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Research article

Pharmacognostic Evaluation and Phytochemical Analysis of Seeds of *Vigna mungo* (L.) Hepper

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Abstract

Vignamungo (L.)Hepperbelonging to the family Fabaceae (Papilionaceae) commonly known as Black gram or Urd. It is an erect hairy annual plant with long twining branches, flowers are small and yellow in color; fruits are cylindrical and pods are hairy containing 1-4 seeds per pod. The present study provides updated information on its pharmacognostic, phytochemical analysis and pharmacological properties. The phytochemical analysis of the powdered seeds revealed the presence of carbohydrates, flavonoids, saponins, tannins, alkaloids, steroids and vitamin C qualitatively, whereas flavonoids was analysed quantitatively too. It can be used medicinally as anti-inflammatory, analgesic, ulcerogenic, hypoglycemic, hepatoprotective, immunostimulatory, anticonvulsant, antioxidant, narcotic activity. Differential extraction yielded aqueous extract 40.16%, methanol extract 15.50%, ethanol extract 12.07%, chloroform extract 9.88%, petroleum ether extract 0.34% and Quantitative pharmacognostic analysis gave moisture content 14.60%, alcohol extractive value 4.00%, water extractive value 23.6%, chloroform extractive value 2.80%, petroleum ether extractive value 0.8%, total ash 2.5%, acid–insoluble ash 0.50% and water soluble ash 1.0%.Water soluble extractives are more than alcohol soluble extractives show more water soluble constituents in the seeds.

Keywords: *Vigna mungo* (L.) Hepper, Pharmacognostic evaluation, Physico-chemical character, phytochemical studies

1. INTRODUCTION

Black gram consists of the seeds of *Vigna mungo*, belonging to the family Fabaceae. It is extremely nutritious. It mainly contains moisture (10.9%), protein (24.0%), fat (1.4%), fiber (0.9%), minerals (3.2%), and carbohydrates (59.6%). It is used as a demulcent, aphrodisiac, in CNS disorders, in diabetes and in hair disorders (1).Black gram [*Vigna mungo* (L.)Hepper] is a vital pulse crop cultivated in summer under a wide range of agro-ecological zones, mostly of rain fed nature. It is cultivated in many South Asian countries, including India, Pakistan, Nepal, Bangladesh, Thailand, Philippines and Korea. It is the least researched crop among pulses, and no international center of CGIAR system has this crop on its consent. Although in a number of countries, it is recognized as a potential crop, but no systematic Pharmacognostic research information is available except few reports worldwide (2).Black gram is mainly cultivated for its protein reach seeds. It is having dietary protein content next to soybean. Although the main producer of this crop is Indian, but the production is limited due to a variety of biotic and abiotic stresses. It is highly susceptible to yellow mosaic virus, fungal pathogen, insects and drought, which result in significant yield losses. To enhance the tolerance of black gram against disease and insects, the classical breeding met with limited success because there is no adequate and satisfactory level of the genetic variability present within the available germplasm (3). Black grams were analyzed for their contiguous and mineral composition, vitamins (niacin and ascorbic acid), protein fractions, amino acid profile of total seed proteins, fatty acid profile of seed lipids, in vitro protein digestibility and certain antinutritional factors. The seed's sample contained minerals such as Na, K, Mg and P in abundance. Albumins and globulins found to be the principal protein of the investigated *Vigna mungo* varieties. The principal oligosaccharide of all the three varieties of *Vigna mungo* was Raffinose. The nutritional contents of *Vigna mungo* contribute to many health benefits to humans (4).

