International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 7 | Issue 2 | APR-JUN | 2017 | 06-13
Original Research Article – Pharmaceutical Sciences

PHYTOCHEMICAL ANALYSIS OF *MUCUNA PRURIENS* AND *HYOSCYAMUS NIGER* SEEDS

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ABSTRACT

Traditionally the seed are used by indigenous peoples at world level for snakebite due to its ability to synthesize antibodies against the antigen i.e venom. The medicinal value of plant depends on chemical substances that elicit physiological actions on the human body. The knowledge on the chemicals define the value of folk medicine. So, Mucuna pruriens and Hyoscyamus niger seeds were analysed for its phytochemicals quantitatively, qualitatively, for its nutrient content, secondary metabolites, antioxidant, antimicrobial activities. The observed FTIR result shows Mucuna pruriens tannin and saponin was found to be higher, while in Hyoscyamus niger seeds, the tannin and phenol was higher. Among the nutrients studied, protein was rich in both the seeds studied. Likewise, the flavonoid content was rich in both the seeds thereby, inducing reducing power and total antioxidant activity in both the seeds. Antibacterial activity was higher in Salmonella typhi with Mucuna pruriens while the other sample showed no positive result with bacteria. So, it is concluded, that both the seeds are therapeutically more important emphasizing further scientific research to validate its potency.

KEY WORDS

Antioxidants, FTIR, HPLC, Seeds

Introduction

The evaluation of plant is based on phytochemical and pharmacological approaches leading to the drug discovery through natural product screening. Hence, we have selected *M. pruriens* and *Hyoscyamus niger* seeds for our present study. *M. pruriens* is a leguminous plant otherwise called as velvet bean is used as a forage, fallow, green manure crop enriched with proteins [1] vitamins such as niacin, ascorbic acid, aminoacids, glutathione, lecithin, gallic acid, beta sitosterol, L-DOPA, synthesizing dopamine linked with mood, sex. The fatty acids present are palmitic, oleic, stearic, behenic, linoleic, linolenic acid. It possesses antioxidant, hypoglycemic, lipid lowering, neuroprotective activities.[2] *Hyoscyamus niger* is analgesic[3,4] in nature mostly used as a hallucinogen and narcotizing agent,[5,6] containing anticholinergic alkaloids tropane, atropin, scopolamine and hyoscine.[7,8] 20% of the plants contain alkaloids which are produced in roots, then transported to parts of the plant. It is used to treat asthma, diarrhea, abdominal pain and urinary incontinence, parkinsons, hysteric disease. Locally, it is used to wipe out parasitic worms, rheumatism, insomnia, removes gas in the alimentary tract, bleeding disorder. While using natural products we must know the limit whether we are making it a poison or drug. Antioxidants are important in the prevention of human diseases by functioning as a free radical scavenger, complexer of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation etc. Antimicrobials from plant origin show therapeutic potential in the treatment of infectious diseases while mitigating side effects of using synthetic antimicrobials. Hence, the present study was designed to analyze phytochemicals qualitatively and quantitatively as well as phytonutrients, secondary metabolites, antioxidant activity.

Methods

Sample collection

Samples were purchased from local herbal shop at Salem, Tamil Nadu, India.

Extract preparation

Aqueous extract was prepared by dissolving 15g of powdered *M. pruriens* and *Hyoscyamus niger* seeds powder in 200ml water or laboratory use and heated on a hot plate (constant stirring) at 30-40°C for 20minutes, cooled and filtered through filter paper and used for qualitative,[9-11] antioxidant, phytonutrient analysis by adopting standard procedures.

QUALITATIVE ANALYSIS

Test for glycosides:

To the extract added aqueous NaOH. Formation of yellow color indicates glycosides.

Test for flavonoids:

In a test tube containing 0.5 ml of extract, 5-10 drops of dilute HCl and ZnCl or magnesium were added the solution was boiled for a few minutes. Presence of reddish pink or dirty brown color confirms flavonoid.

Test for saponins:

In a test tube containing 0.5ml of aqueous extract, a drop of sodium bicarbonate was added, shaken vigorously. Appearance of froth confirms saponins.

Test for steroids:

To 2ml of chloroform extract, 1ml of concentrated H_2SO_4 was added carefully along the sides of the test tube. Appearance of red color in the chloroform layer confirms steroids.

Test for carbohydrate

Molisch test:

To the extract added few drops of alcoholic alpha naphthol solution. Few drops of concentrated sulphuric acid was added along the sides of test tube to get a violet colored ring at the junction.

