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Effect of Certain Anti-diabetic Ayurvedic Drugs Against Microbes Causing Diabetes-Dependent Infections

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The ayurvedic drugs Glymin®, Hyponidd®, Madhumerin®, Mersina® and Limit® are administered as anti-diabetic drugs in the Indian system of medicine in which *Gymnema sylvestre* is a common ingredient. We investigated the antimicrobial properties of the ethanolic extracts of these drugs against common microbes causing diabetes-depended infections using agar-well diffusion method. The zone of inhibition (in the order of high to low) for each drug was: Glymin - S. *aureus,* S. *pyogenes, B. subtilis, Pseudomonas aeruginosa;* Limit - B. *subtilis,* S. *pyogenes, S. aureus, Enterococcus faecalis;* Mersina - *S. aureus, S. pyogenes, B. subtilis, E. faecalis;* Madhumerin - *S. pyogenes, C. albicans, S. aureus*; and Hyponidd - S. *pyogenes*. The minimum inhibitory concentration of the drugs against the test microorganisms were in the range of 75 – 200 mg ml^{-1} (1.9 – 5.0 mg well^{-1}). The Gas Chromatograph – Mass Spectrometric analysis of the ethanolic extracts of the drugs revealed the presence of Ar-tumerone and curlone in Madhumerin; palmitic and benzoic acids in Glymin®; tetradecanoic acid, asarone and caryophyllene in Limit®; α-citral in Mersina® and δ-elemene in Hyponidd® to a high percentage similarity with the internal standards of the instrument. To sum up, this study suggests that the components present in the polyherbal formulations (Mersina®, Glymin® and Limit®) would play a vital role in inhibiting diabetes-depended infections thereby reducing the gravity of the disease with the advantage of reducing treatment cost and the quantity of drug consumption.

Key words: Antimicrobial, Ayurvedic drugs, Diabetes, Diabetes-dependent infections.

Abbreviations: ATCC, American type culture collections; GC-MS, gas chromatograph-mass spectrometry; MIC, minimum inhibitory concentration; MIC, minimum inhibitory concentration; ND, not detected; O D, optical density; RT, retention time and SD, standard deviation.

Diabetes mellitus is a major public health burden, which by the year 2030 would engulf over 366 million people worldwide[1]. Besides the classically used for the treatment of diabetes (insulin, sulphonylureas, biguanides and thiazolidinediones), several species of plants have been described in the scientific and popular literature with hypoglycaemic activity[2]. Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown[2]. Oral administration of Hyponidd, a

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herbo-mineral formulation composed of the extracts of ten medicinal plants (Table 1) at a concentration of 100/200 mg Kg^{-1} for a period of 45 days in streptozotocin-induced diabetic rats resulted in significantly lower levels of blood glucose and increased levels of hepatic glycogen and total hemoglobin[3]. Clinicians very often encounter with the problem that diabetic patients are at higher risk for various infections than non-diabetic patients. Patients with uncontrolled diabetes often develop diabetic complications like foot ulcers, retinopathy, neuropathy and macrovascular complications. Diabetic foot infections are usually polymicrobial in nature due to aerobic (*Staphylococcus* spp., *Streptococcus* spp and *Enterobacteriaceae)*, anaerobic bacteria (*Bacteroides* spp., *Clostridium* spp and *Peptostreptococcus* spp.) and fungi. *Klebsiella pneumoniae* and *Proteus* spp. are the major isolates from diabetic foot infections[4].

Ayurveda, the ancient science of life remains to be one of the most ancient and yet living traditions being practised widely in India, Sri Lanka and in the developed countries. Ayurveda is the corner stone of complementary and alternative system of medicine in India[5]. Ayurvedic plants have been found effective to cure the dysfunctions of heart, kidney, lungs; cardiovascular, skin, nervous, immune systems, and ailments such as cancer, diabetes mellitus, pain and inflammation, *etc.* Plants make over 100,000 bioactive compounds (alkaloids, flavanoids, tannins and phenolics, *etc*.) many, if not most of which have antimicrobial activity[6].

