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Research Article

Phytochemical Screening and Cytotoxic Properties of Ethanolic Extract of Young and Mature Khat Leaves

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The khat plant has been culturally used in many parts of Africa and the Arabian Peninsula for many years to induce psychostimulating effect. Because of the global wide-spreading nature, khat chewing is being considered as a universally growing problem. *Catha abbottii*, *Catha edulis*, and *Catha transvaalensis* are the three species of khat commonly chewed in Saudi Arabia and nearby regions. Khat users usually prefer to chew young leaves over mature ones due to the diverse effects produced by both. Though many of the constituents of khat leaves have been identified, the complete phytochemical profile of young and mature leaves was not performed or compared; also, no evidence is available to affirm the cytotoxicity of young or mature leaves. Therefore, this study aimed to investigate the phytochemical basis of the differential response of the young and mature leaves and to assess the cytotoxicity of young and mature khat leaves. Ethanolic extracts of young and mature leaves of three khat cultivars were subjected to GC-MS. Hierarchical cluster analysis revealed the existence of two major clusters. The extracts of young leaves were found to contain the maximum content of cathinone; however, methoxyamphetamine was found in only one extract of young leaves. Cytotoxicity investigations were also conducted on both types of leaves using three cancer cell lines, human breast adenocarcinoma, human ovary adenocarcinoma, and human colon adenocarcinoma and also normal human fetal lung fibroblast cell line was used. All extracts showed comparable cytotoxicity, IC_{50} ranging from 22–59 μg/mL on the cancer cells; however, we observed more cytotoxicity against normal cells (IC_{50}: 6–41 μg/mL). The predominant cytotoxicity on normal cells may pose many health hazards to khat consumers.

1. Introduction

Khat (*Catha edulis*) has culturally been used in many parts of Africa and the Arabian Peninsula, including Saudi Arabia and Yemen, but it is believed to be a globally growing problem [1–3]. Khat is mainly used to increase mental capacity [4], physical strength [5], and social entertainment [6] and enhance cheerfulness [7] and sexual orgasms [8]. The World Health Organization considers khat a drug of abuse since it causes a range of health problems [9]. There is a

biological plausibility that chronic khat use may induce memory deficits and impair cognitive flexibility [10]. The differential patterns of memory deficits may reflect the differences in dose effect as well as time-dependent impairment [11]. Cathinone, a major constituent of khat, is structurally and functionally similar to amphetamine and cocaine. Cathinone caused the proliferation of gonadotrophs but decreased the lactotrophs and corticotrophs in anterior pituitary sections of animals in high dose and long-term exposure, while an effect of low dose on these cells was insignificant [12]. Few articles contribute to death among khat chewers to khat-induced heart failure, but several other studies have demonstrated that khat chewing has unfavorable cardiovascular effects [13]. Like amphetamine, the reflection of undesired actions of cathinone on the myocardium is observed through variations in heart rate, blood pressure, and vascular actions [14]. However, further studies are needed to address the risk factors in khat chewers that may explain khat-induced cardiotoxicity, cardiomyopathy, and heart failure.

Khat is reported to induce toxic hepatitis together with high-titer anti-nuclear antibody mimicking serologic patterns of autoimmune hepatitis and potentially associated with malignant and malignant oral disorders [15]. There are several pieces of evidence connecting khat chewing to genetic damage of the oral mucosa and cancer [16, 17] Esophageal and gastric carcinoma have been observed in khat chewers in both men and women in Yemen [18]. A similar review of oral cancers presenting over two years in the Asir region of Saudi Arabia showed strong circumstantial evidence linking the long-term use of khat with an increased rate of oral malignancies [19].

Khat young and mature leaves have a different degree of psycho-stimulating and variable biological properties. Khat chewers, however, prefer young leaves over mature for the more desired outcomes and fewer side effects. The young leaves are believed to possess higher central nervous system-(CNS-) stimulating activities than the mature leaves. This difference in CNS-related activities could be due to the presence of the same constituents in both young and mature leaves; however, in different quantities or due to the presence of different constituents should be investigated. No assessment and comparison of the complete phytochemical profiles of young and mature leaves of *Catha edulis* has been conducted. Therefore, we have undertaken this study to investigate the phytochemical constituents of both young and mature khat leaves and to find out the degree of cytotoxicity of their selected extracts.

2. Materials and Methods

2.1. Collection of Plant Material. Young leaves (locally known as Nwaif) and mature leaves (locally known as Gafra) of three cultivars of khat (*Catha edulis*) locally known as Gaifi, Kofat, and Gahasha were collected from the Jazan region of Saudi Arabia and divided into two groups each: young Gaifi (**N1**), young Kofat (**N2**), and young Gahasha (**N3**) for young leaves and mature Gaifi (**G1**), mature Kofat (**G2**), and mature Gahasha (**G3**) for mature leaves. All khat varieties were identified by Dr. Yahiya Masrahi, Department of Botany, Faculty of Science, Jazan University. The collected leaves were washed, dried in the shade at room temperature, and powdered.

