

Full Length Research Paper

Antimicrobial effect of local medicinal plant extracts in the Kingdom of Saudi Arabia and search for their metabolites by gas chromatography-mass spectrometric (GC-MS) analysis

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The present study was designed to evaluate the anti-microbial properties of leaf extract from some local plants in Saudi Arabia belonging to seven different families which are *Rumex vesicarius* from the family *Polygonaceae*, *Datura stramonium* from the family *Solanaceae*, *Zygophyllum coccineum* from the family *Zygophyllaceae*, *Citrullus colocythus* from the family *Cucurbitaceae*, *Lasiurus scindicus* from the family *Poaceae* and *Heliotropium digynum* from the family *Boraginaceae* against seven human pathogenic bacteria and yeast strains including *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella suis* ATCC 13076, *Shigella sonnei* ATCC 11060, and *Candida albicans* ATCC 10231. The compounds were extracted using two different extraction agent water and acetone. The evaluation of the anti-microbial activities of the extracts was examined against all the aforementioned microorganisms by standard agar well diffusion method. The water extracts showed no antibacterial activities in all the different plant families, but as for the acetone's extracts, the antimicrobial activities were moderate to strong (about 18 to 30 mm). The compounds present in the extracts were identified by gas chromatography-mass spectrometry (GC-MS) analysis. The leaf extracts of the following medicinal plants were mainly found to contain compounds like 4-ethyl-2-hydroxycyclopent-2-en-1-one, 2-methoxy-4-vinylphenol, and cyclobutanol for *R. vesicarius*; hexanoic, nonanoic, octanoic, and heptanoic acid for *D. stramonium*; 1-hexyl-2-nitrocyclohexane, 2-octadecyl-propane-1,3-diol, octadecanal, and cyclohexane for *Z. coccineum*; l-(+)-ascorbic acid 2,6-dihexadecanoate, eicosanoic acid, and 2-heptadecenal for *C. colocythus*; butanoic acid, 6-octen-1-ol, 3,7-dimethyl-propanoate, 2-hydroxy-3,5-dimethylcyclopent-2-en-1-one, and alpha-cadinol for *L. scindicus*; and 5H-1-pyridine indolizine, 7-acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo, 1-hexyl-2-nitrocyclohexane for *H. digynum*.

Key words: Antimicrobial activities, medicinal plant extract, agar well diffusion method, gas chromatography-mass spectrometry (GC-MS), Saudi Arabia

INTRODUCTION

Medicinal herbs have a long history in improving human health and curing various diseases. A wide interest has been made for researchers using herbal material in identi-

fication of the active components and verifications of their efficacy. All modern clinical drugs over 50% are of natural product origin. India has an extensive rich heritage of herbal medicine since the time of Ayurveda with medicinal properties. The Indian people have a tremendous passion for medicinal plants and use them for a wide range of health related applications from a common cold to memory improvement and treatment of

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poisonous snake bite (Kumari et al., 2009).

Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products (Ivanova et al., 2005). The history of plants being used for medicinal purpose is probably old as the history of mankind.

The use of medicinal plants in industrialized societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine. Extraction and characterization of several phyto-compounds from these green factories have given birth to some high activity profile drugs (Mandal et al., 2007). Medicinal plants represent a rich source of antimicrobial agents. In the last few years, a numbers of studies have been conducted on medicinal herbs in different countries to prove the efficiency of antimicrobial activity.

With this background, the present study was undertaken with an aim of evaluating the antibacterial activities of the leaf extracts from different local medicinal plants. The advancement of medical sciences has generated various synthetic drugs to tackle complicated diseases. Fortunately, most of the modern synthetic and semi-synthetic drugs owe their origin to the natural precursors, that is, plants, animals, and microorganisms. The evolution of the plant precursor was the combination of traditional plant lore, their chemotaxonomy and medicinal properties. The discovery of new drugs from plant sources is a multidisciplinary effort involving the study of traditional medicine and its biological and toxicological screenings.

