-Methyl- Heptane, 1,7-Dibromo), Cis-1-Methyl-2-Ethylcyclohexane (Cyclopentane, 1,1'-Ethylidenebis); Phenol, 2,6-Bis (1,1-Dimethylethy) (Butylated Hydroxytoluene); Dodecanoic Acid (Lauric Acid); 3-Cyclohexen-1-Ol, 3-Methyl (Thenyldiamine); Triallylmethylsilane (2-Furanmethanol); Tetradecanoic Acid (Myristinic Acid); Eophytadiene (14-Ethylene-14-Pentadecne); Hexadecanoic Acid (Palmitinic Acid); Octa-Sulfur (Sulfur (S8)); Hexadecane, 1-(Ethenyloxy) (Hexadecal Vinyl Ether); 9,12-Octadecadienoic Acid (Z,Z) (Linoleic Acid); 9,12,15-Octadecatrien-1-Ol (11,14,17-Eicosatrienoic Acid); Octadecanoic Acid (Stearic Acid); 1-Propanone (1-Tert-Butyl-3-Pivaloylcyclopropene); Methyl Heptadecan-8(E) (1H-Benzocyclohepten-7-Ol); 9,12-Octadecadienoic Acid (Cis-Linoleic Acid); Bicyclo Dec-2-Ene (Ethyl Linoleolate); 1,2-Cyclohexanediamine, N,N,N',N'etramethyl; Eicosanoid Acid (Arachic Acid); 15-Ydroxypentadecanoic Acid (Pentadecanoic Acid); Pentacosane; 1,2-Benzenedicarboxylic Acid (Bis(2-Ethylhexyl) Phthalate); (Z)-3-(2-Cyanoethenyl)-2,5-Dimetho Naphthalene (2-Propenenitrile,3-(3,8-Dimethoxy-2-Naphthalenyl)); N-Nonadecene; Heptacosane (N-Heptacosane); 1,2-Cyclopentan Ediacetic Acid(Di-Tert-Butyl ester Of .Alpha Alp Ha.'-Diacetyl-4-Oxo-1,2-Cyclopentanediacetic Acid); 3,3'-Dimenthol Cyclohexane (4-(1,1-Dimethylethyl)-1-(2-Propenyl)4-T-Butyl-1-(1-Methylallyl)Cyclone); Dotriacontane; 1,3,12-Nonadecatriene; 1-Docosene; Nonadecane; Hexyl Cyclopentenone; Benzene, 2-Fluoro-1-Methyl-4-Nitro (Toluene, 2-Fluoro-4-Nitro Or 2-Fluoro-4-Nitrotoluene); Toluene, 2-Fluoro-4-Nitro (2-Fluoro-4-Nitrotoluene); Lycopersin (10H-Benzo(B)Xanthene-7,10,12-Trione, 6,11-Dihydroxy-3,8-Dimethoxy-1-Methyl); Stigmasta-5,22 (Stigmastan-3,5,22-Trien); 9-(2,6-Diethylphenyl)-2,8-Dimethyl -9-H-Purin-6-Amine (9H-Purin-6-Amine, 9-(2,6-Diethylphenyl)-2,8-Di Methyl); 5-Epiaplysterylacetate-1 (2,8-Diacetyl-Peri-Xanthenoxanthene 4,10-Quinone); 1,7-Dicyclopentyl-4-N-Octylheptane (Cyclopentane, 1,1'-(4-Octyl-1,7 Heptanediyl)Bis) and Vitamin E (Alpha. Tocopherol) but Ethanediimidic Acid, Dihydrazide;1,2 Cyclohexanediamine, N,N,N',N'-Tetraethyl; Z)-3-(2-Cyanoethenyl)-2,5-Dimethoxynaphthalene; 9 (2,6 Diethylphenyl)-2,8-Dimethyl-9-H-Purin-6-Amine and 25-Epiaplysterylacetate were alkaloids.