2.2. Preparation of Leaves Extract. All dried leaves powder (200 g each) was exhaustively extracted with 80% ethanol in the Soxhlet apparatus for 6 to 8 hours. Colored extracts were evaporated under reduced pressure to get brown viscous masses. Sample **N1**, **N2**, **N3**, **G1**, **G2**, and **G3** were labeled and stored at 4°C in the dark for GC-MS analysis and cytotoxic activity.

2.3. GC-MS Analysis of the Extracts. GC-MS analyses of each extract were carried out on a Shimadzu Gas Chromatograph instrument (Shimadzu GCMS QP2010 with CTC GC PAL Liquid Injector Computer Software Loaded) fitted with a capillary column TR-5MS (30 m × 0.25 mm), with film thickness 0.25 μm. The carrier gas was He, with the flow rate of 1.2 mL/min. The initial temperature was kept at 70°C and then heated at a rate of 15°C per minute to 290°C and held for 16 minutes. The chromatography was coupled with a Shimadzu QP2010 Ultra MS detector, 70 eV.

2.4. Identification of Constituents. GC-MS identified the most constituents by comparing their retention indices with those of authentic standards available in the laboratory or with the retention indices, which were in close agreement with the reference. GC-MS achieved further identification. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using the NIST08 and Wiley 9 built libraries.

2.5. Hierarchical Cluster Analysis (HCA). Due to significant variations of the contents of various types of khat leaves, the most abundant components from all samples were subjected to multivariate chemometric analysis. Hierarchical cluster analysis (HCA) was performed, and agglomeration and dendrograms were developed to assess the relationship between the components of the different types of extracted khat and to find out the proper classification of these studied samples and to detect the proper classification of khat types using SPPS software version 22.0.

2.6. Cell Culture. Three cancer cell lines, MCF7 (Michigan Cancer Foundation-7; human breast adenocarcinoma), A2780 (human ovary adenocarcinoma), and HT29 (human colon adenocarcinoma), were used in this study to assess the cytotoxicity of different ethanolic extracts. Besides, we also used MRC5, normal human fetal lung fibroblast. All cells were obtained from the ATCC (American Type Culture Collection). The three cancer cells were subcultured in the RPMI (Roswell Park Memorial Institute)-1640 media (10% FBS, fetal bovine serum), while MRC5 was maintained in Eagles Minimum Essential Medium (EMEM, 10% FBS), all at 37°C, 5% CO_2 and 100% relative humidity).

2.7. Cytotoxicity Assay. As previously reported by Bkhaitan et al., the cytotoxic activity of the six extracts was evaluated by the MTT assay (MTT is the abbreviation for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The three cell lines and one normal fibroblast were separately cultured in a 96-well (3×10^3/well) and incubated at 37°C overnight. Final extract concentrations were 0, 6.25, 12.5, 25, 50, and 100 μg/mL of DMSO (dimethyl sulfoxide) 0.1%; $n = 3$). Plates were incubated for 72 h, followed by addition of MTT to each well. Then, the plates were incubated for 3 hr, the supernatant was aspirated, and DMSO was added to each well. Absorbance was read on the multiplate reader. The optical density of the purple formazan A_{550} is proportional to the number of viable cells. Extract concentration causing 50% inhibition (IC_{50}) compared to the control cell growth (100%) was determined. GraphPad Software (GraphPad Software, San Diego California USA) was used for analysis.

3. Results

3.1. GC-MS Analysis. Separation and identification of different phytoconstituents present in the closely related genus in mature and young leaves was analyzed by GC-MS. Proportional chromatograms of unlike varieties of khat extracts are shown in Figure 1. The phytochemical composition of the young and mature extracts, including psychoactive cathine and cathinone, is shown in Table 1. The total area percentage for identified components was 63.56%, 53.98%, 62.17%, 36.67%, 52.57%, and 67.12% for **N1**, **G1**, **N2**, **G2**, **N3**, and **G3**, respectively. Cathine, a chief psychostimulant constituent, was found in all three varieties of young leaves in different proportions, viz., 0.55%, 0.77%, and 0.67%, for **N1**, **N2**, and **N3**, respectively, whereas, only 0.35% and 0.16% found in **G1** and **G3** samples, respectively. The lowest amount was found in **G3** and the highest amount, 0.77%, in the extract of **N2**. However, no trace of either cathine or cathinone was found in the extracts of mature **G2**. Cathinone was found in a lesser amount than cathine, 0.05% to 0.26%, in all extracts which were studied.

3.2. Cytotoxicity Studies. The cytotoxicity of the six khat extracts was determined against three cancer cells, in addition to one normal cell line to compare the selectivity of each extract. The IC_{50} of **N1** ranged from 29–56 μg/mL in the three cancer cell lines, and it was 30 μg/mL against MRC5, normal cells, which was similar to its effect on MCF7. **G1** extract showed similar cytotoxicity against the three cell lines, but it was at least two-fold more toxic towards the normal cells compared with the cell lines (Table 2). **N2** and **G2** extracts exhibited similar activity, with the highest effect also on MRC5 normal cells. **N3** extract was the most significant, as it was 4–8 folds more cytotoxic against MRC5 cells compared with MCF7, A2780, and HT29 cells. Extract **G3** was the only one to show less cytotoxicity on MRC5 cells compared to A2780 and HT29 cells.