Fehling's test:

To the extract added equal amount of Fehling's A and B solution and then the tubes were kept in a boiling water bath. Brick red precipitation of cuprous oxide formation confirms reducing sugar.

Test for Tannin and Phenolic compound:

Ferric chloride test:

To the extract added ferric chloride. Greenish black color confirms a positive result.

Potassium dichromate test:

To the extract added potassium dichromate solution. Positive result was confirmed by brown precipitate formation.

Oil test:

Blue color denoted positive result when the extract was mixed 1ml of 1% copper sulphate and 10% sodium hydroxide.

Fourier transform infrared analysis

Sample preparation

For FT-IR analysis, the samples were ground in a mortar and pestle to reduce the average particle size to 1 or 2 microns. The Fourier transform infrared spectrum was recorded using FT-IR - AGILENT - Model Number: CAP-15T at Tamil Nadu Test House PVT Limited, Chennai.

HPLC

High performance Liquid Chromatography was performed at Tamil Nadu Test House PVT Limited, Chennai using AGILENT- Model Number :1100 With DAD.

SECONDARY METABOLITES

The phenol and flavonoid content of aqueous leaf extract was analyzed.

Determination of Total phenol content

Total phenolic content was determined by Folil-ciocalteau method.[12] To 0.1ml of extract add folin ciocalteau reagent (5 ml, 1:10 diluted with distilled water) stand for 5 min and aqueous $NaCo_3$ (4ml, 1M) was added, incubated for 15min.The phenol liberated was determined by colorimetric method at 765 nm. The standard curve was prepared, expressed in terms of Gallic acid equivalent (mg/g of dry mass).

Estimation of flavonoids

Aluminium chloride method [13] was used for the determination of the total flavonoid content. To 0.1ml of extract added 0.1ml of $AlCl_3$ (10%). The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30min of incubation. The concentration of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.

ANTIOXIDANT ASSAYS

Nitric oxide scavenging assay, reducing power, Total antioxidant assay was performed.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was estimated.[14] Sodium nitroprusside (10mM) in phosphate buffered saline was added to 0.1ml of extract and kept at RT for 150min, followed by addition of 0.5ml of Griess reagent. The absorbance was read at 546nm. Quercetin was used as a reference compound.

Reducing power assay

Reducing power assay was performed.[15]To 0.1ml of extract, phosphate buffer (2.5ml, 0.2M, P^H 6.6) and potassium ferriccyanide (2.5ml 1%), incubated for 20min at 50^0C, 1.0 ml of trichloro acetic acid (10%) was added to stop the reaction, centrifuged at 3000rpm for 10min. To 1.5ml of upper layer solution add 1.5ml distilled water and $FeCl_3$ (0.1ml, 0.1%), contents were mixed and incubated for 10 min and the absorbance was measured at 700nm. Vitamin C was used as a positive control.

Total antioxidant capacity

Total antioxidant capacity assay[16] is based on the reduction of Mo (V1) to Mo (V) by the analyte allowing green phosphate/Mo (V) complex at acidic pH. The total antioxidant activity is expressed as equivalents of ascorbic acid.

Analysis of phytonutrients

Total carbohydrates, proteins, aminoacids were performed according to the standard prescribed methods.

Estimation of carbohydrate

The total carbohydrate was estimated.[17] To 0.1 ml of extract, add 4ml of anthrone reagent, heat for 8-10minutes in a boiling water, cooled and read at 630nm using spectrophotometer Schimadzu Model - UV 1800. Glucose forms a standard.

Estimation of protein

The total protein was estimated.[18] To 0.1ml of extract, add 2ml of alkaline copper reagent, mixed well and kept for 10minutes, followed by 0.2ml of Folin ciocalteau reagent (diluted in the ratio of 1: 2), incubated for 30minutes, read at 660nm using spectrophotometer Schimadzu - Model UV 1800. BSA was used as a standard. With the standard graph unknown concentration was studied.

Estimation of amino acids

The amino acid was estimated [19] To 0.1 ml of extract, add 1 ml of ninhydrin solution dissolved in Butanol: Acetone. Close the tubes to avoid evaporation. With mild stirring, heated at 80-100ºC, 4-7 minutes. Cooled and read at 570nm. Tyrosine standard was used.

