



Chemical Composition and Antimicrobial Activity of Volatile Constituents of Cladodes, Fruits peel and Fruits pulp from *Opuntia ficus indica* (L.) Mill. (Prickly Pear) growing in Egypt



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Abstract

Volatile constituents of *Opuntia ficus indica* (L.) Mill (prickly pear or nopal cactus) cladodes, fruits peel and fruits pulp were prepared by hydro- distillation. The chemical composition was investigated by GC/MS analysis. Dodecane found as the major compound in the volatile constituents of fruits peel and cladodes representing (32.3%) and (20.05%) respectively followed by undecane (20.02%) in cladodes, (14.47%) in fruits peel while the fruits pulp volatile constituents contain undecane as a major compound (20.52%) followed by dodecane (16.86%). The antimicrobial activity was carried out for all samples using diffusion agar method against *Aspergillus flavus*, *Candida albicans*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhimurium*. Fruits peel volatile constituents showed antimicrobial activity against all tested microorganisms except *Candida albicans* while fruits pulp volatile constituents showed activity against *Staphylococcus aureus* and gram-negative bacteria and had no activity against fungi and *Bacillus subtilis*. Cladodes volatile constituents had no activity against all tested microorganisms.

Keywords: *Opuntia ficus indica*, Prickly pear, Nopal cactus, Volatile constituents, Dodecane, Undecane, Antimicrobial activity.

Introduction

Opuntia ficus-indica (L.) Mill. commonly called prickly pear or nopal cactus belongs to Cactaceae family, which includes about 1500 species of cactus. *Opuntia* is a large genus of succulent shrubs, native to the new world and now widely grown in the warmer parts of the world due to their unique appearance attractive flowers and delicious fruits [1]. Nopal cactus used in health, cosmetics and nutrition in forms of tea, jam, juice, and oil extracted from prickly pear seeds. Cactus fruits was given as anti-ulcerative and antidiarrheal agents, flowers used as oral anti-hemorrhoid and cladodes syrup as a treatment for whooping cough. On the other hand,

fresh and dry fruits consumed in large amounts as food. *Opuntia ficus indica* was known for its high content in polyphenols exhibiting antioxidant and anti-inflammatory properties [1, 2]. Interestingly, alkaloids, indicaxanthin, neobetanin, and various flavonoids have been isolated from cactus [3], along with polysaccharides, which are abundant in cladodes extracts and have antidiabetic activity [4]. The *Opuntia ficus indica* cladodes contain vitamins and various flavonoids, particularly quercetin 3-methyl ether, a highly efficient radical scavenger [5, 6]. Cladodes extract of *Opuntia ficus-indica* lowers cholesterol level, had antiulcer and anti-inflammatory activity, and the water extract improves wound

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healing [7, 8]. Cactus fruits contain ascorbic acid, vitamin E, carotenoids, fibers, amino acids and antioxidant compounds (phenols, flavonoids, betaxanthin and betacyanin) which responsible for its health benefits such as hypoglycemic and hypolipidemic action, and antioxidant properties [9–11]. In this respect, the fruits of *Opuntia ficus indica* is a valuable source of nutrients [12] as well as anti ulcerogenic [13, 14], antioxidant [5, 13–16], anticancer [16], neuroprotective [17], hepatoprotective [18], and anti-proliferative compounds [19]. Also *Opuntia ficus indica* byproducts have great health and economic value as it contains vitamins, minerals, flavonoids and fatty acids and have different biological activity such as antifungal and antioxidant activity [20]. A few studies have focused on composition of volatile constituents of different cactus pear parts and their by-products. The aim of this study was to characterize *Opuntia ficus indica* volatile constituents of fruits pulp, peel and cladodes.

Experimental:

Plant material:

Voucher specimens deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt (voucher no. 24.12.17.8). Fresh fruits and cladodes of *Opuntia ficus indica* were collected from Orman Botanical garden, Giza, Egypt at July 2016. The plant was authenticated by Eng. Therese Labib Yousef, National Gene Bank and Orman Botanical Garden Consultant.

Hydro-distillation:

1.2 kilogram of fresh fruits peel, 1.1 kilogram of fresh cladodes and 400 gram of fresh fruits of *Opuntia ficus indica* were hydro-distilled to obtain volatile constituents by using Clevenger-type apparatus for six hours then extracted with hexane, filter over anhydrous sodium sulphate and kept in refrigerator.