The chemical compositions of the ethanolic extract of **N1** and **G1** are tabulated in Table 1. The ethanolic extract of **N2** consisted of a variety of 26 chemical constituents (63.56%) including six alkanes (11.32%), three each of alkyl benzenes (7.88%), monoamine alkaloids (1.28%), and acyclic diterpenes (3.98%), two glycosides (10.79%), and one each of alkyl nitrile (2.0%), aromatic ketone (1.54%), aromatic alcohol (2.61%), fatty ester (1.25%), alkenyl ester (2.50%), disaccharide (4.62%), alkyl amide (3.59%), and phytosterol (1.71%). The predominant constituent detected in the **N2** sample was 4-methyl mannitol (8.73%) followed by 1-ethenyl-3-ethyl-benzene (4.97%), galactitol (4.62%), 1-ethenyl-4-ethyl-benzene (3.94%), β-sitosterol (3.71%), (Z)-13-docosenamide (3.59%), 4-methyldodecane (3.12%), and 1-dodecanol (3.09%). The other chemical constituents present in the **N2** sample included 2,2′-azo bis [2-methyl-propane nitrile (1.46%), 2,2-dimethyltetradecane (2.48%), 5-methylundecane (1.54%), 4,6-dimethyldodecane (1.23%), 1-phenyl-1,2-propane-dione (1.51%), 1-phenylpropanol (2.61%), cathinone (0.22%), cathine (0.77%), 3-methoxyamphetamine (0.29%), 1-hydroxy-1-phenyl-2-(acetylamino) propane (1.02%), methyl α-D-glucopyranoside (2.06%), heneicosane (1.09%), isobutyl tetradec-3-enyl fumarate (2.51%), galactitol (4.62%), and phytol (1.10%).

Twenty-three chemical constituents were characterized in the ethanolic extract of mature Kofat (**G2**) amounting 53.98%. There were six alkanes present in maximum percentage (13.50%) along with one alkyl amide (6.92%), two alkyl benzenes (6.64%), two glycosides (5.08%), and two acyclic diterpenes (1.74). Various constituents, viz., alkyl nitrile (1.62%), sesquiterpene (1.04), cycloalkane (4.28), alkyl alcohol (2.69%), fatty ester (1.05%), aromatic ester (1.05%), fatty ester (3.06%), aromatic acid (1.8%), fluoroalkyl ester (1.46), and phytosterol (1.97%) were detected individually. The main phytoconstituents of **G2** were (Z)-13-docosenamide (6.92%), 1-methyl-2-phenyl cyclopropane (4.28%), 3-methyl-5-propylnonane (3.99%), 1-phenyl-3-ethyl-benzene (3.63%), 4-methyl mannitol (3.27%), 1,3-diethenyl-benzene (3.10%), and palmitic acid (3.06%). In addition, tetramethylbutanedinitrile (1.62%), 2,6,10-trimethyldodecane (1.04%), 2,6,7-trimethyldecane (1.74%), 2,6-dimethyldecane (1.61%), 2,7,10-trimethyldodecane (1.38%), methyl-β-D-galactopyranoside (1.81%), 2,6,10,14-tetramethylhexadecane (1.55%), dibutyl phthalate (1.05%), phthalic acid (1.8%), and heptacosyl heptafluorobutyrate (1.46%) were also detected only in the **G2** sample.

The chemical constituents characterized in both the species were 2,2,6-trimethyldecane, 1-ethenyl-3-ethyl-benzene, 1,3-diethenyl-benzene, 1-dodecanol, tetradecanyl acrylate, 4-methyl mannitol, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, (Z)-13-docosenamide, and β-sitosterol, and their amounts varied from 8.73 to 0.19%. Three euphorigenic and psychostimulant monoamine alkaloids cathine (0.77%), cathinone (0.22%), and 3-methoxyamphetamine (0.29%) were only present in young leaves.

The ethanolic extract of **N2** consisted of 26 variety of chemical constituents (62.17%) including six alkanes (11.86%), three each of aromatic compounds (9.76%) two each of sesquiterpenes (3.65%), monoamine alkaloids (0.81%), and one each of alkane nitrile (1.91%), aromatic ester (1.33%), alkyl alcohol (2.54%), fatty acid (1.58%), monosaccharide (6.37%), acyclic diterpenic alcohol (1.92%),

