

EVALUATION OF ANTIBACTERIAL ACTIVITY OF SEED EXTRACTS OF *VIGNA UNGUICULATA*

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ABSTRACT

Aqueous and ethanolic extracts of seeds of *Vigna unguiculata* collected from the local area of Guntur were studied for antibacterial activity. The antibacterial activity was tested against Gram positive bacteria, *Bacillus subtilis* and Gram negative bacteria, *Escherichia coli* by agar well diffusion method. Different concentrations (100 µg/ml, 200 µg/ml and 300 µg/ml) of the extracts were incorporated into the wells. An extra well was bored into which the standard drug tetracycline (300 µg/ml) was poured. Both the extracts showed concentration dependant activity against the microorganisms investigated. The results showed the highest positive antibacterial activity with an inhibition diameter of 22 mm in case of aqueous extract of 300 µg/ml concentration, against the Gram negative bacteria; *Escherichia coli*. The *E.coli* species were found to be more sensitive than that of the *Bacillus subtilis*. The aqueous extract exhibited more antibacterial activity against both the Gram positive and Gram negative organisms than that of the ethanolic extract. The purification and the determination of the active components responsible for the antibacterial activity from *Vigna unguiculata* seeds are under investigation.

Keywords: *Vigna unguiculata*, Seeds, Antibacterial, Nutrient agar medium, Tetracycline, Agar well diffusion method.

INTRODUCTION

Antibacterials are the agents which can effectively treat the infections caused by the various strains of bacteria. The frequency of life threatening infections caused by the pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in developing countries [1]. The antimicrobial properties have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their medicinal properties [2]. The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives and in this, the folk medicine is playing an important role to treat infections [3]. Plants are the cheapest and safest alternative sources of antimicrobials [4, 5, 6]. The antibacterial activity was performed by using different methods like agar-well diffusion method and agar disk-diffusion assay [7]. One of such plants which can be effectively used as an antibacterial agent is *Vigna unguiculata*.

Vigna unguiculata which is most commonly called as "cow pea" is an edible legume of the family Fabaceae with high protein contents. It is a twining bushy glabrous, annual plant. The fruit pods grow up to 90 cm long and the pods are slightly depressed between the seeds. Each pod is 10 – 20 seeded. The seeds vary in size, shape and colour. The seeds are sweet and they have astringent, laxative, diuretic, anthelmintic, antibacterial and galactagogue properties. The seeds also help in relieving the conditions like anorexia, constipation, jaundice and general debility [8]. Hence the present study focused to investigate the role of aqueous and ethanolic extracts of *Vigna unguiculata* seeds as antibacterial agents against some human pathogenic bacteria.

MATERIALS AND METHODS

Plant material

Collection and authentication of plant materials

The seeds of *Vigna unguiculata* belonging to the family Fabaceae were collected in the month of January, 2013 from the local areas of Guntur district, Andhra Pradesh, India. The plant material was identified and authenticated by Dr. Sreenivasa prasanna, M. Pharm., PhD, Department of Pharmacognosy, M. L. College of Pharmacy, Singarayakonda, Andhra Pradesh, India and a voucher specimen (MPESFPS - 02/13) was preserved in the Department of Pharmacology, Malineni Perumallu Educational Society's Group of Colleges (Integrated Campus), M. L. Faculty of Pharmaceutical Sciences, Pulladigunta, Guntur, Andhra Pradesh, India.

Processing of sample

The seeds of *Vigna unguiculata* were collected, pulverized into fine powder and used for extraction.

Preparation of extracts

Preparation of aqueous extract of *Vigna unguiculata* seeds

The powdered dried seeds were subjected to extraction process by maceration with water at room temperature for 48 hours with occasional stirring. The extract was filtered and then concentrated to dryness at room temperature.

Preparation of ethanolic extract of *Vigna unguiculata* seeds

The powdered seeds were loaded into the Soxhlet extractor and subjected to extraction with ethanol. After extraction, the solvent was distilled off and the extract was concentrated to dryness at room temperature.

Phytochemical analysis

The aqueous and ethanolic extracts of *Vigna unguiculata* seeds were subjected to preliminary phytochemical screening.

Test microorganisms

The bacterial strains used in the present study are *Escherichia coli*, a Gram negative, and *Bacillus subtilis*, a Gram positive organism, which were obtained from the Department of Biotechnology, Bapatla College of pharmacy, Bapatla, Andhrapradesh, India.

Screening of Antibacterial activity

The agar well diffusion method was used to determine the inhibitory effects of the seed extracts against the microbes [9, 10]. The bacterial isolates obtained were first grown in nutrient broth for 18 hrs at 37°C. Simultaneously, nutrient agar medium was prepared and it was poured into sterile petri dishes while hot and then they are allowed to set. After this stipulated period, 0.2 ml of the broth culture of the bacteria was aseptically inoculated on this plate using a sterile cotton swab and allowed to dry [11]. Confluent growth is desirable for accurate results. Wells of 6 mm size was made in the agar plates with the help of the sterile stainless steel borer and these wells were loaded with different concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml) of the aqueous and ethanolic extracts of *Vigna unguiculata* seeds. A standard (tetracycline) solution which acts as a positive control was also placed (300 µg/ml) in an extra well along with the extracts. Then these plates were incubated at 38°C for 24 to

48 h. The zones of inhibition were measured in millimeter diameter using meter rule [12].

