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Antimicrobial activity of the ethanolic and aqueous extracts of *Salacia chinensis* Linn. against human pathogens

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ABSTRACT

Objective: To investigate antimicrobial effects of ethanolic and aqueous extracts of *Salacia chinensis (S. chinensis)* Linn. against pathogenic bacteria and fungi. **Methods:** The *Staphylococcus aureus (S. aureus)* (MTCC 96), *Staphylococcus epidermidis (S. epidermidis)* (MTCC 435), *Bacillus subtilis (B. subtilis)* (MTCC 121), *Escherichia coli (E. coli)* (MTCC 443), *Klebsiella pneumoniae (K. pneumoniae)* (MTCC 432), *Proteus mirabilis (P. mirabilis)* (MTCC 1429), *Salmonella paratyphi A (S. paratyphi A)* (MTCC 735), *Salmonella typhimurium (S. typhimurium)* (MTCC 98), *Shigella flexneri (S. flexneri)* (MTCC 1457) and *Pseudomonas aeruginosa (P. aeruginosa)* (MTCC 424), *Candida albicans (C. albicans)* (MTCC 183) and *Cryptococcus neoformans (C. neoformans)* (clinical isolate) were originally obtained from Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh, India. Antimicrobial activity was carried out by disc diffusion and broth dilution methods against pathogens by using crude ethanolic and aqueous extracts. **Results:** Ethanolic extract of *S. chinensis* L. leaves showed significant antimicrobial activity against *S. epidermidis* (33.20 mm), *C. albicans* (30.40 mm) and *C. neoformans* (18.20 mm) mean values were documented. Aqueous extract of leaves showed significant inhibitory activity against *C. neoformans* (19.8 mm) and *S. epidermidis* (17.80 mm) were observed. Based on broth dilution method, the ethanolic extract of crude plant material showed the minimum inhibitory concentration (MIC) values against *S. epidermidis*, *C. neoformans* (256 μg/mL) and *C. albicans* (512 μg/mL), whereas the aqueous extract of *S. chinensis* L. leaves showed significant inhibitory activity against *S. epidermidis* (512 μg/mL) and *C. neoformans* (1024 μg/mL) were observed. **Conclusions:** The present result revealed that ethanolic extract of *S. chinensis* L. possesses significant antifungal activity when compared as the antibacterial activities.

1. Introduction

Medicinal plants are believed to be important source of new chemical substances with potential therapeutic effects [1]. The secondary metabolites of plants were found to be source of various phytochemicals that could be directly used or as intermediates for the production of new drugs. Traditional medicine should be able to play an even greater role in the modern primary healthcare system of the developing countries. The natural ingredients of traditional medicine are believed to be more acceptable to the human body, when compare to modern synthetic drugs. Thus the most important factor needed is to derive the maximum benefit from the traditional system of medicine for providing adequate health care service to rural people [2]. Nature has long been an important source of medicinal agents. An impressive number of modern drugs have been isolated or derived from natural source, based on their use in traditional medicine. Plants have formed a basis for traditional medicine system that has been used for thousands of years in countries with ancient civilizations [3]. The plants have been used traditionally for centuries and modern scientific studies have shown the existence of good correlation between the traditional or folkloric application

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of some of the plants further strengthens the search for pharmacological active components from plants [4, 5, 6].

Salacia chinensis L. which belongs to the family Celestraceae (spike- thorn family), is a small erect or straggling tree or large woody, climbing shrub, native to India including Andaman and Nicobar Islands [7]. *S. chinensis* L. is commonly called as Chinese Salacia, Lolly vine, Lollyberry and locally as Saptrangi in Ayurveda. Salacia spp is an important source of salacinol, mangiferin and ketonolol which are effective as antidiabetic, antiobese, hepatoprotective, hypolipdemic, anticaries, antiulcers, hypoglycemic and antioxidant agent [8]. Root bark was used in the treatment of gonorrhoea, rheumatic, tonic, blood purifiers, amenorrhea, dysmonorrhoea, asthma, skin diseases and ear disease [9]. *S. chinensis* roots have biologically active compounds such as triterpenes, phenolic compounds, glycosides and colouring agents which show various medicinal properties [7]. Over the past 20 years, there has been a lot of interest in the investigation of natural materials as source of new antibacterial agent. There were less research outcomes regarding antimicrobial activity of *S. chinensis* L. therefore an attempt to be made and to explore the antimicrobial properties.

