

## Comparative of Phytochemical and Antimicrobial of *Sesbania Grandiflora* Leaves Extract

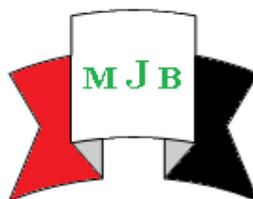
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### **Abstract**

The aim of this study was detection of the bioactive chemical constituents of sesban leaves extract by a qualitative analysis for each ethanol and methanol extract as used the Ethanol extract as antibacterial . The phytochemical analysis of the methanol and ethanol extracts of leaves of *Sesbania sesban* revealed the presence of flavonoids, alkaloids, steroids, phenols, carbohydrates and anthraquinones. While, they realed other chemical compound such as, tannins, saponins, terpenoids , amino acids and phytosterols. From the other hand, the ethanol extract of sesban was used as antibacterial against the gram positive bacteria (*Streptococcus*, *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus*, (ATCC 25923),) and the gram negative bacteria (*Escherichia coli* (ATCC 25922), *Salmonella typhi* (MTCC 733), *Proteus vulgaris* (MTCC 1771), *Pseudomonas aeruginosa* (MTCC 424), *Klebsiella pneumoniae* (ATCC 15380) and was compared with standard drugs such as, gentomacine and pencillin. The result showed greater inhibition zone in *Pseudomonas* (18 mm) at 1000mg/ml while, smaller inhibition zone in *Streptococcus* (10mm) at 500mg/ml.

**Key words:** phytochemical, SESBANIA GRANDIFLORA LEAVES , antibacterial.

### **الخلاصة**

الهدف من هذه الدراسة هو الكشف عن المكونات الكيميائية النشطة بيولوجيا لمستخلص أوراق السيسبان عن طريق التحليل النوعي لكل من مستخلصا الميثانول والايثانول واستخدام الأخير كمضاد للبكتريا. وقد كشف التحليل الكيميائي النباتي لمستخلص الميثانول والايثانول من أوراق السيبان وجود الفلوفونويد، قلويدات، المنشطات، الفينول، الكربوهيدرات والأنتراكينونات. في حين اختلفا في وجود مركبات كيميائية أخرى مثل العفص، الصابونين، تيريبيويدس، الأحماض الأمينية وفيتوسترولس. من ناحية أخرى، فإن استخدام مستخلص الایثانول من أوراق السيسبان كمضاد للبكتيريا ضد البكتيريا ايجابية الجرام (العقدية، المكورات المعوية البرازية (أي تي سي سي سي ٢٩٢١٢)، المكورات العنقودية الذهبية، (أي تي سي سي سي ٢٥٩٢٣)،) والبكتيريا سالبة الجرام (الاشريكية القولونية (أي تي سي سي سي سي ٢٥٩٢٢) والسالمونيلا التيفية (MTCC 733) ، المتقلبة الاعتيادية (MTCC 1771) ، الزائفة الزنجارية (MTCC 424) ، الكلبسيلا الرئوية (أي تي سي سي سي ١٥٣٨٠) ومقارنة ذلك مع الأدوية القياسية مثل، جنتاميسين والبنسلين. وقد أظهرت تثبيط أكبر منطقة عند الزائفة الزنجارية (١٨ ملم) في حين ١٠٠٠ mg/ml، وتثبيط أصغر منطقة في العقدية (١٠ MM) في ٥٠٠ mg/ml لذلك من هذه النتائج يمكن أن نثبت إمكانية استخدام مستخلص الإيثانول من أوراق السيسبان كعلاج للأمراض.

## **Introduction**

Throughout human history, people have relied on natural products and plants to promote and maintain good health, to fight sickness, pain and disease<sup>(1)</sup>. According to World Health Organization (WHO), traditional medicine is estimated to be used by 80% of the population of most developing countries. These plant-based medicines are used for primary health care needs<sup>(2)</sup>.

*Sesbania sesban (L.)*, is a widely available plant; it is an open branching tree tall up to 15m and 39cm in diameter belongs to family Fabaceae<sup>(3)</sup>. The chemical constituents found are galactomannans, linoleic acid, beta-sitosterol and carbohydrates<sup>(4)</sup>. The major contributors of phenolic substances in *S. grandiflora* are simple phenolics acids. Apart from this the other bioactive compounds reported in this plant are saponins<sup>(5)</sup>. Traditionally the plant has been used for the treatment of headache, in fever, as a tonic, in catarrh, as an astringent etc<sup>(6-7)</sup>. Generally bark is used as astringent and used for treatment of small pox, ulcers in mouth and alimentary canal, infantile disorders of stomach, scabies etc. The juice of leaves of the *Sesbania grandiflora* have been reported to have anxiolytic and anticonvulsant anthelmintic demulcent, expectorant, antipyretic, in treatment of bronchitis, cough, vomiting, wounds ulcers, diarrhoea, dysentery etc<sup>(6)</sup>. The flowers have been reported to have antimicrobial activity.