GC/MS analysis and identification:

Analysis was carried out using Shimadzu GCMS-QP2010 (Tokyo, Japan) equipped with Rtx-5MS fused bonded column (30 m x 0.25 mm ID x 0.25 μm film thickness) (Restek, USA) equipped with a split-splitless injector. The capillary column was directly coupled to a quadrupole mass spectrometer (SSQ 7000, Thermo- finnigan, Bremen, Germany). The initial column temperature was kept at 45 °C for 2 min (isothermal), programmed to 300°C at a rate of 50 °C / min, and kept constant at 300°C for 5 min (isothermal). The injector temperature was 250 °C. Carrier gas: He, the flow rate was 1.41 ml/min. All

the mass spectra were recorded applying the following condition: (equipment current) filament emission current, 60 mA; acquisition mass range; 500-35 amu; ionization voltage, 70 eV; anion source, 200 °C. Diluted samples (1% v/v) were injected with split mode (split ratio 1:15). Retention indices (RI) of the sample components were determined based on homologous n-alkane hydrocarbons under the same conditions. The quantitative composition was obtained by peak area normalization, and the response factor for each component was considered to equal 1. Compounds were identified by using library search of National Institute of Standards and Technology (NIST) database [21] as well as by comparing their mass spectral fragmentation pattern with those previously reported [22].

Microorganisms used for antimicrobial activity evaluation:

Aspergillus flavus RCMB002002, *Candida albicans* RCMB 005003(1) ATCC10231, *Staphylococcus aureus* RCMB 010010, *Bacillus subtilis* RCMB 015(1) NRRL B-543, *Escherichia coli* RCMB 010052 ATCC 25955 and *Salmonella typhimurium* CMB 006(1) ATCC 14028 were obtained from Regional center for Mycology and Biotechnology (RCMB). The susceptibility tests were performed according to NCCLS recommendations (National Committee for Clinical Laboratory Standards, 1993). Screening tests regarding the inhibition zone were carried out by well diffusion method [23].

Results and discussion:

Volatile constituent composition

Opuntia ficus indica fruits pulp, cladodes and fruits peel yielded pale yellow, colourless and dark yellow volatile constituents with yield (0.003%), (0.001%) and (0.001%) respectively. The volatile constituent chemical composition was investigated using GC/MS technique (table 1.)

Opuntia ficus indica fruits pulp volatile constituents

The GC/MS analysis of volatile constituents showed 76 compounds representing (93.74%) of the total volatile constituents consisting of (81.25%) hydrocarbons and (12.49%) oxygenated compounds. The major compounds of this volatile constituents are undecane (20.52%), dodecane (16.86%), 2-methyl undecane (3.96%), pentyl cyclohexane (2.98%), 2-Butyloctanol (2.78%), 2-Tridecen-1-ol(2.42%), 2-Hexyl-1-octanol(1.51%) and decanal (0.9%), this results differ from the previous results, which reported that the major isolated compounds are hexadecanoic acid (33%), limonene (5.5%), 1-

Tetradecene (4.6%), 2-Dodecenolactone (4.6%) and squalene (5.3%) [24], 2-Hexen-1-ol (15.99%), 2-Nonen-1-ol (13.98%), 4-Decenoic acid, methyl ester (9.25%), butanoic acid, 2-methyl-, methyl ester (9.1) and 2-Hexen-1-ol, acetate (8.1%)[25], 2-Hexen-1-ol (58%), hexan-1-ol (25.2%) and 2,6- Nonadiene -1-ol [26] and 2-hexanal (44%), hexanol (18.7%) and 3-hexenil acetate (11.1%) and hexanal (2.3%) [27]. There were remarkable differences between our results and the literature, this could be contributed to difference in soil, environmental conditions and to the different extraction technique used, only [24] use hydro distillation and [25, 26, 27] use SPME technique instead.

