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# Effect of Different Extraction Conditions on Total Alkaloids, Total Phenolic and Total Flavonoid Content of *Vigna mungo* L hepper

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## ABSTRACT

*Vigna mungo* L Hepper is an important pulse crop cultivated in India. It is rich source of many nutritional compounds and it is an integral part of diet. Bioactive compounds like phenolic, alkaloids, flavonoids, saponins etc. are present in the seeds. Objective is to determine the content of bioactive compounds present in the black gram seeds and to study effect of sample pretreatment, fractionation on total alkaloid, total phenolic and total flavonoids content. Three types of extracts were prepared by using ethanol as a solvent by using Soxhlet extractor. First Ethanolic extract (EE), second extract prepared from pretreated seeds with an acid (EEH) and third fraction from ethanolic extract (EF) were prepared. Total alkaloid content were determined spectrophometrically by using Bromocresol green using Atropine as a standard. Total phenolic content was estimated spectrophometrically by using Gallic acid as a standard. Quercetin was used as a standard for estimation of total flavonoids content. The total phenolic content of the EE, EF and EEH extract, was 20.0 ± 5.28, 21.03±5.04 and 17.8±5.77 Gallic acid equivalents/g respectively. The total flavonoid content of EE, EF and EEH extract was 166.7± 3.66, 304.2 ±3.48 and 112.5± 3.95 quercetin equivalents/g. The total alkaloid content of EE, EF and EEH extract, was 121.9±3.77, 154.8±3.60 and 202.1±3.49 Atropine equivalents/g. various treatments have effect on extraction of bioactive compounds. Extract from pretreated seeds with acid improved extraction of alkaloids. Fractionation of extract yield higher content of flavonoids and phenolic content than normal ethanolic extract. Hydrolysis of extract results in decreased concentration of flavonoids and phenolic.

**Keywords**: Alkaloid, Bioactive, Flavonoids, Phenolic, *Vigna mungo*

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## 1. INTRODUCTION

*Vigna mungo* is pulse crop extensively cultivated in India. It is called as black gram in English, Masha in Sanskrit. The seeds of black gram are sweet, laxative, aphrodisiac, tonic, appetizer, diuretic, galactagogue and styptic. Seeds are useful in piles, asthma, scabies, leucoderma, gonorrhoea, pains, epistaxis, paralysis, rheumatism and affections of the nervous system, liver and cough. [1] Pure black gram cake known as idli is used as a night diet for diabetics. It is medicinally used both internally and externally, internally used in gastric catarrh, dysentery, diarrhea, cystitis, paralysis, piles, rheumatism and affections of nervous system, in the form of decoction and externally as poultice, also in gastritis, dysentery and rheumatism. [2]

Black gram seeds consist of moisture, proteins, fats, fibers, carbohydrates and minerals. allantoin, glutathione, plant growth regulators and lignin precursors are present in the seed and seedlings. [3] Oil extracted from black gram showed high amount of tocopherol while fatty acid composition of the fat extracted showed substantial amount of unsaturated fatty acids. [4] Saponins are present in the seeds. Shells of black gram and the root of black bean sprout, consist the saponins of soyasaponin I, soyasaponin II, soyasaponin V, saponin A, saponin B, acetylsoyasaponin A(4) and soyasaponin beta(g). [5] Potassium is present in highest amount and Zinc in lowest amounts. The amino acid like cysteine is present in lowest quantity. [6] Phenolic, terpenoids and flavonoids were reported to be present in black gram seed extract. [7] Three anthocyanins, two leucoanthocyanins, two glycoflavones, and five flavonol glycosides were found in their seed-coats, hypocotyls and mature leaves. Cyanidin 3-glucoside, robinin and kaempferol

7-rhamnoside was found in *V. mungo.* The leaves of *V. mungo* contain predominantly robinin and rutin. [8]

Raw material for extraction needs physical treatments. Sample preparation is important and initial step in analytical process. Drying, homogenization, sieving, derivatisation, hydrolysis etc. are steps in sample preparation. Sample preparation improves sample stability, enhances efficiency of the extraction process. Pretreatments may enrich analytes or transform them into derivatives that can be easily analyzed. One of them is hydrolysis. If raw samples subject to prior hydrolysis it will separate aglycones that can increase possibilities of detection and quantification. Less attention is provided on sample preparation than separation and analytical detection. Present work is undertaken to study effect of sample pretreatment and fractionation of extract on extraction of bioactive compounds like flavonoids, alkaloids, etc. present in the black gram seeds. This is done by quantitative determination of total alkaloids, total flavonoids and total phenolic contents of different extracts.