The medicinal value of plants lies in some chemical substances or group of compounds that produce a definite physiological action in the human body. These chemical substances are called secondary metabolites<sup>(8)</sup>. Phytochemicals with biological activity have had great utility as pharmaceuticals and pharmacological

actions. These type of activities' of herbal drugs are due to the presence of various active principals or phytoconstituents like alkaloids, glycosides, reducing sugar, tannins, saponins, resins, phytosterols, flavonoids, organic acids, essential oils, fixed oils etc. Although in recent times, synthetic drugs are used extensively in modern medicine systems<sup>(9)</sup>.

The aim of the present work is to evaluate the phytochemical composition and relationships between total phenolic contents and antibacterial potentials from *S. grandiflora*.

## **Materials and method**

### **Collection of Plant material**

The leaves of sesban plants were collected from garden of veterinary medicine collage University of Kufa from November - December 2012. The studies were conducted from march 2013. It was cleaned, washed, shade dried and powdered for the polyphenols extract study.

### **Preparation of extracts**

Extraction of the plant leaves were done with different solvents based on the polarity of the solvents. Were used as solvents methanol and ethanol

Extraction by methanol is achieved according to (10).

1- Two hundred (200) gm. of sesban leaves were crushed with 400ml of 95% methanol, mixed for 18h in magnetic stirrer at room temperature, then filtered under vacuum using Whitman No.(1).

2-The filtrate residues from step one was mixed again with 200ml of 95% methanol for 18h in magnetic stirrer at room temperature and the filtered was collected as described in step one. The filtrate substance that result from step 1 and 2 was evaporated in incubator

(42°C) to reach one –third of original volumes.

The concentrated extract was separated from low organic materials by addition of chloroform 20:100 (extract: chloroform) in separatory funnel, then the mixture was left for one hour to separate in two layers: lower layer contain chloroform and upper layer contain (total polyphenol ). The upper layer was separated with chloroform 10:100 (extract: chloroform), From the upper layer, total polyphenol was collected and dried at (40°C) then collected as powder.

- Ehtanol extracts was depending on ethanol by using soxhlet apparatus and continuous hot extraction for 72 hours at (60-70 )c° then dried and collected as powder.<sup>(11)</sup>

#### **Phytochemicals detection of the active components of sesban leaves :**

Chemical tests were carried out on the sesban leaves extract by using standard procedures to identify the constituents as follows:

**Detection of tannins:**According to <sup>(12)</sup>.

**Detection of steroids:**According to <sup>(13)</sup>.

**Detection of terpenoids:**According to <sup>(13)</sup>.

**Detection of saponins:**According to <sup>(14)</sup>.

**Detection of flavonide: NaOH Tests, Detection of phenol, Phytosterols: Salkowski Test , Liberman-Burchard's Test, Carbohydrates Molish's Test ,Amino acids Ninhydrin Test and Anthraquinones :** According to <sup>(15)</sup>.

**Detection of alkaloids:** According to <sup>(16)</sup>.

#### **The samples and culture media**

Isolates of bacteria were obtained from laboratory of microbiology of Veterinary Medicine College in Al-Kufa University. Antimicrobial activity of sesban leaves extracts against the gram positive bacteria (*Streptococcus*, *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus*, (ATCC 25923),) and the gram negative bacteria (*Escherichia coli* (ATCC 25922), *Salmonella typhi* (MTCC 733), *Proteus vulgaris* (MTCC 1771), *Pseudomonas aeruginosa* (MTCC 424), *Klebsiella pneumoniae* (ATCC 15380) were studied and compared with standerd drugs such as , pencillin and gentamaicin. The bacterial isolates were grown in Mueller Hinton Agar.

#### **Antimicrobial test**

##### **The disc diffusion method**

This method was used to determine the antimicrobial activity in vitro. In the present study, bacterial isolates were cultured by using sterile cotton swabs, the organisms will be spread across the plate so the whole plate is covered uniformly. Filter discs uniformly loaded with 50 µl of each extraction then and they were placed with sterile tweezers on to the prepared plates, and put standard antibacterial (gentomacine100/ml) and antibacterial (penciline 200/ml) was used as positive control on the prepared plates. The zones of inhibition were measured after of 18 and 24 h on the underside of the Petri dishes using transparent ruler in millimeters <sup>(17,18)</sup>

#### **Results**

In the present investigation, comparison between phytochemical of methanol sesban extract and ethanol sesban extract as shown in **table (1)**

**Table(1) : Showed the comparison phytochemical of sesban extracts**

Chemical group	Methanol extract	Ethanol extract
Tannins	++	+
Flavonoids	+	+
Saponnins	++	+
Alkaloids	+	+
Steroids	++	++
Phenols	++	++
Terpenoids	-	+
Carbohydrates	+	+
Amino acids	+	-
Anthraquinones	+	+
Phytosteolsr	++	+

(+) indicates presence, (++) indicates presence in high levels, (-) indicates absence.

The phytochemical test of the crude methanol and ethanol extracts of *Sesbania sesban* leaves Refers to presence of the same chemical compound such as, flavonoide, alkaloids, steroids, phenols, carbohydrates and anthraquinones. While, different in amount of the other

chemical compound such as, tannins, saponnins, terpenoids, amino acids and phytosterols.