***Opuntia ficus indica* fruits peel volatile constituent**

Chromatographic analysis showed 72 compounds, representing (98.5%) of the total volatile constituents consisting of (90.56%) hydrocarbons and (7.94%) oxygenated compounds. The major compounds of this volatile constituents are dodecane (32.3%), undecane (14.47%), 3, 6-dimethyl undecane (4.9%), 2-methyl undecane (4.62%) and 3-methyl undecane (2.38%), cyclo dodecyl methanol (1.82%), Dodeca-1,6-12-ol, 6,10- dimethyl (1.2%), nopinone (0.72%), Cyclohexanone -2,2-dimethyl-5-(3-methyloxiranyl) (0.49%), 2-isopropyl-5-methyl-1-hexanol (0.25%) and 3-Cyclohexyl propyl alcohol (0.11%). By reviewing the literature, we found that the result differ from our results in which

hexahydrofarnesylacetone constitutes (19.1%) followed by dodecalacton (6.7%), farnesene (5.7%), 2-dodecenolacton (5.6%) and limonene (4.6%) [24].

***Opuntia ficus indica* cladodes volatile constituent**

The GC/MS analysis of the volatile constituents of *Opuntia ficus indica* cladodes showed 65 compounds representing (91.39%) of total volatile constituents, consisting of (82.65%) hydrocarbons and (8.74%) oxygenated compounds. The major identified compounds were dodecane (20.05%), undecane (20.02%), 2-methyl undecane (4.28%), pentyl cyclohexane (3.16%), 2-butyloctyl alcohol (3.0%), 3, 6-dimethyl undecane (2.59%), P-menthane (2.54%) and 2-dodecenol (2.32%). There are no previous studies concerned with the volatile constituents of *Opuntia ficus indica* cladodes.

The plant volatile constituents have beneficial properties such as antioxidant and antimicrobial activities [28]. Therefore, the in vitro antimicrobial activity of volatile constituents of different parts of *Opuntia ficus indica* was tested against two gram-positive bacteria, two gram-negative bacteria and two fungi using agar diffusion technique [23], the minimum inhibitory concentration (MIC) for each volatile constituents against each microorganism was calculated and presented in (Table.2), (figure.1).

Table 1. The chemical composition of *Opuntia ficus indica* volatile constituents of fruits pulp, peel and cladodes.

No	Compound	R.I	Area %		
			Fruits pulp	Fruits peel	cladodes
1	2- Methyl nonane	958	0.08	-	0.03
2	3- Methyl nonane	965	0.06	-	-
3	1- isopropyl -3- methylcyclohexane	978	0.08	-	-
4	P-Menthane	981	0.36	-	2.54
5	Decane	998	2.58	0.35	1.68
6	5-methyldecane	1012	0.08	0.27	0.84
7	2-isopropyl-5-methylcyclohexanol	1013	0.07	-	-
8	2,2-dimethylnonane	1017	0.18	-	-
9	4-methyldecane	1020	1.57	0.46	1.22
10	(1-methylbutyl)cyclohexane	1022	0.07	0.41	-
11	3,7-dimethyldecane	1026	0.52	0.57	-
12	1-cyclohexylbutane	1030	0.5	-	0.29
13	1-isopropyl-1-methylcyclohexane	1035	2.19	0.64	-
14	2-ethylnonane	1036	0.33	-	1.5
15	1-methyl-1-ethylcyclohexane	1038	0.13	-	-
16	1-methyl-3-propylcyclohexane	1038	-	-	1.39
17	1,1-dimethyl-2-propylcyclohexane	1040	-	-	0.04
18	2-ethyl-1,1,3-trimethylcyclohexane	1041	0.09	-	-
19	1-ethyl-2-propylcyclohexane	1043	2.25	0.06	1.88
20	1-isobutyl-2,5-dimethylcyclohexane	1046	0.22	-	-
21	2,5-dimethylcyclohexane	1046	-	-	0.13

