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Pharmacognostic and Phytochemical screening of Cowpea seeds (*Vigna unguiculata*)

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Abstract

Vigna unguiculata (Cow pea) is well known medicinal plant. The drug isolated from this plant is used in different science of medicines like, ayurveda, unani, sidda, allopathic, homeopathic and naturopathic therapy. The present investigation deals with pharmacognostic and phytochemical screening of the seeds of *Vigna uguiculata.* The pharmacognostic studies include physicochemical constant and fluorescence analysis. The petroleum ether, chloroform, methanol and aqueous extracts are subjected to preliminary phytochemical screening. The physicochemical constants obtained were found to be within normal levels prescribed by phytochemical standards. The phytochemical studies of seeds of *Vigna uguiculata* revealed the presence of primary and secondary metabolites like proteins, carbohydrates, glycosides, phenols,flavonoids, saponins, sterols and alkaloids. The flavonoid content of methanol extract was found to be 621± 2.494 µg rutin/g. These studies provide a referential information for identification of this crude drug and the results are helpful for the isolation of medicinally important active components.

Keywords: Vigna unguiculata, Phytochemical screening, Pharmacognostic studies, Total phenol, Flavonoid content.

Introduction

Plants have always been a major component of traditional system of healing in developing countries, which have also been an integral part of their history and culture. Medicinal plants offer an alternative remedies with tremendous opportunities. Many traditional healing herbs and plant parts have been shown to have medicinal value especially in the rural areas and that these can be used to prevent and cure several human diseases. Even today, majority of the world population depends on herbal healthcare practices [1].

Herbal medicines formulation generally involves the use of fresh or dried plant parts. However in developed countries, the key obstacle in acceptance of the alternative medicines is the lack of documentation and stringent quality control. With this back drop, making an effort towards standardization of the plant material to be is extremely important. Thus the research work carried out on traditional medicines need to be documented. The very important aspect in preparation, safety and efficacy of herbal products is the correct knowledge of such drug. The most simple and reliable tool to obtain complete information of crude drug is Pharmacognosy [2,3,4,5]. The standardization process can be achieved by stepwise pharmacognostic and phytochemical studies which helps in standardization and identification of the plant material. To ensure reproducible quality of herbal medicine this will contribute to its safety and efficacy. Correct identification and quality assurance of the starting material is essential [6].

Cowpea (*Vigna unguiculata* (L) Walp.) is one of the most ancient food `source and has probably been used as a crop plant since Neolithic times [7]. Like many other legumes, its seeds are the most valuable part and are well-known due to their ascribed nutritional and medicinal properties. It is used to treat stubborn boils by mixing the seed powder with oil [8]. The seed are used to strengthen the stomach and is also diuretic. Boiled seeds are eaten to destroy worms in the stomach [9]. To treat amenorrhea, infusion of seed can be taken orally whilst powdered roots eaten with porridge & is used to treat chest pain, epilepsy and painful menstruation [10]. Cooked seeds and roots of other herbs are used orally to treat blood in urine and bilharzias [11]. Cowpea (*Vigna unguiculata* (L) Walp.) is known to be an excellent source of protein and also rich in important vitamins, minerals, including soluble and insoluble dietary fiber. The present study was carried out to explore pharmacognostic standards and preliminary phytochemical screening of biologically active compounds from seeds of *Vigna unguiculata*.

Materials and Methods

Collection of Plant material

The seeds of *Vigna uniguculata* were collected from local market in Gulbarga and authenticated from dept of Botany, Gulbarga University, Gulbarga.

Extraction method

The seeds of *Vigna uniguculata* were dried in shade and reduced to coarse powder, and then around 100 grams of powder was subjected to successive (soxhlet) extraction by using different solvents like petroleum ether, chloroform, methanol and finally with deionized water. The extract thus obtained is evaporated to dryness and stored separately at 4^0c for further use.

Pharmacognostic studies

The ash values, extractive value and loss on drying were performed according to the standard methods prescribed in Indian Pharmacopeia [12] and also as per the WHO guidelines [13] on quality control methods for medicinal plants materials. Fluorescence analyses were carried out according to the method of Chase and Pratt 1958 [14] and Kokoski 1995 [15].

Phytochemical screening

Petroleum ether, chloroform, methanol and aqueous extracts were subjected to comparative phytochemical analysis for the presence of various secondary phytoconstituents using standard procedure described by kokatte 1998[16] and Horborne 2003[17]. The extract residues of the plant were subjected to phytochemical screening to screen the presence of various active phytocompounds like phenols, tannins, flavonoids, saponins, alkaloids & primary metabolites like carbohydrates, proteins, lipids.