In vitro studies showed that many medicinal plants like *Mikania glomerata* Spreng, *Psidium guajava*, *Zingiber officinale*, *Syzygium aromaticum*, *Allium sativum*, possess potent antimicrobial agents[3]. Omoregbe et al., (1996)[7] have reported that *Momardica charantia*, *Alstonia boonei*, and *Ocimum bacilicum* showed antimicrobial activities against *Escherichia coli, Salmonella paratyphi* and *Shigella dysenterae*. Similar results were obtained for *Piper regnellii* against *S. aureus* and *B. subtilis*[8]. Ayurvedic drugs Glymin$^{\grave{O}}$, Hyponidd$^{\grave{O}}$, Madhumerin$^{\grave{O}}$, Mersina$^{\grave{O}}$ and Limit$^{\grave{O}}$ are prescribed for diabetes in the Indian system of medicine in which the known anti-diabetic herb, *Gymnema sylvestre* R.Br of Asclepidaceae is a major component. *G. sylvestre* showing potent antimicrobial activity[9] led us to explore the possiblity of administering a single drug formulation with dual capacity for curing both diabetes and diabetes-depended infections. Thus, the specific objectives of this study are: to (a) check the antimicrobial activity of ayurvedic anti-diabetic drugs employing *G. sylvestre* and allopathic modern drug as controls, (b) elucidate the chemical composition of the ayurvedic drugs under study, and (c) identify the ayurvedic anti-diabetic drugs with antimicrobial potential.

MATERIAL AND METHODS

Herbal Material and Extraction Method

Gymnema sylvestre (the positive control) *leaves* were collected from Calicut University Campus during the month of March 2006, and were identified by Dr. A K Pradeep, Curator, Calicut University Herbarium, Department of Botany, University of Calicut, India. The leaves were shade-dried for four days and then powdered using a mixer grinder. 10 g of *G. sylvestre* leaf powder was extracted with ethanol in Soxhlet apparatus (100 ml) for 8 h, and the crude extract so obtained was used for the chemical analysis using Gas Chromatograph – Mass Spectrometry (GC-MS). Evaporated and dried crude extract was considered as 100%. Using sterile distilled water the extract was made into different concentrations ranging from 75 mg ml^{-1} to 200 mg ml^{-1} to perform anti-microbial activity studies.

Drug Samples and Extraction Method

Commonly prescribed ayurvedic anti-diabetic drugs or tablets like Glymin®, Hyponidd®, Madhumerin®, and capsules like Mersina® and Limit® were purchased from Sree Narayana Pharmacy, Kozhikode, India. Allopathic anti-diabetic drug Daonil® was used as a control. Details of the aforesaid drugs are summarized in Table 1. 30 g of the ayurvedic drugs were weighed and mixed with absolute ethanol and made up to 100 ml and introduced into the Soxhlet apparatus and extracted for 8 h. The extract obtained was filtered using syringe driven filter unit of 0.45 microns (Millipore). The filtrate was used for the chemical analysis using GC-MS. The ethanol was evaporated and the dried extract was considered 100%, which was used for antimicrobial studies, after proper dilutions ranging from 75 mg ml^{-1} to

200 mg ml^{-1} using sterile distilled water. The antimicrobial activity of the drug samples were checked by agar-well diffusion method[10] at different concentrations.

Microbial Strains

The common human pathogens used for the antimicrobial activity screening were the bacteria *Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes,* and the yeasts *Candida albicans* and *C. parapsilosis*. All these ATCC microbial cultures were obtained as gift from the Jain Institute of Vocational and Advanced Studies, Bangalore, India.

Culture Media Used

Before testing the antimicrobial activity, the bacterial cultures were grown in Nutrient Agar and then in Nutrient Broth and the Yeast cultures were grown in Sabuourd's Dextrose Agar. For testing the antimicrobial activity of bacteria Muller-Hinton Agar (MHA) and for Yeast Potato Dextrose Agar (PDA) were used. All the media were procured from Hi Media, Mumbai.

Preparation of McFarland's Standard

0.5 mL of 0.048 M $BaCl_2$ was added to 99.5 mL of 0.18 M H_2SO_4 with constant stirring. The standard was distributed into screw-cap tubes of same size and with the same volume as those used in growing the broth cultures. The tubes were sealed tightly to prevent loss by evaporation and are stored for protecting from light at room temperature. The turbidity standard (OD = 0.132 at 600 λ) was vigorously agitated on a vortex mixer before use.

Determination of Antimicrobial Activity

The agar-well diffusion method[10] was followed to check the antimicrobial activity of drug and *G. sylvestre* extracts. The media, MHA (3.8 %) and PDA (3.9 %), glass wares, cork borer (6 mm in diameter) and other accessories were sterilized using an autoclave at 121 °C for 20 min at 15 ψ. 25 ml of medium was poured into each plate (90 mm in diameter) and allowed to solidify. 12 h old respective bacterial cultures with turbidity similar to McFarland's standard (OD = 0.132 at 600 λ) were spread plated into each petriplate using sterilized swabs. Four wells (6 mm in diameter) were made at the four corners of the plate using a sterile cork borer. 25 µl of the drug extract at different concentrations (1.9, 2.5, 3.8, and 5.0 mg $well^{-1}$), *G. sylvestre* leaf extract; sterile distilled water and allopathic drug (controls) were applied to each well in appropriate experimental designs. The plates were incubated at 37 °C for 18 - 24 h. Experiments were repeated at least in triplicate, and the average values with standard deviations (SD) of anti-microbial activity were calculated.

