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Screening of antimicrobial and antioxidant potentials of some medicinal plants from Simaroubaceae family

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Abstract
The use of plants in treatment of burns, dermatophytes and infectious diseases is common in traditional medicines. Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health related quality of human life since their introduction. The development of new antimicrobial agents against resistant pathogens is increasing interest of scintists. In the present study methanolic extracts of leaf, stem and stem bark of *Ailanthus excelsa, Quassia amara and Simarouba glauca* from Simaroubaceae family were used for antimicrobial activity. It was found that most plant extracts studied had antibacterial and antifungal activities. The methanolic extracts of *Quassia* leaf, *Simarouba* leaf and *Simarouba* bark showed strong antibacterial activity against all four bacterial species i.e. *Escherichia coli, Proteus bacilli, Staphylococcus aureus* and *Corynebacterium diphtheria*. All plant extracts were found to show better antifungal activity against *Candida albicans* as compared to *Aspergillus niger*. Antioxidant activity was evaluated by using DPPH scavenging activity. The results suggest that methanolic extracts of leaf, stem and stem bark of *Ailanthus excelsa, Quassia amara and Simarouba glauca* has promising antioxidant activity and could serve as a potential source for natural antioxidants. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

Keywords: Antibacterial, antifungal, antioxidant, medicinal plants, Simaroubaceae

Introduction
Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people everyday [1]. Morbidity and mortality due to diarrhoea continues to be a major problem in many developing countries, specially amongst children. Infections due to variety of bacterial etiologic agents such as pathogenic *Escherichia coli, Salmonella spp., Staphylococcus aureus* are the most common [1].

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health related quality of human life since their introduction [2]. However, over the past few decades, these health benefits are under threats as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions, but also with the continuous use of antibiotics microorganism have become resistant. In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, immunosuppressant and allergic reactions [3]. This has created immense clinical problems in the treatment of infectious diseases. Therefore, there is need to develop alternative antimicrobial drugs for the treatment of infectious diseases; one approach is to screen local medicinal plants for possible antimicrobial properties. Plant materials remain an important resourse to combat serious diseases in the world.

Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine [4]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organisation estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [5]. The pharmacological industries have produced a number of new antibiotics; resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents.

There are many published reports on the effectiveness of the traditional herbs against Gram-positive and Gram-negative microorganisms, and as a result plants are still recognized as the bedrock for modern medicine to treat infectious diseases [6].

Fungi can cause diseases to animals including human directly or by producing mycotoxins. Most fungi are saprophytes, whereas few fungal species are parasitic causing diseases in plants or animals. Around 5.1 million species of fungi are believed to exist on earth; however from these only few are capable of causing diseases in humans and plants.

The free radicals are species with very short half life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. These species may be either oxygen derived (Reactive Oxygen Species, ROS) or nitrogen derived (Reactive Nitrogen Species, RNS). In recent years, many studies evidenced that plants containing high content of antioxidant phytochemicals can provide protection against various diseases induced by free radicals [7]. Large number of scientific studies also revealed that the plant extracts are rich source of natural antioxidant. The present study was focused on the evaluation of the antibacterial, antifungal and antioxidant properties of different parts of *Ailanthus excelsa*, *Quassia amara* and *Simarouba glauca.*

The Simaroubaceae family includes 32 genera and more than 170 species of trees and shrubs of pantropical distribution [8]. It is characterised by its content of bitter substances, mostly responsible for its pharmaceutical properties [9]. Quassinoids can be considered a taxonomic marker of the Simaroubaceae family since it is the most abundant group of natural substances and their synthesis is almost exclusive. According to Polonsky [10] the active component of plants in the Simaroubaceae family is a group of alkaloids known as quassinoids that gives out its distinct bitter taste.

Ailanthus excelsa Roxb. belonging to family Simaroubaceae is commonly known as Maharukha. It is large deciduous tree; bark slightly bitter and leaves pinnately compound. The traditional claims, phytochemical investigation, pharmacological evaluation and some ayurvedic formulations provide the backbone to make this tree, a plant of heaven [11]. Traditionally or in Indian system of medicine, *Ailanthus excelsa* Roxb. is used in treatment of asthma, cough, cancer, diabetes and also used as antispasmodic and bronchodilator [12].

