



RESEARCH ARTICLE



Received on: 06-08-2014
Accepted on: 30-08-2014
Published on: 15-09-2014

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Conflict of Interest: None Declared !

DOI: [10.15272/ajbps.v4i35.564](https://doi.org/10.15272/ajbps.v4i35.564)

Antibacterial Activity of Leaf Extracts of *Aloe Vera*, *Ocimum Sanctum* and *Sesbania Grandiflora* against the Gram Positive Bacteria

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Abstract

Diseases have been managed traditionally using medicinal plants, as they contain components of therapeutic value. In view of this, in the present investigation, phytochemical analysis of aqueous, ethanol and chloroform extracts of some herbal leaves like *Aloe vera*, *Ocimum sanctum* and *Sesbania grandiflora* and their antibacterial activities against Gram positive bacteria like *Staphylococcus aureus*, *S. epidermis*, *Streptococcus mutans*, *S. pyogenes*, *S. pneumoniae*, *Micrococcus luteus*, *Bacillus cerus* and *B. subtilis* were investigated. The phytochemical analysis revealed the presence of triterpenoid, phenol and tannin in all the extracts of the selected plants. All the leaf extracts of the selected plants inhibited the growth of the test organisms and exhibited antibacterial activity. However, the majority of the bacterial species were susceptible to all the extracts of *O. sanctum*, when compared to *A. vera* and *S. grandiflora*. This work supports the traditional use of these plants and the therapeutic value against bacterial pathogens, so that they can be alternate antibacterial agents in future.

Keywords: *Aloe vera*, *Ocimum sanctum*, *Sesbania grandiflora*, antibacterial activity and phytochemicals

Cite this article as:

Jothi Karumari R, Vijayalakshmi K, Tamilarasi L and Ezhilarasi Balasubramanian. Antibacterial Activity of Leaf Extracts of Aloe Vera, Ocimum Sanctum and Sesbania Grandiflora against the Gram Positive Bacteria. Asian Journal of Biomedical and Pharmaceutical Sciences; 04 (35); 2014; 60-63.

INTRODUCTION

Microorganisms such as bacteria, fungi and virus cause serious infections among human beings in tropical and sub tropical countries of the world. They exist in nature in all ecological conditions [1]. Diseases due to the pathogenic bacteria and fungi represent a critical problem to human health and they are one of the main causes of morbidity and mortality worldwide [2]. The resistance developed by the bacteria to various antibiotics restricts the choice of antibiotics for therapy [3]. Antimicrobial resistance is a serious threat to mankind because most of the infection causing bacteria has become multidrug resistant [4]. This adds urgency to the research for new infection fighting strategies.

In recent years, the antimicrobial properties of the medicinal plants are being increasingly reported from different parts of the world [5-7]. Therefore, researchers have turned their attention to medicinal plants to develop better drugs against microbial infections [8]. Hence in the present investigation three medicinal plants *Aloe vera*, *Ocimum sanctum* and *Sesbania grandiflora* were selected to analyze their antibacterial efficacy against few selected Gram positive bacteria. *A. vera* is a green color plant with thick, fleshy, tapered, spiny, marginated and dagger shaped leaves [9] growing from a short stalk near ground level. It is the most widely recognized herbal remedy which helps to stimulate the body's immune system [6]. *O. sanctum* - the sacred plant of India is widely distributed around the world and is used in herbal medicine for the management of various diseases [10]. The tender leaves of *S. grandiflora*, which is a herbal plant is eaten as food in various parts of South Asia and is very effective in curing many diseases [11].

Infections due to variety of pathogenic bacteria like *E. coli*, *V. cholerae*, *Salmonella Sp.*, *Pseudomonas Sp.*, and *Staphylococcus aureus* are most common. *Streptococci* are common food borne pathogenic bacteria which are frequently isolated from various foods. Food poisoning originating from contaminated foods by Gram positive and negative bacteria created concern to the society. *Streptococcus mutans* is one of the main opportunistic pathogen of dental caries [12], which plays a central role in the corrosion of tooth enamel. *Staphylococcus aureus* is one of the normal microbial floras of the skin, upper respiratory and intestinal tracts [13]. Since the medicinal values of plants lie in their phytochemical components [14], in the present investigation the phytochemical analysis was also carried out in different extracts to substantiate their efficacy.

MATERIALS AND METHODS

Plant materials

Three Indian herbal plants *Aloe vera* is belonging to Asphodelaceae family; *Ocimum sanctum* belonging to

Lamiaceae family and *Sesbania grandiflora* belonging to fabaceae family were selected for this study. The plants were collected from the local markets of Chennai and Thriuvallur, Tamil Nadu, India. The botanical identification of the plant samples were carried out and authenticated by Dr. A. Manoharan, Head of the department of plant biology and biotechnology, Presidency College, Chennai - 5.