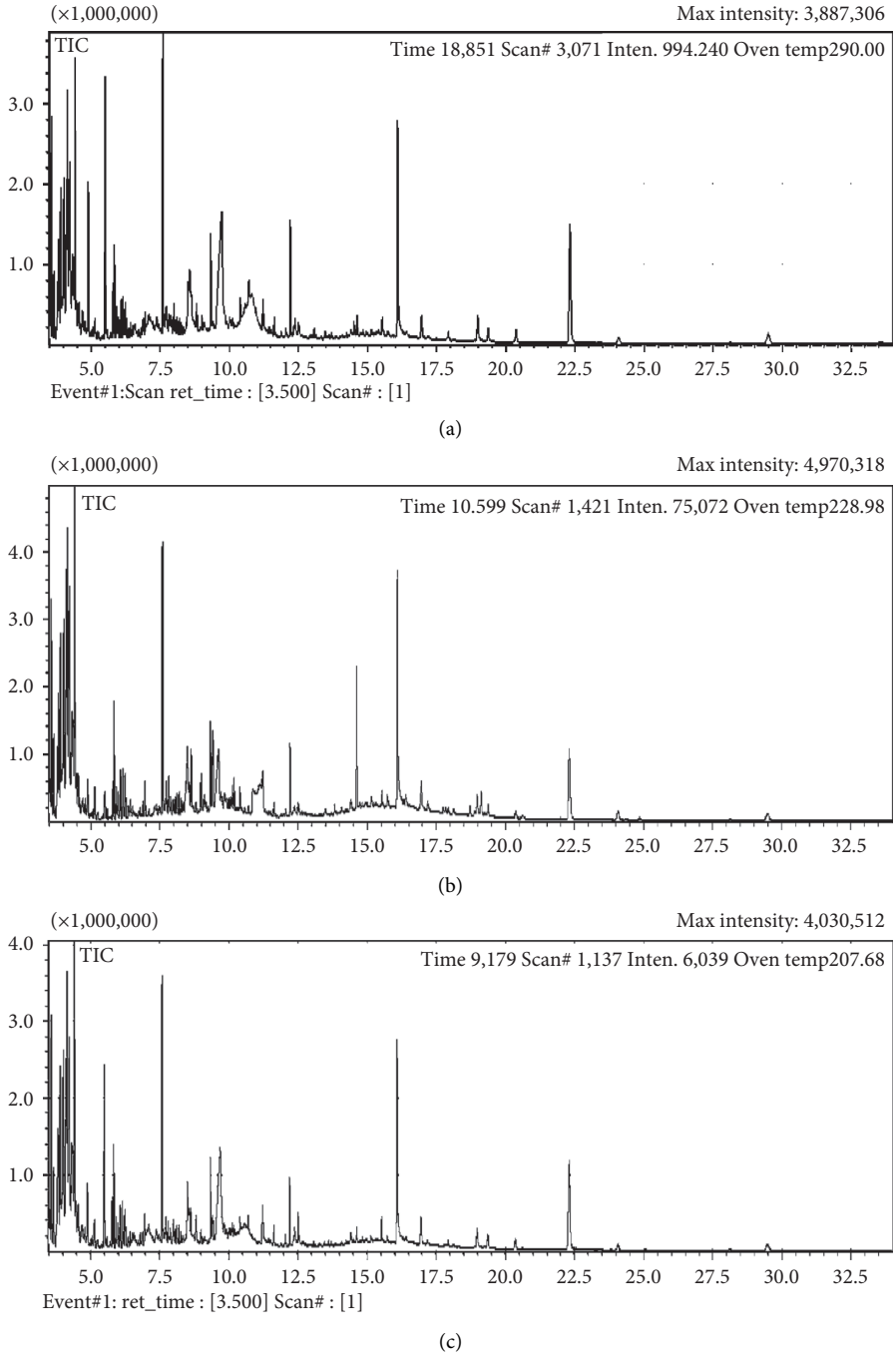


Figure 1: Representative typical GC-MS total ion current (TIC) chromatograms of three ethanolic extracts of khat samples: (a) young Kofat **N1**, (b) mature Kofat **G1**, and (c) young Gahasha **N3**.

diterpene (2.54%), alkyl mercaptan (2.62%), silyloxy alkane (1.93%), disaccharide (3.98%), vit. C ester (1.42%), alkyl amide (3.95%), and phytosterol (4.03%). The predominant constituent detected in the **N2** sample was 4-methyl mannitol (6.37%) followed by β-sitosterol (4.03%), 9-octadecenamide (3.95%), 1-ethenyl-4-ethyl-benzene (3.76%), 1-ethenyl-3-ethyl-benzene (3.24%), and 3-methyl-5-propylnonane (3%). The other chemical constituents present in the **N2** sample included heneicosane (2.89%), 2,2,11,11-tetramethyldodecane (2.4%), tetramethylbutane dinitrile (1.91%), 2,2-dimethyldecane (0.28%), benzene α-hydroxyethyl acetate (1.33%), tert-nonyl mercaptan (2.62%), and 3-ethyl-6-trimethylsilyloxyoctane (1.93%).

Twenty-seven chemical constituents were characterized in the ethanolic extract of **G2** amounting 36.67%. Monosaccharide (7.11%) and disaccharide (10.55%) were present in maximum amount. There were three aromatic compounds (7.71%) and four alkanes with high percentage (6.68%) along with two each of sesquiterpene (2.75%) and monoamine alkaloid (0.44%). Various constituents, viz.,

Table 1: Chemical composition of alcoholic extract of young and mature leaves of Kofat, Gaifi, and Gahasha species.