## 2. MATERIALS AND METHODS

### 2.1 Authentication of plant material

Seeds of *Vigna mungo* were procured from local farmers located near Alandi, Pune. The plant specimen was authenticated as *Vigna Mungo* L. Hepper, family Fabaceae with voucher specimen number DJS 05 from Botanical Survey of India, Pune.

### 2.2 Extraction of Plant material

#### 2.2.1 Ethanolic Extract (EE)

Seeds of *Vigna mungo* were washed properly and foreign organic matter was separated. Seeds were coarsely powdered for extraction. 500gm of coarse material was loaded into Soxhlet apparatus and defatted using Petroleum ether solvent. Exhausted powder was air dried and again extracted into Soxhlet apparatus by using ethanol. Ethanolic extract was collected and concentrated by using Rotary Vacuum evaporator.

#### 2.2.2 Ethanolic Fraction (EF)

EE was shaken with little quantity of water and fractionated with Petroleum ether. Aqueous layer was separated and concentrated by using Rotary Vacuum evaporator.

#### 2.2.3 Ethanolic extract after hydrolysis (EEH)

100 gm of coarsely powdered seeds were refluxed with 400ml of 2N Hydrochloric acid for 30 min. Refluxed material was dried and further extracted with ethanol in Soxhlet apparatus.

### 2.3 Determination of total phenolic contents in the plant extracts

Total phenolic contents were determined by Folin-Ciocalteu's reagent spectrophotometric method. Methanolic solution of the EE, EF and EEH in the concentration of 1 mg/ml was used for the analysis. Reaction mixture was prepared by mixing 0.5ml of Methanolic solution of EE,EF and EEH extracts, 2.5ml of 10% Folin-Ciocalteu's reagent and 2.5 ml 7.5% NaHCO3. Blank was containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent and 2.5 ml of 7.5% of NaHCO3. The samples were thereafter incubated at 45°C for 45 min. The absorbance was determined using UV spectrophotometer at λmax of 765 nm. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract) [9]

### 2.4 Determination of total Alkaloid contents in the plant extracts

Calibration curve for atropine was prepared by using 0.4, 0.6, 0.8, 1 and 1.2 ml of atropine standard solution and transferred each to different separating funnels. Then 5 ml pH 4.7 phosphate buffer and 5 ml BCG solution were added and a mixture was shaken with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine. A part of EE, EF and EEH was dissolved in 2 N HCl and then filtered. One ml of this solution was transferred to a separating funnel and washed three times with 10 ml chloroform. The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and the complex formed was extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking. The extracts were collected in a 10-ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. [10]

### 2.5 Determination of total flavonoids contents in the plant extracts

The total flavonoid content of EE, EF and EEH was determined by the aluminum chloride colorimetric method. Ethanolic solution of the EE, EF and EEH in the concentration of 1 mg/mL was used for the analysis. 50 µL of crude extract were made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution; After 5 min 0.3 mL of 10% $AlCl_3$ solution was added, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1M NaOH solution were added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoids content was calculated from a calibration curve, and the result was expressed as mg Quercitin equivalent per g dry weight. [11]

## 3. RESULTS AND DISCUSSION

Prior to extraction, hydrolysis of raw material may lead to the separation of aglycones. Hydrolysis simplifies the composition of the material, facilitating compound extraction. It will increase the possibilities of detection, quantification and analysis. Hydrolyses require to release insoluble compounds bound to sample matrix components that are not directly extractable by organic solvents. Acid hydrolysis has been traditional approach for the measurement of aglycones and phenolic acids from flavonoids glycosides and phenolic acid esters respectively. Acid treatment leads the separation of the constituting sugars and aglycones. Addition of water to EE causes green colour precipitation. Petroleum ether removed impurities from EE. For the extraction of phenolic compounds from plant material ethanol in combination with water are frequently used. Therefore water was added for facilitation of extraction of phenolic and other water soluble compounds from seeds. [12] EE was dark greenish black in colour with semisolid appearance. EF was greenish black in colour with semisolid appearance. EEH was reddish brown in colour with semisolid nature.

