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A REVIEW ON PHYTOCHEMICAL PHARMACOLOGICAL AND PHARMACOGNOSTICAL PROFILE OF HORSE GRAM

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ABSTRACT

Horse gram is a well known, inexpensive, underutilized source of nutrients like protein, calcium and iron. It is one of the major human nutrients and contains rich source of carbohydrate, fat, protein, vitamins and minerals. Other than its nutritional constituents it has some other nutritive bioactive substances. *Macrotyloma uniflorum* contains major bioactive constituents are acids like phenolic acid, phyticacid, proteinease enzymatic inhibitors have significant metabolic and physiological effects. It is mentioned in the alternative/old medicinal systems as horse gram and is very useful in treating kidney stones, weight loss, diabetes, cold, fever, cholesterol, throat infection, piles etc. mainly for the patients of jaundice and water retention. Used in weight loss diet and also because of rich in proteins(20%), because of low acceptable taste and flavor of cooked products it is used only by the farming community and low income peoples, and called as under utilized, less expensive nutritional plant.

KEYWORDS: *Macrotyloma uniflorum*, Phytoconstituents, Hyperglycemic activity.

INTRODUCTION

Dietary supplements from different plants sources are considered to as phyto pharmaceuticals. Natural products contain essential phytoconstituents which are capable of curing various diseases by acting as a plant medicine and are the source for drug designing processes. Recent researchers reveals that many phytoconstituents such as proteins, vitamins,

minerals, carbohydrates, terpenoids, tannins, phenols, flavonoids can protect human against diseases for which it is studied extensively to establish their efficacy and to understand the underlying mechanism of their action. Secondary plant metabolites have biological properties such as antioxidant activity, anti inflammatory, stimulation of the immune system, anti microbial activity, modulation of detoxification enzymes, decrease of platelet aggregation and modulation of hormone metabolism and anti cancer property. *Macrotyloma uniflorum* also described as *Dolichos biflorus* L. in the literature. Horse gram sprouts are used in eliminating kidney stones. It also helps in lowering cholesterol levels and could play a role in antioxidant. *Macrotyloma uniflorum* is commonly used as food for horses, because of this it named as horse gram. It is mainly cultivated in Myanmar, Sri Lanka, Bangladesh, west indies etc. Kaempferol 3 OB Sitosterol and Stigmasterol were investigated and recently reported the cytotoxicity assessment of the horse gram. It contain essential bioactive components with less anti nutritional factor especially phytic acid, which get degraded due to the enzyme known as phytase produced during sprouting.

Synonym[1]

Macrotyloma uniflorum

Dolichos biflorus

Dolichos benadirianus

Dolichos uniflorus

Common Names[2]

Tamil name : Kaanam, Kollu

Sanskrit name : Kulathika, Sweta beeja, Kulattha

English name :Madras gram, Horse gram, poor man’s pulse

Arabic name : Gaheth, Kultthi, Kulit(Hindi), Habbul Kulth

Chinese name : Bian Dou

Kannada name : Huruli

Malayalam name : Mutira

Manipuri name : Ngakijou manbi

Nepali name : Gahat

Telugu name : Ulavalu.

Classification

Kingdom : Plantae

Subkingdom : Viridiplantae

Infrakingdom : Streptophyta

Superdivision : Emryophyta

Division : Tracheophyta

Subdivision : Spermatophyta

Class : Magnoliopsida

Super order : Rosanae

Order : Fabales

Family : Fabaceae

Genus : Macrotyloma

Species : Macrotyloma uniflorum

Distribution[3]

Origin and distribution

Native place of horse gram is the old world tropics and indigenous to India. Around 2000 BC, there is a investigation report of archaeological department revealed that the use of horse gram as food supplement particularly in India, that the proof of origin. The genus of horse gram Macrotyloma and it contains 25 species indigenous to Asia and Africa. The naturalized or wild horse gram presence is recorded in India and Africa (especially in East, Southern and Central Africa). The main primary origin of *Macrotyloma uniflorum* and its function as cultivated plant is in the plains and hills of low attitude extending southwards in the Western Ghats in south west India, mainly in Sahyadri hills. During the time of Neolithic period, through counter migration of human beings, horse gram cultivation is spread over in all over India, particularly in northern and western parts of the India. Then horse gram is also harvested in West indies, Sri Lanka, Indonesia, Malaysia etc., In India it is grown in the states of Tamil nadu, Andhra Pradesh, Madhya Pradesh, Karnataka, Chhattisgarh, Orissa, Bihar, Jharkhand, Uttar Pradesh and in the foot hills of Uttaranchal and Himachal Pradesh.