2. Materials and Methods

2.1. Collection of plant materials

The fresh aerial parts of the *S. chinensis* L. were collected from evergreen and moist, deciduous forest of Ponmudi hills in Kerala. The plant species was primarily identified by Dr. E.S.Santhoshkumar, Scientist, Tropical Botanical Garden and Research Institute Palode, Thrivanandhapuram, Kerala. The aerial parts of the plants were allowed to dry in shade for two weeks.

2.2. Preparation of extracts

The aerial parts of *S. chinensis* L. were shade dried and pulverized. 250 g of powdered material was packed in Soxhlet apparatus and subjected to continuous hot percolation for 8 h using 450 mL ethanol (75% V/V) as solvent. The ethanol extract was concentrated under vacuum and dried in a dessicator. Aqueous extract made by cold maceration method. About 50 g of powedered material mixed with 300 mL of distilled water and kept for 7 days at room temperature. The extract obtained from water was filtered through Whatmann filter paper No.1 and residue water content was evaporated (40 ℃) with heating mantle. The obtained extracts were stored in refrigerator and were dissociated in dimethyl sulfoxide for prior to use.

2.3. Microorganism tested

A total of 12 microorganisms were used to assess the antimicrobial properties, it includes, three gram- positive bacteria, *Staphylococcus aureus* (*S. aureus*) MTCC 96, *Staphylococcus epidermidis* (*S. epidermidis*) MTCC 435, and *Bacillus subtilis* (*B. subtilis*) MTCC 121; seven gram-negative bacteria, *Escherichia coli* (*E. coli*) MTCC 443, *Klebsiella pneumoniae* (*K. pneumoniae*) MTCC 432, *Proteus mirabilis* (*P. mirabilis*) MTCC 1429, *Salmonella paratyphi* A (*S. paratyphi* A) MTCC 735, *Salmonella typhimurium* (*S. typhimurium*) MTCC 98, *Shigella flexneri* (*S. flexneri*) MTCC 1457 and *Pseudomonas aeruginosa* (*P. aeruginosa*) MTCC 424; two fungus, *Candida albicans* (*C. albicans*) MTCC 183 and *Cryptococcus neoformans* (*C. neoformans*) clinical isolate. The microorganisms were originally obtained from Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh, India.

2.4. Minimum Inhibitory Concentration- Disc diffusion method

Antimicrobial activities of ethanolic and aqueous extracts of *S. chinensis* were determined by disc diffusion [10] and broth dilution methods. Sterile Hi-sensitivity agar (Himedia-M 486) (PH-7.2) was prepared and poured into the plates. The depth of the medium should be ~ 4 mm. Three to four similar colonies of pure cultures were inoculated with tryptone soy broth (Himedia- M 323), incubated at 37 ℃ for 2-8 h and the inoculums size was adjusted to yield uniform suspension containing 105-106 cells/mL (McFarland's standard). The agar surfaces of the plates were swabbed with test culture in three directions turning the plates to 60° between each swabbing. Confluent growth is desirable for accurate result. The sterile discs were (6 mm; Himedia) used for the loading crude plant extracts (ethanol and aqueous). Five different concentrations were prepared (250, 500, 750, 1000 and 1250 μg) and loaded in appropriate disc. The impregnated discs were incubated at 37 ℃ for an hour. The dried discs were placed over the surface of swabbed medium with equal distance to avoid the overlapping of the zone of inhibition. Pre-diffusion time was given to the swabbed plates in refrigerator condition for 5 min. The plates were incubated at 37 ℃ for 16-18 h during which the activity was evidenced by the presence of zone of inhibition surrounding the discs. Each experiment was done in triplicate. A panel of antibiotics was used against each microbial strains and which antibiotic given sensitive with particular organism is used as a control.

2.5. Minimum Inhibitory Concentration- Broth dilution Method

Tube dilution method was used to determine the minimum inhibitory concentration (MIC) of the extracts in Muller Hinton broth (Himedia-M 391) and Sabouraud Dextrose Broth (Himedia-M 033) as specified by National Committee for

Clinical Laboratory Standard (NCCLS, 1998). A total of 10 mL of each broth was dispensed into separate test tube and was sterilized at 121 ℃ for 15 min and then allowed to cool. Two-fold serial dilutions of the extracts in the broth were made from the stock concentration of the extracts to obtain 8- 4096 μg/mL for ethanolic and aqueous extracts. About 0.1 mL of the standardized inoculums of the microbes was inoculated into the different concentration of the extracts in the broth. The test tubes of the broth were incubated at 37 ℃ for 24 h and 30 ℃ for 1-7 days for bacteria and fungi respectively and observed for turbidity. The lowest concentration that showed no turbidity in the test tube was recorded as the MIC.

