

Phytochemical Analysis and Antimicrobial Activity of Spaghetti Squash towards Some Pathogenic Microbes

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Abstract: *The present study of Spaghetti squash plant seed extract was screened to detect the presence of the active metabolites like alkaloids, saponins, tannins, phylobtannins, steroids, glycosides and terpenoids. The solvents used for extraction of Spaghetti squash seed were methanol, chloroform, acetone, benzene and petroleum ether. The extracts were tested against Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus and Escherichia coli microorganisms using well diffusion method at different concentrations: 75 µg/mL, 150 µg /mL and 250µg /mL). The methanol and acetone extracts were showed strong inhibition compared to other extracts on all bacterial strains. Our study also showed that species, strains and concentrations of Spaghetti squash extract are some of the factors that may influence the sensitivity of the tested bacteria. A significant correlation was observed between zone of inhibition and concentration of extract.*

Keywords: Spaghetti squash, Extract, Strains, Phytochemical analysis, Antimicrobial activity

1. Introduction

In nature many plants and plants seed provided source of medicine at the earlier times. Medicinal plants are the foundation of many important drugs of the modern world. Plants are now playing an important role in many medicines like allopathic medicine, herbal medicine, homoeopathy and aromatherapy. Many of these local medicinal plants are used as spices and food items. Many Researchers have estimated that each serving increase in fruits, vegetables, herbs and spices decreases the risk of cancer by 15%, heart disease by 39% and death by any causes 20%. This idea has been established by epidemiology study (Steimez, 1996). Pumpkin is grown for use as a vegetable and as medicine. Traditionally, it has been used as medicine in many countries such as China, Argentina, India, Brazil, Korea, Mexico and Iran (Popovic, 1971; Jia et al., 2007; Adolfo and Michael, 2007), since pumpkin seeds are rich not only in proteins but also a rich source of antioxidants vitamins such as carotenoids and tocopherols (Stevenson et al., 2007) and minerals, and low in fats and calories. Some of them are used in traditional medicine and some are still not explored scientifically for their medicinal values. So, the present study of Spaghetti squash plant extract taken for phytochemical analysis study as well as antimicrobial activities.

2. Materials and Method

2.1 Collection of Plant Seed and Strains

The Spaghetti squash plant seeds were obtained from Tamil Nadu Agriculture University, Coimbatore. Bacterial strains Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus and Escherichia coli were obtained from Vivek Laboratory, Kanyakumari. All bacterial strains were subculture frequently every 15days and maintained on nutrient agar slants.

2.2 Preparation of Extract

The whole plant seeds were cleaned and shade dried for 10 days. The dried seed were pulverized by an electrical blender to get powder form. Then 50ml of solvents were separately mixed in 5g of seed powder. The mixture was kept in the dark area for 3days at room temperature. After 3days, mixture was filtered through whatmen No-1 paper. All the extract was kept 4°C for further uses.

2.3 Preliminary Screening of Phytochemicals

The different qualitative tests were performed for establishing profile of given extract for its chemical composition. The following tests were carried out on extract to detect various phyto constituents present in Spaghetti squash plant seeds extracts.

1. Tannin

Equal volume of extract and distilled water was boiled at 100°C for 10min in water bath. Then it was filtered after that adds 5-6 drops of 1% ferric chloride. Green coloration indicated the presence of tannin.

2. Saponin

Extract was diluted with distilled water (1:4) ratio. The mixture was shaken vigorously. The result observed on standing for a stable froth formation.

3. Terpenoid

Extract, chloroform and conc.H₂SO₄ were carefully added (5:2:3). Reddish brown coloration interface was indicated the presence of terpenoid.

4. Alkaloids

Mayer's reagent 2-3 drops was added in 1ml of extract. Reddish precipitate indicated the presence of alkaloid.

5. Steroid

Acetic acid 2ml was added in 500 μ l extract and 2ml conc.H₂SO₄ added mixture. Violet to blue indicated the presence of steroids.

6. Flavonoid

Conc.HCL 6-8 drops was carefully added in equal volume of extract and 1N NAOH added mixture. Yellow to orange coloration indicated the presence of flavonoids.

7. Reducing sugar

1ml of extract was dissolved in 10ml distilled water. Then equal volume of fehling's solution A & B were added. Then the mixture was boiled at 100⁰C in water bath. Brick red precipitate indicated the presence of reducing sugar.

2.4 Antimicrobial activity determination

Antibacterial activity was determined by using the agar-well diffusion method (Naz et al., 2012). Mueller Hinton Agar was used as media.