Plants are considered to be Natures Green Pharmacy, which provides drugs to maintain the good health and restore the failing health of human beings. Most flora of the Kingdom of Saudi Arabia are reputed for their use in folk medicine, in this respect, it is necessary to analyse and compile the medicinal properties and their effect against various human pathogenic bacteria and yeasts. However, in this preliminary study, some ideas might be re-evaluated about the safe and harmless natural medicines produced by the local plants in Saudi Arabia. Pharmacological industries have produced a number of new antibiotics in the last three decades, but still the resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). Such a fact is very important, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. From 1980 to 1990, Montelli and Levy (1991) documented a high incidence of resistant microorganisms in clinical microbiology in Brazil. This fact has also been verified in other clinics around the world. The problem of microbial resistance is growing and

the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem.

Nature is an excellent source of medicinal agents and an impressive number of modern drugs have been isolated from natural resources. Plants contain numerous biologically active compounds, many of which have been shown to have anti-microbial activity. Thus, over 50% of these modern drugs are of natural origin and as such these natural compounds play an important role in drug development in pharmaceutical industry (Jeyachandran et al., 2007).

Diseases spread by microbes are the number one cause of death worldwide. This may be due to the increase in incidence of multiple drug resistance. Microbial resistance to almost all anti-microbial agents has been reported (Truiti et al., 2003). This resistance is largely due to indiscriminate use of anti-microbial drugs commonly used in treatment of these diseases. A part from resistance, some antibiotics has side effects which limit their usage. So, there is an urgent need to discover new spectrum of anti-microbial agents with minimal side effects, and higher plants are potential source of novel anti-microbial prototypes (Maureer et al., 1996). The present study describes the evaluation and phytochemical screening of anti-microbial potential plant species *Antigonon leptopus* against four common human dental pathogens. *A. leptopus* (family: Polygonaceae) commonly known as "coral bells" or Mountain rose is a known medicinal plant.

The plant parts were utilized extensively by the Chinese and Indians. As part of search for anti-microbial compounds from these plants, they extracted and screened the flowers for anti-microbial activity; it was found out that the alcoholic extract showed the anti-microbial activity. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The present study has been undertaken with an objective to determine the antibacterial and antiyeast activities of the leaf extract of seven local medicinal plants in Saudi Arabia. The selected medicinal plants were collected from nearby desert region of Riyadh. Antibacterial activity was carried against six human pathogenic Gram-positive and Gram-negative bacteria. The objective of this research was to evaluate the potential of plant extracts and phytochemicals on standard microorganism strains as well as multi-drug resistant bacteria, which were isolated from hospitals.

MATERIALS AND METHODS

Collection of medicinal plants

All plants were collected locally from the desert areas of Riyadh, Saudi Arabia. The freshly collected whole plants of *Rumex*

Table 1. Antibacterial activity of leaf extract from different medicinal plants in Saudi Arabia.

*Leaf extract using acetone	<i>S. suis</i> (mm)	<i>P. aeruginosa</i> (mm)	<i>E. coli</i> (mm)	<i>S. aureus</i> (mm)	<i>B. subtilis</i> (mm)	<i>S. sonnei</i> (mm)	<i>C. albicans</i> (mm)
<i>R. vesicarius</i>	-	-	++ (18)	-	-	-	++ (20)
<i>D. stramonium</i>	-	-	-	+++ (25)	++++ (30)	-	+++ (24)
<i>Z. coccineum</i>	-	-	-	-	-	-	++ (19)
<i>C. colocythus</i>	-	++ (18)	+ (9)	++++ (31)	-	-	-
<i>L. scindicus</i>	-	++ (17)	++++ (28)	-	-	-	+ (10)
<i>H. digynum</i>	+ (10)	++ (16)	+++ (23)	-	-	++ (13)	+ (9)

++++, Very strong activity; +++, good activity; ++, moderate activity; +, weak activity; -, no activity. *, No antibacterial activities was observed when water used for leaf extract (data not shown).

vesicarius, *Datura stramonium*, *Zygophyllum coccineum*, *Citrullus colocythus*, *Lasiurus scindicus*, and *Heliotropium digynum* were stored by the Department of Botany and Microbiology, College of Science, King Saud University. This study was carried out from 5th September, 2011 to 1st December, 2011.