22	3,7,11-trimethyl-1-dodecanol	1050	0.43	-	0.32
23	5,6-dimethylundecane	1055	0.89	-	-
24	2-methyldecane	1062	2.33	0.99	2.44
25	3-methyl decane	1068	-	0.57	-
26	3,4-dimethylundecane	1069	1.44	-	-
27	2,3,3 tri methyl octane	1071	-	0.05	-
28	7-methyl-1-undecene	1072	-	-	0.24
29	Ethylcyclooctane	1076	-	-	0.22
30	1-ethyl-1,4-dimethylcyclohexane	1076	0.28	0.28	-
31	(2-methylbutyl)cyclohexane	1092	0.28	0.41	-
32	Undecane	1100	20.52	14.47	20.02
33	2-isopropyl-5-methyl-1-hexanol	1101	-	0.25	-
34	2-isopropyl-5-methyl-1-hexanol	1104	0.47	-	0.77
35	2,3,6,7-tetramethyloctane	1106	0.32	0.13	-
36	2-butyl octanol	1116	2.78	1.65	3.0
37	2-hexyldecanol	1119	1.89	1.38	0.45
38	2,6,11-trimethyldodecane	1122	0.93	-	-
39	2,3,5,8-tetra methyl decane	1125	-	1.47	-
40	2-tridecen-1-ol	1126	2.42	-	-
41	2-dodecenol	1126	-	-	2.32
42	2-methyl-1-octanol	1128	0.41	0.21	-
43	Pentylcyclohexane	1133	2.98	1.93	3.16
44	Hexylcyclopentane	1137	1.16	0.73	1.17
45	1-ethyl-2-propylcyclohexane	1140	-	0.22	-
46	1-methyl-3-pentylcyclohexane	1146	-	0.56	-
47	4-ethyl decane	1148	-	0.47	0.74
48	2-hexyl-1-octanol	1148	1.51	-	0.9
49	6-methylundecane	1153	2.05	1.77	2.07
50	5-methylundecane	1156	1.5	1.45	1.68
51	4-methylundecane	1158	2.32	1.97	2.37
52	2-methylundecane	1162	3.96	4.62	4.28
53	Dodecanal	1164	0.9	-	0.9
54	3-methylundecane	1169	2.04	2.38	2.15
55	2,5-dimethylundecane	1174	0.36	0.41	0.42
56	4-pentenyl cyclohexane	1175	-	0.45	-
57	Dodeca-1,6-12-ol, 6,10- dimethyl	1179	-	1.2	-
58	1-methy-1-cycloundecene	1180	1.06	-	-
59	1-methyl-4-(1-methylbutyl cyclohexane)	1188	-	1.33	-
60	Nonadecene	1191	0.22	-	-
61	1-methyl-2-pentylcyclohexane	1195	0.58	0.84	-
62	Dodecane	1200	16.86	32.3	20.05
63	Nopinone	1204	-	0.72	-
64	3,5,5-trimethyl-5-vinylcyclohexanone	1205	0.4	-	-
65	2,3,7-trimethyldecane	1206	0.2	0.32	0.19
66	2,4-dimethyldodecane	1208	0.27	-	-
67	6-methyldodecane	1212	2.56	-	-
68	3,6- dimethylundecane	1212	-	4.9	2.59
69	(4-methylpentyl)cyclohexane	1213	0.53	-	0.59
70	5- methyl-2- undecane	1218	-	0.29	-
71	3,7- dimethylundecane	1220	-	-	0.28
72	Cyclohexanone -2,2-dimethyl-5-(3-methyloxiranyl)	1221	-	0.49	-
73	Cyclododecylmethanol	1222	1.21	1.82	-
74	Dodecane , 1- ethenyloxy	1225	-	0.11	-
75	3,8- dimethylundecane	1227	-	-	0.04
76	(4- methylpentyl) cyclohexane	1238	-	1.15	-
78	1-butyl-2-propylcyclopentane	1241	-	0.46	-

79	Pentylcyclopentane	1241	0.2	-	0.22
80	3-Cyclohexylpropyl alcohol	1243	0.07	0.11	0.08
81	5-methyltetradecane	1247	0.05	-	-
82	4- ethyl undecane	1247	-	0.08	0.06
83	6-methyl dodecane	1251	-	0.37	0.22
84	2,4-dimethylundecane	1253	0.14	1.46	0.81
85	4-methyldodecane	1258	0.13	0.81	0.33
86	2-methyldodecane	1263	0.35	0.57	0.29
87	6,9 dimethyl tetradecane	1269	-	0.13	0.06
88	2,7,10-trimethyldodecane	1272	0.06	0.15	0.07
89	Tridecane	1299	0.18	0.42	0.19
90	2- methyl tridecane	1361	-	0.07	0.04
91	2,6,10-trimethyldodecane	1374	0.04	0.04	0.04
92	Tetradecane	1396	0.4	0.98	0.45
93	2- methyl tetradecane	1461	-	0.28	-
94	Pentadecane	1499	0.41	1.03	0.46
95	2- methyl pentadecane	1560	-	0.08	0.04
96	Hexadecane	1596	0.34	0.79	0.79
97	2-methylhexadecane	1660	0.03	0.05	0.03
98	Heptadecane	1697	0.59	0.71	0.36
99	2,6,10-trimethylhexadecane	1703	0.1	-	0.2
100	2,6,10,14- tetramethyl hexadecane	1703	-	0.22	-
101	Octadecane	1795	-	0.56	0.32
102	Nonadecane	1896	0.26	0.47	0.29
103	Eicosane	1993	0.22	0.36	0.24
104	Henicosane	2099	0.19	0.33	0.21
105	Docosane	2201	0.16	0.24	0.18
106	Tricosane	2298	0.14	0.18	0.16
107	Tetracosane	2392	0.1	0.14	0.21
108	Pentacosane	2497	0.07	0.11	-
109	Hexacosane	2599	0.05	-	0.05
110	Heptacosane	2696	0.04	-	0.04

