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SEARCH FOR ANTIMICROBIAL POTENTIALS FROM *SIMMONDSIA CHINENSIS*

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ABSTRACT

Seeds of *Simmondsia chinensis* were extracted with pet ether, benzene, chloroform, ethyl acetate, methanol and distilled water for evaluating its antimicrobial activity and phytochemical analysis. The purpose of screening is to justify, authenticate the use of Indian Medicinal plants in ethnomedicinal or folklore as traditional treasure to cure various ailments. In present investigations attempts were made to screen the Indian Medicinal Plants as antibiotics. The extracts were tested against seven bacteria and three fungi through disc diffusion assay where standard tetracycline and mycostatin (for bacteria and fungi, respectively) were used. The results showed that all the extracts possess good antimicrobial activity against selected test bacteria and fungi. The preset results therefore offer a scientific basis for traditional use of the various extracts of *Simmondsia chinensis.* All the extracts possessed antimicrobial potential against all test bacteria and fungi which explains that their use in daily life will generate a resistance or immunity to fight against microorganisms. Phytochemical analysis revealed the presence of terpenoids, flavonoids, tannins and alkaloids.

INTRODUCTION: According to World Health Organization, medicinal plants are the most excellent source to acquire a variety of newer herbal 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 realize their properties, safety and usefulness.

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of immense implication in therapeutic treatments. In the preceding years, a number of studies have been conducted in different countries to establish such efficiency. Several plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant [1].

Since the preceding decade, the rise in the failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the selection of quite a few medicinal plants for their prospective antimicrobial activity [2, 3].

The microorganisms have developed resistance to many antibiotics because of arbitrary use of antimicrobial drugs that create a big dilemma in the treatment of infectious diseases [4]. With the augment in the resistance of many microorganisms to the presently used antimicrobials and the high cost of production of synthetic compounds; in addition to many side effects; there is a need to look for the alternatives.

Plants have provided a good source of antiinfective agents; emetine, quinine, berberine, tannins, terpenoids, alkaloids and flavonoids continue to be highly efficient instruments in the fight against microbial infections [5].

Alpinia galangal is traditionally used in the treatment of various ailments across India, China and Southeast Asian Countries. In India it is a reputed drug in indigenous system of medicine and largely used as antibacterial and antiseptic. In southern India, the rhizome has been used as a domestic remedy for bacterial infections [6].

Sulphurized liquid wax of *Simmondsia chinensis* is used in the treatment of psoriasis[7]. Furthermore, dermatological research suggests that the plant may be used to reduce inflammation [8].

Therefore, in present project attempts have been made to medicinal plants *Simmondsia chinensis* for evaluation of antimicrobial potentials.

FIGURE 1: (A) *SIMMOND CHINENSIS.*

MATERIALS AND METHODS:

Collection: Plant sample (*Simmondsia chinensis*) and its seed pods were harvested from female jojoba bushes from CAZRI AND REGIONAL RESEARCH STATION NBPGR JODHPUR RAJASTHAN in mid April and early June 2010 and stored on ice.

Identification: The sample was authenticated and was given identification number. The sample was authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

Sources of test organisms: Bacteria-Pure culture of all test organisms, namely *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Shigella sonnei* and *Trichophton rubrum* and fungi *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* were obtained through the courtesy of Mahatma Gandhi Institute of applied Sciences (MGiaS), Jaipur, which were maintained on Nutrient broth media.

Culture of Test Microbes: For the cultivation of bacteria, Nutrient Agar Medium (NAM) was prepared by using 20 g Agar, 5 g Peptone, 3 g beef extract and 3 g NaCl in 1 L distilled water and sterilized at 15 lbs pressure and 121°C temperature for 25-30 min. Agar test plates were prepared by pouring approximately 15 mL of NAM into the Petri dishes (10 mm) under aseptic conditions.

A saline solution was prepared (by mixing 0.8% NaCl) in distilled water, followed by autoclaving and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24-48 h. To prepare the test plates, in bacteria, 10-15 mL of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant.

Preparation of test extracts: Crushed powder (50 g) of the species was successively soxhlet extracted with ethanol. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were pooled individually. Each filtrate was concentrated to dryness in vitro and redissolved in respective solvents, out of which 80 mg/10 disc i.e. 8 mg/disc concentration were stored at 4°C in a refrigerator, until screened for antibacterial activity.

Bactericidal assay: For both, bactericidal in vitro Disc diffusion method was adopted (Gould and Bowie, 1952), because of reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whatmann No. 1 paper (5mm in diameter), which were containing

1mg, 5mg and 10mg of the text extracts and reference drugs (tetracycline and mycostatin for bacteria and fungi, respectively) separately. Such treated discs were air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h in case of bacteria and 37˚C for 48h in case of fungi, after which the zones of inhibition could be easily observed.

Five replicates of each test extract were examined and the mean values were then referred. The Inhibition Zone (IZ) in each case were recorded and the Activity Index (AI) was calculated as compared with those of their respective standard reference drugs (AI = Inhibition Zone of test sample/Inhibition zone of standard).