1. Agar well diffusion

The MHA plates were prepared by pouring 20ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 minutes and 0.1 % inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. Wells were bored into the medium using a sterile 6 mm diameter cork borer. Then different concentrations of extracts (75,150 and 250 μ g/ml) were loaded on well separately. The solvent was allowed to evaporate on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37⁰C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

3. Results

To study the antibacterial activity of the seed extracts of Spaghetti squash in different solvents (chloroform, acetone, benzene, petroleum ether and methanol). The concentration chosen were 75 μ g , 150 μ g and 250 μ g/ml the experiment was performed using well diffusion method. The antibacterial activity was tested against the pathogenic organism *Pseudomonas aeruginosa*, *Proteius vulgaris*, *Staphylococcus aureus* and *Escherichia coli* were shown in Table 2. In Table 2, all the bacterial strains were found to be sensitive to acetone and methanol. It was observed that zone of inhibition was seen for all the organisms of both extracts. But, it is evident that the methanol extracts were comparatively more effective than acetone extracts. A higher zone of inhibition was seen for the organism *Pseudomonas aeruginosa* and

Staphylococcus aureus. In the well diffusion method a higher zone of inhibition was observed in 150 μ g /ml and 250 μ g /ml of seed extracts of acetone and methanol. Table (2) showed the phytochemical screening of the seed extracts of Spaghetti squash. Steroids only present in the extracts.

Table 1: Phytochemical analysis of Spaghetti squash seed extract

S.No	Phytochemical Tests	Results
1	Alkaloids	-
2	Saponins	-
3	Tannins	-
4	Flavonoids	-
5	Phlobatannins	-
6	Steroids	+
7	Glycosides	-
8	Terpenoids	-

(+) Phyto constituent present

(-) Phyto constituent absent

Table 2: Antibacterial activity of different extracts of Spaghetti squash seed extracts

Name of the bacterial strain	Different extract	Zone of inhibition (mm)		
		Extract concentration		
		75 μ g/ml	150 μ g/ml	250 μ g /ml
<i>Pseudomonas aeruginosa</i>	Methanol	-	20	24
	Chloroform	-	-	-
	Acetone	13	18	20
	Benzene	-	-	-
	Petroleum ether	11	15	17
<i>Proteius vulgaris</i>	Methanol	-	-	5
	Chloroform	-	-	-
	Acetone	-	-	9
	Benzene	-	-	-
	Petroleum ether	-	-	-
<i>Staphylococcus aureus</i>	Methanol	17	20	22
	Chloroform	-	-	-
	Acetone	14	16	21
	Benzene	-	-	-
	Petroleum ether		7	11
<i>Escherichia coli</i>	Methanol	-	6	8
	Chloroform	-	-	-
	Acetone	-	-	10
	Benzene	-	-	-
	Petroleum ether	-	-	-

(-) no inhibition

4. Discussion

Plants and their products have been used for many years for human health. There are still many plants which have various medicinal values but still not explored and used. Plants contain many novel compounds with medicinal values which need scientific exploration. The antibacterial compounds extracted from these plants might inhibit bacteria by a different mechanism to that of currently used antibiotics and have therapeutic values as antibacterial agents. Methanol and acetone extracts have showed good antibacterial activity against *Pseudomonas aeruginosa* and

Staphylococcus aureus strain. Out of the two doses (150µg and 250µg) of extracts, higher doses showed greater antibacterial activity. The chloroform and benzene extracts did not inhibit growth against Escherichia coli, Pseudomonas aeruginosa and Proteus vulgaris. Methanol extract of Manglefera indica seeds was done against 61 bacterial strains. The extract showed potent antibacterial activity against all strains tested (Vaghasiya, 2011). The present study we tested the methanolic extract of Spaghetti squash and it showed potent antimicrobial activities. Ethanol extract is more likely to be selected for further pharmaceutical experimentation for human and animal use. This is because methanol is risky due to its high toxicity and not applicable for usage. It is noticed in this study, as expected, that inhibition of microorganisms is directly proportional with the concentration of the extract. The use of Mueller Hinton's agar along with a unified inoculum size (0.5 McFarland) follows the standard methods (Clini, 2011). But is it expected that antimicrobial activity and inhibition zone diameter might have been affected by factor related to its diffusion in agars such as the Mueller Hinton's. Generally, the methanol extract of Spaghetti squash exhibited higher antibacterial effect compared with other extracts. The extraction of the biologically active compounds from the plant material depends on the type of solvents used in the extraction procedure. According to Parekh et al. (2006), methanol, Ethanol and water are the most commonly used solvents for determining the antimicrobial activity in plants.

5. Conclusion

The present study investigates the seed Spaghetti squash contain potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against various diseases.

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