***Vigna mungo* (L.) Hepper** (Synonyms: *Phaseolus mungo* Linn.) belonging to the family Fabaceae (Papilionaceae) commonly known as Eng: Black gram, Hin: Urd. It is an erect hairy annual plant with long twining branches; leaves are trifoliate; leaflets are ovate, entire: flowers are small and yellow in color; fruits are cylindrical and pods are hairy with a short, hooked beak containing 1-4 seeds per pod. Seeds are generally black with a white hilum producing from seeds. It is widely distributed in India, Pakistan, Nepal, Bangladesh, Thailand, Philippines and Korea (5). In the different system of traditional medicines, it has been recognized as the treatment of different diseases and ailments of human beings (6).The leaves of *Vigna mungo* (L.) Hepper show significant anti-inflammatory, analgesic, ulcerogenic activity and the young leaves are used as a vegetable. The antiatherogenic nature of *PhaseolusMungo*L. has been reported. Black gram fiber exhibits significant hypoglycemic action in experimental animals (7).The seeds of *Vigna mungo* (L.)Hepper showed significant hepatoprotective, immunostimulatory (8), anticonvulsant (9), and antioxidant activity, etc. (10) and on the other hand, roots of *Vigna mungo* (L.) Hepper showed significant narcotic and anti-inflammatory activity (5). The seeds of *Vigna mungo* (L.) Hepper contains isoflavones such as genistein, 2-hydroxygenistein, 2-hydroxydaidzein, kievitone, cyclokievitone, 5-deoxykievitone, 2-hydroxydihydrodazein, isoferreirin, eurenol, glycinol, demethylvesititol, kievitonehydrate, 4-O-methylkievitone, cyclokievitonehydrate and 5-deoxykievitonehydrate. It also contains hemicelluloses A, hemicelluloses pectin, starch & hexosans (11).

2. MATERIALS AND METHODS

Plant collection, identification and extraction

The seeds of *Vigna mungo* (L.) Hepper were purchased from Maliyana, Meerut District, Uttar Pradesh, India in August 2012. It was authenticated by Dr. A. K. Gupta (Reader) Dept. of Botany Meerut College Meerut (U.P.) India. The plant having a voucher specimen's number MCM/Bot-2 was deposited in the Department of Botany, Meerut College Meerut (U.P.) India, for future reference. The seeds were dried under shade and made into fine powder using pestle and mortar. These fine powders are analyzed for following Pharmacognostic parameters.

Pharmacognostic evaluation of the plant

Determination of Extractive values

For the determination of Organoleptic characters such as color, nature, taste and yield of the extracts, 100g of dried and powdered plant material was successively extracted in the soxhlet extractor using petroleum ether, chloroform, ethanol (99%, v/v) and distilled water solvents in the increasing order of polarity for 24h. The Resulting liquid extracts were evaporated to dryness under reduced pressure. The yield of the extracts was calculated using the following formula (12).

$$\text{Extractive value (\%)} = \frac{\text{Residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

Alcohol soluble extractive value

It accurately weighed 5 gm coarse and air dried powdered drug was macerated with 100ml ethanol (99%) in a stoppered flask for 24 hrs. with frequent shaking for 6 hrs. It was then filtered rapidly through filter paper taking precautions to prevent excessive loss of ethanol. The volume was made up to 100ml with ethanol. The residue was evaporated in a flat bottom shallow dish, dried at 105 0C, weighed and kept in desiccators. Average extractive value in percentage w/w (on the dry basis) was calculated concerning to air dried drug (Table-1) (13).

Water soluble extractive value

5 gm coarse and air dried drug material was macerated with 100ml water in a stoppered flask for 24 hrs.with frequent shaking for first 6 hrs. The extract was filtered rapidly through filter paper taking precaution to prevent excessive loss of solvent. The residue was evaporated in a flat bottom shallow dish, dried at 105 0C weighed and kept in a desiccator. Average extractive value in Percentage w/w (on the dry weight basis) was calculated concerning air dried drug (Table-1) (13).

Chloroform soluble extractive value

It accurately weighed 5 gm coarse and air dried powdered drug was macerated with 100ml chloroform in a stoppered flask for 24 hrs.with frequent shaking for 6 hrs. It was then filtered rapidly through filter paper taking precautions to prevent excessive loss of

chloroform. The volume was made up to 100ml with chloroform. The residue was evaporated in a flat bottom shallow dish, dried at 105 0C, weighed and kept in desiccators. Average extractive value in percentage w/w (on the dry basis) was calculated with reference to air dried drug (Table-1).

Petroleum ether soluble extractive value

5 gm coarse and air dried drug material was macerated with 100ml petroleum ether in a stoppered flask for 24 hrs.with frequent shaking for first 6 hrs. The extract was filtered rapidly through filter paper taking precaution to prevent excessive loss of solvent. The residue was evaporated in a flat bottom shallow dish, dried at 105 0C weighed and kept in a desiccators. Average extractive value in Percentage w/w (on dry weight basis) was calculated concerning air dried drug (Table-1).