- **F2 analysis showed 6 compounds include:** Ammonia; Ethanol; Pronarcol (Ethoxyethane); Freon 20 (Trichloro Methane Or Chloroform); Phenol, 2,6-Bis(1,1-Dimethylethyl) (4-Methyl- (CAS) Or 4-Methyl-2,6-

Di-Tert-Butylphenol) and 2,3-Tetramethylcyclopropanoic acid but never each weren't alkaloids.

- **F3 analysis showed 6 compounds include:** Ammonia; Ethanol; Ethane; Ethyl Acetate (Acetidin); 2-Butyl-3, 4,5,6-Tetrahydropyridine and Hexadecanoic Acid (Palmitic Acid) but never each weren't alkaloids.
- **F4 analysis showed 18 compounds include:** Ammonia; Ethane, 1,1'-oxybis; Methane dichloride (dichloromethane or R 30 or Freon 30); Ethanol, 2-ethoxy; Freon 20; Benzene, methyl (Toluene); Ethanoic Acid Butanol Ester (Acetic Acid, Butyl Ester); Butyrate Hydroxytoluene Phenol, 2,6-Bis(1,1-Dimethylethyl)-4-Methyl(4-Methyl-2,6-Di-Tert-Butylphenol); Hexadecane; 2-Nor-Hexylthiop (Hene (Thienyl)Hexane); N-Docosane; Hexadecanoic Acid, Methyl Ester; Octacosane; Octadecanoic acid, Methyl Ester (Methyl Stearate); 3-Tridecanone; 4-(2'-Hydroxy-3'-Isopropylaminopro Proxy)Phenyl acetic Acid Ethyl N,N-Dimethyloxamate (Acetic Acid, (Dimethyl amino) Oxote or Oxalic Acid, Dimethyl); Benzenedicarboxylic Acid, Bis(2-Ethylhexyl) Ester (Phthalic Acid, Bis(2-Ethylhexyl) Ester) and 2H-Azirin-1-N-3-Amine, N,N,2,2-Tetramethyl (2,3-Dehydropiperidin-6-One (6-Amino-5-Pentenoic-.Alpha.-Lactame 3 or (Dimethyl amino)-2,2-Dimethyl-[1- (15)-N]-2H-Azirine) but Ethanediimidic Acid, Dihydrazide; 1,2-Cyclohexanediamine, N,N,N',N'-tetra methyl; Z)-3-(2-Cyanoethenyl)-2,5-Dimethoxy naphthalene; 9-(2,6-Diethylphenyl)-2,8-Dimethyl-9-H-Purin-6-Amine and 25-Epiaplysteryl acetate were alkaloids (Tables 7 and 8).

Table 7: Compound determination result of GC-MS analysis of alkaloid fractions (F) of *F. asepala.*

N.	F1 Compounds	Rt	%	N.	F1 Compounds	Rt	%
1	Ethanediimidic acid, Di hydrazide	1.33	0.52	41	Lycopersin	52.13	1.66
2	Ammonia	1.35	0.2	42	Stigmasta-5,22-Dien-3-Ol, Acetate,	52.6	1.73
3	Ethanol	1.39	2.26	43	9-(2,6-Diethylphenyl)-2,8-Dimethyl -9-H-Purin-6-Amine	53.44	0.3
4	Ethene, Ethyloxy	1.44	7.97	44	5-Epiaplysterylacetate-1	53.67	2.81
5	Acetic acid, Ethyl ester	1.7	0.3	45	A.-Tocopherol	53.95	8.14
6	2-Octylthiophene	19.15	0.25		F2 Compounds	Rt	%
7	Bicyclo[3.2.0]Hept-2,6-Diene-1,2,3 ,4,4,5,6-D(7)	20.16	0.15	1	Ammonia	1.32	4.22
8	Cyclopentane, 1,1'-Ethylidenebis	20.53	0.52	2	Ethanol	1.39	19.18
9	Phenol, 2,6-Bis(1,1-Dimethylethy)	21.12	3.87	3	Pronarcol	1.44	65.3
10	Dodecanoic Acid (Cas)	22.39	0.22	4	Freon 20	1.74	3.11

