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REVIEW ARTICLE

Review on Standardization and Phytochemical of *Vigna unguiculata*

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ABSTRACT

Vigna unguiculata belongs to the family *fabaceae* is commonly known as kulathi, Horse gram in English. It is mainly distributed in Asia, Nigeria, Delta State, Africa, USA and dry savanna region of Northern Ghana. *Vigna unguiculata* has been of keen interest in phytochemical and Ayurvedic research due to its excellent medicinal values. In the present review an attempt has been made to gather the information related to phytochemical composition, physicochemical properties, effect of soil on cultivation, Traditional/Ayurvedic uses and part wise chemical constituents, nutrients composition, mineral/protein profile amino acid content antioxidant activities & antiradical activities. Traditionally it is used in treating diseases such as - cholesterol reduction, it is very useful in blood, haemorrhoids, tumors, scrofula, bronchitis, heart disease, nephrolithiasis, urolithiasis, leucorrhoea, menstrual disorders, colic, worms, splenomegaly, cough, asthma, strangury, hiccough, ophthalmopathy, fever, urticaria and rheumatoid arthritis etc. The plant exhibit wide array of phytoconstituents like proteins, amino acids and vitamins - thiamin, riboflavin, and niacin are water soluble compounds which are responsible for varied potent physiological and pharmacological activities. This review is in a narrative format and consists of publications pertinent to *V. unguiculata* available in public domain.

KEYWORDS

Vigna Unguiculata, Phytochemistry, Chemical Constituents, Ayurvedic Uses

INTRODUCTION

Kulathi (*Vigna unguiculata*) is a leguminous plant which belongs to the family *Fabaceae*. It is widely grown all over the world though it is perceived to have originated from Africa.[24] It is a major staple food crop in sub-Saharan Africa, especially in the dry savanna regions of West Africa & Brazil.[1,23,45] Nigeria is one of the world prime producers of Kulathi.[12,19] *V. unguiculata* forms part of the human diet in African countries due to it has high amount of protein (23%), carbohydrates (56%) and fibre (4%) that can fulfill the human essential amino acid necessities when complemented with cereals.[4,29]

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The seeds & leaves are a major source of plant proteins and vitamins for man, feed for animals[13]. The little leaves and immature pods are eaten as vegetables. The Kulathi plant has the ability to tolerate drought and fix atmospheric nitrogen in the soil enhanced by the rhizome.[6,17,32,33,34,43] Breeding system studies of seed have high variability observed in seed setting among accessions with low pod set (0.00-0.40 pod/flower) were separated from inbreeding accessions, including cultivated Kulathi, which displayed high pod set (0.70-1.00 pod/flower).[42] Kulathi also suppresses weed because of its quick growth and establishment and control soil erosion to some extent. The economic uses of cowpea makes it a choice crop for serving food security needs of societies. Some health benefits of cowpea include, toning the spleen, stomach

and pancreas helps induce urination and relieves damp conditions like leucorrhoea. Kulathi is rich in potassium with good amount of calcium, magnesium and phosphorus. It also has small amount of iron, sodium, zinc, copper, manganese and selenium. Kulathi is rich in vitamin A & C and also has appreciable amount of thiamin, riboflavin, niacin, vitamin B_6 and pantothenic acid as well as small amount of foliate. Kulathi shoots and leaves are rich sources of calcium, phosphorous and Vitamin B.[3] These nutrients provided by cowpea makes it extremely valuable especially where many people cannot afford animal proteins such as meat and fish. We can evaluate nutritional composition and functional properties of some recombinant inherited lines of Kulathi to establish potential usage[1,11,26,31]. Kulathi (*Vigna unguiculata*), is also known as black eye pea is a legume of African origin that is useful as a rotational cover crop to help meet a cash crop, nitrogen needs to control erosion to improve soil properties. Used as a cover crop an encourage populations of beneficial insects to protect cash crops from insect pests. Its drought tolerance makes it valuable in rain fed agriculture or in unirrigated fallow fields. It is good for fixing atmospheric nitrogen for increasing soil organic matter content and improving soil structure after soil incorporation useful to increase phosphorus availability in the soil. Good tolerates heat and drought, moderate shade, and low fertility soils eaten as a vegetable & Kulathi can also be used for the production of high quality food when mixed with crops such as corn or it can be used for rotational grazing. In soils low in phosphorus, the roots of kulathi develop effective mycorrhizal associations, improving the soil available phosphorus content. Some new kulathi cultivars have been bred especially for the ability to take up soil phosphorus, so that it can be made available for following crops. The seeds of kulathi major source of protein and other nutrients. It is cultured for its undeveloped pods and full-grown seeds and is eaten by people all around the world, especially in the developing nations.[5, 30, 40] Its amino acid complements those of cereals, while mineral contents: calcium and iron are higher than that of meat, fish and egg and the iron content equates that of milk; the vitamins- thiamin, riboflavin, niacin (water soluble) and their levels compare with that found in lean meat and fish which make them. Many researchers have showed that daily consumption of 100–135gm of dry beans reduces serum cholesterol level by 20% thereby, reducing the risk for coronary heart diseases by 40%. Besides its health related benefits, beans are inexpensive, considerably cheaper and due to their physicochemical and functional attributes, legume starches can be used as nutritional ingredients in the same way as starches from cereals and tubers.[9,35,36,37] Centres of plant domestication areas and hatched areas indicated that the regions of important seed-crop domestication and vegecultural crops, respectively. Accepted primary domestication centres were found in West African savanna and woodlands *Vigna unguiculata*, 3,700 yr BP; (years Before the Present). This evolutionary sequence of seed size increase before the rise of non-shattering is by no means universal. Grain size development in pearl millet, it did not occur until 2,000 years after domestication. Data from India and sub-Saharan Africa also propose that the raise in seed size in pulses was delayed by millennia after the beginning of cultivation and may not have been part of the preliminary domestication.[33]