Quassia amara commonly known as bitterwood or Amargo is a small evergreen shrub growing only about 3 meter in height. It bears a small drupe, red flowers and compound, alternate leaves. *Quassia amara* Linn. Being an ethnomedicinal plant proved to have anti-diabetic properties. Quassin constituents in it are one of the bitterest substances found in nature and it has been used in management of type 2 diabetes [13].

Simarouba glauca, commonly known as 'Laxmitaru' or 'paradise tree'. The specific name *glauca* means covered with bloom which refers to the bluish green foliage. This is evergreen tree, grows to a height of 12-15 m with large circular crown. The bark and leaf extract of *Simarouba* is well known for its different types of pharmacological properties such as antihelmenthic, antiparasitic, antidysentric and anticancerous [14]. Joshi and Joshi [15] speculated that the chemicals present in leaf, fruit, pulp and seed of *S. glauca* are known to posses the medicinal properties such as analgesic, antimicrobial, antiviral, astringent, stomachic and vermifuge.

Materials and Methods

Collection of Plant Material: Fresh plant material of *Ailanthus excelsa*, *Quassia amara* and *Simarouba glauca* (leaf, stem and stem bark) was collected from nurseries. Plant material was first washed and then sundried followed by oven drying at temperature of 80ºC and then powdered using grinder.

Preparation of Extracts: Plant extracts were prepared by taking 80% of methanol as a solvent and kept in water bath for 2 hours at 60ºC. The extracts were filtered using filter paper. The filtered extracts were kept for complete evaporation of solvent in an oven at 40ºC and concentrated material was collected and weighed to obtain extracted values. The methanolic extracts of *Ailanthus* leaf (AL), *Ailanthus* stem (AS), *Ailanthus* bark (AB), *Quassia* leaf (QL), *Quassia* stem (QS), *Quassia* bark (QB), *Simarouba* leaf (SL), *Simarouba* stem (SS) and *Simarouba* bark (SB) obtained in concentrated form were dissolved in 25% of dimethyl sulfoxide (DMSO) and used to test the antibacterial, antifungal and antioxidant activity against the test organisms. The preliminary antimicrobial activity was done at different concentrations (1%, 2%,4% & 5%) of extracts. Preliminary analysis confirms the activity of most of the extracts at 2% level against the test organisms.

Microorganisms Used: Gram-positive bacteria (*Staphylococcus aureus* and *Corynebacterium diphtheria*), Gram-negative bacteria (*Escherichia coli* and *Proteus bacilli)* and fungal strains *Aspergillus niger* and *Candida albicans* obtained from department of Microbiology, Smt. CHM college, Ulhasnagar were used for evaluating antimicrobial activity.

Antimicrobial Activity: The antibacterial and antifungal activities of the above plant parts extract against four pathogenic bacteria (two Gram-positive and two Gram-negative) and two pathogenic fungi were investigated by agar well diffusion test.50 ml of sterile Nutrient Agar media was bulk seeded with 1ml of saline having bacterial suspension. The Potato Dextrose Agar (PDA) medium was poured into sterile petri plates and allowed to solidify and the fungal inoculum was seeded on PDA medium. The wells of 9mm were made on the medium using a sterile borer and 20µl of the extracts were added to respective pores. The petri plates with organisms containing extracts were incubated at 37ºC for 24 hours. After incubation they were observed for the presence of clear zones of inhibition around the well indicating antibacterial and antifungal activity. DMSO was used as negative control.

Antioxidant Assay: The *in vitro* antioxidant activity of test extracts was estimated using standard 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method. The reaction mixture contains 1.8 ml of 0.5 mM solution of DPPH and 0.2 ml of extracts. The mixture was then vigorously shaken and incubated for 10 minutes at room temperature in dark and the antioxidant activity of each extract was quantified by decolourisation at 540nm. A blank was prepared without adding extract. Lower the absorbance of the reaction mixture indicated higher free radical scavenging activity [16].

Results and Discussion

The antimicrobial activity of plant parts from three medicinal plant species has been evaluated *in vitro* against pathogens including four bacterial species (*Escherichia coli, Proteus bacilli, Staphylococcus aureus* and *Corynebacterium diphtheria*) and two fungal species (*Candida albicans* and *Aspergillus niger*). In general, most of the different plant part extracts exhibited broad spectrum of antimicrobial activity.