GC-MS Analysis

The GC-MS (GC version 3800, Front Injector 1079; MS 1200 L Single Quadrapole, USA) analysis of the ayurvedic drug samples was conducted at Sophisticated Test and Instrumentation Centre (STIC), Cochin University of Science and Technology (CUSAT), Kochi, India. The automatic injection system injects 1µl of the sample into the VF 5 MS column and low bleed 5% Phenyl, 95% dimethylpolysiloxane, 30 m in length, ID 0.25 mm and 0.25 µm thickness were used with helium as the carrier gas at a constant flow of 1.0 ml min^{-1}. The column oven temperature were: at time 0 min 70 °C , at time 15 min 220 °C (10 °C min^{-1} with 0 min hold), and at time 24 min it was 300 °C (20 °C min^{-1} with 5 min hold). Other internal controls were: ion source pressure, 57 m Torr; manifold pressure, 1.55×10^{-5} Torr, Collision Cell pressure, 0.112 m Torr, Probe inlet pressure, 504.904 Torr, Turbopump Speed, 100% and Temperatures: manifold 40.3°C, ion source 280.3 °C, transfer line 279.6 °C

Identification of the Compounds

The similarities of the components in the samples were identified by matching their mass spectra with those recorded in the MS library of STIC, CUSAT.

RESULTS

Antimicrobial Activity

Ethanolic extracts of various ayurvedic anti-diabetic drugs have exhibited higher degree of antimicrobial activity as compared with the control allopathic drug. The drug Glymin was found effective against *S. aureus*, *S. pyogenes*, *B. subtilis* and *P. aeruginosa* with a zone of inhibition of 13 mm, 12 mm, 11 mm and 8 mm (Fig. 1 A-D), respectively at the highest concentration (200 mg ml^{-1}) tested; Limit Cap

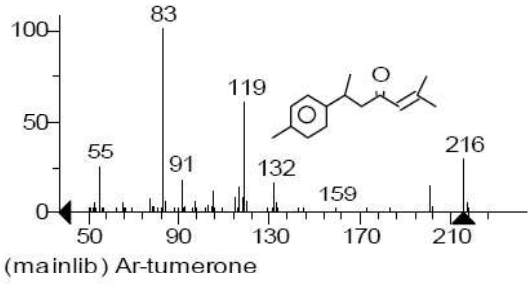

Fig. 5A: Mass spectrum of drug sample Madhumerin with known library at RT 12.821 min and scan number 604.

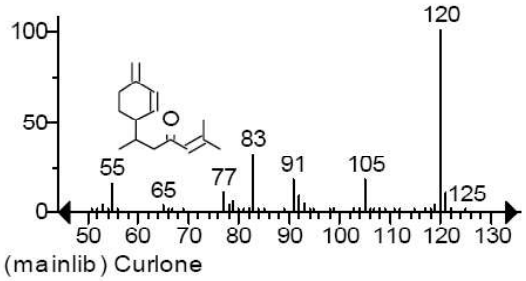

Fig. 5B: Mass spectrum of drug sample Madhumerin with known library at RT 13.269 min and scan number 632.

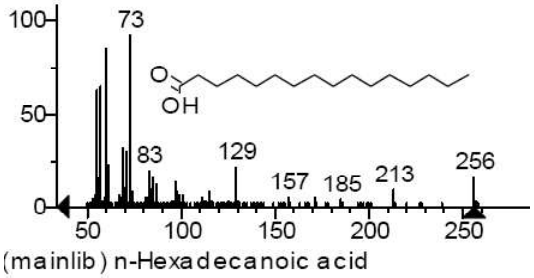

Fig. 6A: Mass spectrum of drug sample Glymin with known library at RT 16.583 min and scan number 839.

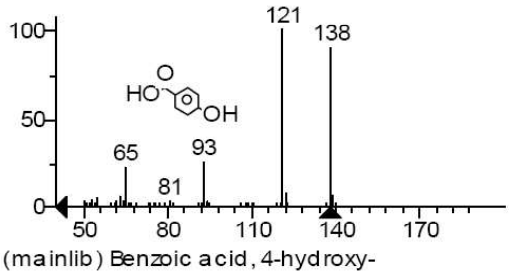

Fig. 6B: Mass spectrum of drug sample Glymin with known library at RT 12.744 min and scan number 600.