11	3-Cyclohexen-1-Ol, 3-Methyl	25.45	5	5	Diethoxyethane	2.36	0.39
12	Triallylmethylsilane	25.69	0.35	6	Phenol, 2,6-Bis(1,1-Dimethylethyl)	21.08	3.74
13	Tetradecanoic Acid	26.8	0.66	7	2,2,3,3-Tetramethylcyclopropanoic Acid	25.4	4.05
14	Eophytadiene	28.24	0.85		F3 Compounds	Rt	%
15	Hexadecanoic acid	31.22	8.62	1	Ammonia	1.33	3.51
16	Octa-Sulfur	32.5	0.7	2	Ethanol	1.39	20.41
17	Hexadecane, 1-(Ethenyloxy)	33.62	0.28	3	Ethene	1.45	70.54
18	9,12-Octadecadienoic acid (Z,Z)	36.11	7.74	4	Ethyl Acetate	1.7	1.32
19	9,12,15-Octadecatrien-1-Ol	36.32	5.47	5	2-Butyl-3,4,5,6-Tetrahydropyridine	1.94	25.39
20	Octadecanoic Acid	36.86	3.22	6	Hexadecanoic acid	30.8	2.29
21	1-Propanone				F4 Compounds	Rt	%
22	Methyl Heptadecan-8(E)	37.6	0.24	1	Ammonia;	1.36	0.45
23	9,12-Octadecadienoic acid	38.62	0.25	2	Ethane,1,1-Oxybis	1.46	3.98
24	Bicyclo[[7.1.0]Dec-2-Ene	38.8	0.45	3	Methane Dichloro	1.52	21.65
25	1,2-Cyclohexanediamine, N,N,N',N'etramethyl	41.23	1.34	4	Freon 20	1.75	46.22
26	Eicosanoic acid	41.54	0.27	5	Ethanol, 2-Esthoxy		
27	15-Hydroxypentadecanoic acid	43	30.45	6	Benzene, Methyl	2.78	0.39
28	Pentacosane			7	Ethanoic acid, Butanol ester	3.33	0.6
29	1,2-Benzenedicarboxylic acid	45.01	0.23	8	4-Methyl-2,6-D I-Tert-Butylphenol	21.08	0.77
30	(Z)-3-(2-Cyanoethenyl)-2,5-Dimetho Xynaphthalene	45.56	0.16	9	Hexadecane	23.03	0.54
31	N-Nonadecene	47.21	0.76	10	2-Nor-Hexylthiop Hene	25.39	1.67

32	Heptacosane	47.53	1.2	11	N-Docosane	27.41	0.57
33	1,2-Cyclopentan Ediacetic acid	48.67	0.43	12	Hexadecanoic acid, Methyl ester	29.98	4.99
34	3,3'-Dimenthol Cyclohexanol	48.8	0.97	13	Octacosane	31.54	0.51
35	Dotriacontane	49.06	0.37	14	Octadecanoic Acid	36.39	3.37
36	1,3,12-Nonadecatriene	49.78	0.94	15	3-Tridecanone	39.12	1.62
37	Hexyl Cyclopentenone	50.6	2.81	16	3-Tridecanone; 4-(2'-Hydroxy-3'-Isopropylaminopro Proxy)Phenylacetic Acid	43.48	0.96
39	Benzene, 2-Fluoro-1-Methyl-4-Nitro or Toluene, 2-Fluoro-4-Nitro	51.62	1.57	17	Benzenedicarboxylic Acid, Bis (2-E Thylhexyl) Ester	44.98	0.97
40	Toluene, 2-Fluoro-4-Nitro	51.8	1.37	18	6-Amino-5-Pentenoic-.Alpha.-Lactame 3 $$ (Dimethylamino)-2,2-Dimethyl-[1- (15)-N]-2h-Azirine	47.56	2.14