Table 1 illustrates that QL, SL and SB showed strong activity against all tested bacterial strains. Whereas AS and QS show poor activity only against *Corynebacterium diphtheria,* AB

and QB showed good activity against *Corynebacterium diphtheria* while poor activity against *E. coli* and *P. bacilli*, AL and SS showed poor activity against all tested bacterial strains. Amongst all plant part extracts QL showed strongest activity (38mm in diameter) against *S. aureus.* Similar to the present findings, Santhosh *et al*. [17] suggested *Simarouba glauca* has promising antioxidant activity and could serve as a potential source for natural antioxidants. Ethyl acetate and petroleum extracts of *S. glauca* leaves were tested against *Staphylococcus, Salmonella, Bacillus, Klebsiella, Pseudomonas sp*. and *E. coli* by disc diffusion method and it showed no activity towards all the tested organisms [17].

Antifungal assay showed that amongst all plant part extracts AS was not effective against both tested fungal species (Table 2). QL and SL showed strong activity against *Candida albicans*, SB and AL showed good activity and AB, QB, SS and QS were found to be with least activity against *Candida albicans.* All the plant part extracts were found to be with poor activity or no activity against *Aspergillus niger*. It has been reported by Ratha *et al*. [18] that chloroform fraction of methanol extract of *Ailanthus excelsa* leaf was proved to have antifungal activity against *A. fumigatus, A. niger, A flavus* and *P. notatum* which may be due to the presence of triterpene compound [18]. Similarly, antifungal activity of various extracts of *Ailanthus excelsa* bark against fungal strains *A. terrus, A. niger* and *A. Flavus* were reported by Patil *et al*. [19]. In their findings, petroleum ether extract showed less activity, methanolic extract exhibited moderate activity, whereas ethyl acetate showed good activity against the test organisms. According to Mikawlrawng *et al*. [20] the leaf extracts of *Simarouba glauca* has antifungal properties. They found extracts of this plant is more effective against *Aspergillus parasiticus* as compared to *Fusarium oxysporum*.

Table 3 illustrates the percentage inhibition of DPPH radicals. Antimicrobial activity was found to be effective at 2% level of drug concentration hence antioxidant activity was performed at the same concentration of all the plant part extracts. The present study revealed that different parts of all the three plants showed strong (above 90%) free radical scavenging activity. QL was found to show strongest (94.09%) scavenging activity amongst all extract. DPPH is relatively stable nitrogen centred free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichiometric ally depending on the number of electrons taken up. Substances capable of donating electrons/hydrogen atom are able to convert DPPH (purple) into their non radical form 1,1-diphenyl-2-picrylhydrazine (yellow), a reaction which can be followed spectrophotometrically [21].

Table 1: Antibacterial activity (Zone of inhibition in mm) of different plant parts of *Ailanthus excelsa, Quassia amara* and *Simarouba glauca*

Bacterial species	AL	QL	SL	AS	QS	SS	AB	QB	SB
Escherichia coli	15	30	29	-	-	17	14	11	32
Proteus bacilli	13	20	23	-	-	16	18	12	33
Staphylococcus aureus	13	38	31	-	-	16	-	-	34
Corynebacterium diphtheria	23	32	33	15	18	19	28	24	32

*Zone of inhibition including well size 9mm.
- indicates no activity

Table 2: Antifungal activity (Zone of inhibition in mm) of different plant parts of *Ailanthus excelsa, Quassia amara* and *Simarouba glauca*

Fungal species	AL	QL	SL	AS	QS	SS	AB	QB	SB
Candida albicans	22	34	33	-	12	12	15	14	29
Aspergillus niger	22	12	11	-	-	11	-	-	12

*Zone of inhibition including well size 9mm.
- indicates no activity

Table 3: DPPH radical scavenging activities of different plant parts of *Ailanthus excelsa, Quassia amara* and *Simarouba glauca*

Plant extracts	% Inhibition
Ailanthus leaf (AL)	93.84
Quassia leaf (QL)	94.09
Simarouba leaf (SL)	90.22
Ailanthus stem (AS)	92.18
Quassia stem (QS)	90.06
Simarouba stem (SS)	93.29
Ailanthus bark (AB)	91.36
Quassia bark (QB)	90.04
Simarouba bark (SB)	91.67

Conclusion

Antimicrobial activity of three plants was screened against common pathogens. In general, methanolic extracts of the selected plants appeared to be effective source of active antimicrobial agents and all the extracts showed promising antioxidant activity by DPPH, hydroxyl radical scavenging assay. From the results obtained, it is clear that these compounds could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases. Further studies are needed to better evaluate the potential effectiveness of the crude extract as the antimicrobial agents.

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