Preparation of leaf extract

Fresh leaf parts of the plant materials were washed under running tap water and then with distilled water twice, and was air dried for about 1 week and then they were homogenized to fine powder and were stored in airtight bottle. The air dried and powdered leaf material (20 g) was extracted with 200 ml acetone and distilled water using Erlenmeyer flask in a shaking incubator at 28°C for 2 days. The acetone and water extract was filtered and evaporated until dryness. The extract was stored at 4°C until further use.

Antimicrobial activities of the leaf extracts from medicinal plants

Pathogenic microorganisms

Seven human pathogenic microorganisms (which were *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella suis* ATCC 13076, *Shigella sonnei* ATCC 11060, and *Candida albicans* ATCC 10231) which were collected from American Type Culture Collection, ATCC were kindly provided by Dr. Ismet Ara, King Saud University, Riyadh. The pathogenic microorganisms were maintained in nutrient agar broth and were slants as working cultures and were stored at -20°C as 30% glycerol stock for long time use.

Determination of antimicrobial activities by agar well diffusion method

Standard agar-well diffusion method (Collins et al., 1995) was employed to determine the antimicrobial activities for both acetonic and aqueous plant extracts. One milliliter of acetonic extracts of the given leaves was used against the test microorganisms. Approximately, 10 ml of sterile Muller-Hinton agar (MHA) was poured into sterile culture plates and allowed to set. About 10 ml of the antibiotic medium seeded with 0.1 ml of a 24 h old culture of bacteria isolates was layered onto the MHA and allowed to set. The seed medium was then allowed to dry at room temperature for about 30 min. Using a sterile straw, wells of about 8 mm in diameter were punched on the plates. About 0.1 ml of each dilution of the extracts was dispensed into the wells and the plates were incubated

at 37°C for 48 h. At the end of the period, inhibition zones formed on the medium were evaluated in milliliter.

Gas chromatography-mass spectroscopy (GC-MS) analysis of crude extract

GC-MS analysis was performed to identify the active antibacterial compound in the plant leaves extract. Identification of several compounds was done by injecting 1 µl of sample into a RT x-5 column (30 × 0.32 nm) of GC-MS model (Perkin Elmer, Clarus 500, USA) and helium (3 ml/min) was used as a carrier gas. The following temperature gradient program was used: 75°C for 2 min followed by an increase from 75 to 175°C at a rate of 50°C per min and finally 7 min at 175°C. The m/z peaks representing mass to charge ratio characteristics of the antimicrobial fractions were compared with those in the mass spectrum library of the corresponding organic compounds (Pandey et al., 2010). The chemical components of the extracts were analyzed in the central laboratory of King Saud University, Riyadh, Saudi Arabia. Identification of the chemical constituents of extracts were made using Perkin Elmer (Clarus 500, USA) gas chromatography coupled with (Clarus 500, USA) mass spectrometer (MS). The compounds were identified by comparing them with the mass spectrum and they were matched with the inbuilt library.

RESULTS AND DISCUSSION

Antibacterial activity of the leaf extract

The results obtained showed that concentrated acetonic extracts of *R. vesicarius*, *D. stramonium*, *Z. coccineum*, *C. colocythus*, *L. scindicus*, and *H. digynum* had inhibitory effects (9.0 to 30 mm) on most of the seven tested microorganisms as represented in Table 1. While the concentrated aqueous extract showed no inhibitory effects on the tested microorganisms (data not shown). *Z. coccineus* L. is a desert shrub up to 75 cm high, green glabrous, leaves compound, with a pair of bright green, glabrous, cylindrical fleshy leaflets at least 10 mm long terminating a slightly longer, terete, fleshy petiole. Capsule is club-shaped, 5-angled, 8 to 10 mm long, with obtuse apex (Migahid, 1988). This group of plants distributed in North Hijaz, South Hijaz, Northern region, Western Najd, and Eastern region of Saudi Arabia. In Saudi Arabia, a shrub likes desert medicinal plant *Z.*

coccineus L. (Family Zygophyllaceae was reported to produce zygophyllin, quinovic acid, saponins, tannins, resins, and wax (Al-Kahtani et al., 2000). Fruits of this plant are used in the treatment of rheumatism, gout, asthma, hypertension, as a diuretic and an antidiabetic (Baulos, 1983).