Description[4]

Horse gram is a twining annual forming dense growing 35-65 cm height. Trifolate leaves are 2-8 cm long and 3-5cm wide. Leaflets are ovate, rounded at the base, slightly tapering or pointed, terminal leaflet symmetrical, laterials asymmetrical, 3-5.5cm long, 3-4cm broad,

softly woolly on both surfaces, fimbriolate, palerbeneath. Yellow or greenish yellow flowers with violet blot on the standard are borne singly. Pods are 5-8cm long and 3-9mm wide with 5-8 seeds. *Macrotyloma uniflorum* is the cultivated annual form which has wider pods. It is drought resistant but cannot withstand water logging.

Flowers

Inflorescence an axillary, bracts upto 4mm long. Flowers bisexual, papilionaceous; calyx pubescent, lobes triangular lanceolate, upper pair entirely fused. Corolla with cream, yellow or greenish yellow standard, often with a small purple blotch inside, obovate oblong. Wings and keel greenish yellow, stamens 10,9 fused and 1 free.

Fruits

Fruits a linear oblong pod 3-8cm * 4-7mm, upcurved towards apex, acuminate, densely hairy when young. Later more sparsely, margins glabrous, smooth or warty. Fruits are dehiscent, 5-10 seeded. Seeds are trapezoidal, oblong or rounded reniform. They are pale to dark reddish brown, speckled or mottled with black and orange –brown or all black.

Leaves

Alternate, trifoliate, stipules lanceolate, 4-10 mm long, striated. Petiole 1-7cm long, rachis 2.5-10mm long, leaflets ovate-rhombic, obovate or elliptical, apex rounded to acute, base rounded, lateral leaflets asymmetric, hairy to glabrescent on both surfaces.

Pods

Pods 6-8cm long and 4-8mm wide with 6-7 seeds. Var.uniflorum is cultivated annual form which has wider pods.

Seed

Flattened small seeds with light red, brown, grey black or mottled testa with photo and thermo sensitive nature.

IMAGES OF *Macrotyloma uniflorum*

Whole plant of Macrotyloma uniflorum

Flower of Macrotyloma uniflorum

Pods of *Macrotyloma uniflorum*

Seeds of *Macrotyloma uniflorum*

VARIETIES[5]

Four varieties of horse gram have been distinguished:

- M.uniflorum - var.uniflorum
- M.uniflorum - var.stenocarpum
- M.uniflorum - var.verrucosum
- M.uniflorum - var.benadirianum

Ethanomedicinal Uses[6]

Macrotyloma uniflorum have antihyperglycemic properties and also have qualities which reduce insulin resistance. Antihypercholesterolemic, anti microbial, antiobesity, antihelminthic, analgesic, anti inflammatory, antidiabetic, anticholilithiatic, antihistamine, anti peptic ulcer, anti oxidant, anti obesity, anti urolithiatic against calcium oxide crystals, anti urolithiatic against calcium phosphate crystals, anti urolithiatic against uric acid crystals, diuretic, haemolitic, hepatoprotective.

Phytoconstituents

Phenolic acids namely 3,4 dihydroxybenzoic, 4 hydroxybenzoic, vanillic, caffeic, p – coumaric, ferulic, syringic and sinapic acids.

Flavonoids Compound

Cotyledon - Kaempferol 6.0 c, Quercetin 9.7c, seed coat – quercetin 129.5 a, kaempferol – 117.2 a.

Bio active compounds

Horse gram contains various compounds that can cause various biological actions in the humans, including both useful and harmful side effects. The undesirable ones are to be considered as anti nutrients or anti nutrimental factors or compounds. The antinutritional compounds found in pulse crops can be categorized as protein ANCs include alkaloids, phytic acids, phenolic compounds such as tannins and saponins. The presence of antinutritional factors, such as phenols, tannins, phytic acids and flatulence causing oligosaccharides are now being considered as potential anti oxidants. These anti nutrients causes a number of positive health effects such as a decrease risk of intestinal diseases (gallstones, diverticulosis, constipation and colon cancer), coronary heart disease, prevention of dental caries and treatment of diabetes. saponins and another common class of antinutrients compounds have been reported to show hypocholesterolemic as well as anticarcinogenic effects.

Phytic Acids

Phytic acid or inositol hexaphosphate IP6 is a simple ringed carbohydrate with six phosphate groups attached to each carbon (a bio active sugar molecule) and a major form of phosphorylated inositol. Phytic acid exists in the form of free acids, phytate, or phytin and all all of these forms are interchangeable. The concentration of phytic acid in horse gram revealed a significant quantity in embryonic axe fraction.