Distribution

It is native of India and is distributed throughout the tropical regions. It occurs all over India up to an altitude of 1600m. It is cultivated in Tamil Nadu, Uttar Pradesh, Chota Nagpur and part of Assam.[7]

Botanical Classification

Family: Fabaceae

Sub-family: Fabiodeae

Genus: Vigna

Species: Vigna unguiculata

Taxonomy and nomenclature

Latin name: Vigna unguiculata

Synonyms: Kulattha, Kulatthika, Tamrabeeja, Khalva, vardhipatraka

Figure 1: *Vigna Unguiculata*

Common Names[15,7]

Sanskrit: Khalva, Vardhipatraka

Bengali: Kulattha, Kalaya

English: Horse gram

Gujrati: Kalathi, Kulathi

Hindi: Kulathi, Kurathi

Kannada: Huruli, Hurali

Kashmiri: Kath

Malayalam: Mudiraa

Marathi: Kulitha

Punjabi: Lodhar

Tamil: Kollu, Kaanam

Telugu: Ulavalu

Urdu: Kulthi

Other Names

Cowpea, asparagus bean, black-eyed pea, catjang, catjang cowpea, Chinese long bean, clay pea, cow-pea, cream pea, crowder pea, pea bean, purple-hull pea, southern pea, sow pea, yard-long bean, dolique asperge, dolique mongette, haricot asperge, haricot etc.

Ecology

A branched, sub-erect, annual, with small trifoliate leaves, bearing when mature, narrow, flat, curved pods, 3.5-5.0 cm long, tipped with a persistent style. The pods contain 5-6 flattened, ellipsoid seed, 3-6mm long.[7]

Cultivation

An annual branched, sub-erect or twining, downy or glabrescent herb, cultivated all over India.[15] The observed 6.20 value of the pH of the soil indicate that the soil is slightly acidic and this can be attributed to the high rainfall prevalent in the area leading to leakage of the basic cations from the surface area of the soil. The low organic matter content and total nitrogen could be attributed to the effects of soil erosion, outflow and bush burning predominant in the study area. Similarly, the low exchangeable cations may be due to the low clay activity and low organic content of the soil. This observation could be as a result of basic macronutrients such as N, P, K, Ca, Mg, S and the biological production of acid which limits plant growth and development.[2]

Physiology

Excellent as cover crops to suppress weeds, provide erosion control, and attract beneficial Insects very good for weed suppression by quick growth and establishment good for fixing atmospheric nitrogen, and for increasing soil organic matter content and improving soil structure after soil incorporation useful to increase phosphorus availability in the soil tolerates heat and drought, moderate shade, and low fertility soils good forage eaten as a vegetable use in rotations with annual crops including vegetables, herbs, cut flowers, annual ornamentals, and root crops such as dry land taro.