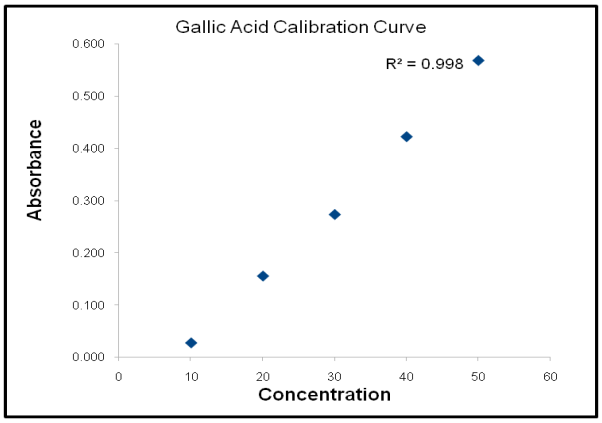


**Figure I Gallic Acid Calibration Curve for determination of Total Phenolic Content**

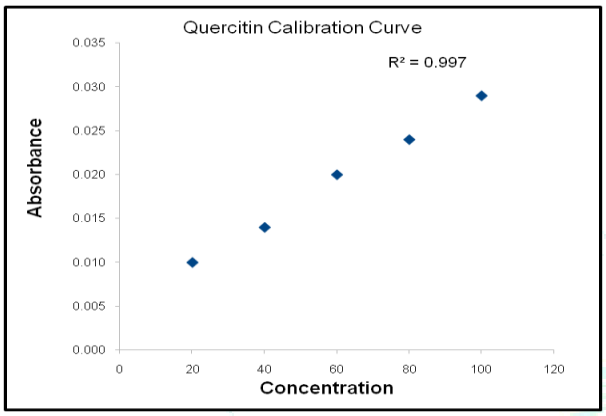


**Figure II Quercetin Calibration Curve for determination of Total Flavonoid Content**

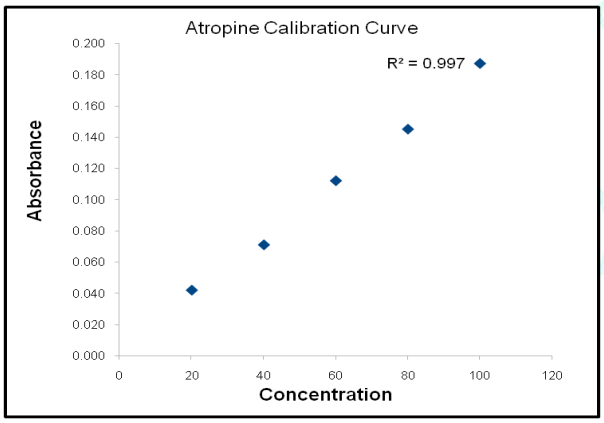


**Figure III Atropine Calibration Curve for determination of Total Alkaloid Content**

**Table 1: Comparison of extraction condition effects of *Vigna mungo* seed extracts (mg/ml)**

| Extract/Parameter | EE | EF | EEH |
|---|---|---|---|
| Total Phenolic content | 20.0 ± 5.28 | 21.03±5.04 | 17.8±5.77 |
| Total Flavonoid content | 166.7± 3.66 | 304.2 ±3.48 | 112.5± 3.95 |
| Total Alkaloid content | 121.9±3.77 | 154.8±3.60 | 202.1±3.49 |

The total phenolic content of the EE, EF and EEH extract, calculated from the calibration curve ($R^2$ = 0.998), was 20.0 ± 5.28, 21.03±5.04 and 17.8±5.77 Gallic acid equivalents/g respectively (Figure I). EF showed highest phenolic contents than EEH. This may be due to hydrolysis techniques are not always efficient and destroy some phenolic compounds. Addition of water content in preparation of EF caused precipitation of phenolic compounds. The total flavonoid content of extracts, calculated from the calibration curve Figure II ($R^2$=0.997), was 166.7± 3.66, 304.2 ±3.48 and 112.5± 3.95 Quercetin equivalents/g. Hydrolysis of sample does not prove to efficient in extraction of flavonoids. EF showed highest concentration of flavonoids. The total alkaloid content of *Vigna mungo* EE, EF and EEH, calculated from calibration curve (Figure III) ($R^2$=0.997), was 121.9±3.77, 154.8±3.60 and 202.1±3.49 Atropine equivalents/g. Extract after hydrolysis showed 202.1µg/ml of alkaloids, while EF contains 154.8 µg/ml of alkaloid content. Hydrolysis of *Vigna mungo* seeds improved the alkaloid contents. Refer Table I for the comparative effect of treatments on Total Phenolic, Alkaloid and Flavonoid content.

Extraction of bioactive compound requires specific treatments. Present study concludes fractionation of ethanolic extract of *Vigna mungo* yield higher concentration of phenolic and flavonoids. Acid hydrolysis improved yield of alkaloids from the seeds.

## 4. CONCLUSIONS

Various treatments have effect on extraction of bioactive compounds. Extract from pretreated seeds with acid improved extraction of alkaloids. Fractionation of extract yield higher content of flavonoids and phenolic content than normal ethanolic extract. Hydrolysis of extract results in decreased concentration of flavonoids and phenolic.

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