Phenolic Compounds

Phenolic compounds have a greatest beneficial interest on human health due to presence of antioxidant property, such as protection of oxidative damage. The principal phenolic compounds of horse gram seeds are flavonols such as quercetin, kaempferol, and myricetin, vanillic, p – hydroxybenzoic, and ferulic acids. The phenolic acids are a large family of secondary metabolites having either derivatives of benzoic acid or of cinnamic acid, which

are commomly found as esters of caffeic and quinic acids and are responsiblr for various beneficial effects in a multitude of diseases.

Flatulence Factors

Raffinose family oligosaccharides (raffinose, stachyose and verbascose) alpha galactosyl derivative of sucrose, a low molecular weight causes accumulation of gas, discomfort, diarrhea, pain and cramps after digestion. The cotyledon fractions contain higher concentrations of oligosaccharides (raffinose, stachyose and verbascose), accounting for 39% of the total soluble sugars.

Proteinase Inhibitors

Protease is a group of enzymes whose catalytic function is to hydrolyze (breakdown) peptide bonds of proteins. proteases, is an indispensable for maintenance and survival of the organisms but have potentially damaging role in higher concentrations, thus its activities nedto be regulated. trypsin, chymotrypsin, elastase, chymase, cathepsin G, plasmin, thrombin and subtilisin are classical examples of functional serine proteinases.

PHARMACOLOGICAL IMPORTANCE

Hypoglycemic Activity[7]

Macrotyloma uniflorum (Lam.) Verdc. (Leguminosae) seeds, known as the poor man's pulse crop in India, have been used as a food and also used in the traditional method for treatment of kidney stones, diabetes, obesity, etc. *Macrotyloma uniflorum* α-amylase inhibitor (MUAI) inhibited both the mouse pancreatic and human salivary α-amylase in a non-competitive manner with K_i values of 11 and 8.8 µM and IC_{50} value of 30 and 12.5 µg/mL, respectively. It decreased the serum glucose level in the treated diabetic mice. Histological findings suggested minimum pathological changes in the treated diabetic mice as compared to the diabetic control.

Antioxidant Activity[8]

Total phenolics and the antioxidative properties of two varieties of horse gram (*Macrotyloma uniflorum*) were studied. The raw and dry-heated seed samples were extracted successively with methanol and 70% acetone separately. After removing the solvents, the extracts were freeze-dried. The black seeds contained relatively high levels of total phenolics and tannins than the brown seeds with respect to the treatments and solvents extraction. The extracts were subjected to assess their potential antioxidant activities using systems such as α,α-diphenyl-β-

picrylhydrazyl (DPPH•), ABTS• $^{+}$, FRAP, linoleic acid emulsion, O2- and OH•. The superoxide anion radical-scavenging activity was found to be higher in 70% acetone extract of the both raw and dry heated seeds of the respective varieties at the concentration of 600 µg in the reaction mixture. The DPPH radical and ABTS cation radical-scavenging activities were well proved and related with the ferric-reducing/antioxidant capacity of the extracts. Interestingly, among the various extracts, 70% acetone extracts of dry-heated samples of brown variety, and raw and dry-heated samples of black variety showed significantly ($P < 0.05$) higher hydroxyl radical-scavenging activity. In general, all extracts exhibited good antioxidant activity (53.3–73.1%) against the linoleic acid emulsion system but were significantly ($P < 0.05$) lower than the synthetic antioxidant, BHA (93.3%).

Antiinflamatory Activity[9]

Two sets of experiments were conducted in rats which were fed with boiled and unboiled horse gram and black gram, at a dose of 100 mg/100 g body weight, for 21 days and 60 days. The results showed that horse gram supplementation for 21 days and 60 days significantly increased the activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathioneperoxidase and showed no significant changes in the activities of the inflammatory mediators such as cyclooxygenase, lipoxygenase, myeloperoxidase, nitric oxide synthase, monocyte chemoattractant protein-1(MCP-1), tumor necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β), etc. However, the black gram (with skin and without skin) supplementation significantly increased activities of the inflammatory mediators and showed a significant decrease in the antioxidant enzymes in both the 21-day and 60-day experiments. Thus, these preliminary results demonstrate the anti-inflammatory and antioxidant potential of horse gram.