Determination of total phenolic content

Total phenolic content was estimated according to the method of Alfawaz, M. A., 2006 [18], using folin ciocalteu reagent (FCR) and gallic acid as standard. 10 mg of extract was included in 10 ml volumetric flask, then added with 0.4 ml reagent of FCR and incubated for 4-8 mins. Furthermore, the solution was added with 4.0 ml of 7% Na2Co3, then added distilled water. After 2 hr of incubation, the solution absorbance was measured at 750nm wavelength versus a blank consisting distilled water and FC reagent. Concentration of phenolic compound was calculated according to the following equation that was obtained from the standard gallic acid graph. Total phenolic content was expressed in gallic acid equivalent (GAE) of each gm extract of dry weight. Concentration of phenolic compounds was calculated according to the following equation that was obtained from standard Gallic acid graph.

Absorbance =0.002x+0.004 (R^2= 0.994)

Estimation of flavonoids

Firstly 2ml of the sample solution was accurately removed in a volumetric flask(10ml) by adding 0.6ml of NaNO2 (5%) solution, shaken up and then allowed to stand for 6 min. secondly, 0.5ml of the Al(No3)3 (10%) solution was added to the volumetric flask, shaken and was left to stand for 6 min. Finally, 3.0ml of NaoH (4.3%) solution was added to the volumetric flask, followed by addition of water to the scale, shaken and left to stand for 15min before determination. Using the sample solution with coloration as reference solution and 500nm as determination wavelength, the coloration method was used to determine the content of flavonoids in the sample by uv detector [19]. Concentration of flavonoid content was calculated according to the following equation obtained from standard routine graph.

Absorbance = 0.001x-0.004 (R^2= 0.993).

Results

Physicochemical studies

The physical appearance, color and percentage yield are recorded in table. 1. The petroleum ether extract of cowpea seed was found to be semisolid yellow with 3.0% yield where as chloroform, methanol and aqueous extract appeared as semisolid brown with 5.0%, 7.5%, and 11.0% respectively.

Table 1. Shows nature and percentage yield of extract

S.No.	Extracts	Nature of extract	Color	Percentage yield
1	Petroleum ether	Semi-solid	Yellowish	3.0%
2	chloroform	Semi-solid	Brown	5.0%
3	Methanol	Semi-solid	Brown	7.5%
4	Water	Semi-solid	Brown	11.0%

Total ash, acid insoluble ash, water soluble ash and moisture content of the seed powder of cowpea were done and the results are tabulated in table 2. The total ash value, acid insoluble ash and water soluble ash was found to be 3.33%, 1.33% and 0.66% respectively. The moisture content was determined by loss on drying and found to be 6.2%.

Table 2. Shows physiochemical parameters

S.No.	Parameters	Percentage of Ash
1	Total Ash	3.33%
2	Acid insoluble Ash	1.33%
3	Water soluble Ash	0.66%
4	Loss on drying (%w/w)	6.2%

Fluorescent Studies

Behavior of cowpea seed powder with different chemical reagents was detected under daylight and UV light to detect the fluorescent compounds (Table 3). The cowpea seed powder as such appears white color under both day light and UV light. But when treated with a different reagent shows a broad range of color. Seed powder when treated with 1N HCl, 1N aqueous NaoH and 1N methanolic NaoH appears as pink, reddish and lemon yellow under both day and UV light. Whereas powder appears as brown color under day light and light brown under UV light when treated with $Fecl_3$ respectively.

Table 3. Fluorescence analysis of powder

S.No.	Treatment	Ordinary light	UV light
1	Powder as such	White color	White color
2	Power + 1N Hcl	Pink color	Pink color
3	Power + 1N NaoH (water)	Reddish	Reddish
4	Power + 1N NaoH (methanol)	Lemon yellow	Lemon yellow
5	Power + $fecl_3$	Brown color	Light brown
6	Power + Chloroform	Cream color	Light yellow
7	Power + Methanol	Cream color	Cream color
8	Power + Petroleum ether	Cream color	Cream color
9	Power + Acetone	Cream color	Cream color
10	Power + Conc. H_2SO_4	Dark blue color	Dark Blue

Qualitative phytochemical screening

The qualitative analysis of primary and secondary metabolites of seed extract revealed the presence of protein, carbohydrates, glycosides, saponins, sterols and flavonoids and alkaloids. The petroleum ether and chloroform responded positively to the sterol and alkaloid tests indicating the presence of sterols and alkaloids, where as methanol extract shows positive response for the test of Glycosides, saponins, carbohydrates, phenols, flavonoids and protein . The aqueous extracts have showed positive results to the glycosides, saponins, carbohydrates, phenols and flavonoid tests (Table 4).