The results of the antimicrobial activities with different conc. Of the ethanol sesban extract against the bacteria were shown in table (2).

**Table (2): Effect of ethanol extract of sesban plant and standard drugs on Pathogenic organism**

Test organism	standard drugs		Ethanol 500mg/ml	Ethanol 750 mg/ml	Ethanol 1000mg/ml
	Gn.	Pen.			
Streptococcus	27	12	10	11	14
Pseudomonas	25	-	13	15	18
Staphylococcus	22	23	13	13	15
Salmonella	28	-	11	15	15

Escherichia coli	30	34	13	13	15
Klebsiella	28	20	12	16	16
Proteus vulgaris	30	25	-	12	16
Enterococcus	25	20	14	15	14

Values are inhibition zone (mm) .

In the investigation of antibacterial has been shown significant to all bacteria above.

### **Discussion**

Phytochemical analysis is the characterization of an active principle responsible for some toxic or beneficial effect shown by a crude plant extract. Alkaloids are heterogeneous group compounds which contain one or more nitrogen atom in acyclic system. These are widely used for medicinal purposes and have positive or negative effects to human beings<sup>(19)</sup>. Alkaloids are reported to have analgesic, anti-inflammatory function and help to alleviate pain, develop resistance against diseases and endurance against stress<sup>(20)</sup>. A better precipitation of alkaloids was obtained in the methanol and ethanol extracts of leaves extracts of *Sesbania sesban*. The result coincides with the view of Jain *et al.*<sup>(21)</sup>, who found high degree of alkaloid precipitation in the methanol extract of *Cocculus hirsutus*.

Harborne<sup>(22)</sup> qualified flavonoids as being probably the most useful class of secondary plant constituents from a systematic point of view. The flavonoids are the compounds structurally derived from the parent substance flavone, and contain conjugated aromatic systems<sup>(19)</sup>. Flavonoids have been referred to as nature's biological compound because of their inherent ability to modify the

reaction taking place in the body due to allergies, virus and carcinogens. They show anti-inflammatory, antimicrobial and anticancer activity<sup>(23)</sup>.

Flavonoids are found in methanol and ethanol extracts of leaves extracts of *S.sesban* and show different degree of precipitation.

Phenols are reported as antitumour agents and exhibit antioxidant properties<sup>(24)</sup>. The methanol and ethanol extracts of leaves of *S.sesban* showed a better precipitation of phenolic content. Similar results were reported for methanol leaf extracts of *oxalis corniculata*<sup>(25)</sup>.

Phytosterols were found to be present in all the five extracts of the plant parts. Sterols and triterpenes are based on the cyclopentane perhydrophenanthrene ring system. In recent years, an increasing number of these compounds have been detected in plant tissues. These phytosterols are probably ubiquitous in occurrence in higher plants and occur as both free and as simple glucosides<sup>(19)</sup>. Similar observations are made from the plant parts of *Ichnocarpus frutescens*<sup>(26)</sup>. Today, natural products derived from plants are being tested for the presence of new drugs with new modes of pharmacological action, utilizing the special feature of higher plants to produce a large number of secondary metabolites<sup>(27)</sup>.

Highly significant degree of activity was observed against the test bacteria *Pseudomonas* with 18 mm in diameter followed by *Proteus vulgaris* and *Klebsiella* with 16 mm in diameter at 1000 mg/ml of the extract. In most of the bacteria examined, a better zone of inhibition was obtained at 500mg/ml and 750 mg/ml of the extract. When compared to the standard drugs, the plant extract showed a substantial amount of inhibition in the case of *Escherichia coli* (13 mm), *Enterococcus faecalis* (14.5 mm). A fluctuating trend of inhibition zone was found against some pathogens in the analysis. Similar fluctuation trend of inhibition zone was reported by<sup>(28, 29)</sup>. This may be due to the fact that at higher concentrations, the rate of diffusion may perhaps be varied and hence, it might not be available to react with the microorganisms.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay and in the recent years several reports available on the antibacterial activity of plant extracts on human pathogenic bacteria<sup>(30)</sup>. The beneficial effects of treatment can be achieved with leaves extract of *S.sesban* for various bacterial infectious diseases like pneumonia, diarrhoea, urinary tract infection and even some skin disease. The broad antibacterial activities could be as a result of the plant secondary metabolites like alkaloids, flavonoids, tannins, phytosterols etc., present in the extracts.<sup>(31)</sup> reported that tannins had been widely used topically to sprains, bruises and superficial wounds as such, it could be probable that tannins and other plant phenols from this extract were responsible for these broad activities. Some of these observations have helped in identifying the active

principle responsible for such activities and in developing drugs for the therapeutic use in human beings.

### **Conclusion**

Phytochemical study showed the presence of phytochemicals such as alkaloids, flavonoids, phenols and phytosterols in *Sesbania sesban* which might be responsible for their therapeutic effects. It further reflects a possibility for the development of many more novel chemotherapeutic agents or templates from the plant which in future may serve for the production of improved therapeutic plant based drugs. In conclusion, the stem extract of *Sesbania sesban* possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial and fungal diseases.

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