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# Chemical Examination of Bio-Active Constituents from Seeds of *Sesbania sesban* Linn

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Due to various medicinal properties of seeds and roots of *Sesbania sesban* we report the chemical examination of seeds of *Sesbania sesban* Linn.

## INTRODUCTION

*Sesbania sesban* Linn[1,2] belongs to natural order leguminosae and occurs in plains of Himalayas to Ceylon and North West. It is commonly known as Jayanti in Hindi. The Ayurvedic system of medicine describes its seeds to be stimulant, astringent and is useful in chronic ulcers, diarrhoea, excessive menstrual flow and in small pox eruptions. Its bark is an astringent. Roots are useful in treatment of fevers, ulcers, diabetes and relieves throat troubles. Its leaves are useful in hydrocele and in all pains and inflammations.

## EXPERIMENTAL

About 3 kg of air dried and powdered seeds of *Sesbania sesban* was extracted with petroleum ether (40–60°C) in a soxhlet extractor for 3 days. The petroleum ether extract (2 L), after being concentrated under reduced pressure to 100 mL was kept overnight in refrigerator. A yellow coloured deposit was separated out at the bottom of the flask. It was separated by filtration and its study was in progress. The fatty material (28 g) thus obtained from petroleum ether extract was saponified by caustic potash (15 g) in 95% alcohol (500 mL) for 5 h. After removal of excess of alcohol, the soap formed was cooled and dissolved in water. The soap solution was shaken thoroughly with ether in a separating funnel. After removal of the unsaponified matter (ether distilled off, study is in progress), fatty acids were liberated from soap solution by addition of conc. $H_2SO_4$ and were extracted with ether. The excess of acid was removed by washing the ethereal extract with water and then dried over anhydrous sodium sulphate. The mixed fatty acids (16.50 g) thus obtained by distillation with ether were found to have S.V. 211.2, S.E. 241.9 and I.V. 20.7. The mixed fatty acids were further separated into solid and liquid fatty acids by "Twitchells lead salt alcohol"[3] process as modified by Hilditch[4] and co-workers. The methyl esters of both solid and liquid fatty acids were prepared. The results were recorded as below.

| Fraction | Quantity (mg) | I.V. | S.V. | S.E. |
|---|---|---|---|---|
| Solid | 11.24 | 2.80 | 171.02 | 169.01 |

The methyl esters were fractionally distilled and were identified by their saponification and iodine values. Further confirmation of methyl esters was done by their Co-paper and Co-TLC[5] with authentic samples.

The observations and results are recorded below:

| Acid | Wt. of methyl esters (mg) | $R_f$ values of methyl esters | Weight of acid (mg) | % of acid in mixed acids |
|---|---|---|---|---|
| Palmitic | 3.74 | 0.42 | 4.32 | 23.39 |
| Stearic | 6.79 | 0.28 | 6.22 | 39.49 |
| Oleic | 0.95 | 0.50 | 0.28 | 1.98 |
| Linoleic | 4.95 | 0.56 | 4.62 | 25.39 |

The defatted seeds (20 g) obtained above were subjected to test for its amino acid composition. This was done by hydrolysing the defatted material by 6 N HCl for 24 h at 110°C. The hydrolysate was dissolved in water (50 mL), filtered and concentrated to dryness. The excess of acid was removed by repeated evaporation and finally dissolved in 10% isopropanol. The solution thus obtained was subjected to paper chromatography and identity of amino acids was confirmed by co-chromatography with authentic specimens. The results are tabulated as below:

| S. No. | Amino acids identified | $R_f$ reported[5, 6] | $R_f$ observed |
|---|---|---|---|
| 1. | Lysine | 0.48 | 0.50 |
| 2. | Cysteine | 0.28 | 0.27 |
| 3. | Alanine | 0.61 | 0.62 |
| 4. | Arginine | 0.57 | 0.57 |
| 5. | Glycine | 0.55 | 0.56 |
| 6. | β-Phenyl alanine | 0.56 | 0.55 |
| 7. | Valine | 0.41 | 0.40 |
| 8. | Leucine | 0.76 | 0.75 |
| 9. | Tyrosine | 0.64 | 0.65 |
| 10. | Glutamic | 0.51 | 0.50 |
| 11. | Aspartic | 0.44 | 0.45 |
| 12. | Histidine | 0.79 | 0.78 |
| 13. | Citruline | 0.25 | 0.25 |
| 14. | Norvaline | 0.65 | 0.64 |