STASTICAL TOOL

The Mean and Standard deviation (S) was calculated by using the following formula:

Mean = Sum of x values / n (Number of values)

$$s = \frac{\sqrt{\sum(X-M)^2}}{n-1}$$

Antibacterial assay

Preparation of Inoculum:

The *Escherichia coli, Bacillus punilus, Salmonella typhimurium, Staphylococcus aureus* were pre-cultured in nutrient broth overnight in a rotary shaker at 37°c, centrifuged at10,000rpm for 6min, pelletwas suspended in double distilled water and the cell density was standardized spectrometrically (nm).

Preparation of test sample:

For the anti-microbial tests, extracts were diluted in dimethyl sulfoxide (DMSO): Methanol (1/1: v/v) solvent to a concentration of 20mg/ml. Anti-bacterial activity was measured using agar dilution technique. Briefly the extracts were dissolved in dimethyl sulfoxide (DMSO, Merck) and serially diluted in molten Muller Hinton Agar (MHA, Sigma) in petri dishes (100mm×15mm). The solvent did not exceed 1% concentration and did not affect the growth of the organisms. For bio-assays, suspension of approximately 1.5×10^8 bacterial cell /ml in sterile normal saline were prepared and about1.5ml of was uniformly seeded on Muller-Hinton-Agar medium with 3-4mm thickness in 12cm×1.2cm glass petri dishes, left aside for 15min.and excess of suspension was then drained and discarded properly. Wells of 60mm in diameter and about 2cm apart were punctured in the culture media using sterile cork boreos. Well were filled with 0.1ml of each 20µg/ml concentration of each sample (2µg/well) and incubated at 37 °c for 48hrs.Bio-activity was determined by measuring diameter of inhibition zones(DIZ) in mm.

Results

The results obtained from the Qualitative and Quantitative analysis was shown below.

Qualitative analysis

Table 1 shows the result of phytochemicals present in *Mucuna pruriens* and *Hyoscyamus niger* seeds. All the

phytochemicals were found to be present in both *Mucuna pruriens* and *Hyoscyamus niger* seeds.

Table 1. Qualitative analysis of Phytochemicals in *Mucuna pruriens* and *Hyoscyamus niger* seeds

Phytochemicals Tested	*Mucuna pruriens* seeds	*Hyoscyamus niger* seeds
Glycosides	++	++
Flavonoids	+++	++
Saponins	++	++
Steroids	++	++
Phenols	++	++
Alkaloids	++	++
Molish's test	+++	++
Fehling's test	+++	++
Oil test	+	+
Ferric chloride test	++	++
Potassium dichromate test	+++	++

+++ Stronger, ++ Moderate, + Mild reaction

Quantitative analysis using FTIR

Table 2 shows the results of *Mucuna pruriens* and *Hyoscyamus niger* seeds. From the result, we could observe that, *Mucuna pruriens* contain higher amount of saponin (42.3), flavonoids (36.78) and moderate amount of tannin (12.3), lesser amount of phenol (5.6) and alkaloid levels were found to be very low (1.89). While in *Hyoscyamus niger* seeds phenol (45.83) was observed in higher quantity, moderate amount of tannin (12.3) with lesser amount of flavonoid (5.6). The saponin and alkaloids were found to be below the quantification limit.

Table 2. FTIR spectrum results of *Mucuna pruriens* and *Hyoscyamus niger* seeds

Phytochemicals	Spectrum results of *Mucuna* ***pruriens*** seeds	Spectrum results of *Hyoscyamus* ***niger*** seeds
Total phenol as gallic acid equivalent	05.60	45.23
Total tannin as tannic acid equivalent	12.30	12.30
Total flavonoids as quercetin equivalent	36.78	05.60
Saponins	42.30	BQL(LOQ:0.1)
Alkaloids	01.89	BQL(LOQ:0.1)

BQL - Below Quantification Limit, LOQ – Limit of Quantification

Quantitative analysis using HPLC

The results of flavonoids analysed in *Mucuna pruriens* seeds through HPLC was shown in Fig.1 and Table.3. Similarly, the HPLC results of *Hyoscyamus niger* seeds was depicted in Fig.2 and Table.4.