RESULTS AND DISCUSSION

Physical properties of the extracts

The extracts obtained showed better yields. The color, texture and the percentage yield of the aqueous and ethanolic extracts of *Vigna unguiculata* were tabulated in table 1.

Phytochemical analysis

Preliminary phytochemical screening of aqueous and ethanolic extracts of *Vigna unguiculata* seeds was performed by using various phytochemical identification tests. These tests revealed the presence of carbohydrates, alkaloids, glycosides, saponins, tannins and polyphenols in both the aqueous and ethanolic extracts of *Vigna unguiculata*. Whereas the aqueous extract showed the flavonoids in addition and the steroids were observed only in the ethanolic extract. However the fixed oils were not detected in both the extracts. The

results of preliminary phytochemical screening of aqueous and ethanolic extracts of *Vigna unguiculata* seeds were showed in table 2.

Antibacterial activity

Both the aqueous and ethanolic extracts showed better action against the Gram positive bacteria (*Bacillus subtilis*) than that of the Gram negative organisms (*Escherichia coli*) when compared to that of the standard drug tetracycline. The standard drug tetracycline showed a zone of inhibition of 20 mm. The aqueous extract of *Vigna unguiculata* showed better action against *Bacillus subtilis* than that of the ethanolic extract. Whereas in case of a Gram-negative bacteria (*Escherichia coli*), the standard drug tetracycline showed a zone of inhibition of 24 mm. An increase in the antibacterial activity was observed with respect to the increase in the dose of the extracts. The aqueous extract of *Vigna unguiculata* showed better action against *Escherichia coli* than the ethanolic extract. The results indicating the antibacterial activity of aqueous and ethanolic extracts of seeds of *Vigna unguiculata* against *Bacillus subtilis* and *Escherichia coli* were tabulated in table 3.

Table 1: Physical properties of *Vigna unguiculata* seed extracts

Plant part	Type of Extract	% Yield	Texture	Colour
<i>Vigna unguiculata</i> seeds	Aqueous extract	14.4	Gel	Creamish
	Ethanolic extract	10.2	Semisolid	Brownish

Table 2: Phytochemical analysis of *Vigna unguiculata* seed extracts

Phytochemicals	Aqueous Extract	Ethanolic Extract
Carbohydrates	+	+
Fixed oils	-	-
Glycosides	+	+
Alkaloids	+	+
Flavonoids	+	-
Tannins	+	+
Saponins	+	+
Steroids	-	+
Polyphenols	+	+

Presence: +, Absence: - .

Table 3: Antibacterial activity of Aqueous and Ethanolic Extracts of Seeds of *Vigna unguiculata* against *Bacillus subtilis* and *Escherichia coli*

Bacteria	Concentration of the plant extracts (µg/ml)	Zone of Inhibition(in mm)		Zone of Inhibition of Positive Control (Tetracycline 300 µg/ml) (in mm)
		Aqueous Extract	Ethanolic Extract	
<i>Bacillus subtilis</i> (Gram Positive Organism)	100	12	10	-
	200	14	14	-
	300	18	16	20
<i>Escherichia coli</i> (Gram Negative Organism)	100	16	14	-
	200	18	16	-
	300	22	20	24

Microgram/milliliter: µg/ml, Millimeter: mm.

These results were interesting because in the traditional method of treating a bacterial infection, decoction of the plant parts or boiling the plant in water is employed whereas according to the present study, preparing an extract with an organic solvent was showed a better antibacterial activity [13]. In the whole study it was observed that the plant extracts showed high activity against a Gram positive organism than a Gram negative organism. The plant parts which were used for the study may contain the bio-components whose antibacterial potentials are highly comparable with that of the tetracycline, against all Gram negative and Gram positive bacteria tested. The activity of the plant parts against both the Gram negative and Gram positive bacteria may be an indicative of the presence of broad spectrum antibiotic compounds in the plant [14]. Today most pathogenic organisms are becoming resistant to antibiotics [15]. To

overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency [16]. Thus *Vigna unguiculata* could become a promising natural antibacterial agent with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. If plant extracts are to be used for medicinal purposes, the issues of safety and toxicity should be monitored.

CONCLUSION

Thus from the present study it was concluded that both the aqueous and ethanolic extracts of seeds of *Vigna unguiculata* possesses good antibacterial activities which supports the traditional folklore. Further work should be done for the isolation and characterization of active principles responsible for the antibacterial activity and to establish the effectiveness

and pharmacological rationale for the use of *Vigna unguiculata* as an Antibacterial drug.

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