Table 4. Qualitative analysis of primary and secondary metabolites of seed extract

S.No.	Tests	Pet ether	Chloroform	Methanol	Water
I	**Test for sterols**				
	A)Salkowaski Test	+ ve	+ ve	-ve	-ve
	B) Leibermann burchart	+ ve	+ ve	-ve	-ve
II	**Test for Glycosides**				
	A) Keller-killaini test	-ve	-ve	+ ve	+ ve
III	**Test for Saponins**				
	A) Foam test	-ve	-ve	+ ve	+ ve
IV	**Test for Carbohydrates**				
	A) Fehlings test	-ve	-ve	+ ve	+ ve
	B) Benedicts test	-ve	-ve	+ ve	+ ve
	C) Molisch test	-ve	-ve	+ ve	+ ve
	D) Barfoed's test	-ve	-ve	+ ve	+ ve
V	**Test for Alkaloids**				
	A) Dragendroff"s Test	+ ve	+ ve	-ve	-ve
	B) Mayer's Test	+ ve	+ ve	-ve	-ve
	C) Wagner's Test	+ ve	+ ve	-ve	-ve
VI	Test for Phenol				
	A) Ferric chloride test	-ve	-ve	+ ve	+ ve
	B) Lead acetate Test	-ve	-ve	+ ve	+ ve
	C) Gelatin Test	-ve	-ve	+ ve	+ ve
VII	**Test for Flavanoids**				
	A) Ferric chloride test	-ve	-ve	+ ve	+ ve
	B) Lead acetate Test	-ve	-ve	+ ve	+ ve
VIII	**Test for Proteins**				
	A) Millon's test	-ve	-ve	+ ve	-ve
	B) Biuret test	-ve	-ve	+ ve	-ve

The Quantitative analysis of methanol extract for the presence of flavonoid is carried out and found to be 621± 2.494 µg rutin/g (Table 5).

Table 5. Quantitative estimation of Phenols and Flavonoid content

	Phenols (µg rutin/g)	Flavonoid(µg rutin/g)
Methanol extract	80	621± 2.494

Discussion

The pharmacognostic evaluation of crude drug plays an important role in judging the censoring acceptability or rejection of crude drugs in the market. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs (20). Equally important in the evaluation of crude drugs is the ash value, acid insoluble ash value and water soluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica (21). The ash value of cowpea seed powder is 3.33%. This ash value is indicative of the impurities present in the drug. Since the ash value is constant for a given drug, this value is also one of the diagnostic parameters of the drug. In the present study, the cowpea seed powders have more acid insoluble ash value than water soluble ash value. The ash value is generally the index of the purity as well as identity of the drug. In herbal drugs, variable limit of water are present. An excess of water in medicinal plant material encourages microbial growth and deterioration following hydrolysis. Estimation of moisture content is important for the material which absorbs moisture easily or deteriorates quickly in the presence of water. In the present study the moisture content was found to be 6.2%.

The physicochemical parameters such as percentage of ash, extractive value, foreign matter content and loss on drying were determined in triplicate and the results are in line with the findings of kalaskar & surana et al., 2012[22].

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Many phytocompounds fluoresce when suitably illuminated. The fluorescent color is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent (23). Florescence analysis of powders gives a clue if powder is in adulteration thus can be used as a diagnostic tool for testing the adulteration. Presence or absence of certain important compounds in an extract is determined by color reactions of the compounds with specific chemicals which act as dyes. This procedure is pre-requisite before going for detailed phytochemical investigation. In the present study the florescent analysis of seed powder of cowpea showed a broad range of color spectrum under day light and UV light when treated with different chemicals. According to kalaskar et al 2012 [24] bark powder was analysed for their fluorescence analysis. The dried bark material was analysed under visible light, short and long UV light after treatment with various acids, alkalis & other reagents.

The phytochemical screening of cowpea seed was undertaken for the identification of different chemical constituents present in different extracts. The petroleum ether, chloroform, methanol and aqueous extract of cowpea seed obtained by successive soxhlet extraction showed the presence of phenols,flavonoids, sterols, saponins, protein and carbohydrates but alkaloids were positive only for petroleum ether and chloroform extracts.

Based on the result obtained from preliminary phytochemical screening, the methanol extract is further subjected to quantitative estimation of phenols and flavonoids. The quantitative estimation phenol and flavonoid in the methanol extract of cowpea seed powder was found to be 80±2.867 mgGAE/g and 621± 2.494 µg rutin/g, repectively. Earlier reports also suggest that the phenol and flavonoid content is in much higher concentrations in the methanol extract (25).

Conclusions

Cowpea (*Vigna unguiculata* (L) Walp.) is one of the most ancient food sources and has probably been used as a crop plant since Neolithic times. The pharmacognostic investigation on physicochemical characteristics and fluorescence analysis shows the authentic properties of the crude drug which will prevent adulteration, substitution and has a crucial role in standardization of crude drug. These parameters will also guide in the proper identification of the *Vigna unguiculata* seeds from other species of Cowpea as well as help in authentication of the purity of the plant. Ash value is a criterion to judge the identity and purity of crude drugs. Extractive value is used for evaluating a crude drug as it gives idea about the nature of chemical constituent, soluble in that particular solvent which is used for extraction. The phytocontituents of the Cowpea (*Vigna unguiculata* (L) Walp.) indicates that it is a good source of secondary metabolites. From these above findings it helps for establishing adequate profile of the plant species and creates path for further studies of other biologically active compounds present in *Vigna unguiculata* seeds.

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