Phytochemical Screening:

1. **Test for Reducing sugars** (Fehling's Test): The aqueous ethanol extract (0.5gm in 5 ml of water) was added to boiling fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

2. **Test for Terpenoids** (Salkowski test): To 0.5 gm each of the extract was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoides.

3. **Test for Flavonoids**: 4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red colour was observed for flavonoids and orange color for flavons.

4. **Test for Tannins**: About 0.5 g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

5. **Test for Saponins**: To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

6. **Test for Alkaloids**: Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's regent. The alcoholic extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The sample was then observed for the turbidity or yellow precipitation.

RESULTS:

Antimicrobial Activity: The profile of the medicinal plants used in the present investigation. The results of antimicrobial activity of the crude extracts of Selected Indian Medicinal Plant (*Simmondsia chinensis*) showed good antimicrobial activity against selected test bacteria and fungi (**Table 1**). Overall, these extracts showed appreciable activity against selected test bacteria and fungi and hence, it justifies its use in our traditional system of medicine to cure various diseases (**fig. 2**).

While screening the extracts of *Simmondsia chinensis*, good antimicrobial activity against all the selected bacteria and fungi was observed. The various extracts were found active against all the bacteria and fungi tested. Results comparable to the standards, were found against, *P. aeruginosa* (9mm), *E. coli* (9.6mm), *K. pneumoniae* (10.6mm), *A. niger* (8.5mm), *A. flavus* (11mm) [Pet ether extract], *P. aeruginosa* (9.6), *E. coli* (9mm), *C. albicans* (8mm), *A. niger* (7.5mm) [Benzene extract], *P. vulgaris* (9mm), *E.coli* (8.6mm), *C.albicans* (9.3mm), *A. niger* (7mm) [Chloroform extract], *P. vulgaris* (8.3mm), *T. rubrum* (8.3mm), *K. pneumoniae* (9mm), *S. sonnei* (8.6), *A. niger* (9.5mm), *A. flavus* (9.5mm) [Ethyl acetate extract], *P. vulgaris* (8mm), *S. aureus* (14mm), *E. coli* (8mm), *K. pneumoniae* (9.3mm), *A. niger* (7.6mm) [Methanol extract], *T. rubrum* (8.3mm), *K. pneumoniae* (10mm) and *C. albicans* (8mm) [Aqueous extract].

TABLE 1: ANTIBACTERIAL EFFICACY IN TERMS OF INHIBITION ZONE OF *EMBELIA RIBES* AGAINST SELECTED BACTERIA AND FUNGI

Simmondsia chinensis Extracts	Measure	Bacteria						Fungi			
		P. vulgaris	*P. aeruginosa*	*T. rubrum*	*S. aureus*	*E. coli*	*K. pneumoniae*	*S. sonnei*	*C. albicans*	*A. niger*	*A. flavus*
	Standard I.Z.	9.1	9.9	10.9	17.9	9.8	12.3	11.9	11.4	9.7	11.8
Pet ether	I.Z. (mm)	6.3	9	--	8	9.6	10.6	13.3	17.3	8.5	11
	A.I.	0.692	0.909	--	0.446	0.979	0.861	1.117	1.517	0.876	0.932
Benzene	I.Z. (mm)	9.3	9.6	13.6	8	9	17	16	8	7.5	6.5
	A.I.	1.021	0.969	1.247	0.446	0.918	1.382	1.344	0.701	0.773	0.550
Chloroform	I.Z. (mm)	9	19	12.3	10.6	8.6	12.3	16	9.3	7	6
	A.I.	0.989	1.919	1.128	0.592	0.877	1.000	1.344	0815	0.721	0.508
Ethyl Acetate	I.Z. (mm)	8.3	14	8.3	8.6	13	9	8.6	7.5	9.5	9.5
	A.I.	0.912	1.414	0.761	0.480	1.326	0731	0.722	0.657	0.979	0.805
Methanol	I.Z. (mm)	8	13.3	7.5	14	8	9.3	7.3	6	7.6	6.3
	A.I.	0.879	1.343	0.688	0.782	0.816	0.765	0.613	0.526	0.783	0.533
Distilled Water	I.Z. (mm)	9.6	14.3	8.3	8.6	11.3	10	7	8	14.3	13.6
	A.I.	1.054	1.444	0.761	0.480	1.153	0.813	0.588	0.701	1.474	1.152

I.Z. = Inhibition zone, A.I. = Activity index. *I.Z. in mm are the mean value of the triplicates.

