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Antibacterial Activity and Phytochemical Analysis of *Vigna aconitifolia*.

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ABSTRACT

The unmethodical and indiscriminate use of commercial antimicrobial drugs has led to the development of multidrug resistance, complicating the choice of empirical therapy. Characteristics of *Vigna aconitifolia* (moth bean) sprouts and seeds with reference to its antimicrobial activity and phytochemicals have been investigated. Moth beans in local language are also known as Matki. Both extracts of Matki were able to inhibit the growth of *Salmonella and Proteus*. Phytochemicals like Tannins, Flavonoids, Saponins, Steroids, Carbohydrates, and Coumarins were detected in *Vigna aconitifolia*.

Keywords: *Vigna aconitifoli*, moth beans, phytochemical, antimicrobial activity

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INTRODUCTION

The Legumes (including alfalfa, clover, lupines, green beans and peas, peanuts, soybeans, dry beans, broad beans, dry peas, chickpeas, mung bean, lentils and moth bean) are an important meal of the human diet in all over the world, especially in the developing countries. India is as one of the important legume producing nation accounts 29% of world area and 19% of world production [1]. The nutritional importance of legumes, are due to presence of low fat, dietary fiber, high protein content and many micronutrients [1].

Vigna aconitifolia (matki) is a drought-resistant legume, commonly grown in arid and semi-arid regions of India. Phytochemicals are the bioactive compounds that occur naturally in plants. Leguminous seeds are important source of proteins and source of natural antioxidants. Legumes contain a number of phenolic compounds such as flavonoids (stress protection) and phenolic acids (exert preventive activity against infectious and degenerative diseases, inflammation and allergies) [2].

The emerging resistance of many Gram positive and negative enteric pathogens continues to pose threat, yet such problem is unparallel with the discovery of alternative agents to battle the issue [3]. Recent bans and restrictions on the use of animal antibiotic growth promoters stimulated interest in bioactive secondary metabolites of plant source as alternative performance enhancers [3]. Enzymes, peptides, and polyphenols extracted from matki beans can possess both antimicrobial and antifungal activities. The mung (*Vigna radiata*) has been studied extensively and shown to have many different medicinal properties but the matki which belongs to the same family is not that widely researched. Evaluation of phytochemical compositions of the different moth bean accessions remains unexplored and hence scanty [4]. Hence the present study focused on determination of the phytochemical constituents and the antimicrobial activity of *Vigna aconitifolia*.

MATERIALS AND METHODS

Plant Materials

Matki sprouts and dried matki seeds were procured from local market and ground to fine paste and powder by using mechanical grinder.

Test Organisms

Gram negative bacteria like; *Escherichia coli*, *Salmonella typhimurium*, *Salmonella paratyphimurium*, *Proteus*, *Klebsiella*, *Shigella*, *Pseudomonas* Gram positive bacteria like; *Staphylococcus aureus* and fungus like *Candida* were used as test organisms to determine the antimicrobial activity of matki. These test organism were procured from the sister institute, Dr. D. Y. Patil Medical Research Center, Pune, India

Preparation Of Extracts

30g of matki seeds powdered and 30g of sprout paste were used for extraction in 300ml of absolute ethanol using a soxhlet apparatus. The ethanol content was evaporated to 1/4th the volume (diluted extracts) and this was used as extracts for all the studies.

Phytochemical Analysis

Qualitative phytochemical analyses for the extracts were performed according to [6] and the protocols for each test, are mentioned in the table 1.

Table 1: Protocols for Phytochemical analysis of *Vigna aconitifolia*

Phytochemical Test	Protocol
Tannins (<i>Braymer's Test</i>)	500µl extract + 500µl H ₂ O + 1 drop FeCl ₃ (5%)
Flavonoids	1000µl extract + 500µl ammonia solution + 100µl H ₂ SO ₄ (conc.)
Saponins (<i>Foam Test</i>)	(a) 500µl extract + 500µl H ₂ O + heat (b) 500µl extract + Olive oil (few drops)
Steroids (<i>Salkowski Test</i>)	500µl extract + 500µl CHCl ₃ + 500µl H ₂ SO ₄ (conc.)
Phlobatannins (<i>Precipitate Test</i>)	500µl extract + 500µl HCl (1%) + heat
Carbohydrates (<i>Molisch's Test</i>)	500µl extract + 2500µl H ₂ O + 1 drop Ethanol α-naphthol (20%) + 500µl H ₂ SO ₄ (conc.)
Glycosides (<i>Liebermann's Test</i>)	500µl extract + 500µl CHCl ₃ + 500µl CH ₃ COOH
Coumarins	500µl extract + 700µl NaOH (10%)
Anthocyanins	500µl extract + 500µl HCl (2N) + NH ₃