Traditional & Modern Uses of Different Parts of Plant

Seed: Roasted seeds are used to treat neuritis, insomnia, weakness of memory, dyspepsia, indigestion, needles in limbs and sensation of pins, periodic palpitation, congestive cardiac failure etc. it is an admirable medicine for stomatitis, corneal ulcers, coleic diseases, kwasiorkar, marasmus.[15] *Vigna unguiculata* is a rich source of amino acid and protein & some of the amino acids play an important role in the management of sickle cell disease.[10] Kulathi seed have cardioprotective potency & also preventing cardiovascular diseases. [11]

Leaf: Decoction of leaves is used to treat as hyperacidity, nausea and vomiting.[15]

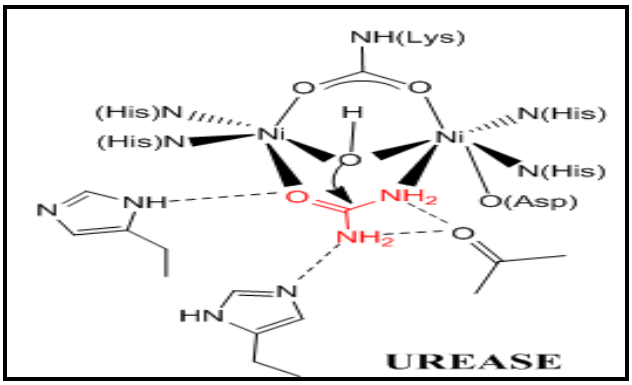
Phytochemistry

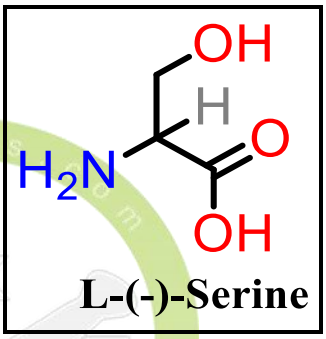
***Major Chemical Constituents* - Enzyme Urease and oil**

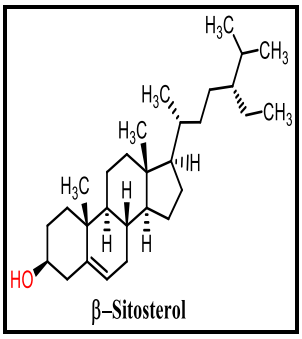
Leaf & Stem: Genisteine, dalberegioidin, kievitone, phaseollidin, coumesterol, psoralidin, lectin like glycoprotein dolichin A and dolichin B, Isoferreirin. *Vigna unguiculata* have high concentration of malaic, malonic, citric and oxalic acids that are used in medicine & also found calcium content ranges from 58 to 287 mg/100g while the iron content is about 2.5 to 10.5 mg/100g.[3] Manganese toxicity symptoms first appearing on the mature leaves of Kulathi are dark-brown spots representing deposits of oxidized Manganese and phenolics.[25]

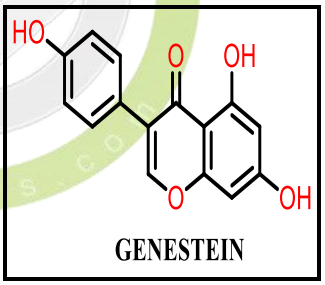
Seed: The whole seeds of Kulathi reported to contain about 0.18%–0.59% tannins, phenolic acids, such as p-hydroxybenzoic acid, protocatechuic acid, 2,4-dimethoxybenzoic acid, and cinnamic acid derivatives, such as p-coumaric acid, caffeic acid, cinnamic acid and ferulic acid.[11] Urease, protease[21], strepogenin, β-sitosterol, genistein, 2' –hydroxygenistein, dalbergioidin, kievitone, phaseollidin, isoferreirin, coumesterol, psoralidin, 5-O-α-L rhamnopyranosyl (1→2)-O-β-D-glucopyranoside, phyto-haemagglutinins, β-N-acetyl glucosaminidase, α & β-galactosidase, α-mannosides, β-glucosides, β-sitosterol.[28] 5-hydroxy-7,3,4-trimethoxy-8-methyl isoflavone - 5-neohesperidoside, D-glucose, D-galactose, L-rhamnose, D-arabinose and L-ascorbic acid and amino acids viz., glycine, alanine, cysteine, serine and aspartic acid.[4,15] Chlorogenic acid & Caffeic acids present in methanolic extract of *V. unguiculata.*[35] Amino acid analysis of the seed extract of *V. unguiculata* showed the presence of the following amino acids; aspartate (27.8%), threonine (3.3%), serine (2.6%), glutamine (43.5%), proline (17.6%), glycine (9.5%), alanine (18.7%), cysteine (3.6%), valine (8.0%), methionine (3.2%), isoleucine (5.3%), leucine (5.4%), tyrosine (0.5%), phenylalanine (5.5%), histidine (4.5%), lysine (0.5%), arginine (14.3%) and tryptophan (0.5%). The proximate analysis of the extract indicated appreciable content of protein (23.65%), moisture (12.85%), ash (3.4%), fats and oil (4.5%) and fiber (4.8%).[10]