Results, more than the standard, were observed for *S.sonnei* (13.3mm), *C.albicans* (17.3mm) [Pet ether extract], *P.vulgaris* (9.3mm), *T.rubrum* (13.6mm), *K.pneumoniae* (17mm), *S.sonnei* (16mm) [Benzene extract], *P.aeruginosa* (19mm), *T.rubrum* (12.3mm), *K.pneumoniae* (12.3mm), *S.sonnei* (16mm) [Chloroform extract], *P.aeruginosa* (14mm), *E.coli* (13mm) [Ethyl acetate extract], *P.aeruginosa* (13.3) [Methanol extract], *P.vulgaris* (9.6mm), *P.aeruginosa* (14.3mm), *E.coli* (11.3mm), *A.niger* (14.3mm) and *A.flavus* (13.6mm). The pet ether extract of the plant showed no activity against *T.rubrum.*

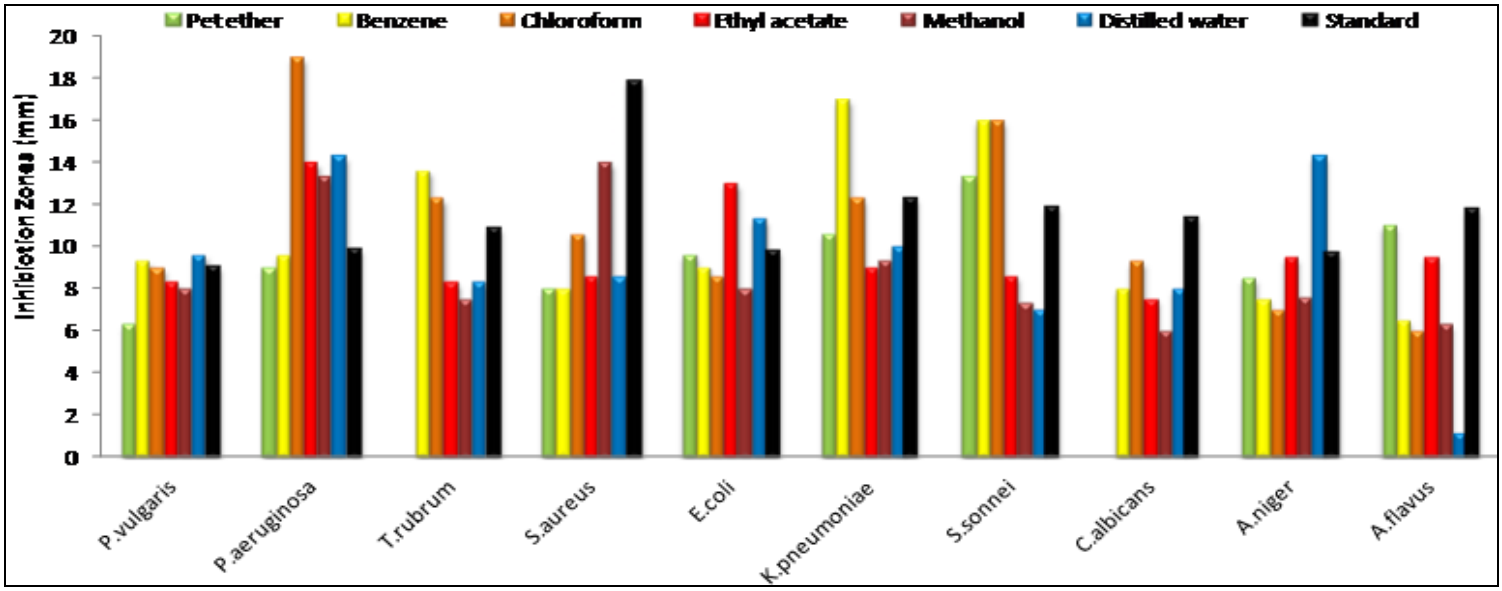

FIGURE 2: ANTIMICROBIAL POTENTIAL OF *SIMMONDSIA CHINENSIS* AGAINST SELECTED TEST MICROORGANISMS IN TERMS OF INHIBITION ZONE

Phytochemical Analysis: The phytochemical analysis of the plant exhibited a number of phytochemicals. Terpenoids, flavonoids, tannins and alkaloids were reported in both the plants. Saponins were found absent in the plant (**Table 2**).

TABLE 2: RESULTS OF PHYTOCHEMICAL STUDY

Plants → / Tests ↓	*Simmondsia chinensis*
Reducing sugars	++
Terpenoids	++
Flavonoids	++
Tannins	++
Saponins	--
Alkaloids	++

DISCUSSION: The antimicrobial screening of the plants shows its high potential to be used as therapeutic agents. The plant shows antimicrobial activity more than the standards (tetracycline and mycostatin, for bacteria and fungi, respectively) for almost all the bacteria tested. Very little work has been done on the biological activity and plausible medicinal applications of medicinal plants and hence extensive investigation is needed to exploit their therapeutic utility to combat diseases.

The present results therefore offer a scientific basis for traditional use of the various extracts of *Simmondsia chinensis*. These results explain that Indian Medicinal Plants have potentials as antimicrobials. Further, more or less the selected Indian Medicinal Plant have also possessed antimicrobial potential against all test bacteria and fungi which explains that their use in daily life will generate a resistance or immunity to fight against microorganisms.

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