Fig.1 HPLC chromatogram of *Mucuna pruriens* seeds -Flavonoids

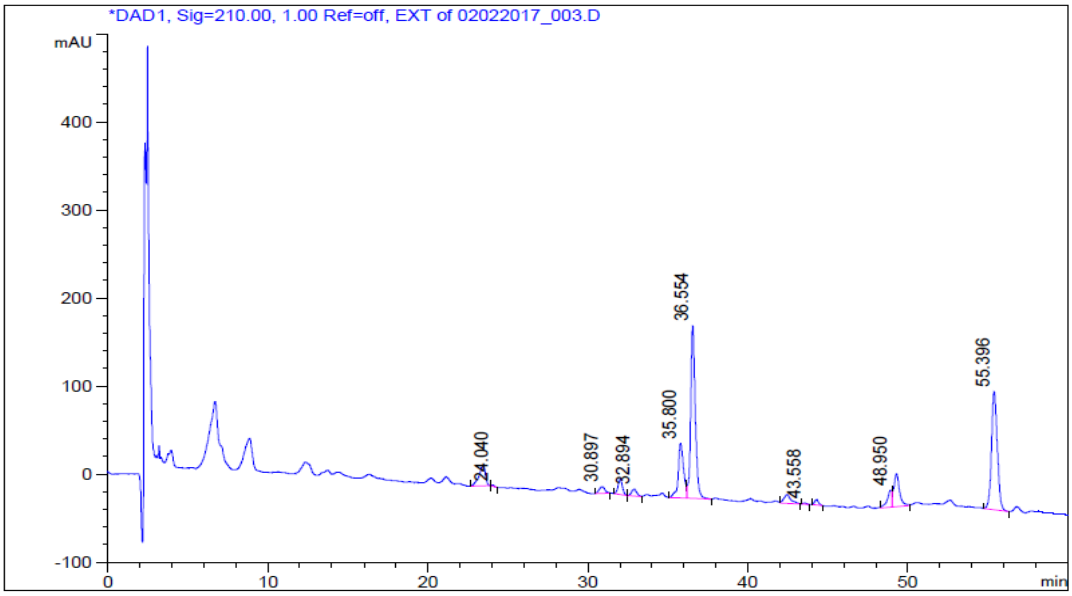

Table.3. Details of area percent / peak for *Mucuna pruriens* seeds

Peak	RT (min)	Height	Area	Area%
1	23.451	023.747	0658.421	05.522
2	24.040	001.358	015.762	00.132
3	30.897	007.757	0197.284	01.655
4	32.026	017.253	0330.087	02.769
5	32.894	007.815	0172.766	01.449
6	35.800	062.035	1460.992	12.254
7	36.554	195.768	3933.778	32.994
8	42.436	009.852	0298.065	02.500
9	43.558	001.503	0020.476	00.172
10	44.298	005.909	0103.672	00.870
11	48.950	019.156	0390.825	03.278
12	49.296	037.429	0923.408	07.745
13	55.396	134.084	3417.135	28.661

Fig.2. HPLC chromatogram of *Hyoscyamus niger* – Flavonoids

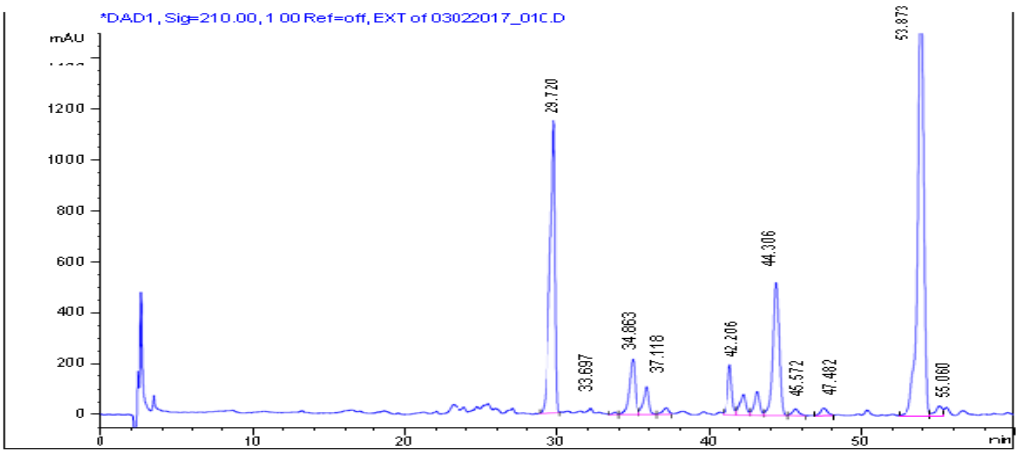

Table.4. Details of area percent / peak for *Hyoscyamus niger*

Peak	RT (min)	Height	Area	Area%
1	29.720	1.152e3	27910.453	25.434
2	33.697	7.542	115.362	0.105
3	34.863	216.757	5658.413	5.516
4	35.845	108.735	2524.251	2.300
5	37.118	24.244	599.446	0.546
6	41.299	197.594	3992.832	8.639