S. no	Retention time	Chemical constituents	% area Kofat		% area Gaifi		% area Gahasha	
			Young (**N1**)	Mature (**G1**)	Young (**N2**)	Mature (**G2**)	Young (**N3**)	Mature (**G3**)
1	3.52	2,2′-Azo bis [2-methyl-propane nitrile]	2.0	—	—	1.43	1.88	2.0
2	3.56	Tetramethylbutanedinitrile	—	1.62	1.91	—	—	—
3	3.6	2,6,10-Trimethyldodecane	—	1.04	0.98	0.66	1.14	0.95
4	3.83	2,2-Dimethyldecane	—	2.75	0.28	—	0.36	0.22
5	3.83	2,2-Dimethyltetradecane	2.48	—	—	1.77	—	—
6	3.84	2,2,11,11-Tetramethyldodecane	—	—	2.4	—	—	—
7	3.9	2,2,6-Trimethyldecane	1.86	2.11	1.83	1.42	5.34	4.56
8	4.0	4-Methyldodecane	3.12	—			3.86	—
9	4.02	3-Methyl-5-propylnonane	—	3.99	3	2.32	—	3.6
10	4.1	3,6-Dimethylundecane	—	—	—	—	—	1.41
11	4.11	2,6,7-Trimethyldecane	—	1.74	—	—	—	—
12	4.15	1-Ethenyl-3-ethyl-benzene	4.97	3.63	3.24	2.52	3.86	—
13	4.16	1-Methyl-2-phenylcyclopropane	—	4.28	—	—	—	—
14	4.22	1-Methyl-1-p-tolyethyl butyrate	—	—	—	—	—	3.77
15	4.23	1-Ethenyl-4-ethyl-benzene	3.94	—	3.76	—	4.71	—
16	4.24	1-Phenyl 1-butene	—	—	—	2.95	—	—
17	4.33	5-Methylundecane	1.54	—	1.46	1.13	1.93	—
18	4.34	2,6-Dimethyldecane	—	1.61	—	—	—	—
19	4.36	2,7,10-Trimethyldodecane	—	1.38	2.67	2.09	2.03	—
20	4.37	4,6-Dimethyldodecane	1.23	—	—	—	1.5	1.08
21	4.42	1,3-Diethenyl-benzene	2.92	3.01	2.76	2.22	3.21	6.01
22	4.9	1-Phenyl-1,2-propanedione	1.51	—	—	—	0.72	0.5
23	5.5	1-Phenylpropanol	2.61	—	—	—	—	—
24	5.5	Benzene α-hydroxyethyl acetate	—	—	1.33	—	—	—
25	5.51	α-Hydroxyethyl benzene acetate	—	—	—	—	1.91	—
26	6.6	Cathinone	0.22	—	0.26	0.09	0.16	0.16
27	7.0	Cathine	0.77	—	0.55	0.35	0.67	0.05
28	7.1	3-Methoxyamphetamine	0.29	—	—	—	—	—
29	7.3	p-α-Dimethyl phenyl ethyl amine	—	—	—	—	—	1.9
30	7.6	1-Dodecanol	3.09	2.69	2.54	2.44	2.89	2.09
31	8.4	Methyl β-D-galactopyranoside	—	1.81	—	—	—	—
32	8.5	1-Hydroxy-1-phenyl-2-(acetylamino) propane	1.02	—	—	—	—	—
33	8.5	N-Crotonyl-N-(4-methoxyphenyl)-aminomalonic acid, diethyl ether	—	—	—	1.44	—	—
34	8.57	Methyl α-D-glucopyranoside	2.06	—	—	—	—	—
35	8.57	Octyl-β-D-glucopyranoside	—	—	—	—	—	1.91
36	8.57	Capric acid (decanoic acid)	—	—	1.58	1.71	—	—
37	8.6	Heneicosane	1.09		2.89	—	—	—
38	8.63	Methyl 6-O-[1-methylpropyl]-β–D-galactopyranoside	—	—	—	3.74	—	—
39	9.3	Tetradecanyl acrylate	1.25	1.05	—	1.31	1.02	—
40	9.4	2,6,10,14-Tetramethylhexadecane	—	1.55	—	1.13	—	—
41	9.7	4-Methyl mannitol	8.73	3.27	6.37	7.11	6.52	6.16
42	10.39	Neophytadiene			2.54	1.26	—	1.18
43	10.5	3,7,11,15-Tetramethyl 2-hexadecen-1-ol	1.33	0.19	1.92	3.35	—	2.27
44	10.7	Isobutyl tetradec-3-enyl fumatate	2.51	—	—	—	—	—
45	10.8	Galactitol	4.62	—	3.98	10.55	—	—
46	10.84	Tert-nonyl mercaptan	—	—	2.62	—	—	—
47	10.86	Dibutyl phthalate	—	1.05	—	—	—	—
48	10.92	3-Ethyl-6-trimethylsilyloxyoctane	—	—	1.93	—	—	—
49	11.12	Palmitic acid (n-hexadecanoic acid)	—	3.06	—	—	—	2.95
50	11.21	Ascorbic acid 2,6-dihexadecanoate	—	—	1.42	2.69	—	—
51	12.2	Phytol	1.1	—	—	—	—	—
52	12.52	Stearic acid	—	—	—	—	—	6.58
53	14.62	Phthalic acid (1,2-benzenedicarboxylic acid)	—	1.8	—	—	—	—
54	16.0	(Z)-13-Docosenamide	3.59	6.92	—	—	4.99	15.7
55	16.08	9-Octadecenamide	—	—	3.95	5.54	—	—
56	16.93	Pentatriacontane	—	—	—	—	1.07	—

Table 1: Continued.