Among the Arabs, the seeds are reputed as anthelmintic (Kirtikar and Basu, 1981) and also used in rheumatism, gout, cough, asthma, and diuretic (Al-Kahtani et al., 2000). *H. digynum* (Forssk.) Asch. ex C. Christens. (= *H. luteum* Poir.): family Boraginaceae, a richly branched, hairy yellow-green small shrublet, 20 to 40 cm high, retrorsely tomentellous, canescent. Leaves small, 1 to 1.5 cm long, sessile or short petioled, ovate-lanceolate, petiole not exceeding 1 to 2 cm in length, inflorescence dense cyme, 1 to 4 cm long. Corolla pale-yellow with dark throat, one and half times as long as calyx, abruptly constricted to a short tube not exceeding the calyx, lobes narrowly linear. Stigma long, conical, half as long as the style, hirsute at apex. Nutlets typically hairy in sand (Migahid, 1988).

The plants are distributed in North Hijaz, Eastern Najd and Eastern region of Saudi Arabia. The phytochemical screening of the aerial parts showed the presence of alkaloids, flavonoids, tannins, sterols and/or interpenes, volatile oils and volatile bases (Mohammad and Al-Yahya, 1990). Nine more fatty acids have also been reported (Ismail 1984). The plant is used in folk medicine for the treatment of dog bite and skin diseases in Saudi Arabia (Mohammad and Al-Yahya, 1990). The use of plant compounds for pharmaceutical purposes has gradually increased in Brazil. According to World Health Organization (Santos et al., 1995), medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants.

Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (Almagboul et al., 1985; Artizzu et al., 1995; Ikram and Inamul., 1984; Izzo et al., 1995; Kubo et al., 1993; Shapoval et al., 1994; Sousa et al., 1991). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant.

These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (Jansen et al., 1987), as well as in tannin (Saxena et al., 1994). The antimicrobial properties of plants have been investigated by a number of researchers world-wide, especially in Latin America. In Argentina, a research tested 122 known plant species

used for therapeutic treatments (Anesini and Perez, 1993). It was documented that among the compounds extracted from these plants, 12 inhibited the growth of *S. aureus*, 10 inhibited *E. coli*, and 4 inhibited *A. niger*, and also it was reported that the most potent compound was the one extracted from *Tabebuia impetiginosa*. The antimicrobial properties of compounds obtained from *Parthenum argentatum* against *C. albicans*, *Torulopsis hansemula*, *K. pneumoniae* and *P. aeruginosa* was detected (Martinez et al., 1994, 1996).

It was observed that the substances extracted from nine known plants in Uruguai did not show any activity against *C. albicans* and *Saccharomyces cerevisiae*, but inhibited the growth of *B. subtilis*, *E. coli* and *P. aeruginosa* (Alonso-Paz et al., 1995). Many studies have been conducted in Brazil. The inhibitory activity of *Vatairea macrocarpa* on *Klebsiella* species and *S. aureus* was observed (Matos et al., 1988) and the inhibitory activity of extracts from *Eucalyptus* species against soil fungi (Bruna et al., 1989).

A more detailed study on antimicrobial compounds was done evaluating extracts from 120 plant species from 28 different families (Santos et al., 1990). It was documented that 81 extracts obtained from 58 plants were active against *S. aureus*, and five extracts from four other plants inhibited the growth of *P. aeruginosa*. Another study (Lemos et al., 1992) detected the antibacterial and antifungal (*C. albicans*) activity of essential oils obtained from *Croton triangularis* leaves. Extracts from *Lippia gracilis* and *Xylopia sericea* showed antifungal activity. The investigation of antimicrobial activity as well as cell toxicity of extracts from 30 plant species against five bacterial species and two fungal species was studied (Nascimento et al., 1990).