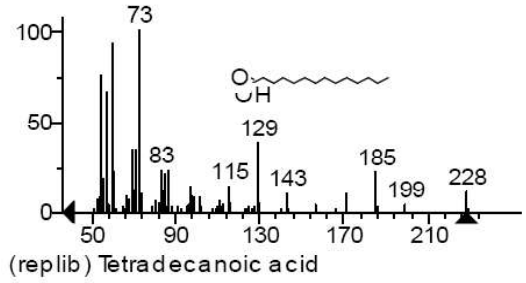

Fig. 7A: Mass spectrum of drug sample Limit with known library at RT 14.08 min and scan number 682.

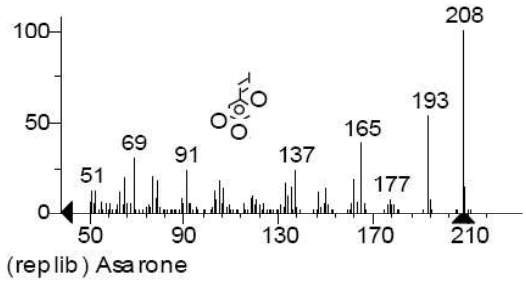

Fig. 7B: Mass spectrum of drug sample Limit with known library at RT 11.349 min and scan number 512.

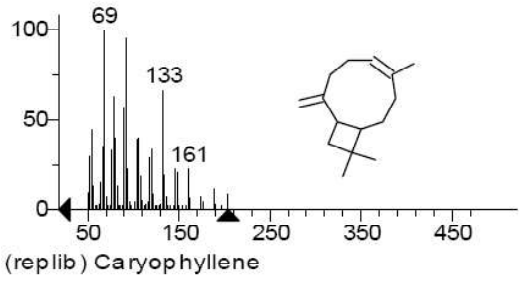

Fig. 7C: Mass spectrum of drug sample Limit with known library at RT 9.855 min and scan number 419.

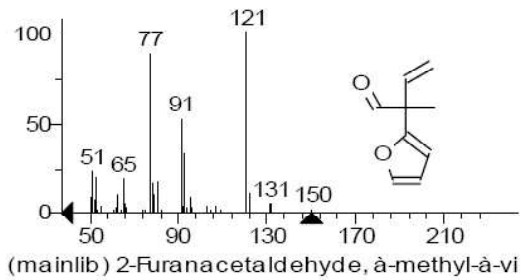

Fig. 8A: Mass spectrum of drug sample Hyponidd with known library at RT 12.841 min and scan number 603.

Table 1. Ayurvedic drug samples used for checking the chemical composition and anti-microbial activities. The weight (in mg) of each plant component (extract/powder) in the capsule/tablet is given in brackets against that plant/ drug.

S No.	Drug	Batch No.	Company	Mfg Date	Exp Date	Composition
1	Glymin®	5CHF	Kerala Ayurveda Pharmacy Limited	Dec-05	Nov-08	*Salacia oblonga* (300) *Syzygium cumini* (200) *Pterocarpus marsupium* (200) *Gymnema sylvestre* (100) *Tinospora corditolia* (100) *Emblica officinalis* (100) *Curcuma longa* (100) *Processed white betumin* (30)
2	Limit® Capsules	259	Ayulabs Pvt. Ltd.	Oct-05	Sep-09	*Momordica charantia* (60) *Enicostema littorale* (50) *Gymnema sylvestre* (50) *Eugenia jambolana* (50) *Withania somnifera* (50) *Trigonella foenum* (50) *Trivangibhasma* (50) *Myristica fragrans* (50) *Trikatu* (50) *Suddha shlajith* (40)
3	Mersina® Capsules	Mer-25	J & J De Chane, Hyderabad, India	Nov-05	Oct-08	*Gymnema sylvestre* (75) *Momordica charantia* (81) *Cassia auriculata* (63) *Syzygium cumini* (96) *Phyllanthus emblica* (48) *Melia azadirachta* (30) *Trigonella foenum* (15) *Coccinia indica* (93) *Tinospora cordifolia* (63) *Potassii cordifolia* (12) Preservatives: Methyl paraben, Propyl paraben and Bronidol
4	Hyponidd®	HY278H	Charak Pharm-aceutical Pvt Ltd	Jul-05	Jun-08	*Momordica charantia* (12) Purified Asphaltum & Yashad Bhasma (37.5) *Swertia chirata* (15) *Melia azadirachta* (75) *Pterocarpus marsupium* (75) *Tinospora cordifolia* (75) *Gymnema sylvestre* (112.5) *Enicostemma littorale* (112.5) *Emblica officinalis* (150) *Eugenia jambolana* (150) *Cassia auriculata* (225) *Curcuma longa* (300) *Curcuma longa* (70) *Shudh guggul* (60) *Abrak Bhasam* (50) *Strychnos nosvomica* (30) *Tribang Bh.*(30)

Table 1. Cont.