R.I: Retention index

Table 2. MIC (ug/ml) of *Opuntia ficus indica* volatile constituents of fruits pulp, peel and cladodes against different types of microorganisms.

Microorganism	Reference std		Samples		
	Gentamycin	Ketoconazole	Fruits pulp	Fruits peel	cladodes
<i>Aspergillus flavus</i> (002002)	-	1	NA	5000	NA
<i>Candida albicans</i> (1) ATCC10231	-	2	NA	NA	NA
<i>Staphylococcus aureus</i> (010010)	1	-	5000	5000	NA
<i>Bacillus subtilis</i> (1)NRRL B-543	2	-	NA	2500	NA
<i>Escherichia coli</i> (010052) ATCC 25955	2	-	2500	2500	NA
<i>Salmonella typhimurium</i> 006(1) ATCC14028	0.5	-	5000	5000	NA

NA: NO Activity, Ketoconazole: positive control for fungi, Gentamycin: positive control for bacteria

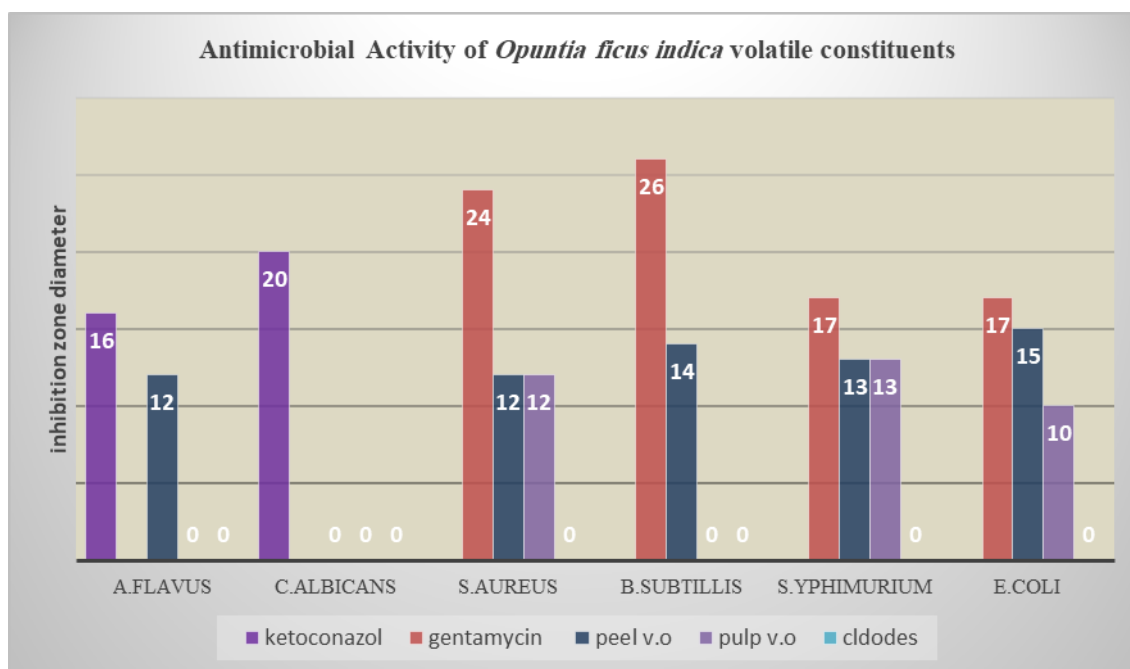


Figure 1 : Antimicrobial activity of *Opuntia ficus indica* volatile constituents

Antimicrobial activity

The antimicrobial activity of fruits pulp volatile constituents shows highest activity on *E.coli* then *Staphylococcus aureus* and *Salmonella typhimurium* and finally it has no activity against *Candida albicans*, *Bacillus subtilis* and *Aspergillus flavus*, while the fruits peel volatile constituents shows the highest activity against *Aspergillus flavus* then *E.coli*, *Salmonella typhimurium* and weak activity against *Staphylococcus aureus* and *Bacillus subtilis* and has no activity against *Candida albicans* and finally cladodes volatile constituents has no activity against all the tested microorganisms.