It was concluded that ethanol extracts from 70% of the plants were toxic to cell and only one of the species of *Combretum duarteianum* showed antimicrobial activity. The toxicity of extracts from *Arthemus sativa*, which is known to have antimicrobial activity, was also studied (Carvalho et al., 1988). The antimicrobial activity from *Mikania triangularis*, known as "thin leaf guaco", was tested against five genera of bacteria and three genera of yeast, and it showed that it had activity against *Bacillus cereus*, *E. coli*, *P. aeruginosa*, *S. aureus* and *Staphylococcus epidermidis* (Cruz et al., 1996). Effects of phytochemical were conducted (Izzo et al., 1995; Jansen et al., 1987) and the antimicrobial activity of anacardic acid on *S. aureus*, *Brevibacterium ammoniagenes*, *Streptococcus mutans* and *Propionibacterium acnes* was observed. Later, the bactericidal activity of anacardic acid and totarol on methicillin resistant strains of *S. aureus* (MRSA) and the synergistic effect of these compounds associated with methicillin (Muroi and Kubo, 1996) was tested.

The results observed for each acetonic extract of plant *R. vesicarius* showed a very good activity against *E. coli* ATCC 25922 and *C. albicans* ATCC 10231; *D.*

stramonium was extremely active against *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633 and *C. albicans* ATCC 10231; *Z. coccineum* appeared to have a moderate activity against *C. albicans* ATCC 10231; *Citrullus colocythus* showed moderate inhibitory property against *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 10231 and a very promising result against *S. sonnei* ATCC 11060; *L. scindicus* was moderate against *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922, but showed a very good inhibitory activity against *C. albicans* ATCC 10231; extract of *H. digynum* inhibited all the pathogenic microbial strains except for *B. subtilis* ATCC 6633 and *S. sonnei* ATCC 11060 (Table 1). However, in this study, the acetonic extract of *H. digynum*, *L. scindicus* and *D. stramonium* had shown an excellent result against *E. coli*, as for *R. vesicarius*, *C. colocythus* and *Z. coccineum*, the inhibitory effect was very weak (Table 1).

Dock Sorrel or *R. vesicarius* L. family: Polygonaceae (a.k.a. Hummayd, Hammad) is a Middle Eastern herb traditionally used in its folk medicine for the treatment of a variety of liver, stomach, respiratory and aches. It is a shrubby annual plant which is used in salads or added to acidify goats milk for the preparation of Iqitos Iqt which are dried milk shreds. Fresh leaves are used like spinach in soups and to accompany meat (Tukan et al., 1998). It appears as an interesting herb, because a recent study identifies various active compounds extracted from leaves using ethyl acetate and n-butanol. A variety of phenolics, including derivatives of apigenin, quercetin, and diosmetin have been identified (El-Hawary et al., 2011), and were identified using liquid chromatography with mass spectroscopy with an electrospray ionization (ESI) system.

Researchers assessed both the antioxidant potential and its liver (hepatoprotective) benefits using extracts from the roots, leaves and fruits in a rat model. An essential oil from the fruits and its lipid composition were also determined. The research builds on parallel studies of the antibacterial properties (Mostafa et al., 2011), the flavonoids and anthroquinones (Saleh et al., 1993), and the mineral composition, and it appears as a good source of calcium, iron, magnesium and potassium (Alfawaz, 2006). Like most sorrels and docks, it has a high acid content. For the various acids, the contents are, citric acid: 270 to 300 mg/100 g leaves, malic acid: 5530 to 5620 mg/100 g leaves and oxalic acid: 2840 to 3260 mg/100 g. In our study, we have observed that in the leaf extract of *R. vesicarius*, 4-Ethyl-2-hydroxycyclopent-2-en-1-one, 2-Methoxy-4-vinylphenol and cyclobutanol were present as major compounds (Table 2). It was reported that the extract of *Angelica sinensis* produced cyclobutanol, and it was active against bacteria (Deng, 1992). This finding strongly supported that antibacterial components were present in our extracted compounds from *R. vesicarius*. In our study, *D. stramonium* was extremely active against *S. aureus* ATCC 25923, *B.*

subtilis ATCC 6633 and *C. albicans* ATCC 10231. During GC-MS analysis, leaf extract of this plant produced hexanoic, nonanoic, octanoic, and heptanoic acid. Similarly, the inhibitory effects of plant methanolic extract of *D. stramonium* L. and leaf explant callus against bacteria and fungi were studied by Alireza et al. (2010). In their study, it was found out that the methanolic extracts of leaf were most effective against *B. subtilis* and *S. epidermidis*. Their results also showed that the active compound was atropine alkaloid.