S. no	Retention time	Chemical constituents	% area Kofat		% area Gaifi		% area Gahasha	
			Young (**N1**)	Mature (**G1**)	Young (**N2**)	Mature (**G2**)	Young (**N3**)	Mature (**G3**)
57	16.95	Heptacosyl heptafluorobutyrate	—	1.46	—	—	—	—
58	16.96	1-Triacontanol	—	—	—	1.38	—	—
59	22.3	β-Sitosterol	3.71	1.97	4.03	3.61	2.8	2.07
60	24.04	Lupeol	—	—	—	1.23	—	—
		Total detected compounds (%)	63.56	53.98	62.17	36.67	52.57	67.12

Table 2: Cytotoxic activity of the six extracts against three cell lines and one normal fibroblast (MTT 72 h $IC_{50} \pm SD$ μg/mL).

Ethanolic extract of khat	IC_{50}			
	MCF7	A2780	HT29	MRC5
N1	29.91 ± 0.03	53.78 ± 7.45	56.58 ± 4.44	30.63 ± 1.59
G1	29.23 ± 0.48	20.97 ± 5.03	59.33 ± 0.74	15.29 ± 2.51
N2	29.34 ± 0.53	52.12 ± 4.41	56.63 ± 6.99	22.94 ± 1.20
G2	51.11 ± 9.05	36.83 ± 2.89	59.41 ± 0.38	36.22 ± 6.31
N3	22.65 ± 3.51	23.74 ± 7.35	51.22 ± 0.28	6.15 ± 0.33
G3	54.90 ± 4.08	38.47 ± 0.19	39.22 ± 0.10	41.48 ± 1.25

alkane nitrile (1.43%), alkyl ester (1.31%), acyclic norditerpene (1.13%), alkyl alcohol (1.38%), and pentacyclic triterpene (1.23%) were detected individually. The main phytoconstituents of **G2** were galactitol (10.55%), 4-methyl mannitol (7.11%), 9-octadecenamide (5.45%), methyl 6-O-[1-methylpropyl]-β–D-galactopyranoside (3.74%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (3.35%), ascorbic acid 2,6-dihexadecanoate (2.69%), 3-methyl-5-propylnonane (2.32%), 2,7,10-trimethyldodecane (2.09%), 1-ethenyl-3-ethyl-benzene (2.52%), 1-phenyl 1-butene (2.95%), 1,3-diethenyl benzene (2.22%), and 1-dodecanol (2.44%). In addition, 2,2′-azobis [2-methyl] propane nitrile (1.43%), 2,2-dimethyltetradecane (1.77%), N-crotonyl-N-(4-methoxyphenyl)-aminomalonic acid, diethyl ether (1.44%), tetradecanyl acrylate (1.31%), 2,6,10,14-tetramethyl pentadecane (1.13%), 1-triacontanol (1.38%), and lupeol (1.23%) were also detected in the **G2** sample.

The chemical constituents characterized in both the species were 2,6,10-trimethyldodecane, 2,2,6-trimethyldecane, 3-methyl-5-propylnonane, 2,7,10-trimethyldodecane, 1-ethenyl-3-ethyl-benzene, 5-methylundecane, 1,3-diethenyl benzene, cathinone, cathine, 1-dodecanol, capric acid (decanoic acid), 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 4-methyl mannitol, neophytadiene, ascorbic acid 2,6-dihexadecanoate, 9-octadecenamide, and β-sitosterol, and their amounts varied from 10.55% to 0.09%.

The chemical compositions of **N3** consisted of a variety of 21 chemical constituents (52.57%) including six alkanes (12.56%), three each of aromatic compounds (11.78%), two each of sesquiterpenes (3.17%) and monoamino alkaloid (0.83%). Alkane nitrile (1.88%), phenyl ketone (0.72%), aromatic ester (1.91%), aliphatic alcohol (2.89%), alkyl ester (1.02%), monosaccharide (6.52%), alkyl amide (4.99%), and phytosterol (2.8%) were present individually in young leaves. The predominant constituent detected in the sample was 2,2,6-trimethyldecane (5.34%), 4-methyldodecane (3.86%), 1-ethenyl-3-ethyl benzene (3.86%), 1-ethenyl-4-ethyl benzene (4.71%), 1,3-diethenyl benzene (3.21%), 4-methyl mannitol (6.52%), and (Z)-13-docosenamide (4.99%). The other chemical constituents present in the **N3** sample included 2,2′-azobis [2-methyl]-propane nitrile (1.88%), 2,6,10-trimethyldodecane (1.44%), 2,2-dimethyldecane (0.36%), 5-methylundecane (1.93%), 2,7,10-trimethyldodecane (2.03%), 4,6-dimethyldodecane (1.5%), 1-phenyl 1,2-propanedione (0.72%), cathinone (0.16%), cathine (0.67%), 1-dodecanol (2.89%), tetradecanyl acrylate (1.02%), pentatriacontane (1.07%), and β-sitosterol (2.8%).

