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Phytochemicals in *Macrotyloma uniflorum* – A Review

Mohanraj R

Houston Community College, Houston, TX, USA

***Correspondence:** Remya Mohanraj, Houston Community College, Houston, TX, USA

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Abstract

Macrotyloma uniflorum (horse gram), is an underutilized legume that has high nutritional value. It is highly drought resistant and the seeds possess medicinal properties by virtue of the wide array of phytochemicals harbored in them. This article attempts to present a comprehensive review of the phytochemicals present in the seeds of M. uniflorum. Information presented in this chapter has been compiled from various published sources.

Keywords: horse gram, *Macrotyloma uniflorum*, phytochemicals, review

Introduction

Horse gram (Macrotyloma uniflorum (Lam.) Verdcourt (Syn., *Dolichos uniflorus* Lam., *Dolichos biflorus* auct. non L.)) [1] belonging to Fabaceae, is protein-rich and is amenable for cultivation in dry conditions and marginal soil fertility [2]. Flowers together in the leaf axils without a common peduncle [3]. Seeds of horse gram are ovoid in shape and colored pale fawn, light red, brown, or black [4]; reddish brown or grey [5]; pale brown, medium brown and blackish brown [6] (Figure 1). In ayurvedic medicine, whole seeds of horse gram, is advocated in the treatment of renal stones, piles, oedema, etc. [7]. The seeds have been reported to be a significant source of iron, molybdenum [7, 8] and protein [9].

Traditional medicinal use of horse gram seeds is emphasized by its mention in Charak Samhita (renowned text on Indian medicinal system) as a remedy for piles, hiccup, abdominal lump, bronchial asthma, for causing and regulating perspiration [10]. Kirtikar et al. in their book Indian medicinal plants, note Sanskrit writers' recommendation of horse gram's use as a demulcent in calculous affections cough, etc. [3]. The seeds find use in herbal medicine not only as tonic, astringent, diuretic but also as a remedy for asthma, bronchitis, urinary discharges, hiccoughs, ozoena, heart trouble and brain diseases [11]. *M. uniflorum's* utilization in Indian traditional medicine in treatment of urinary stones was reported by Ravishankar et al. [12]. A soup of the seeds could be heat generating and help dilute renal stones [13]. The figure shows two traditional South Indian dishes made using these seeds (Figure 2a and 2b). It's common knowledge in certain parts of south India to consume Kollu Rasam especially when affected by common cold (Figure 2b) (Figure 2).

Figure 1: Seeds of *M. uniflorum.*

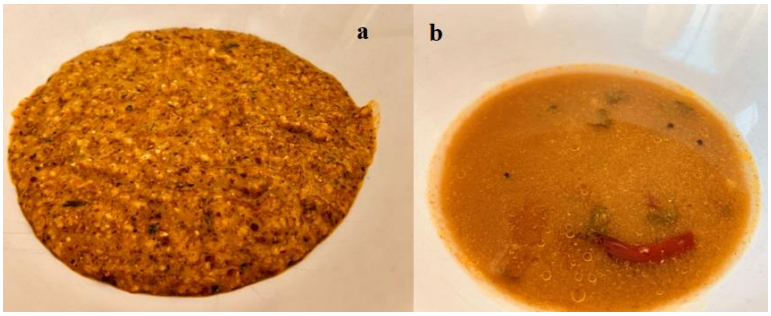

Figure 2: (a) Kollu chutney (b) Kollu rasam- South Indian dishes made using horse gram.

Various studies have corroborated the anti-hypercholesterolemic effect [14], anti-microbial activity [15–17] anti-helmintic activity [18, 19] anti-inflammatory [20], anti-diabetic [21], anti-oxidant [22, 23] and anti-urolithiatic activity [13, 24–26] of *M. uniflorum* seeds. Auxilia et al. reported that dolichins from the horse gram plant possess anti-HIV properties [27].

From the food composition table published by The National Institute of Nutrition (ICMR) by sampling whole horse gram seeds from different geographical regions of India, the protein content of horse gram seeds is comparable to other legumes and almost twice the protein content as of most cereal grains [28]. Although rich in proteins, intake of these seeds is restricted to certain groups because of its less acceptable taste and flavor and has therefore been an underutilized food legume [29].