Table 5. Analysis of Secondary metabolites, Phytonutrients, Antioxidant activity in *Mucuna pruriens* and *Hyoscyamus niger* seed

Compounds / activity studied	*Mucuna pruriens* seeds (mg/g)	*Hyoscyamus niger* seeds (mg/g)
Phytonutrients		
Protein	27.50±1.410	15.50 ±1.410
Amino acid	04.58±2.960	02.55± 0.000
Carbohydrate	00.59±1.279	02.50± 0.282
Secondary metabolites		
Total phenolics	2.6±0.141	01.46 ±0.083
Flavonoids	4.7±0.000	11.80 ±0.070
Antioxidant activity		
Nitric oxide	02.40±0.00	03.30 ±0.00
Reducing power	35.50±0.00	36.00± 0.00
Total antioxidant	54.75±0.00	32.25 ±2.12

Values are Mean ± SD for three experiments

Table. 6. Antibacterial activity of *Mucuna pruriens* and *Hyoscyamus niger* seed

Test organism	NCIM	*Mucuna pruriens* zone diameter (mm)	*Hyoscyamus niger* seed zone diameter (mm)
Escherichia coli	2065	1.4	Nil
Bacillus pumilus	2327	0.9	Nil
Salmonella typhii	2501	1.6	Nil
S taphylococcus aureus	5345	1.4	Nil

Nil- No zone was observed

Quantitative analysis

The results of secondary metabolites, phytonutrients, antioxidant activity of *Mucuna pruriens* and *Hyoscyamus niger* seeds was shown in Table.5.

Table.5 shows the results of biochemical parameters analysed in *Mucuna pruriens* and *Hyoscyamus niger* seeds. *Mucuna pruriens* seed contain higher protein content (27.50±1.410 mg/g), moderate levels of amino acid (04.58±2.960 mg/g) and very low levels of carbohydrate (00.59±1.279 mg/g). Among the secondary metabolites assessed, flavonoid was found to be higher (4.7±0.000mg/g) compared to total phenolics (2.6±0.141mg/g). The antioxidant (54.75±0.00 mg/g), reducing power activity (35.50±0.00 mg/g) was higher compared to nitric oxide scavenging activity (02.40±0.00mg/g). Likewise, with *Hyoscyamus niger* seeds, the protein content was higher (15.50 ±1.410mg/g), whereas, the aminoacid and carbohydrate content was lower (02.55± 0.000, 02.50± 0.282mg/g). Among the secondary metabolites studied, the flavonoid content was found to be higher (11.80 ±0.070mg/g), compared to phenolics (01.46 ±0.083mg/g). Similar to *Mucuna pruriens* seeds, the antioxidant (32.25 ±2.12mg/g), reducing power activity (36.00± 0.00mg/g) was found to be higher with *Hyoscyamus niger* seeds, and nitric oxide scavenging activity (3.30 ±0.00) was reduced.

Antibacterial activity

Table.6 shows the results of antibacterial activity of *Mucuna pruriens* and *Hyoscyamus niger* seeds.

The result shows that the antibacterial activity was found to be higher with *Mucuna pruriens* seeds compared to *Hyoscyamus niger* seeds as no zone of inhibition was observed. The zone of inhibition observed for *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* was 1.4mm, 1.6mm, 1.4mm while *Bacillus pumilus* showed 0.9mm. The obtained results show that it could be a better inhibitory agent for *E. coli* causing anemia / kidney failure, *Salmonella typhi* causing typhoid, *Staphylococcus aureus* causing skin and soft tissue infections, *Bacillus pumilus* causing sepsis.

Conclusion

In India, *Mucuna pruriens* is considered an aphrodisiac, diuretic, emmenagogue, nerve tonic, uterine stimulant. *M. pruriens* seed powder revitalize the harmonic balance of male reproductive hormones in infertile men, activates the enzymes of metabolic pathways and energy metabolism. *Hyoscyamus niger* seeds are poisonous to rodents, fish, pigs, children. Any poison at right dose could act as a drug. From our studies, we could find that both the samples were therapeutically beneficial due to its antioxidant activities induced by phytochemicals.

Acknowledgement

The author wish to acknowledge Beloved Honorable Vice-Chancellor Prof. Dr.C.Swaminathan Avl and Respected Registrar Dr. M.Manivannan, Periyar University, Salem, Tamil Nadu, India for their administrative support as well as for offering lab facilities and also the authors thank Tamil Nadu Test House PVT Ltd, Chennai for performing analysis.

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