2.6. Determination of Activity Index

The activity index of the crude plant extract was calculated as;

$$\text{Activity Index (AI)} = \frac{\text{Zone of inhibition of the extract}}{\text{Zone of inhibition obtained for standard antibiotic Drug}}$$

3. Results

The antimicrobial activities of the extracts (ethanolic and aqueous) at different concentrations were screened by the disc diffusion method and the mean value of zone of inhibition was assessed in millimeter diameter. The results are given in the tables- 1 and 2. The minimum inhibitory concentration (MIC) was determined by the broth dilution method and the results are given in tables- 3.

The ethanolic extracts (*S. chinensis* L.), showed significant antimicrobial activity against *S. epidermidis* (33.20±1.85 mm, AI-0.83), *C. neoformans* (18.20±1.28 mm, AI-0.52), and *C. albicans* (30.40±1.78 mm, AI-1.26). Ethanolic extracts exhibits significant antifungal activity when compared with the control antifungal agents (Ketoconazole). Ethanolic extract showed significant antibacterial activity against *S. epidermidis* and the results were similar to the zones produced by the control antibacterial agent (Amoxycillin/Clavulanic acid).

In the disc diffusion method the ethanolic extract showed

Table 1
Antimicrobial activity of ethanolic extract of *Salacia chinensis* L.

Name of the microorganism	Zone of inhibition in mm					
	Ethanolic extract		Mean±SEM	Standard antibiotics (μg/disc)	Zone in mm	Activity Index (AI)
	Minimum	Maximum				
Staphylococcus aureus	9	15	12.80±1.20	Amoxycillin/Clavulanic acid (30)	40	0.32
Staphylococcus epidermidis	30	40	33.20±1.85	Amoxycillin/Clavulanic acid (10)	40	0.83
Bacillus subtilis	-	-	-	Ciprofloxacin (5)	32	0.00
Escherichia coli	-	-	-	Ciprofloxacin (5)	30	0.00
Klebsiella pneumoniae	-	-	-	Chloramphenicol (30)	30	0.00
Proteus mirabilis	9	15	12.0±1.14	Lomefloxacin (10)	25	0.48
Salmonella paratyphi A	11	16	13.60±0.81	Co-trimoxazole (25)	28	0.48
Salmonella typhimurium	-	-	-	Chloramphenicol (30)	29	0.00
Shigella flexneri	10	14	11.80±0.66	Lomefloxacin (10)	36	0.32
Pseudomonas aeruginosa	-	-	-	Lomefloxacin (10)	30	0.00
Candida albicans	25	35	30.40±1.78	Nystatin (100)	24	1.26
Cryptococcus neoformans	15	22	18.20±1.28	Ketoconazole (10)	35	0.52

Table 2
Antimicrobial activity of aqueous extract of *Salacia chinensis* L.

Name of the organism	Zone of inhibition in mm					
	Aqueous extract		Mean±SEM	Standard antibiotics (μg/disc)	Zone in mm	Activity Index(AI)
	Minimum	Maximum				
Staphylococcus aureus	9	13	11.8±0.8	Amoxycillin/Clavulanic acid (30)	40	0.29
Staphylococcus epidermidis	15	20	17.8±1.02	Amoxycillin/Clavulanic acid (10)	40	0.44
Bacillus subtilis	-	-	-	Ciprofloxacin (5)	32	0.00
Escherichia coli	9	9	9.0±0.0	Ciprofloxacin (5)	30	0.30
Klebsiella pneumoniae	8	8	8.0±0.0	Chloramphenicol (30)	30	0.26
Proteus mirabilis	-	-	-	Lomefloxacin (10)	25	0.00
Salmonella paratyphi A	-	-	-	Co-trimoxazole (25)	28	0.00
Salmonella typhimurium	8	8	8.0±0.0	Chloramphenicol (30)	29	0.27
Shigella flexneri	8	8	8.0±0.0	Lomefloxacin (10)	36	0.22
Pseudomonas aeruginosa	8	12	8.8±0.8	Lomefloxacin (10)	30	0.29
Candida albicans	-	-	-	Nystatin (100)	24	0.00
Cryptococcus neoformans	17	22	19.8±1.02	Ketoconazole (10)	35	0.56

Table 3
Minimum inhibitory concentration of extract of *Salacia chinensis* L.