Keeping the above background in mind, the present chapter is aimed at presenting a comprehensive review of various published reports on phytochemicals in *M. uniflorum* seeds. The review intends to provide the necessary background information for scientists to carry out studies on this valuable and yet underutilized legume. Electronic literature search was conducted to look for various recent studies that had analyzed the phytochemicals from *M. uniflorum*.

Methods of Phytochemical Analysis

Phytochemicals help defend plants against biotic and abiotic stresses [30, 31]. They have varying effects as antioxidants, enzyme action modulators, immune stimulators, hormone metabolism modulators, anti-bacterial and antiviral agents [32, 33]. Extensive investigations on the use of secondary plant metabolites as a source of pharmaceutical compounds are being carried out [34]. Isolation and purification of therapeutic biomolecules from medicinal plants opens avenues for the discovery of new drugs. *M. uniflorum* contains several phytochemicals which could be attributed to its beneficial effects on human health.

Analysis of phytochemicals could be done qualitatively or quantitatively. Preliminary qualitative analyses include various biochemical tests performed to test the presence of phytochemicals. For instance, some of the available tests include Mayer's, Dragendorff's reagent for alkaloids [35, 36]; test using hydrochloric acid for flavonoids [37]; frothing test for saponins [38]; Liebermann–Burchard test, Salkowski's test for phytosterols [39, 40]; Lowry assay for proteins

[41]; Millon's test with acetic acid for tannins and phenolic compounds [42]; Barfoed's test, Fehling's test, Benedict's test for carbohydrates [43–45]; shaking with distilled water and observing the layer of foam formed for saponins [46]; Salkowski test for terpenoids [47]; using Folin-Ciocalteu reagent for phenols [48]; alkaline reagent test for flavonoids [49]. One or more of the available methods could be chosen depending on the conditions.

De Silva et al. in their review describe gas chromatography (GC), gas chromatography coupled with mass spectrometry (GC-MS), liquid chromatography (LC), high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC) as qualitative and quantitative techniques for screening phytochemicals from plant materials [50]. Chromatography includes a variety of separation techniques that are based on partitioning different components of a sample between a mobile phase and a stationary phase. The mobile phase is either a gas or liquid [51]. During chromatography, the components of a mixture are separated according to their difference in affinities for a stationary phase and different chromatographic techniques use different stationary phase and operational conditions [52]. Physicochemical characteristics and instrumentation availability guide the choice of chromatographic system and qualitative and quantitative information is obtained from the retention time and peak area data from the chromatogram [51].

Structure determination of the isolated compounds could be carried out using a variety of techniques including UV-visible, infrared (IR), nuclear magnetic resonance (NMR), and mass spectroscopy. Structural determination relies on a spectrum that indicates the amount of radiation absorbed after passing electromagnetic radiation through an organic molecule [53, 54].

Phytochemicals in *Macrotyloma uniflorum*

This section discusses the various phytochemicals that have been reported mainly from the seeds of *M. uniflorum*. Since other reviews on the subject are available, the present review is focused on including more recent studies.

Aqueous extracts of *M. uniflorum* were qualitatively analyzed for various phytoconstituents by Patel et al. and they reported carbohydrates, alkaloids, flavonoids, saponins, phytosterols and phenolic compounds from the extracts [55]. Studies carried out by Ojha et al. showed that germination and fermentation of *M. uniflorum* seeds significantly reduces anti-nutritional factors and increases bioactive components. In addition, it was found that antioxidant content decreased during soaking and increased during roasting, germination and fermentation [56].

Manikandan et al. through phytochemical screening observed alkaloids, flavonoids, phenols, tannins, saponins, glycosides, steroids, terpenoids, proteins and carbohydrates from various solvent extracts of *M. uniflorum* seeds. Among the various solvents, ethanolic extract was reported to be very effective followed by methanol, aqueous, chloroform and hexane extracts [57]. Venugopal et al. reported an RF value of 0.9 in TLC plates from petroleum ether, acetone, ethanol, chloroform, ethyl acetate, methanol and water extracts of *M. uniflorum*. The authors also reported the presence of terpenoids through UV-visible spectroscopy and qualitative analysis of acetone, chloroform, methanol and aqueous extracts [58].