*Note: These tests were performed for both seed and sprout extracts

Antimicrobial Assay [5]

The bacteria and yeast were inoculated in Luria Bertani and Sabouraud Broth (both from HiMedia) respectively. The antimicrobial activity was checked by well diffusion method. Briefly, overnight grown microbial cultures were spread on sterile Mueller-Hinton agar (HiMedia) plates, wells were bored and 100µl of extract samples were added to the respective well. Plates were incubated for 15min at 4°C for pre-diffusion and then incubated at 37°C for 18-20 hr. Zone of inhibition of the test organism were measured to indicate the antimicrobial activity of the extract.

RESULTS

Phytochemical Analysis

Preliminary phytochemical analysis of ethanol extracts of *Vigna aconitifolia* sprouts and seed powder was performed by using various qualitative tests. These tests revealed the presence of Tannins, Flavonoids, Saponins, Steroids, Carbohydrates, Coumarins in both the extracts of *Vigna aconitifolia*, but at varying intensity. Tannins, Flavonoids was detected more in the sprout extract whereas, the powder extract showed more of the Steroids, Carbohydrates, and Coumarins. However, Phlobatannins, Glycosides, and Anthocyanins were not detected in both extracts. The results of phytochemical screening of both extracts of *Vigna aconitifolia* seeds are shown in table 2.

Table 2: Phytochemical analysis of *Vigna aconitifolia* (matki)

Phytoconstituents	Observations	Sprout extract	Powder extract
Tannins (<i>Braymer's Test</i>)	Green precipitate	+++	+
Flavonoids	Yellow coloration	+++	+
Saponins (<i>Foam Test</i>)	Froth and emulsion	++	++
Steroids (<i>Salkowski Test</i>)	Reddish brown ring at junction	+	+++
Phlobatannins (<i>Precipitate Test</i>)	No red precipitate	Negative	Negative
Carbohydrates (<i>Molisch's Test</i>)	Reddish violet ring at junction	+	+++
Glycosides (<i>Liebermann's Test</i>)	No colour change	Negative	Negative
Coumarins	Yellow coloration	+	+++
Anthocyanins	No colour change	Negative	Negative

Antimicrobial Assay

The powdered extract of Matki showed antimicrobial activity against almost all the organisms except *Shigella*. Both species of *Salmonella* were highly sensitive to the powdered extract. Same was seen with *Proteus*. The results indicating the antibacterial activity of both extracts of *Vigna aconitifolia* against all test organisms are tabulated in table 3 and Figure 1.

Table 3: Diameter of Zone of inhibition against different organisms

Microorganism	Sprout extract	Powder extract
<i>Escherichia coli</i>	14	15
<i>Shigella</i>	13	-
<i>Klebsiella</i>	12	11
<i>Proteus</i>	-	18
<i>Salmonella typhimurium</i>	12	21
<i>Salmonella para typhimurium</i>	-	20
<i>Staphylococcus</i>	10	12
<i>Pseudomonas</i>	15	13
<i>Candida</i>	13	12