5	Madhumerin®	works, 4541	Kashmir ayurvedic Amristsar, India	Nov-05	Oct-08	*Shudh Shingraf* (30) *Shud Silajith* (30) *Azadirachta indica* (30) *Trigonella foenum-graecum* (60) *Rasanjan sudh* (20) *Gymnema sylvestre* (30) *Momordica charantia* (30) *Syzygium cumini* (30) *Ext. Giloy* (20) *Terminalia chebula* (20)
6	Daonil ®	296163	Aventis, Goa	Jul-05	Jun-08	Glibenclamide I.P (5)

Table 3. The minimum inhibitory concentration of the drug samples against each of the test microorganisms used

Drugs	MIC of test microorganisms used in mg ml^{-1}					
	S. aureus	*S. pyogenes*	*B. subtilis*	*P. aeruginosa*	*E. faecalis*	*C. albicans*
Glymin	75	150	150	200	nd	nd
Limit	75	75	75	nd	200	nd
Mersina	75	75	75	nd	75	nd
Hyponidd	nd	200	nd	nd	nd	nd
Madhumerin	100	150	nd	nd	nd	200

ND = not detected

S. pyogenes (13 mm) (Fig. 4 A-B) and *C. albicans* (8 mm). Other controls (Daonil® and sterile distilled water) did not show any inhibition against any of the microbes tested. In fact, all the microbes were tested against all the drug samples, Table 2 presents the data of only those microbes which are inhibited by the drugs. The minimum inhibitory concentration (MIC) of the drug samples tested against each microorganism is listed in Table 3.

Chemical Analysis

GC-MS analyses of the ayurvedic anti-diabetic drugs led to the elucidation of the main components with their percentage similarity with the library and retention time (RT) listed in Table 4. However, this study permits only data obtained with ethanolic extracts of the samples and it may not represent all the components present in the sample, for instance *G. sylvestre* did not yield any peak.

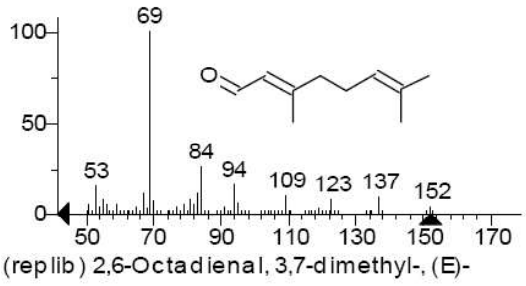

Fig. 9 A. Mass spectrum of drug sample Mersina with known library at RT 7.799 min and scan number 291.

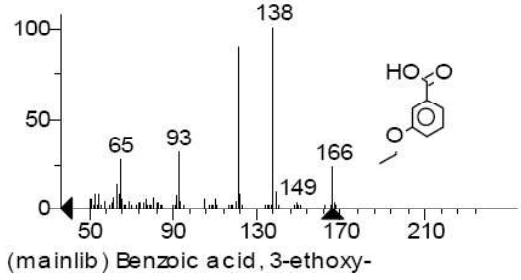

Fig. 9 B. Mass spectrum of drug sample Mersina with known library at RT 12.791 min and scan number 603.

Table 2. Antimicrobial activities (performed in triplicates) of the ethanolic extracts (mg well^{-1}) of the drug samples against the positive control (*G. sylvestre* leaf), as revealed by the size of inhibition zone (mm) on Muller-Hinton Agar (MHA) medium in Petri-plates of 90 mm diameter after overnight incubation at 37 °C.