Table 8: Alkaloid determination results of GC-MS analysis of alkaloid fractions of *F. vaillantii* and *F. asepala.*

%	Rt (min)	Alkaloid	Fraction	N.
5.73	49.59	2,3-Dehydropiperidin-6-One $$ 6-Amino-5-Pentenoic-.Alpha.-Lactame	1 *F. VA*	1
2.86	51.07	Tertio-Butyl-4 Cyclohexanoxime (Azomycin) $$ 1H-Imidazole, 2-nitro-	1 *F. Va*	2
4.09	53.75	1,10-Dicyanodecane(Azomycin)	1 *F. Va*	3
0.26	21.5	Pentanamide, 4-oxo-N-phenyl- $$ Levulinanilide	4 *F. Va*	4
2.91	25.39	6-n-butyl-2,3,4,5-tetrahydropyridine	4 *F. Va*	5
4.7	39.15	Phenol, 4-(2-Aminopropyl)-	4 *F. Va*	6

		$$ Phenol, P-(2-Aminopropyl)-, P-Hydroxyamphetamine $$ Dl-P-Hydroxyamphetamine		
2.73	43.49	Glycine, N-Acetyl-, Ethyl Ester (CAS) $$ N-Acetyl glycine Ethyl Ester $$ Ethyl Acetamidoacetate $$ Ethyl N-Acetylglycinate $$ Acetylglycine Ethyl	4 *F. Va*	7
0.72	1.33	Ethanediimidic acid, dihydrazide (CAS) $$ oxamidebishydrazone $$ Oxalamidrazone $$ Oxalic acid bisamidrazone	1 *F. as*	1
0.85	28.24	Neophytadiene $$ 2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	1 *F. as*	2
1.34	41.23	1,2-Cyclohexanediamine, N,N,N',N'-tetra methyl-	1 *F. as*	3
0.16	45.56	(Z)-3-(2-cyanoethenyl)-2,5-dimethoxynaphthalene $$ 2-Propenenitrile, 3-(3,8-dimethoxy-2-naphthalenyl)-, (Z)	1 *F. as*	4
2.9	51.62	Toluene, 2-fluoro-4-nitro $$ 4-t-Butyl-1-(1-methylallyl)cyclohe xanol Benzene, 2-fluoro-1-methyl-4-nitro $$ 2-Fluoro-4-nitrotoluene Cyclohexane, 1-butyl-4-(1,1-dimet hylethyl)	1 *F. as*	5
0.3	53.443	25-Epiaplysterylacetate-1 (10-Quinone) $$ 9-(2,6-diethylphenyl)-2,8-dimethyl -9-h-purin-6-amine	1 *F. as*	6
0.96	43.48	4-(2'-Hydroxy-3'-Isopropylaminopropoxy) Phenyl acetic Acid	4 *F. as*	7
2.14	47.557	2,3-Dehydropiperidin-6-One $$ 6-Amino-5-Pentenoic-.Alpha.-Lactame	4 *F. as*	8

Antifungal assay: According to the analysis of variance there was a significant difference related to antifungal effects on *A. flavus* and *A. niger* in terms of all traits except Fraction/Time (F/T). There was a significant

difference between fractions, concentration, time of growth, fraction/concentration, concentration/time and fraction/concentration/ time on the *A. flavus* and *A. niger* growth (Table 9).

Table 9: Analysis of variance for antifungal effect of alkaloid fractions of *F. vaillantii* on the *A. flavus* and *A. niger*.