Antimicrobial Activity[10]

Plant oxidized fatty acids, phyto-oxylipins, have had a great impact on plant physiology. Phyto-oxylipins are a group of biologically active molecules that play a decisive role in plant defense against plant pathogens (Feussner and Wasternack, 2002;). Purification and characterization of the metabolites by plant Lipoxygenases were well studied when compared to the physiological role of Lipoxygenases. We doubted the role of Phyto -oxylipins in providing resistance to the Horse gram (Dolichos biflorus) plant against the common plant pathogens and play a key role in plant defense responses. Hence we made an attempt to find

out the antimicrobial activity of primary phyto-oxylipins generated from Dolichos biflorus Lipoxygenase against some of the common bacterial and fungal strains in vitro.

Anti Urolithiatic Activity[11]

The effect of oral administration of aqueous and alcohol extracts of Macrotyloma uniflorum (Fabaceae) seeds on calcium oxalate urolithiasis has been studied in male albino wistar rats. Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphate. Supplementation with aqueous and alcohol extract of Macrotylomauniflorum seeds significantly reduced the elevated urinary oxalate showing a regulatory action on endogenous oxalate synthesis. The increased deposition of stone forming constituents in the kidneys of calculogenic rats was significantly lowered by curative andpreventive treatment using aqueous and alcohol extracts. The results indicate that the seeds of M. uniflorum are endowed with significant antiurolithiatic activity and it also indicate that the alcoholic extract of M.uniflorum shows better anti urolithiatic activity than aqueous extract

Anti Histaminic Activity[12]

Suralkar et al., screened the ethanolic extract of M.*uniflorum* seeds for their anti histaminic activity by using histamine induced contraction on goat tracheal chain preparation and histamine induced bronchoconstriction in Guinea pigs. Histamine induced contraction of isolated goat tracheal chain preparation was significantly protected against histamine induced bronchospasm as indicated by delay in the preconvulsiveddyspnoea time (PCT) following the exposure of histamine aerosol.

Anti Helmintic Activity[13]

The seeds of M.*uniflorum* have anthelmintic activity which can be beneficial in eliminating worms. Philip, et al. tested the alcohol extracts of M.*uniflorum* seeds for their anthelmintic activity. These extracts exhibited potent anthelmintic activity against *Pheretima posthuma* and its activity was comparable with that of the standard, albendazole.

Anti Choliolithic Activity[14]

Bigonia, et al. found that M.*uniflorum* seed exerted antilithogenic influence by decreasing the formation of lithogenic bile in mice. Both the methanolic and acetone extracts (ME and AE) were capable of decreasing cholesterol hyper secretion into bile and increasing the bile acid output. The maximum effect was found in the AE as it decreased the papillary proliferation of

gallbladder and hepatic fatty degeneration. Antioxidant property of polyphenol and tannin inAE may provide its potential antilithogenic effect.

Anti-Histaminic Activity[15]

Suralkar et al., screened the ethanolic extract of *M. uniflorum* seeds for their anti-histaminic activity by using histamine induced contraction on goat tracheal chain preparation and histamine induced bronchoconstriction in Guinea pigs. Histamine induced contraction of isolated goat tracheal chain preparation was significantly subdued by the ethanolic extract of *M. uniflorum* seeds. The guinea pigs were significantly protected against histamine induced bronchospasm as indicated by delay in the preconvulsivedyspnoea time (PCT) following the exposure of histamine aerosol.

Anti-Peptic Ulcer Activity[16]

Panda, et al., evaluated the antiulcer activity of the hydroalcoholic extract of the seeds of M. uniflorum (MUSE) and p-coumaric acid against indomethacin (non-steroidal anti-inflammatory drug) and absolute ethanol (necrotizing agent) induced ulcers in rats. A dose-dependent decrease in the ulcer index could be seen in both models after the pre-treatment with MUSE and p-coumaric acid. MUSE and p-coumaric acid elicited significant antioxidant activity by attenuating the ulcer elevated levels of malondialdehyde and restored the ulcerdepleted levels of reduced glutathione and the antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase.

Hemolytic Activity[17]

The 1-butanol extract showed the significant hemolytic activity by mouse erythrocytes. Kawsar, et al. reported the presence of compounds such as methyl ester of hexadecanoic, ethyl ester of hexadecanoic acid mixture and n-hexadecanoic could be constituted a possible chemotaxonomic marker.

Hepatoprotective Activity[18]

Parmar, et al. discovered the significant hepatoprotactive properties of *M. uniflorum* seeds against D-Galctosamine and paracetamol induced hepatotoxicity in rats.