		Concentration of the drugs applied per well on the MHA plate											
Drugs	Microorganisms	5. 0 mg well^{-1}			3. 8 mg well^{-1}			2. 5 mg well^{-1}			1. 9 mg well^{-1}		
		Control	Drug	SD	Control	Drug	SD	Control	Drug	SD	Control	Drug	SD
	S. aureus	29	13	.57	15	11	.57	13	9	.00	11	7	.00
	S. pyogenes	nd	12	.57	nd	9	.00	nd	Nd	nd	nd	Nd	nd
Glymin	*B. subtilis*	28	11	.00	12	9	.56	10	Nd	nd	nd	Nd	nd
	P. aeruginosa	28	8	.57	11	Nd	.56	10	Nd	nd	nd	Nd	nd
	S. aureus	29	10	.00	15	9	.00	13	9	.00	11	7	.00
	S. pyogenes	nd	13	.57	nd	11	.55	nd	8	.00	nd	7	.00
Limit	*B. subtilis*	28	13	.57	12	12	.57	10	11	.55	nd	11	.55
	E. faecalis	nd	7	.00	nd	Nd	nd	nd	Nd	nd	nd	Nd	nd
	S. aureus	29	14	1.0	15	13	1.0	13	12	.00	11	11	.55
	B. subtilis	nd	18	.57	nd	16	.57	nd	14	.55	nd	12	.00
	E. faecalis	Nd	11	.00	nd	10	nd	nd	8	.00	nd	7	.00
Mersina	*S. pyogenes*	nd	9	.00	nd	Nd	nd	nd	Nd	nd	nd	Nd	nd
	S. aureus	29	8	.00	15	8	.00	13	7	.00	11	Nd	nd
	S. pyogenes	nd	13	.56	nd	11	.00	nd	Nd	.00	nd	Nd	.00
Hyponidd	*C. albicans*	nd	8	.00	nd	Nd	.00	nd	Nd	.00	nd	Nd	.00

Madhumerin ND = not detected SD = Standard Deviation

Table 4. Gas Chromatograph Mass Spectrometry (GC-MS) analyses of the drug samples at various retention time (RT) (in minutes) and Peak Scan with their percentage similarity with the known library components

S No.	Sample	Major Components	Known activity	Peak Scan & RT (Min)	Percentage Similarity with known Component	Common similar components
1	Madhumerin	Ar-Tumerone	Anticancer, antitumor, antiinflammatory	604 & 12.821	94.5	*Curcuma longa*
		Curlone	-	632 & 13.269	84.0	*Curcuma longa*
		Palmitic acid	Antioxident, Hemolytic, Nemoatocide Pesticide	839 & 16.583	69.2	*Cucumis sativus* *Terminalia catappa* *Nicotiana tabacum* *Azadirachta indica*
2	Glymin	Benzoic acid, 4-hydroxy-	Antibacterial Antiyeast fungicide pesticide antiseptic	600 & 12.744	30.6	*Citrus aurantium* *Ocimum gratissimum* *Melia azedarach* *Nicotiana tabacum* *Camellia sinensis*
		Benzoic acid, 3-hydroxy-, methyl ester	Antibacterial Antiyeast fungicide pesticide antiseptic	478 & 10.786	20.9	*Citrus aurantium* *Ocimum gratissimum* *Melia azedarach* *Nicotiana tabacum* *Camellia sinensis*
3	Hyponidd	Ä-Elemene	Anticancer, apoptotic, VEGF-inhibitor	488 & 10.993	2.41	*Melia azedarach* *Trigonella foenum-graecum* *Micromeria croatica*
		Asarone	Antipyretic, Fungicide	512 & 11.349	42.1	
4	Limit capsule	Tetradecan-oic acid	Antioxident, Cancer-preventive	682 & 14.083	80.7	*Varola surinamensis* *Cocus nucifera* *Daucus carota* *Calendula officinalis* *Terminalia catappa L*
		Caryophyll-ene	Antibacterial, anticarcinogenic,antitumor,antiinflammatory	419 & 9.855	17.0	*Ocimum basilicum* *Salavia officinalis* *Ocimum gratissimum* *Eucalyptus citriodora* Ocimum basilicum
5	Mersina	2,6-Octadienal,3,7dimethyl(E)-		291 & 7.799	37.9	*Citrus aurantium* *Citrus limon* *Micromeria juliana*

The drug Madhumerin revealed components with 94.5% similarity with Artumerone at an RT of 12.821 min and 84% similarity with curlone at an RT of 13.269 min (Fig. 5A & 5B). Drug Glymin revealed components with 69.2% similarity with n-hexadecanoic acid (palmitic acid) at an RT of 16.583 min. Glymin showed 30.6% similarity with 4-hydroxy- benzoic acid at an RT of 12.744 min. (Fig. 6A, 6B). Drug Limit capsule revealed components with 80.7% similarity with tetradecanoic acid (myristic acid) at an RT of 14.083 min. Limit capsule showed 42.1% similarity with Asarone at an RT of 11.349 and 17.0% similarity with Caryophyllene at an RT of 9.855 min (Fig. 7A, 7B & 7C). Drug Hyponidd revealed components with 2.41% similarity with cyclohexene, 4-ethenyl-4-methyl-3-(1-methyletheenyl)-1-(1-methylethyl)-,(3R-trans)-(δ-elemene) at an RT of 10.993 min (Fig. 8A). Drug Mersina revealed components with 37.9% similarity with 2,6-octadienal, 3,7-dimethyl-, (E)-(α-citral) at an RT of 7.799 min and 8.12% similarity with 3-ethoxy-benzoic acid, at an RT of 12.791 min (Fig. 9A & 9B).