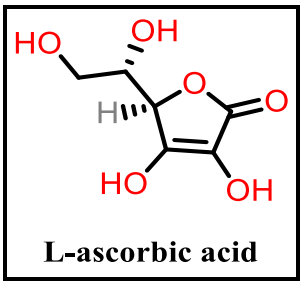
Structures of Chemical Constituents[15]

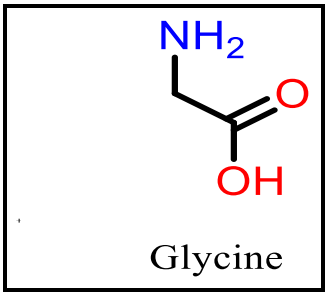


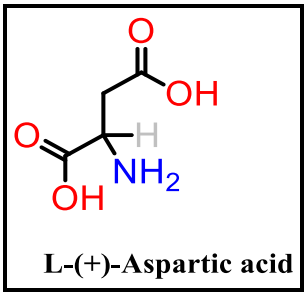


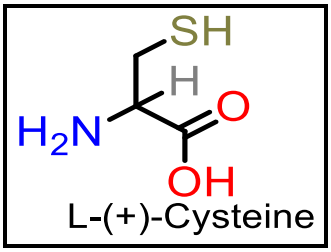


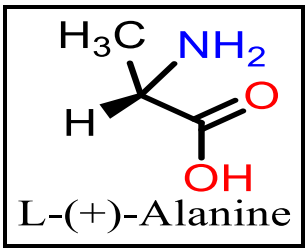












Phytochemical Analysis & Antibacterial Activity of *Vigna unguiculata*

Preliminary phytochemical screening of aqueous and ethanolic extracts of *Vigna unguiculata* seeds was done by many phytochemical identification tests. These tests exposed the presence of carbohydrates, alkaloids, glycosides, saponins, tannins and polyphenols in both the extracts of *Vigna unguiculata*. While the aqueous extract showed the flavonoids in addition and in the ethanolic extract steroids were observed. Still the fixed oils were not detected in both the extracts. The results of preliminary phytochemical screening of both the extracts of *Vigna unguiculata* seeds were showed that both the extracts have better action against the Gram positive bacteria than that of the Gram negative bacteria. When compared to that of the standard drug tetracycline. The standard drug tetracycline shows a zone of inhibition of 20 mm. The aqueous extract of *Vigna unguiculata* showed better action against *Bacillus subtilis* than that of the ethanolic extract. Whereas in case of a Gram-negative bacteria (*Escherichia coli*), the standard drug tetracycline showed a zone of inhibition of 24 mm. An increase in the antibacterial activity was observed with respect to the increase in the dose of the extracts. The aqueous extract of *Vigna unguiculata* showed better action against *Escherichia coli* than the ethanolic extract. This study concludes both the aqueous and ethanolic extracts of seeds of *Vigna unguiculata* possess excellent antibacterial activities which supports the traditional myths.[8]

The gamma irradiated and unirradiated seed coats extract from *Vigna Unguiculata* investigated for their Antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Klebsiella pneumonia* and Norfloxacin was used as a standard.[27]

The purified Vu-Defr was analyzed by circular dichroism (CD), and its biological activity was tested against L. amazonenis for recombinant and natural defensins activity. The results of all experiments were compared with the results from the natural defensin (Vu-Def). The by Circular Dichroism (CD) spectra of both peptides presented good superimposition indicating that both peptides present very similar secondary structure and that the Vu-Defr was correctly. These results suggested the potential for plant defensins to be used as new antiparasitic substances.[16]