Toxicological Studies[19]

Kawsar, et al. analyzed the aerial parts of M. uniflorum for their cytotoxicity effects. Crude extracts of dichloromethane (CH2Cl2), ethyl acetate (EtOAc), 1-butanol (1-BuOH) and

aqueous (H2O) were screened by using the brine shrimp lethality bioassay technique. Most of the extracts were found to be non-toxic and this indicates that the ethnobotanical use (oral applications) of the *M. uniflorum* is justified.

1. **Nutritional Values of *M. uniflorum* (Lam.) Verdc:** *M. uniflorum* seeds are known as the poor man's pulse crop in Asian countries, especially India. It is commonly used for both food and fodder. The use of dry seeds of horse gram is limited due to their poor cooking quality. Recently, the US National Academy of Sciences recognized this legume as an upcoming potential food resource.
2. **Methods of consuming:** Seeds are the edible part of the plant and consumed as a whole (boiled) seed, as sprouts, as a curry or as whole meal in Asia, popular especially in southern Indian states. Procedures such as de-husking, germination, cooking, and roasting can be undertaken to enhance the nutritional quality of horse gram.
3. **Protein content:** Horse gram is the most protein-rich lentil found on the planet. The seeds have twice the protein content as of cereal grains. Mean protein value of horse gram seeds is almost equivalent to winged bean (Psophocarpus tetragonolobus), gram (*Cicer arietinum*) and soybean (*Glycine max*).
4. **Carbohydrate (CHO) content:** *M. uniflorum* seeds contain common and abundant forms of CHO, viz sugars, fibers, and starches. The digestibility of starches as a legume is lower than that of cereal. Contain less carbohydrate (55- 65%) and energy compared to cereals. CHO available in *M. uniflorum*seeds has low glycemic index.
5. **Fatty acid content:** Saturated fatty acids level in the seeds of *M. uniflorum* is considerably low. It is about 72.49% unprocessed seeds and about 71.99% in toasted seeds. Seeds are rich sources of Linoleic acid, an essential fatty acid. Raw seeds contain 45.58% and toasted seeds contain 40.33% of Linoleic acid.
6. **Dietary fiber content:** Whole grains are the best sources to get fiber into a balance diet. Fibers are of two types, soluble and insoluble. Horse gram seed contains 28.8% total dietary fibers, mainly insoluble dietary fiber (IDF) 27.82% and soluble dietary fiber (SDF) 1.13% with IDF: SDF 24.6 [68]. Horse gram flour contains 16.3% total dietary fiber (14.9% insoluble and 1.4% soluble and 2.2% resistant starch) [69]. Seeds of M. uniflorum contain more insoluble dietary fiber than kidney bean (*Phaseolus aconitifolius*).

7. **Micronutrient content:** Horse gram has the highest calcium content among pulses. As a legume, Horse gram is deficient in methionine and tryptophan, though it is an excellent source of iron and molybdenum.
8. **Anti-oxidant source:** The unprocessed horse gram seed is loaded in polyphenols, flavonoids and proteins, the major anti-oxidants which are also available in fruits and other food materials. The greater part of anti-oxidant properties is limited to the outer coat of seed and its removal would eliminate these properties.

1. Green synthesis of silver nanoparticles using Macrotyloma uniflorum[20]

Green synthesis of noble metal nanoparticles is a vast developing area of research. In this paper we report the green synthesis of silver nanoparticles using aqueous seed extract of *Macrotyloma uniflorum*. The effect of experimental parameters such as amount of extract, temperature and pH on the formation of silver nanoparticles was studied. The as prepared samples are characterized using XRD, TEM, UV–Visible and FTIR techniques. The formation of silver nanoparticles is evidenced by the appearance of signatory brown colour of the solution and UV–vis spectra. The XRD analysis shows that the silver nanoparticles are of face centered cubic structure. Well-dispersed silver nanoparticles with anisotropic morphology having size ∼12 nm are seen in TEM images. FTIR spectrum indicates the presence of different functional groups in capping the nanoparticles. The possible mechanism leading to the formation of silver nanoparticles is suggested.

CONCULUSION

Macrotyloma uniflorum contained carbohydrates, alkaloids, proteins, glycosides, saponins, phenolics, flavonoids, tannins, steroids, gum, reducing sugars and triterpenoids. It exerted many pharmacological activities including antimicrobial, antidiabetic, antioxidant, anti inflammatory, anti histamine, heptoprotective, cytotoxic, hypolipidemic, gastro protective, anti urolithic, anti helmintic and many other pharmacological effects. This paper will highlight the chemical constituents and pharmacological effects of Macrotyloma uniflorum.

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