Conclusion:

The study of the chemical composition of volatile constituents of *Opuntia ficus indica* fruits pulp, fruits peel and cladodes revealed that the fruits pulp volatile constituents has major hydrocarbon fraction of (81.25%) with undecane as the major compound of (20.52%) and oxygenated fraction of (12.49%) with 2-butyloctyl alcohol as the major compound (2.78%), while the fruits peel volatile constituents has major hydrocarbon fraction of (90.56%) with dodecane as the major compound of (32.3%) and oxygenated fraction of

(7.94%) with cyclododecyl methanol as the major compound (1.82%) and the cladodes volatile constituents has major hydrocarbon fraction of (82.65%) with dodecane as the major compound of (20.05%) and oxygenated fraction of (8.74%) with 2-butyloctyl alcohol as the major compound (3.0%).

Finally, from the study we concluded that the volatile constituents of *Opuntia ficus indica* fruits pulp and fruits peel could be used as a natural antimicrobial agent especially in gastrointestinal tract infection and antidiarrheal agents as reported [1, 2].

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التركيب الكيميائي والتأثير المضاد للميكروبات للطيارة المستخلصة من القشور والثمار والسيقان الورقية لنبات الاوبنتيه فيكس انديكا (التين الشوكي) المزروع في مصر

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التين الشوكي ينتمي الي العائلة الشوكية ويتميز بأزهاره وثماره ذات الألوان الجذابة وهو من الفواكه المفيدة جدا والتي تستخدم في الصناعات الدوائية ومستحضرات التجميل , ونظرا لقيمته الغذائية العالية يتم استهلاكه كفاكهة ويتم الاستفادة من الثمار صناعيا في صناعة المربي والعصائر واستخراج الزيوت. تستخدم ازهار نبات التين الشوكي كمادة قابضة وتخفض النزف في حالات البواسير وتستخدم لعلاج الاسهال والتهاب القولون , كما يستخدم عصير السيقان الورقية لعلاج السعال الديكي. ويحتوي النبات علي نسب عالية من المواد الفينولية والفلافونيدات والتي لها تأثير مضاد للاكسدة والالتهاب. ويهدف هذا البحث الي دراسة التركيب الكيميائي والتأثير المضاد للميكروبات للمركبات الطيارة المستخلصة من القشور والثمار والسيقان الورقية لنبات التين الشوكي .

تم تقطير المركبات الطيارة من قشور وثمار وسيقان نبات الاوبنتيه فيكس انديكا والتعرف علي مكوناتها باستخدام التحليل الكروماتوجرافي الغازي لكتلة الطيف . وكشف هذا ان السدوديكان هو المركب الرئيسي في قشور الثمار بنسبة 32.3% والسيقان بنسبة 20.05% , يليه مركب الانديكان بنسبة 20.02% في السيقان و 14.47% في قشور الثمار. بينما كان الانديكان هو المركب الرئيسي في الثمار بنسبة 20.52% يليه دوديكان بنسبة 16.86% . اما عن التأثير المضاد للميكروبات فان المركبات الطيارة للقشور لها فاعلية ضد كل الكائنات الدقيقة التي تم اختبارها ماعدا فطر الكانديدا البيكانس. في حين ان المركبات الطيارة للثمار لها فاعلية ضد كل الكائنات الدقيقة التي تم اختبارها ماعدا الفطريات والباسيلس سباتيليس. اما المركبات الطيارة للسيقان فليس لها أي فاعلية علي الكائنات الدقيقة التي تم اختبارها.

بناءا علي نتائج البحث تظهر قدرة المركبات الطيارة لقشور وثمار التين الشوكي كمواد مضادة للميكروبات خاصة في حالات عدوي المعدة والامعاء .