Twenty-two chemical constituents were characterized in the ethanolic extract of mature Gahasha (**G3**) amounting 67.12%. There were five alkanes present in maximum percentage (10.87%) along with two alkyl amides (17.6%), monoamino alkaloid (0.22%), and fatty acid (9.53%). Alkane nitrile (2%), sesquiterpene (0.95%), aromatic ester (3.77%), aromatic compound (6.01%), phenyl ketone (0.5%), alkyl amide (1.9%), aliphatic alcohol (2.89%), glucoside (1.91%), monosaccharide (6.16%), diterpene (1.18%), diterpenol (2.27%), and phytosterol (2.07%) were detected individually. The main phytoconstituents of leaves were (Z)-13-docosenamide (15.7%), 2,2,6-trimethyldecane (4.56%), 3-methyl-5-propylnonane (3.6%), 1-methyl-1-p-tolyethyl butyrate (3.77%), 1,3-diethenyl benzene (6.01%), 4-methyl mannitol (6.16%), and stearic acid (6.58%). In addition, 2,2′-azobis [2-methyl]-propane nitrile (2%), 2,6,10-trimethyldodecane (0.95%), 2,2-dimethyldecane (0.22%), 3,6-dimethylundecane (1.41%), 4,6-dimethyldodecane (1.08%), 1-phenyl 1,2-propanedione (0.5%), cathinone (0.17%), cathine (0,05%), p-α-dimethyl phenyl ethyl amine (1.9%), 1-dodecanol (2.89%), octyl-β-D-glucopyranoside (1.91%), neophytadiene (1.18%), 3,7,11,15-tetramethyl 2-hexadecen-1-ol (2.27%), palmitic acid (2.95%), and β-sitosterol (2.07%) were also detected in the sample. The chemical constituents characterized in both the species were 2,6,10-trimethyldodecane,

2,2-dimethyldecane, 2,2,6-trimethyldecane, 2,2′-azobis [2-methyl]-propane nitrile, 4,6-dimethyldodecane, 1,3-diethenyl benzene, 1-phenyl 1,2-propanedione, 1-dodecanol, 4-methyl mannitol, (Z)-13-docosenamide, and β-sitosterol, and their amounts varied from 15.7% to 0.05%. Two euphorigenic and psychostimulant monoamine alkaloid cathine (0.67%) were found in young leaves and in minor traces in mature leaves; cathinone was present in both types of leaves in almost the same concentrations.

Chemometric multivariate analysis using hierarchical cluster analysis (HCA) was performed using the nearest and furthest neighbor cluster methods to analyze and classify the six samples of extracts based on the 60 different components detected in these samples.

Based on GC-MS results and the hierarchical cluster analysis in the table of the agglomeration schedule (Table 3) for cluster solution, there is a sudden jump/gap in the distance coefficient. The solution before the gap indicated a good solution. Accordingly, we could determine two major clusters of samples based on the furthest neighbor clustering method. The first cluster consists of **N1**, **N3**, **N2**, and **G2**, and the second cluster consists of **G1** and **G3**. In the first cluster, **N1** and **N3** combined and showed similar characteristics and therefore can be included in the same subcluster leaving **N2** and **G2** forming the second subcluster in this first group. The hierarchical cluster analysis results are shown in Table 3 and Figure 2.

In previous cytotoxicity studies [20,21], the methanolic extract of *Catha edulis* showed cytotoxicity on MCF 7 and HL60 cells with IC_{50} 33–200 μg/ml, respectively. However, the effect of those extracts was not tested in normal cells to evaluate the possible adverse effect of chewed khat on the health of normal living cells. Thus, khat extracts in this study showed comparable cytotoxicity on the cancer cells, but more interestingly, they were more cytotoxic against the normal cells, which could be associated with the different mouth cavity lesions.

4. Discussion

Out of total 26, 26, and 20 chemical constituents present in ethanolic extracts of young leaves, 6, 4, and 2 different chemical entities were found in **N1**, **N2**, and **N3**, respectively. These chemical compounds were not found in other extracts and were unique for them. **N1** contains six unique compounds: 1-phenyl propanol, cathine, 1-hydroxy-1-phenyl-2-(acetylamino) propane, methyl α-D-glucopyranoside, isobutyl tetradec-3-enyl fumarate, and phytol. **N1** has almost equipotent cytotoxic activity on both normal MRCS (30.63) and human breast adenocarcinoma, MCF7 (29.91), and observed the least activity on HT29. It has no advantage over cancerous cells. **N2** has four unique compounds: 2,11,11-tetramethyldodecane, benzene α-hydroxyethyl acetate, tert-nonyl mercaptan, and 3-ethyl-6-trimethylsilyloxyoctane. It is more cytotoxic to normal cells than MCF7. **N3** has α-hydroxy ethyl benzene acetate and pentatriacontane. It was cytotoxic compared to all other extracts, and was found that its activity is more on normal cells. **N1** was the least cytotoxic among the three, and **N3** is the most toxic among all extracts.