Name of the microorganism	Concentration of extracts (in μg/mL)											
	4096	2048	1024	512	256	128	64	32	16	8	Control	MIC in (μg/mL)
Ethanol extract												
Staphylococcus aureus	−	−	+	+	+	+	+	+	+	+	+	2048
Staphylococcus epidermidis	−	−	−	−	−	+	+	+	+	+	+	256
Proteus mirabilis	−	−	+	+	+	+	+	+	+	+	+	2048
Salmonella paratyphi A	−	−	+	+	+	+	+	+	+	+	+	2048
Candida albicans	−	−	−	−	+	+	+	+	+	+	+	512
Cryptococcus neoformans	−	−	−	−	−	+	+	+	+	+	+	256
Aqueous extract												
Staphylococcus epidermidis	−	−	−	−	+	+	+	+	+	+	+	512
Cryptococcus neoformans	−	−	−	+	+	+	+	+	+	+	+	1024

≥ 15 mm mean zone of inhibition documented, further those microorganisms were tested for MIC by broth dilution technique. The result revealed that 256 μg/mL were obtained as MIC value against *C. neoformans* and *S. epidermidis*, 512 μg/mL was documented against *C. albicans*. The result indicated that the ethanolic extract possesses significant inhibitory activity against both fungi and bacteria.

The aqueous extracts (*S. chinensis* L.) showed significant antimicrobial activity against *C. neoformans* (19.8±1.02 mm, AI−0.56) and *S. epidermidis* (17.8±1.02 mm, AI−0.44). Aqueous extract exhibits significant antibacterial and antifungal activity and it was interestingly noted that the activity very nearer to zones produced by the control antibiotics (Amoxycillin/Clavulanic acid and Ketoconazole).

An equal and more than 15 mm mean zone of inhibition were documented in disc diffusion method, further tested for MIC by broth dilution technique. The result revealed that 512 μg/mL was obtained as MIC value against *S. epidermidis* and 1024 μg/mL was observed against *C. neoformans*. The result indicated that the aqueous extract possesses significant antimicrobial activity against a few organisms.

4. Discussion

Recently there has been considerable interest in the use of plant material as an alternative method to control pathogenic microorganism [11] and many components of plant products have been shown to be specially targeted against resistant pathogenic bacteria [12]. The emergence of multidrug resistant strain of many pathogens is a serious threat and makes chemotherapy more difficult. The toxicity of new generation antibiotics discourages their use in treatment. Moreover, the current cost of most of the chemotherapeutic agents is unbearable to the public especially in developing countries like India [13]. Therefore attempts must be directed towards the development of effective natural, non−toxic drugs for treatment.

The present work was a pioneer attempt to explore the antimicrobial property of *S. chinensis* L. (celestraceae). There were no extensive research outcomes in antimicrobial activity and a few reports only on the basis of phytopharmacological aspects of *S. chinensis* roots, antifungal activity and cytotoxic activities. The ethanolic extract of *S. chinensis* exhibited significant antimicrobial activity against *S. aureus* (33.20 mm), *C. albicans* (30.40 mm), *C. neoformans* (18.20 mm) and results were more than the control antimicrobials. The minimum inhibitory concentration result also indicated that the ethanolic extract possesses significant inhibitory activity against both fungal and bacteria. The results of aqueous extract also showed considerable antimicrobial activity against both bacteria and fungi in disc diffusion and broth dilution methods. The antimicrobial property of *S. chinensis* probably associated with the presence of phenolic derivatives and triterpenes in leaves, [14,15] and stems α−glucosidase inhibitor salacinol, dimer (II), octaacetate, hexamethyl ether, friedelane−type triterpenes, salasones D and E, norfriedelane−type triterpenes, salaquinone B, polyacylated eudesmane−type sesquiterpine, salasol B; Two new friedelane−type triterpenes, salasones D and E, new norfriedelane−type triterpenes, salaquinone B and a new polyacylated eudesmane−type sesquiterpine, salasol B, two new triterpenoids, named 7α, 21α − dihydroxyfriedelane−3−one (1) and 7α, 29− dihyroxyfriedelane−3−one (2) and 21α, 30−dihyroxyfriedelane−3−one, friedelane− type triterpenes, salasones A, B, and C, norfriedelane−type triterpenes, salaquinone A, acylated eudesmane−type sesquiterpine, salasol A [16] and 3β, 22β−dihyroxyolean− 12− en− 29− oic acid, tingenone, tingenine B, regeol A, triptocalline A, and mangiferin. [17]