Selection of bacterial strains, growth medium and standard drug

Authentic pure cultures of Gram positive bacterial strains were selected for the study. *Staphylococcus aureus* (ATCC No. 6538P, MTCC No. 737), *Staphylococcus epidermis* (ATCC No. 155, MTCC No. 435), *Streptococcus mutans* (ATCC No. 25175, MTCC No. 497), *Streptococcus pyogenes* (ATCC No. 14289, MTCC No. 442), *Streptococcus pneumoniae* (ATCC No. 33400, MTCC No. 655), *Micrococcus luteus* (ATCC No. 9341, MTCC No. 1541), *Bacillus cerus* (ATCC No. 11778, MTCC No. 430), *Bacillus subtilis* (ATCC No. 6633, MTCC No. 441) were the eight Gram positive bacteria used for this study.

Nutrient agar medium was selected as growth medium for bacterial strains, while Ciprofloxacin was used as the standard drug to compare the efficacy of the selected plant parts [15].

Preparation of extracts

Freshly collected leaves of *O. sanctum* and *S. grandiflora* were washed; shade dried, powered in an electrical blender and stored air tight for extract preparation. Whereas, for *A. vera* the spines of the leaf were chopped and the upper layer of the skin called rind was opened longitudinally to collect the gel. Then the gel was homogenized to make a crude paste and was used for extraction.

To prepare aqueous extract, 100 gms of dry leaf powder of *O. sanctum* and *S. grandiflora* were soaked in 1.5 liter of distilled water for 24 hours. Whereas, 100gms of crude paste of *A. vera* gel was soaked in 300 ml of distilled water for 24 hours with frequent mixing. This preparation was filtered using Whatman filter paper and the filtrate was concentrated at 100°C in a water bath.

To prepare ethanol and chloroform extracts, 100 gms of dry leaf powder of *O. sanctum* and *S. grandiflora* were soaked in 1 liter of ethanol and chloroform for 15 days, while 100 gms of crude paste of *A. vera* gel was soaked in 300 ml of ethanol and chloroform for 15 days. The filtrate obtained after filtration was concentrated at 70 - 80°C and 60 - 70°C respectively in a water bath.

Phytochemical screening

Phytochemical analysis of secondary metabolites was carried out qualitatively according to the standard methods [16 and 17].

Antibacterial assessment

Antibacterial activity of the extracts was evaluated by using paper disc agar diffusion method. The leaf extracts were used in the concentration of 50,100 and 150 µl disc and the standard drug ciprofloxacin was used in 50 µl / disc concentration.

Individual suspensions of microorganisms were added to sterile nutrient agar medium at 45°C and poured into sterile petri dishes for solidification. Sterile Whatman filter paper discs of 5 mm diameter were dipped in different concentrations of the extracts, standard and blank and were placed on the surface of agar plates. These plates were left in room temperature for pre-incubation for an hour.

These plates were then incubated at 37±1°C for 24 hours. The inhibition zone formed around the discs after 24 hours were measured using ruler in millimeter and expressed in percentage in triplets. The microbes were considered susceptible, less susceptible or resistant to a particular plant extract based on the diameter of inhibitory zone formed.

RESULTS

Phytochemical screening of secondary metabolites (Table 1) revealed the presence of triterpenoids, phenol and tannin in all the extracts of the selected plants. Whereas, presence and absence of steroid, flavonoid, alkaloid, saponin and acid showed variation in different extracts.

S. No.	Secondary metabolites	Aqueous extract			Ethanol extract			Chloroform extract		
		A. V	O. S	S. G	A. V	O. S	S. G	A. V	O. S	S. G
1	Steroid	-	-	+	+	+	+	+	+	+
2	Triterpenoid	+	+	+	+	+	+	+	+	+
3	Flavonoid	+	+	+	-	-	+	+	-	+
4	Phenol	+	+	+	+	+	+	+	+	+
5	Tannin	+	+	+	+	+	+	+	+	+
6	Alkaloid	+	+	+	+	+	+	+	-	+
7	Saponin	+	+	+	+	+	+	-	-	+
8	Acid	+	+	+	+	-	-	-	-	-

Table 1: Qualitative analysis of secondary metabolites in different extracts of selected leaves

A.V- *Aloe vera*; O.S- *Ocimum sanctum*; S.G- *Sesbania grandiflora*

(+) Present; (-) Absent

The extracts of the selected leaves were effective against the screened microbial organisms. Since all the Gram positive bacterial organisms exhibited dose dependent sensitivity in all the extract concentrations of selected leaves, the highest extract concentration (150µl) was alone taken to compare the antibacterial efficacy.