DISCUSSION

Recently, much attention has been directed towards the biologically active compounds isolated from popular plant species. The use of medicinal plants plays a pivotal role in alleviating the basic health needs of developing countries, and they offer a new source of antibacterial, antifungal and antiviral agents with significant activities against infective microorganisms[11]. Several plant species have been described in the scientific and popular literature, owing to their hypoglycemic activity[2]. The infections associated with diabetes make the situation of the diabetics worse. *Gymnema sylvestre* is known to possess anti-diabetic principles[12]. The focus of this study is to identify an ayurvedic drug that could be used with dual quality that is effective for the cure of both diabetes and diabetes-associated infections. As much research work has not been carried out on most of the ayurvedic drugs being traded, this study makes an attempt to unveil the hidden properties of the commercially available anti-diabetic ayurvedic drugs in which *G. sylvestre* is an ingredient.

In the present study, the common ayurvedic anti-diabetic drugs, *viz*., Mersina, Limit, Glymin, Hyponidd and Madhumerin have exhibited antimicrobial activities against common pathogens causing diabetes-related infections at a range of 75 to 200 mg ml^{-1} concentrations (1.9 – 5.0 mg per 6 mm well on the plates). In comparison, the most susceptible microorganism towards the treatment of the drugs were *S. pyogenes, S. aureus,* and *B. subtilis*, while *E. coli*, *K. pneumoniae,* and *Candida parapsilosis* was not inhibited by the drugs. The drug Glymin was found to be effective against *S. aureus*, *S. pyogenes*, *B. subtilis* and *P. aeruginosa* with a zone of inhibition of 13 mm, 12 mm, 11 mm and 8 mm, respectively at the highest concentration (5 mg $well^{-1}$) tested; Mersina showed high activity against *S. aureus* (14 mm), *S. pyogenes* (18 mm), *B. subtilis* (18 mm) and *E. faecalis* (11 mm) at 5 mg $well^{-1}$. Limit Cap also showed high activity against *S. aureus* (10 mm), *S. pyogenes* (13 mm), *B. subtilis* (13 mm) and showed moderate activity against *E. faecalis* (7 mm) at 5 mg $well^{-1}$. Madhumerin showed activities against *S. aureus* (8 mm), *S. pyogenes* (13 mm) and *C. albicans* (8 mm), while the drug Hyponidd showed moderate activity against *S. pyogenes* (9 mm) at a concentration of 5 mg $well^{-1}$. Omoregbe et al., (1996) have reported that *M. charantia*, *Alstonia boonei*, and *Ocimum bacilicum* showed antimicrobial activities against *E. coli, Salmonella paratyphi* and *Shigella dysenterae*. Defensins from *Trigonella foenum-graecum*, a major component of drug Mersina, Limit and Madhumerin, inhibited mycelial spread of *Rhizoctonia solani* and also the germination of spores of *Pheaoisariopsis personata* revealing its antifungal property[13]. Chemical compounds extracted from *Curcuma longa* has been reported to be potent inhibitors of inflammation. Curcumin and other semi-synthetic analogues (sodium curcuminate, diacetyl curcumin, triacetyl curcumin and tetrahydro curcumin) showed inhibitory activities against cotton pellet and granuloma pouch inflammations, carrageenin-induced paw edema in rats[14]. *C. longa* preparations were shown to have anti-protozoal activity against *Leishmania amazonensis,*

nematocidal activities against *Paramecium caudatum* and *Toxocara canis* and anti-bacterial activities against *Staphylococcus albus* and *S. aureus*[14]. *C. longa* is a major component of Glymin, Hyponidd and Madhumerin. Glymin, Limit and Mersina, but not *G. sylvestre*, showing zone of inhibition against *S. pyogenes* and *E. faecalis* suggests that the antimicrobial property may be due to synergistic effects of different plants in the drug rather than a specific plant or a specific phytochemical.