The photometric[7] method was adopted for the quantitative estimation of amino acids (expressed in mg of glycine per 16 mg of nitrogen). The results were recorded in the following table.

| Amino acids | Quantity (expressed in mg) | Amino acids | Quantity (expressed in mg) |
|---|---|---|---|
| Lysine | 2.01 | Leucine | 2.79 |
| Cysteine | 1.06 | Tyrosine | 1.30 |
| Alanine | 0.82 | Glutamic | 2.34 |
| Arginine | 1.21 | Aspartic | 1.82 |
| Glycine | 1.81 | Histidine | 0. 79 |
| β-Phenyl alanine | 1.90 | Citruline | 0.09 |
| Valine | 2.01 | Norvaline | 0.21 |

The seed extract of the plant with 35% ethanol at 10°C for 24 h was tested for their antimicrobial activity against following bacterial and fungal species.

| Bacterial species | Fungal species |
|---|---|
| *Proteus vulgaris* | *Aspergillus flavus* |
| *Escherichia coli* | *Aspergillus niger* |
| *Salmonella stanley* | |
| *Bacillus anthracis* | |
| *Klebsiella pneumoniae* | |
| *Staphylococcus qureus* | |

The antimicrobial activity was tested by filter paper disc method[8] and soft nutrient agar (2%) petri plates previously seeded with the test species were used. The "oxide nutrient broth" and "Sabaraud's broth" agar media[9] were used to check antibacterial and antifungal activities respectively. The activities of various microbes were expressed in terms of the diameter of the zone of inhibition.

The results were tabulated as below:

## ANTIBACTERIAL ACTIVITY

| Bacterial species | Diameter of zone of inhibition (mm) | |
|---|---|---|
| | Ethanolic ext. from seeds of *S. sesban* | Control* |
| *P. vulgaris* | 12 | 18 |
| *E. coli* | 8 | 27 |
| *S. stanley* | 11 | 25 |
| *B. anthracis* | 16 | 22 |
| *K. pneumoniae* | 14 | 15 |
| *S. qureus* | 12 | 30 |

*Streptomycin and Acromycin 400 ppm against gm. positive and gm. negative bacteria.

## ANTIFUNGAL ACTIVITY

| Fungal Species | Diameter of zone of inhibition (mm) | |
|---|---|---|
| | Ethanolic ext. from seeds of *S. sesban* | Control* |
| *Aspergillus niger* | 12.6 | 20 |
| *Aspergillus flavus* | 8.2 | 19 |

*β-naphthol (1000 ppm).

## RESULTS AND DISCUSSIONS

Our results indicated the presence of four fatty acids *viz.* palmitic, stearic, oleic and linoleic acid, from seeds of *sesbania sesban.* Amino acids identified from seeds of *sesbania sesban* were lysine, cysteine, alanine, arginine, glycine, β-phenyl alanine, valine, leucine, tyrosine, glutamic, aspartic, histidine, citruline

β-phenyl alanine, valine, leucine, tyrosine, glutamic, aspartic, histidine, citruline and norvaline. Out of these only leucine, glycine, valine and β-phenyl alanine were found to predominate over rest of amino acids. The ethanolic extract of the seeds of the plant was tested for their antimicrobial efficacy and their activity varied widely in the degree of their susceptibility to gram positive and gram negative bacteria. The extract was found to be more active against *Proteus vulgaris, Klebsiella pneumoniae, Bacillus anthracis* and *Aspergillus niger.*

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