Sharma et al. reported compounds like syringic acid hexoside, [6]-gingerol, tyramine, rutaecarpine, asperuloside, abscisic acid, proline betaxanthin and prostaglandin E1 from methanolic extracts of *M. uniflorum* through liquid chromatography mass spectrometry and proximate analysis [59]. Jaya et al. through qualitative phytochemical analysis of acetone, chloroform, ethanol and aqueous extracts of *M. uniflorum* seeds reported the presence of carbohydrate, protein, alkaloids, flavonoids, tannins, steroids, phenols, glycosides and saponins. Analysis of seed powder by FTIR spectra showed peak values representing alkenes, and alkanes [60].

Patangare et al. attempted quantifying the physiochemical properties of *M. uniflorum* and reported, "moisture content (8.05 ± 0.6), ash (3.91 ± 0.10), total crude fibre (3.9 ± 0.04), crude carbohydrate (58.2 ± 0.16), crude fat (0.45 ± 0.02), and crude protein (21.87 ± 1.07). Potassium (762 mg/100 g), phosphorus (321 mg/100 g), calcium (239 mg/100 g) and sodium (11.5 mg/100 g)" [61].

Rao et al. evaluated *M. uniflorum* for the presence of alkaloids, saponins, tannins, flavonoids, steroids and phenols. They reported antioxidant and anticancer activity from seed coat extracts [62]. A study to determine secondary metabolites, anti-oxidant and cytotoxic activity of seed coat of *M. uniflorum* were carried out by Chakraborty et al. in

petroleum ether, chloroform, ethanol, and aqueous extracts. The results indicated that ethanolic extracts from seed coat had high levels of alkaloid, phenol, flavonoid, tannin, saponin, and terpenoid and had anti-oxidant and cytotoxic activity against B16F10 and B16BL6 cell lines [63].

Thippeswamy et al. estimated ascorbic acid concentrations in moisture free and rehydrated horse gram and reported 17.25 mg and 20.97 mg per 100 g respectively [64]. Phytochemical screening using TLC and HPTLC performed by Zhu et al. confirmed the presence of isoflavone, daidzein, genistein in the ethanolic extract of *M. uniflorum*. FTIR spectra of the methanol and aqueous extracts of fresh and dried horse gram sprouts showed the presence of alkyl halides, aromatics, esters, alkanes, amides, alkenes, phosphines, alcohols, nitro compounds, and carboxylic acids [65]. Methanol extract from fresh horse gram sprouts had the highest amount of total soluble sugars, proteins, flavonoids, terpenoids and less phytic acid. Terpenoids were present in both fresh and dried horse gram sprouts [66]. Suriyavathana et al. reported alkaloids, flavonoids, saponins, phenols, glycosides, tannins, terpenoids from aqueous extracts of seeds following phytochemical screening [67].

Studies by Ramachandraiah et al. revealed the anticoagulant, anti-platelet, and clot dissolving properties of aqueous extract of *M. uniflorum* seeds. Analysis by SDS-PAGE indicated the presence of a high level of monomeric proteins [68].

Valli et al. reported phytochemicals like alkaloids, saponins, terpenoids, glycosides, steroids, triterpenoids, resin, quinone, proteins, amino acids, carbohydrates, flavonoids, cardiac glycosides, phenols, fixed oils, fats and fatty acids from the methanol and aqueous of *M. uniflorum*. Lesser amount of phytic acid was observed in fresh and dried horse gram sprouts and their methanol and aqueous extracts revealed the presence of terpenoids. The authors also carried out GC-MS analysis of methanol extract from fresh horse gram sprouts and showed the presence of various compounds [69].