Determination of ash value

Total ash value

Two grams of dried and powdered plant material was taken in the pre-weighed clean sintered silica crucibles. Then, they were incinerated by gradual increasing of the temperature (400-500^{0}C) in the muffle furnace till white ash obtained until constant weight of ash obtained. The crucible was cooled to room temperature in a desecrator and weighed the ash and calculated the % of total ash with reference to the air dried sample of the crude drug using following formula (Table-2) (12, 14).

$$\text{Total ash Value (\%)} = \frac{Z - X}{Y} \times 100$$

Where, X= Weight of the crucible; Z = Weight of the crucible with ash; Y = Weight of the powder taken (g).

Acid insoluble ash value

The total ash content of the plant material obtained was boiled for 15min, after adding 25ml of 25 %(v/v) HCl in to a 100 ml beaker and was allowed to cool. It was filtered through a Whatman filter paper No. 44 (ash less) and wash the residue twice with hot water. The insoluble ash thus retained on filter paper along with paper was ignited in a preweighed sintered crucible (1000^{0}C). Then the crucible along with the residue was weighed and calculated the acid insoluble ash content using the following formula (Table-2) (12, 14).

$$\text{Acid insoluble ash Value (\%)} = \frac{a}{Y} \times 100$$

Where, a= weight of the residue; Y= Weight of powder taken (g)

Water soluble ash value

The total ash value was determined using 2 g of the air-dried powdered sample. The total ash was boiled for 5 minutes with 25 ml of distilled water; the insoluble matter was collected on an ash less filter paper, washed with hot distilled water, and ignited for 15

minutes at a temperature not exceeding 4500C. The weight of the insoluble matter was subtracted from the weight of the total ash; the difference in weight represents the water-soluble ash. The percentage of the water-soluble ash was calculated with reference to the air-dried powdered plant sample. It was calculated by using following formula (Table-2) (15).

$$\text{Water insoluble ash Value (\%)} = \frac{a}{Y} \times 100$$

Where, a= Weight of the residue; Y= Weight of powder taken (g)

Water soluble ash Values (%) = Total ash value ─ Water insoluble ash value.

Fluorescent studies of powder drugs

A lot of herbs show fluorescence when the cut surface or powder is exposed to UV light and this can be useful in their identification. The fluorescence character of the plant powders (40 mesh) was studied both in daylight and UV light (254 nm and 366 nm) and after treatment with different reagents like sodium hydroxide, hydrochloric acid, nitric acid and ferric chloride etc.(Table-3) (16).

Phytochemical Screening

The seeds were collected and dried in shade and reduced to coarse powder. The powdered seeds were extracted with Petroleum ether, Chloroform, Ethanol, Methanol and Distilled water in Soxhlet apparatus. The extracts were filtered and solvent removed by distillation under reduced pressure. The percentage yields were calculated and the extracts were further subjected to phytochemical tests for Alkaloids, Glycosides, Flavonoids, Carbohydrates, and Tannins (Table-4) (13, 14).

Result and Discussion

Results of Organoleptic study such as extraction yield of seeds extract of *Vigna mungo* (L.) Hepper are shown in Table 1. The extraction yield of different solvents varied from 0.8% to 23.6% in cold maceration process and 0.34% to 40.16% in hot extraction process and could be ranked from high to low i.e. aqueous extracts > ethanol > chloroform> petroleum ether. The percentage of extraction yield will increase with the ratio of solvents, temperature and sample extraction (18). The crude extracts of *Vigna mungo* (L.) Hepper have exhibited a wide range of colour. The petroleum ether and chloroform extracts are yellowish green and greenish brown colour whereas ethanol and aqueous extract are dark brown and brown colour. Further the tastes of petroleum ether and chloroform extract are pungent bitter while the ethanol extract is bitter and aqueous extract is sweet in taste. Similarly the nature of these extracts varies from oily (petroleum ether), waxy (chloroform), resinous (ethanol) and sticky (aqueous) respectively.

Table 1: The Extractive values of the seeds powder of *Vigna mungo* (L.) Hepper by hot extraction method and cold maceration.