Sources of changes	DF	Sum of squares	
		A. flavus	***A. niger***
Fraction (F)	3	2.309	10.480
Concentration(Co)	3	62.185	46.731
Time (T)	3	110.336	83.878
F*Co	9	7.308	19.396
F*T	9	0.481	1.374
Co*T	9	21.443	12.433
F*Co*T	27	7. 630	5.988
Error	12	9.393	16.56
Total	191	221.087	196.841

Based on the results of mean comparisons related to the growth of *A. flavus* and *A. niger*, the fractions of F1, F2, F3 and F4 had antifungal effects on the both *Aspergillus* while the fractions of F1, F2 and F3 had the highest antifungal effects on the *A. flavus*. Related to concentrations for this effect, the concentration 0.12 mg/ml indicated the highest antifungal effect on the *A. flavus* and *A. niger* and concentrations 0.04 mg/ml and 0.08 mg/ml had lower than effects. The analysis of data also strongly indicated that antifungal effects of all concentrations of alkaloids on the *A. niger* are more than on the *A. flavus*. In terms of alkaloid Fraction/Concentration (F/C) interaction for antifungal effects, the highest value was observed in F1C0.04 mg/ml, F3C0.08 mg/ml and F3C0.12 mg/ml, for *A. niger* and F1C0.08 mg/ml, F1C0.12 mg/ml and F3C0.12 mg/ml, for *A. flavus*. The analysis of the results suggested that fraction/ concentration/ time interaction was significant for the all fractions, concentrations and times (4 days). The analysis of the data for three ways interaction of alkaloid fraction (F)/time (D) /concentration (C), also strongly indicated that fungal growth reached its minimum value at secondary days for all concentrations and fractions (Table 10).

Table 10: Mean values of alkaloid fraction (F)/time (D)/concentration (C) interaction in term of antifungal effects of *F. vaillantii* on the *A. niger* and *A. flavus*.

Sources of changes	DF	Sum of squares	
		A. flavus	***A. niger***
Fraction (F)	3	2.309	10.480
Concentration (Co)	3	62.185	46.731
Time (T)	3	110.336	83.878

F*Co	9	7.308	19.39
F*T	9	0.481	1.374
Co*T	9	21.443	12.433
F*Co*T	27	7. 630	5.988
Error	12	9.393	16.56
Total	191	221.087	196.841

Discussion

In this study, total alkaloids of *F. vaillantii* and *F.asepala* were 10.64 mg/g and 8.4 mg/g respectively and they were lower reported procedures. Total alkaloids of *F. agrarian* and *F. septum*, from the air-dried aerial parts were 88 mg/g and 83 mg/g respectively. Total quinolizidine alkaloid contents were 4.26 mg/g (*F. capreolata*) and 5.21 mg/g (*F. bastardi*). Total alkaloid of ethanol and methanol extracts of Synadenium grantii were 29.31 mg/g and 26.68 mg/g respectively. The TLC results showed 7 different alkaloids with different Removing Factor (RF) values using following solvent system: Chloroform: Ethyl acetate: Methanol: Ammonium hydroxide. Our TLC results showed 1, 2, 5 and (3, 2) alkaloids in all alkaloid fractions of roots, stems, leaves and fruits of *F. vaillantii* and *F. asepala* respectively. Rf values of roots, stems and 2 alkaloids of leaves of *F. vaillantii* and *F. asepala* were similar but other had different Rf in all fractions. The isoquinoline alkaloids protopine, cryptopine, sinactine, stylopine, bicuculline, adlumine, parfumine, fumariline, fumarophycine, fumaritine, dihydric fumariline, parfumidine and dihydrosanguinarine have been determined and identified by Gas Chromatography-Mass Spectrometry (GC-MS) in the 8 species of *Fumaria* genus except for *F. valiant* (*Fumaria agrarian, F. bastardii, F. capreolata, F. sepium, F. densiflora, F. faurei, F. officinalis subsp. officinalis, F. parviflora, F. petteri subsp. calcarata and F. macrosepala*) Some quaternary protoberberine alkaloids of Mahoniam anipurensis such as berberine, jatrorrhizine, palmatine, umbellate, coloumbamine, etc. were separated and characterized by Thin Layer Chromatography (TLC) and Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Isoquinoline alkaloids allocryptopine, chelidonine, protopine, bicuculline, sanguinary, cheleritrine, stylopine, and hydrastine were identified from four *Fumaria* species (*F. vaillantii Loisel, F. parviflora Lam., F. rostellata Knaf and F. jankae Hausskn*.) by HPLC-DAD method. Protopine and sanguinary were present in all extracts. Bicuculline and stylopine were found in *F. vaillantii* and *F.parvifolia*.