Singh et al. carried out qualitative estimation of amino acids by paper chromatography and reported the presence of various amino acids like DL-alanine, arginine, L-glutamic acid, hydroxy proline, isoleucine, L-leucine, phenyl alanine, tryptophan, tyrosine, valine from the seeds of *M. uniflorum* [70]. The ethanolic and water extracts of *M. uniflorum* seeds had alkaloids, flavonoids, phenolic compounds, tannins and fixed oils and fats [71].

Panda et al. investigated the cardioprotective activity of *M. uniflorum* seed extract and phenolic acids, p-coumaric acid and ferulic acid in rats. They also reported isolation and quantification of these phenolic acids by HPLC [72]. Chakraborty et al. reported that methanol and ethanol extracts of *M. uniflorum* exhibited significant antimicrobial, antioxidant and anticancer activity. Their study also reported propanedioic acid from methanolic extract and hydroxyurea from ethanol extract [34].

Bharathi et al. performed phytochemical screening and GC-MS analysis on the seed and leaves formulation of *M. uniflorum* and reported the presence of steroids, alkaloids, sugars, phenolics, saponins, tannins and 38 phytocompounds [73]. According to Kachru et al., GC-MS analysis of methanol extract of *M. uniflorum* seeds showed the presence of mome-inositol, hexadecanoic acid, methyl ester, octadecanoic acid and gamma tocopherol. Qualitative analysis showed presence of proteins, flavonoids, phenols, triterpenoids, glycosides, tannins and sterols in different extracts of seeds and leaves [74].

Panda et al. evaluated the protective effects of hydroalcoholic extracts of *M. uniflorum* seeds and ferulic acid against hyperlipidemia and cardiac abnormalities in rats. They had isolated and quantified ferulic acid from seed extracts using HPTLC and HPLC respectively. UV-visible, IR and NMR spectroscopies for major functional groups confirmed the presence of the isolated constituent [75].

Giresha et al. estimated total phenolics from the extracts of *M. uniflorum* and observed that the seed coat had higher concentration of phenolics and phytochemicals (in aqueous extract) than pulp. They also reported that ethanolic and aqueous extracts contained higher concentration of flavonoids, alkaloids, tannins, saponins, phenols and quinones as compared to non-polar solvent extracts [20]. Phytochemical studies by Parvathiraj et al. indicated the presence of carbohydrate, steroid, tannins, phenol, protein, amino acid. Glycosides, flavonoids and saponins were reported to be absent. The authors also reported antibacterial activity of alcoholic and aqueous extracts against nine human pathogens [76].

Suriyamoorthy et al. reported amino acids, proteins, cardiac glycosides, saponins and steroids from petroleum ether and chloroform extracts of *M. uniflorum*. Ethanolic extracts showed the presence of amino acids, proteins, cardiac glycosides, saponins, oils, fat and steroids, whereas aqueous extracts showed tannins, phenols, amino acid, proteins, cardiac glycosides and steroids [77]. Das et al. analyzed the chemical contents of ethanolic extracts of seeds of *M. uniflorum* using GC-MS and identified twenty-eight compounds out of which, mome-inositol and ethyl alpha-d-glucopyranoside were the main ones [78].

Bigoniya et al. analyzed phytochemicals from the seed extracts of *M. uniflorum* qualitatively and quantitatively. The presence of chlorogenic acid and ferulic acid was indicated by TLC; quercetin and chlorogenic acid by HPTLC [79]. Bolbhat et al. analyzed the seeds of eight *M. uniflorum* mutants for the presence of N, P, K, Mg, Zn, Cu, Fe, Mn and reported variations in the amount of macro and micro minerals in the mutant seed material which were attributed to the altered genetic composition [80].

Conclusion

As could be seen from the present review, various studies have been carried out to qualitatively and quantitatively analyze the array of phytochemicals present in horse gram seeds. Studies reporting the bioactivities of the crude extracts as well as isolated compounds lends credence to the traditional medicinal uses of this underutilized legume. Its significance from a nutritional as well as medicinal point of view has been well established. Emergence of newer technologies present more opportunities for the isolation and purification of biomolecules. Further investigations on deducing structures and narrowing down on the exact mechanism of action of the various phytochemicals could open new avenues in the designing of novel drugs from these plant-based compounds.

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