Comparison of the antibacterial efficacy of the selected herbal leaves of the present investigation revealed that

the aqueous extract of *O. sanctum* leaves is more effective against all the selected bacteria than the other two leaves (Table 2). Similarly, the ethanol and chloroform extracts of *O. sanctum* were also more effective showing maximum inhibitory zone against most of the bacteria used for the study. Among the three leaf extracts tested, ethanol extract of *A. vera* and chloroform extract of *S. grandiflora* exhibited maximum inhibition against *S. aureus*, *S. pneumonia* and *B. subtilis* are more susceptible to the ethanol extract of *S. grandiflora*. Similarly *S. mutans* was more sensitive towards the chloroform extract of *A. vera*. The higher efficacy of the aqueous extract of *O. sanctum* as an alternate herbal antibacterial agent replacing synthetic ciprofloxacin is suggested from this investigation.

S.No.	Gram positive bacteria	Zone of inhibition in percentage(%) at 150µl concentration								
		Aqueous extract			Ethanol extract			Chloroform extract		
		A.V	O.S	S.G	A.V	O.S	S.G	A.V	O.S	S.G
1	<i>S. aureus</i>	63	87	63	61	54	56	66	64	71
2	<i>S. epidermis</i>	58	74	52	62	74	63	72	92	60
3	<i>S. pyogenes</i>	58	97	58	63	87	60	74	82	76
4	<i>S. mutans</i>	66	95	73	61	82	79	76	71	63
5	<i>S. pneumonia</i>	62	92	63	74	87	89	76	90	71
6	<i>M. luteus</i>	67	97	64	58	79	76	68	77	65
7	<i>B. cereus</i>	64	74	55	61	87	74	69	77	73
8	<i>B. subtilis</i>	67	77	65	76	63	79	68	82	78

Table 2: Assessment of antibacterial efficacy of selected leaves in different extracts against selected Gram positive bacteria at 150µl concentration.

A.V- *Aloe vera*; O.S- *Ocimum sanctum*; S.G- *Sesbania grandiflora*

DISCUSSION

Plant extracts are potential sources of antimicrobial compounds especially against bacterial pathogens [18]. A medicinal herb can be viewed as a synthetic laboratory as it produces and contains a number of chemical compounds. Earlier report [19], which recorded all the microorganisms to be more susceptible to the higher concentration of the extracts, is in support of the present investigation.

The *A. vera* gel extracts were proved to possess compounds with antimicrobial properties which could be used as an antimicrobial agents [20]. Aqueous extract of *O. sanctum* exhibited growth inhibition on selected microbes in an earlier work [21], is in support of the present report. Presence of flavonoids and tannins are suggested to be likely responsible for the antimicrobial activity of *S. grandiflora* [22]. Hence, the presence of secondary metabolites such as steroid, triterpenoid, flavonoid, phenol, tannin, alkaloid, saponin and acid in the three herbal leaves of the

present investigation may be responsible for the antibacterial activity of the extracts. However, the exact metabolite responsible for the antibacterial efficacy has to be screened further. In an earlier study, it has been reported that different extracts of the same plant differ in their constituents [23] and there by their efficacy is in favor of the present findings.

Though all the bacteria tested in the present investigation were sensitive to all the plant extracts, their effectiveness varied in different extracts. The difference in the antimicrobial efficacy of the plant extracts is suggested to be depended on the variation in their phytochemicals. Less effectiveness of some of the plant extracts against the microbes may be due to the absence or insufficient concentrations of the antibacterial constituents [18]. An earlier report suggested that a greater number of extracts to be active against Gram positive bacteria [24], probably due to the absence of the outer of the bacterial cell wall. Better antibacterial efficacy of *O. sanctum* extracts against most of the bacterial strains selected in the present study may be due to presence of high concentrations of the antibacterial components in them when compared to *A. vera* and *S. grandiflora*.

CONCLUSION

Ancient medicinal systems are mainly dependent on medicinal plants. There are numerous varieties of plant species showing different range of medicinal properties to cure various diseases. Natural bioactive constituents found in parts of plants are phytochemicals. These constituents are deterrents to parasites and microbes. The present study revealed that *O. sanctum* leaf extracts could be a better alternate against most of the Gram positive bacteria analyzed in the present investigation, when compared to other leaves. In future further screening of antibacterial components of these plants will lead to establish pharmaceutically important therapeutics for the formulation of a new drug in the health care needs.

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