GC - MS analysis of *F. asepala* extract determined four known alkaloids includes fumariline, dihydrofumariline, fumaritine and oxyberberine. 6-amino penicillin acid is considered as important compound in the varieties of β-Lactam types and traded in the first of antibiotics. Results of GC-MS analysis of alkaloid fractions of *F. vaillantii* and *F. asepala* were unknown and very different from reported procedures. 6-amino-5-pentenoic-.alpha.-Lactame 6-amino-5-pentenoic-.alpha.-lactame, tertio-butyl-4 cyclohexanoxime (azomycin) and 1, 10-dicyanodecane (azomycin) in fraction 1 of *F. vaillantii* and 6-amino-5-pentenoic-.alpha.-Lactame in fraction 4 *F. asepala* from lactam groups are reported in this paper are new compounds in two *Fumaria*. α-Lactams have often been postulated as intermediates in numerous processes. Lactams contain an amide group as part of a cyclic structure. Traditional antibiotics e.g. penicillin, penems and cephalosporin's are beta-lactam compounds. *Fumaria* species have antibacterial effects. Lactams in *Fumaria* can be because of their antibacterial effects. Aziridine alkaloids also belong to a rare and somewhat neglected group of natural products which are known to play a seminal role in the secondary metabolism of some micro-organisms, plants and various marine organisms. The aziridine-containing compounds have been of interest as both immune-modulatory and anticancer agents since the late 1950. A number of compounds of α-lactams (*Aziridinones*) are reported as carcinogen, cysteine protease inhibitors, antibacterial, antifungal, anticancer, antileishmanial, and antimalarial agents are *F. vaillantii* and *F. asepala* carcinogen? There require future research for response. The methanolic extracts and various fractions of *F. indica* were inactive against the *A. niger A. flavus* and 4 other fungi. The all alkaloid fractions in varies concentrations exhibited broad spectrum inhibition against *A. flavus* and *A. niger*. The minimum concentration and the maximum fungicide concentration against the test *Aspergilluses* were 0.04 mg/ml and 0.12 mg/ml of the all fractions, respectively. Our results showed that *F. valiant* have four type alkaloids with high antifungal effects. These results are according to the results of other researchers as, the alkaloid of *F. indicia* significantly inhibited spore germination of *Erysiphe, Fusarium* and *Penicillium* species. Some alkaloids of Corydalis longipes were found effective the against spore germination of some fungi.

Conclusion

The use of quaternary method of alkaloid extraction showed that *F. vaillantii* has four types of alkaloids, lipophilic compounds and non-basic alkaloids (F1), quaternary benzophenantridine alkaloids, protopine alkaloids and tertiary bases soluble in ether (F2), quaternary protoberberine alkaloids bases soluble in ether (F3) and non-polar compounds and highly polar quaternary alkaloids bases soluble in chloroform (F4), and all four types have antifungal activates and inhibit the growth of A. niger and A. flavus. Traditional antibiotics penicillin, penems and cephalosporin's are beta-lactam compounds. Fumaria speciese have antibacterial effects. Lactams in Fumaria can be because of their antibacterial effects. α-Aziridinones (Lactams) alkaloids also belong to a rare and somewhat neglected group of natural products which are known to play a seminal role in the secondary metabolism of some micro-organisms, plants and various marine organisms. A number of compounds of α-Lactams (Aziridinones) are reported as carcinogen, cysteine protease inhibitors, antibacterial, antifungal, anticancer, antileishmanial, and antimalarial agents are *F. vaillantii* and *F. asepala* carcinogene? There require future research for response. *F. vaillantii* and *F. asepala* have some quaternary protoberberine alkaloids but they are different from other reported compounds. A number of compounds of α-Lactams (Aziridinones) presented in alkaloids of *F. vaillantii* and *F.asepala*.

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