The use of Hyphenated Techniques in Relative Phytochemical Studies of Legumes

These 'hyphenated techniques' are enabling compounds to be detected in plant extract more effectively than ever before. The application of GC-MS and LC-MS in comparative phytochemical studies in legumes is reviewed both from selected research and with and importance on nitrogen-containing & phenolic compounds. The use of GC-MS has provided an extensive data set on the occurrence of quinolizidine alkaloids in legumes and also provides the means to separate the numerous isomeric forms of polyhydroxy alkaloids and hydroxypipecolic acids as their volatile derivatives. LC-MS is enabling the metabolic profiles of intact flavonoid glycosides to be obtained from small fragments of material while recent methods to analyze non-protein amino acids by LC-MS without derivatisation hold much promise in surveys of this important taxonomic characters.[15]

Qualitative Analysis

Physiochemical parameters of *Vigna unguiculata*[7,44]

Sr. no	Parameters	Data base of Medicinal plant[7]	Ayurvedic Pharmacopoeia of India[44]
1	Foreign matter	-	Nil
2	Total Ash content	NMT 5 %	NMT 5 %
3	Acid Insoluble ash	NMT 1%	NMT 1%

4	Alcohol soluble extractive	NLT 3%	NLT 3%
5	Water soluble extractive	NLT 12%	NLT 12%

Quantitative Analysis

Acetylene Reduction by Rhizobium in Pure Culture

Examine the effects of different media supplements on *Rhizobium* strain 32H1 *(Vigna unguiculata*). We observed that the in presence of succinic acid, glutamine and asparagines were equivalent but glutamine in presence of sodium succinate was extensively lesser. The significant increase in acetylene reduction when succinic acid was added with glutamate suggests that an available carbon source was also needed. Since slow growing rhizobia do not usually grow on disaccharides, the sucrose in the callus medium would be ineffective. Callus provides substances for growth or nitrogenase production.[20]

Acetylene Reduction by Legume Root Nodule Protoplasts {Effect of phospho (enol) Pyruvate and Succinate on Acetylene Reduction by Root Nodule Protoplast of V. unguiculata}

The acetylene reduction observed in these experiments occurred within the cytoplasm of intact protoplasts. Maximum rates of reduction estimated 2-5% of that observed in fresh nodules. In this experiment rate of reduction of 12-13nmol acetylene produce per mg dry weight per hour were observed which positively with the reported value 46nmol.[47]

Effect of Temperature on (Vigna unguiculata) Starch

Cowpea starch has vital functional properties like its non-ionic character (high fiber content), high density (1.01–1.08 g/mL), water activity from 0.62 to 0.65 and resistance to lots of bacteria and fungi. Available Starch content pyrodextrinization of kulathi (*Vigna unguiculata*) starches depends on treatment intensity. Temperature is the most important factor affecting AS (available starch) content, with higher temperatures ensuing in lower AS content.[9]

Effect of Dimethoate and UVB Radiation (280-315nm), singly and in Combination, on $^{14}CO_2$ Released by Kulathi Seedlings during Photorespiration (high light) and Respiration (darkness)

Photorespiration and dark respiration were significantly stimulated when seedlings were grown in the medium supplemented with growth stimulatory concentration (50 ppm) of dimethoate and together with UV-B a marginal decrease was noticed. Growth inhibitory concentrations (100 and 200 ppm) of dimethoate treated kulathi seedlings showed that decrease in $^{14}CO_2$ release in light as well as in dark and the effect was further intensified, when seedlings were exposed to combined doses of dimethoate and UV-B, except at 200 ppm dimethoate and UV-B in darkness. The ratio of $^{14}CO_2$ release in light (photorespiration) to dark was more than unity; however, 200ppm dimethoate together with UV-B treated seedlings. The study showed that kulathi (*V. unguiculata*) seedlings is highly sensitive to both dimethoate and UV-B exposures. High concentrations of insecticide dimethoate reduced the growth of seedlings due to greater accumulation of dimethoate in root as it was in direct contact with pesticide.[46]

Legume Nitrogen Fixation, Drought and Transpiration to Soil Dehydration

Legumes are very important in most of the cropping systems due to their ability to fix atmospheric nitrogen and it increases the crops production. Cowpea has ability to fix atmospheric nitrogen transporting as amide which is advantageous to develop sustainable agriculture practices in dry land regions. The drought sensitivity of cowpea was associated with the high xylem sap ureide concentration. The grain legume species can be useful for decreased sensitivity of N_2 fixation to soil drying for regions where drought is a recurring problem.[43]

Effect of NaCl Paclobutrazol and their Combination on SOD & POX Activity in Leaves of Vigna unguiculata (Superoxide Dismutase & Peroxidase)