S.No.	Nature of Extract	Values (% w/w) by hot extraction	Values (% w/w) by cold maceration
1.	Petroleum ether	0.34	0.80
2.	Chloroform	9.88	2.80
3.	Ethanol (99%)	12.07	4.00
4.	Methanol	15.50	10.50
5.	Aqueous	40.16	23.60

The physico-chemical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs (17).The total ash is particularly important in the evaluation of purity of drug i.e. the presence or absence of foreign organic matter such as metallic salts or silica (12). The total ash content of the *Vigna mungo* (L.) Hepper seed was 2.5%, whereas the water insoluble ash was more than that of acid insoluble ash at 1.5% and 0.5% respectively (Table 2). In the present investigation considerable amount of total ash was noticed in seed. Findings can be employed as quality parameter to evaluate *Vigna mungo*(L.) Hepper biomass for any adulteration.

Table 2: Ash value of *Vigna mungo* (L.)Hepper seeds

S.No.	Physical contents	Value (%w/w)
1.	Total ash value	2.5
2.	Acid insoluble ash	0.5
3.	Water soluble ash	1.0
4.	Water insoluble ash	1.5

The fluorescence analysis is adequately sensitive and enables the precise and accurate determination over a satisfactory concentration range. The fluorescence colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The colour of the extracts from organic and inorganic solvents was observed both ordinary and UV light (19). The fluorescence analysis of *Vigna mungo* (L.) Hepper seed treated with different chemical reagents are tabulated in Table 3.

Table 3: Fluorescent studies of powder of *Vigna mungo* (L.) Hepper seeds

S.No.	Solvents Treatment	Visible light	Short UV (252 nm)	Long UV (366 nm)
1	Drug as such	White	Light yellow	Alice blue
2	Drug + 1M.H_2SO_4	Sandy brown	Yellow green	Dark khaki
3	Drug + 1M.HCl	Wheat	Khaki	Dark Khaki
4	Drug + 1M.NaOH in water	Khaki	Green	Dark green
5	Drug + KOH 50% Soln.	Green	Light green	Dark khaki

6	Drug + Ammonia soln.	Khaki	Lawn green	Khaki brown
7	Drug + Picric acid	Yellow	Lime green	Dark slate gray
8	Drug + $FeCl_3$ 5% soln.	Olive	Green	Black
9	Drug + Iodine soln. (5%)	Black	Gray	Black
10	Drug + Petroleum ether	Yellow	White	Blue violet
11	Drug + Chloroform	Yellow	White	Blue violet
12	Drug + Methanol	Light yellow	Yellow green	Gray

Phytochemical screening of *Vigna mungo* (L.) Hepper seed extracts was done with petroleum ether, chloroform, ethanol, methanol and water. The study shows presence of alkaloids, tannin, flavonoids, saponins, glycosides, terpenoids and ascorbic acid. Main attraction of phytochemical screening is presence of flavonoids, saponins, ascorbic acid, tannins and phenols in maximum of extracts. The phytochemical screening of chemical constituents in *Vigna mungo* (L.) Hepper study showed that seeds were rich in flavonoids, saponins, ascorbic acid, tannins and phenols (Table 4). They were known to show medicinal activity as well as exhibiting physiological activity.

Table 4: Phytochemical Screening of Various extracts of *Vigna mungo* (L.) Hepper Seeds

Various Extracts / Phytochemicals	**Petroleum ether extract**	**Chloroform extract**	**Ethanol extract**	**Methanol extract**	**Aqueous extract**
Alkaloids **Dragendorff's test**	-	-	+	-	+
Flavonoids **Shinoda test**	-	-	+	+	+
Steroids **Salkowski reaction**	-	-	-	+	+
Tannins & Phenols **5% $FeCl_3$ Solution**	-	+	+	-	+
Glycosides **Liebermann's test**	-	-	+	+	-
Saponins **Heamolytic test**	+	-	+	-	+
Ascorbic acid	-	+	+	-	+

Conclusion

The pharmacognostical, physico-chemical and preliminary phytochemical analysis on the seeds of *Vigna mungo* (L.) Hepper evolved from the present investigation provide useful information and authentication of the plant. The phytochemical investigation can further be isolated and undergo further pharmacological evaluation of the active principles present in the seeds of *Vigna mungo* (L.) Hepper which will be of massive use for the researchers and also in the field of Indigenous system of medicine.

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