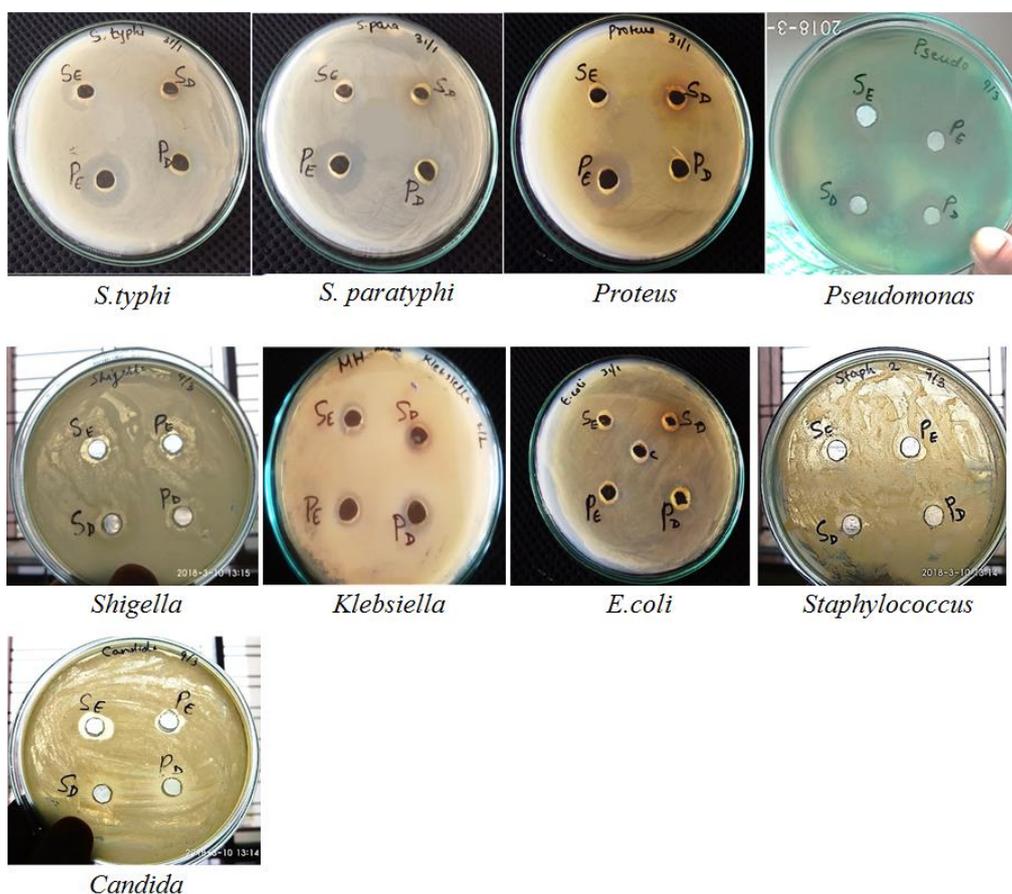


Figure1: Antibacterial activity of matki extracts against different microorganisms

DISCUSSION

Present study showed the presence of Tannins, Flavonoids, Saponins, Steroids, Carbohydrates, Coumarins in the matki seeds. A similar study with matki also showed the presence of alkaloids, flavonoids and tannin [7, 8]. The seed powder extract of *V. aconitifolia* showed a significant effect against gram negative bacteria *Salmonella* and *Proteus*. However, it did not show any effect on the *Shigella*. The anti-bacterial activity of various plants belonging to same genus has been reported earlier including *Vigna radiate* [9], *Vigna unguiculata* [10]. The presence of flavonoids, saponins and alkaloids itself indicates the possession of antibacterial activity [11]. The various phytochemicals are related with many diseases and health related problems to cure and prevent various physiological disorders [12]. Thus the presence of tannins, flavonoids, saponins and steroids in the matki points out at its different medicinal properties like anti-obesity, anti-cancerous, anti-oxidative, anti-diabetic, anti-inflammatory

activity, anti-hypertensive activity etc. Anti-inflammatory activity and anti-hypertensive activity was confirmed by us in a different study [13].

Many bacterial and fungal pathogens are responsible for many harmful diseases in humans and animals. Now, the plants extracts are explored as natural antimicrobial alternative to control the harmful microbes. Extracts of matki seeds and sprouts had different level of antimicrobial activity. *Salmonella* and *Proteus* were strongly inhibited by the seed extract. Similar results were obtained in a study, where *Vigna radiata* sprouts (mung bean) extract showed antimicrobial activity against Gram negative enteric bacteria like *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella spp.* [12,14]. However, the *in vitro* antibacterial activity of *Vigna aconitifolia* root extract showed significant antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus wernerii*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Proteus mirabilis* [15].

CONCLUSION

From the present study it was concluded that both the seed and sprout extracts of *V. aconitifolia* possesses good antibacterial activities. Thus further work is needed to determine the principle component for this antimicrobial activity and to establish the rationale for its use as an antimicrobial drug. Tannins, flavonoids, steroids, saponins, carbohydrates and coumarins have been found to be present in both the extract. Seed powered extract showed the highest zone of inhibition against *Salmonella typhimurium*, *Salmonella paratyphimurium* and *Proteus*.

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