Paclobutrazol [(2RS; 3RS)-1-(4-chlorophenyl)-4,4- dimethyl-2-(1H-12,4-triazol-1-yl)-pentan-3-ol], a triazole compounds are used as fungicides, which have plant growth regulating as well as stress protecting properties and it is reported to reduce gibberellic acid biosynthesis and increase in abscisic acid and cytokinin contents. For Protection of plants from actually dissimilar stress by triazoles is mediated by a reduction in free-radical breaking and increase in antioxidant potential. Triazoles affect the isoprenoid pathway and change the level of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels. Triazole treated plants have a more efficient free-radical scavenging system that enables them to detoxify active oxygen. The present study deals with the salt stress ameliorating properties of paclobutrazol in *Vigna unguiculata* plants under pot culture to estimate the stress ameliorating ability of paclobutrazol & plant already have activities of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX). Plant treat with NaCl with paclobutrazol increased these parameters to a larger extent when compared to NaCl stressed plants. The results showed that the paclobutrazol moderately ameliorated the effects of NaCl stress in *V. unguiculata* and increase the growth and antioxidant enzyme activities.[38]

Genetic Diversity of Six Vigna Species by STMS Analysis (Sequence Tagged Microsatellite Site)

A high level of genetic diversity was identified within 15 accessions of yardlong bean & 6 cultivated *Vigna* species by using STMS analysis based on cowpea *Vigna unguiculata* ssp. *unguiculata* primer-pairs. STMS primers considered from the cowpea genomes were exceedingly transferable to other *Vigna* species. STMS analysis with these primers also exposed a very high level of difference between six *Vigna* species, which clustered into three groups at a 0.5 coefficient of correspondence. Group A represented from African *Vigna* species while groups B and C represented the Asian *Vigna* species. The variation detected using STMS a primer was quite high compared to previous studies variation within species of legumes. This technique is very specific in detecting repetitive DNA in the genome. Microsatellite sequences are rich throughout plant genomes, are hyper variable and are thus more likely to mutate through evolutionary forces. This analysis separated the Cow pea accessions into three groups but the clustering of accessions was not linked to geographical origin. Cow pea is found widely cultivated in Asia, especially Southeast Asia with accessions exhibit a high level of morphological diversity. STMS analysis clearly separated *Vigna* species into the African and Asian *Vigna* groups.[39]

Effect of Fumigation and Moisture Content on the Seedling of Vigna unguiculata

The fumigation of Cowpea seeds with a 1:1 mixture of carbon tetrachloride and ethylene dichloride at a concentration of 20 cm^3 of total storage volume. The effect of fumigation was seedlings arising from seed with higher moisture content; a quarter of the seedling in group weighed less than 0.2 gm. Plants grown from seeds with the lower moisture content did not show to suffer so hugely from fumigation though their general growth was retarded.[14]

Supercritical Carbon Dioxide Extraction using a Customized Supercritical Fluid Apparatus

This present study investigates application of supercritical fluid carbon dioxide extraction (SCFE-CO2) for removing fats from black-eyed pea (*Vigna unguiculata*) to produce high protein-low fat diet products. Supercritical fluid carbon dioxide extraction (SCFE-CO_2) has been found to be an alternative extraction method. This method has several advantages such as simple separation of the carbon dioxide (CO_2) from the extract and leaving no solvent residue in the extract. 20 g of black-eyed peas were used for each experiment. Extraction pressure was varied from 25 to 35 MPa and temperature varied from 40 to 60^0C. The flow rate of CO_2 was varied from 10 to 20 g/min. The static stage was 60 min

for all experiments, while the dynamic stage was 180 min and 240 min, for black-eyed pea & light yellow transparent oil with the characteristic odor was obtained. The *Vigna* seeds after SCFE-CO_2 process became a brittle chalky white color which indicated less or no oil content remain in the raw material.[22]

CONCLUSION

Plants were mainstay of medicine and credited with mystically & almost super natural power of healing. Plants have a vast potential for their use as a curative medicine. However, the ultimate objective of their use is that they should interact directly with our body chemistry. In present scenario, traditional knowledge system in our country is fast eroding and there is an urgent needs to inventoried record all ethno-botanical and cultural information among the diverse ethnic communities for the effective utilization of traditional medicine. The knowledge of the properties of medicinal plants has likely been on to native by their elders or is based on experience. The name and parts of the plant studied, the spectrum of activities, and methods used are discussed in this review article. The present paper reveals the literature up to date review on Ethno-Phyto medicinal research out come and traditional uses of this plant.

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