The chemical analysis of the ayurvedic drug samples revealed that they contain components with high percentage similarity with known compounds such as Tumerone (94.5%), Curlone (84%), Palmitic acid (69.2%), and Tetradecanoic acid (80.7%). The samples also showed components with moderate similarity with 4-hydroxy benzoic acid (30.6%), asarone (42.1%), and caryophyllene (17%). Tumerone and *â*-caryophyllene , which is a major component of the drugs Glymin, Hyponid and Madhumerin, are the components in *C. longa*[15]. Apart from this, tumerone is known to exhibit anticancer and anti-inflammatory activities[16] and caryophyllene have anti-bacterial, anti-inflammatory, and anticancer[17] properties. Tumerone and curlone, the major components of turmeric oil, showed potent anti-microbial activity against *Aspergillus flavus*, *A. parasiticus*, *Fusarium moniliforme* and *Penicillium digitatum* by spore germination method[18] and also exhibited anti-venom activity[14]. In fact, palmitic acid, an active principle of *Phyllanthus emblica, Azadirachta indica* and *Melia azadirachta*[16], the major component in the drugs Mersina and Hyponid, showed nematocidal and pesticidal activities[19] coupled with anti-bacterial activities against *E. coli*, *B. subtilis* and *C. albicans*[20]. Tetradecanoic acid (myristic acid), a phytochemical present in *Phyllanthus emblica, Azadirachta indica* and *Momordica charantia* which are the major components of the drugs Mersina and Hyponidd showed anti-oxidant and anti-cancer properties[16]. Asarone was reported to have slight activity against the yeasts *C. albicans*, *C. parapsilosis* and *C. kruseii*[21]. The essential oils isolated from the leaves of *Sesuvium portulacastrum*, with *trans*-caryophyllene as one of the components, showed anti-bacterial activities against *Acetobacter calcoacetica, B. subtilis, Clostridium sporogenes, C. perfringens, E coli, S. typhi, S. aureus* and *Yersinia enterocolitica*[22]. Alpha-pinene, beta-myrcene, beta-pinene, limonene and β-caryophyllene, the major components of *Pistacia lentiscus* have shown anti-bacterial activities against *E. coli*, *S. aureus* and *B. subtilis* in a synergistic manner[23]. However, the ethanolic extracts of *G. sylvestre* did not yield any peaks which may be due to its interaction with the column or due to the non-evaporation at the temperature maintained in the GC instrument. High-performance liquid chromatography and atmospheric pressure ionization mass spectrometry studies revealed that *G. sylvestre* possess a mixture of triterpene glucuronides named gymnemic acids[24]. Murakami et al., (1996)[25] reported the presence of new saponins, gymnemosides a and b, and gymenmic acid V as the active principles. As the data presented here are those with ethanolic extracts only, a comprehensive study employing various solvent extracts (polar, non-polar and neutral) would reveal the exact performance of drugs studied.

The drugs used in this study are being traded as anti-diabetic drugs in Indian markets, but not as diabetes-dependent anti-microbial agents. In fact, our novel attempt reveals that these anti-diabetic drugs are also useful to treat diabetes-dependent microbial infections in conjunction. In our study, the MIC was at a range 1.9 – 5.0 mg well^{-1}, which varies from drug to drug and pathogen to pathogen. This comparatively higher concentration might be attained in the body of diabetics by the cumulative effect of the continuous use of the drug, as the ayurvedic treatment is a long-term process. Moreover, some drugs tested in our study did not show any inhibition even at the highest concentration (5 mg well^{-1}), *i.e*., thus the inhibitory effects showed by other drugs guide us to choose the right anti-diabetic drug with dual effect. Biologically active, plant-derived chemicals are expected to play an increasingly significant role in the commercial development of pharmaceuticals[5]. This study suggests that the components present in the polyherbal formulations (Mersina Cap, Glymin Tab and Limit Cap) would play a vital role in inhibiting diabetes-depended infections thereby reducing the gravity of the disease. It also suggests that taking a drug with dual effect such as Mersina

Cap, Glymin Tab and Limit Cap would reduce the treatment cost and the quantity of drug consumption.

CONCLUSIONS

This pilot study shows that Mersina Cap, Glymin Tab and Limit Cap are the most promising anti-diabetic ayurvedic drugs which could be used as dual drugs against diabetic–dependent infections too. Comparatively high MIC might be attained in the diabetics by the cumulative effects of the drug intake over a period, as the ayurvedic treatment is a long-term process. For comprehensive profiling of the chemistry of these drugs, extraction has to be done in polar, nonpolar and neutral solvents.

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