It is concluded that this study would lead to the establishment of some valuable compound that has to be used to formulate new, different and more potent antimicrobial drugs of natural origin. Further studies are needed to identify the biologically active compounds and to evaluate the efficiency of the compound against pathogenic microorganisms associated with various human diseases.

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Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Katrin Basah, Berna Elya, Juheini Amin, dan Muhamad Iqbal Julian. Activity of ethanolic extracts from Justicia gendarussa Burm. Leaves on decreasing the uric acid plasma. *Makara, Sains* 2011; **15**(1): 67-70.

[2] Ghani A. In Traditional Medicine. Jahangirnagar University, Savar, Dhaka: 1990.p.15-40.

[3] Craggy GM, Newman DJ, Snader KM. Natural products in drug discovery and development. *J. Nat. Prod* 1997; **60**: 52-60.

[4] Abba D, Inabo HI, Yakubu SE, Olonitola OS. Phytochemical analysis and antibacterial activity of some powdered herbal preparation marketed in Kaduna Metropolis. *Science world J* 2009; **4**(1): 23-26.

[5] Abalaka ME, Olonitol OS, Onaolapo JA, Inabo HI. Determination of activity, time survival and pharmacokinetics of extracts from Momordica charantia on some bacterial pathogens. *Int J P App Scs* 2009; **3**(3): 6-13.

[6] Egharevba HO, Kunle OF. Preliminary phytochemical and proximate analysis of the leaves of Piliostigma thionningii (schumach.) Milne- Redhead. *Ethanobotanical Leaflets* 2010; **14**: 570-577.

[7] Deokate UA, Khadabadi SS. Phytopharmacological aspects of *Salacia chinensis*. *J of Pharmacognosy and Phytotherapy* 2012; **4**(1): 1-5.

[8] Paarakh PM, Patil LJ, Thanga SA. *J of Natural Remedies* 2008; **8**(2): 116-131.

[9] Singh A, Duggal S. Salacia species: Hypoglycemic principles and possible role in diabetes management. *Integrative Med* 2010; **2**(4).

[10]Bauer, Kriby, Sherris and Truck. *American J of Clinical Pathology* 1996; **45**: 493.

[11]Aqil F, Khan MS, Owais M, Ahmad I. Effects of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin-resistant *Staphylococcus aureus*. *J of Basic Microbiology* 2005; **45**: 106-114.

[12]Nostro A, Cellini L, Di Bartolomeo S et al., Effects of combining extracts (from propolis or Zingiber officinale) with clarithromycin on Helicobacter pylori. *Phytotherapy Research* 2006; **20**(3): 187-190.

[13]Gopalakrishnan Sarala, George Shibumon and Benny PJ. Antimicrobial effect of Punica granatum on pyogenic bacteria. *J of Pharma and Biomed Sci* 2010; **3**(06).

[14]Pongpiriyadacha AKT, Mastuda Y, Yoshikawa HMJ. Structures of new friedelane-type triterpenes and eudesmane-type sesquiterpene and aldose reductase inhibitors fron *Salacia chinensis*. Morikawa. *Nat Prod* 2003; **66**(90): 1191-1196.

[15]Yoshikawa MZ, Yi W, Tao N, Seikou MH. New triterpene constitutents, foliasalacins A1-A4, B1-B3, and C, from the leaves of *Salacia chinensis*. *Chem Pharma Bull* 2008; **56**(7): 915-920.

[16]Tran TM, Nguyen THA, Vu DT, Tran VS. Study on chemical constituents and cytotoxic activities of *Salacia chinensis* growing in Vietnam Z. *Naturforsch* 2010; **65b**: 1284-1288.

[17]Kishi A, Morikawa T, Yosikawa M. Structures of new friedelane- and norfriedelane-type triterpenes and polycylated eudesmane-type seaquiterpene from *Salacia chinensis* Linn (S.prinoides DC., Hippocrateceae) and radical scavenging activities of principal constituents. *Chem pharma* 2003.