Crude leaf extract of *L. scindicus* showed antibacterial activity against *E. coli*, *P. aeruginosa*, and *C. albicans*, and in the whole extracts, several compounds were found, such as butanoic acid, 3-methyl-,3,7-dimethyl-6-octenyl ester, 6-Octen-1-ol,3,7-dimethyl-propanoate, 2-Hydroxy-3,5-dimethylcyclopent-2-en-1-one, and alpha-cadinol. Similarly, it was reported that foliar application of 3-(3-indolyl) butanoic acid was used as a technique for preventing and treating bacterial wilt (Matsuda, 2000). Antibacterial compounds for *Ralstonia solanacearum* having substantially (S)-3-(3-indolyl) butanoic acid of a specified structure or its salt as active component can selectively inhibit the growth of *R. solanacearum* and suppress bacterial wilt dependably even if used at relatively lower concentration (Matsuda, 2000). The GC-MS detected several organic compounds; they were identified using a National Institute of Standards and Technology (NIST) spectral library. High quality spectral matches were obtained for some. A table list of these compounds not previously identified in our tested medicinal plant extracts (Table 2) and further studies will clarify the novelty and purity of these compounds.

Conclusion

The acetonic extracts of various leaves were investigated individually for antimicrobial activity by well diffusion method, and tested against selected species to find the inhibitory activities of the pathogenic microbes. The acetonic extract of all the six plant leaves showed considerably high activity against most of the test organisms, especially *E. coli* as compared to the aquatic extracts. These results are promising and encouraging, due to the fact that the plants are rich antibacterial sources that should be considered; as such, further study should be carried out to confirm the purification of the antibacterial compounds. In this study, it was also concluded that different compounds were found in each plant extract, and these data proved that each medicinal plant might have the different medicinal values.

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Table 2. GC-MS analysis of the leaf extracts collected from medicinal plants in Saudi Arabia.

Plant's name	GC-MS compound name	Rev
<i>R. vesicarius</i>	4-Ethyl-2-hydroxycyclopent-2-en-1-one	951
	Plant 4h-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	923
	2-Methoxy-4-vinylphenol	921
	Cyclobutanol	915
	1,3-Dioxolane,2,4,5-trimethyl-	906
<i>D. stramonium</i>	Hexanoic acid	973
	Nonanoic acid	959
	Octanoic acid	964
	Heptanoic acid	955
	1,3-Dioxolane,2,4,5—trimethyl	930
<i>Z. coccineum</i>	1-Hexyl-2-nitrocyclohexane	898
	2-Octadecyl-propane-1,3-diol	889
	Octadecanal	886
	Cyclohexane,1-(1,5- dimethylhexyl)-4-(4-methylpentyl)	879
	2-Octadecyl-propane-1,3-diol	877
	2(3h)-Furanone,3-(15-hexadecynylidene)	850
<i>C. colocythus</i>	l-(+)-Ascorbic acid 2,6-dihexadecanoate	934
	l-(+)-Ascorbic acid 2,6-dihexadecanoate	942
	Eicosanoic acid	923
	2-Heptadecenal	918
	2-(1-4-Cyano- 1,2,3,4etrahydronaphthyl)propanenitrile	917
<i>L. scindicus</i>	Butanoic acid ,3-methyl-,3,7-dimethyl-6-octenyl ester	960
	6-Octen-1-ol,3,7-dimethyl-propanoate	951
	2-Hydroxy-3,5-dimethylcyclopent-2-en-1-one	936
	Alpha-cadinol	934
<i>H. digynum</i>	5H-1-Pyridine	956
	Indolizine	951
	7-Acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo	945
	1-Hexyl-2-nitrocyclohexane	908

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