Similarly, we found some rare compounds in the extracts of mature leaves. **G1**, **G2**, and **G3** have 7, 5, and 4 of such uncommon constituents in them, respectively. **G1** contains seven such unique compounds: 2,6,7-trimethyldecane, 1-methyl-2-phenylcyclopropane, 2,6-dimethyldecane, methyl β-D-galactopyranoside, dibutyl phthalate, phthalic acid (1,2-benzenedicarboxylic acid), and heptacosyl heptafluorobutyrate. It is more cytotoxic to normal cells than other cancerous cells. **G2** has five unique compounds: 1-phenyl 1-butene, N-crotonyl-N-(4-methoxyphenyl)-aminomalonic acid diethyl ether, methyl 6-O-[1-methylpropyl]-β-D-galactopyranoside, 1-triacontanol, and lupeol. It has almost similar cytotoxic activity on both normal and A2780 cells but observed the least effect on HT29 cells. **G3** contains four rare compounds: 3,6-dimethylundecane, 1-methyl-1-p-tolyethyl butyrate, p-α-dimethyl phenylethylamine, and stearic acid. It also has almost similar cytotoxic activity in both normal and cancerous cells, but the minimum activity was observed in HT29 cells.

No compounds were common between **N1** and **G1** or **N3** and **G3** but two common chemical constituents in **N2** and **G2** extracts were observed: 1-hydroxy-1-phenyl-2-(acetylamino) propane and 9-octadecenamide. Similarly, sixteen unique compounds were present in **N1**, and none were found in **G1**; thirteen individual compounds were found in **G1**, but none observed in **N1**. **N2** has six unique compounds, but the same was not found in **G2**; **G2** has nine other distinct compounds that were absent in **N2**. **N3** has eight unique compounds, but they were not found in **G3**; however, **G3** has ten other compounds that were absent in **N3**. The extracts of mature and young leaves have an almost different set of chemical constituents. It could be the reason why there is a difference in their cytotoxicity. Further study is required to identify the exact role of each of these chemical constituents found in these extracts.

In terms of the presence of cathinone and cathine, we found that the maximum content of cathinone in **N1** (0.22) and **N2** (0.26) and **N3** is only 0.16. Cathinone was not found in mature leaves **G1** but was found in **G2** and **G3** (0.09 and 0.16, respectively). Similarly, the presence of cathine was less in mature leaves **G2** (0.35) and **G3** (0.05) but was not found in mature **G1** compared to young leaves **N1**. The maximum cathine was found in **N1** (0.77). Methoxyamphetamine (0.29) was found only in the **N1** extract. It could be the reason for the variation in psychostimulant activities in young and mature leaves.

Isolating and characterizing the different chemical constituents in the ethanolic extract of both mature and young leaves of *Catha edulis* and matching its cytotoxic activity is the strength of this study; however, identifying the biological activities of each constituent found in these extracts requires further study.

Table 3: Agglomeration schedule.

Stage	Cluster combined		Coefficients	Stage cluster first appears		Next stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	1	5	77.860	0	0	4
2	3	4	91.668	0	0	4
3	2	6	124.046	0	0	5
4	1	3	126.395	1	2	5
5	1	2	167.067	4	3	0

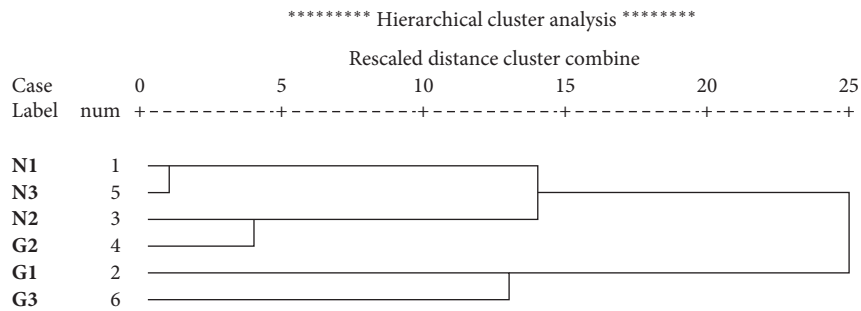


Figure 2: Dendrograms using complete linkage illustrating the average linkage between groups of complexes (hierarchical cluster analysis and rescaled distance cluster combine) for compounds detected by GC-MS in the six khat leaves extracts under study.

5. Conclusions

GC-MS investigations revealed and identified several remarkable phytochemicals with significant variations among them in the young and mature ethanolic extracts of the three khat cultivars. The study confirmed the presence of psychoactive cathine and cathinone in high quantities in the young leaves compared to matured leaves. Ethanolic extracts of khat showed significant cytotoxicity, IC_{50} ranging from 22–59 μg/mL on the cancer cells, compared to previous claims ($IC_{50:}$ ranging from 33–200 μg/mL); however, these extracts were also exhibiting cytotoxicity against the normal cells (MRC5 IC_{50}: 6–41 μg/mL). Hence, the substantial cytotoxic effect on normal cells may pose many hazards to the health of khat consumers. Therefore, awareness campaigns on stopping the usage of khat should be implemented and facilitated in affected societies.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All the authors declare that there are no conflicts of interest.

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