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Kaempferol and Stigmasterol Content Variations in Diverse Cowpea (*Vigna unguiculata*) Seed Varieties

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Abstract

The present study deals with the examining the various six varieties (Konkan Sadabahar, Poosa Kolum, PCP-97021, DCP-2, CP-20116 and CH-9863) of *Vigna unguiculata* (cow pea) seeds to find out the best source for Kaempferol and Stigmasterol content. The described study revealed the presence of maximum content of Kaempferol in Pusa Kolum(2.42%) whereas Stigmasterol was highest in CH-9863 variety(2.922%) among all samples used. Since both secondary metabolites are useful in the treatment of Alzheimer, these varieties can be used by pharmaceutical industries.

Keywords: *Vigna unguiculata, Kaempferol, Stigmasterol, cowpea*

Introduction

Plant cells contain far more compounds than are produced by the basic metabolism. Many of these are highly toxic and are often stored in specific vesicles or in the vacuole. Several studies indicate that this kind of storage, functions as a detoxification of the plant on one hand and generates on the other hand as reservoir of Polyphenols, Terpenes (Sterols), Waxes, alkaloids and pigments are just some key words that illustrate what is meant. As phyto sterols are the equivalents of animal cholesterol, they can in principal influence metabolic processes, where cholesterol is involved. They are extensively used in the food industry and as dietary supplement. High cholesterol levels have long been discussed to increase the risk of developing Alzheimer's disease. Stigmasterol, a phytosterol has an effect on a variety of molecular processes, it lowers enzyme activity and inhibits the formation of proteins implicated in the development of Alzheimer's disease [1], and also it alters the structure of the cell membrane. Polyphenols are other secondary metabolites of plants which are generally involved in defense against ultraviolet radiation or aggression by pathogens [2] in food, polyphenols may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability. Towards the end of 20th century, epidemiological studies and associated meta-analyses strongly suggested that long term consumption of diets rich in plant polyphenols offered some protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [3]. Polyphenols (Flavonoids) from fruits and vegetables seem to be invaluable potential agents in neuro protection by virtue of their ability to influence and modulate several cellular processes such as signaling, proliferation, apoptosis, redox balance and differentiation [2]. They are a large group of low molecular weight, ubiquitously distributed, poly phenolic secondary metabolites. These compounds play a significant role in various stages of plant growth and their existence in the environmental stresses. More than 6,500 flavonoids have been identified, they protect plants against various biotic and abiotic stresses and exhibit adverse spectrum of biological functions and play an

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important role in the interaction between the plant and their environment, it absorbed the harmful UV radiation induced cellular damage. Flavonoids are not essential for plant survival, but they are bioactive and influence the transport of the plant hormone, auxin. Numerous preclinical studies have shown that Kaempferol and its some glycosides have a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, cardio protective, neuro-protective, anti-diabetic, anti-osteoporotic, estrogenic/ anti-estrogenic, anxiolytic, analgesic and anti-allergic [2]. Apart from that, they are responsible for flower colors, protecting the plants from microbes and insects. Kaempferol is a strong antioxidant and helps to prevent oxidative damage of our cells, lipids and DNA. It seems to prevent arteriosclerosis by inhibiting the oxidation of low density lipoprotein and the formation of platelets in the blood. Studies have also confirmed that Kaempferol acts as a chemo preventive agent, which means that it inhibits the formation of cancer cells.

Cow pea (*Vigna unguiculata*) is drought tolerant grain legume which has great agronomic interest as food and fodder The grain constitutes an important source of dietary protein and secondary staple carbohydrate Apart from this the seed are rich in flavonoids such as Kaempferol, and terpenes like Stigmasterol [3]. Its roasted seeds are used to treat neuritis, insomnia, weakness of memory, indigestion, dyspepsia, sensation of pins and needles in limbs, periodic palpitation, congestive cardiac failure etc. it is an excellent medicine for stomatitis, corneal ulcers, coleic diseases. Kaempferol and Stigmasterol have widely reviewed in our laboratory *in vivo* as well as *in vitro* [4-10]. The presence of Kaempferol and Stigmasterol is reported in *Vigna unguiculata* [3,11]. In the interest of obtaining good sources of Kaempferol and Stigmasterol, seed varieties of Cowpea (*Vigna unguiculata*) have been subjected to probing for detecting Stigmasterol and Kaempferol content in them, we have selected 6 varieties of cowpea namely Konkan Sadabahar, Poosa Kolum, PCP-97021, DCP-2, CP-20116 and CH-9863 for further analysis of Kaempferol and Stigmasterol content.

Materials and Methods

Six seeds varieties of *Vigna unguiculata* (Konkan Sadabahar, PoosaKolum, PCP-97021, DCP-2, CP-20116 and CH-9863) were collected from Agricultural University, Dapoli (Longitude 73.189, Latitude 17.752). The quantitative estimation of Kaempferol and Stigmasterol were carried out with the help of High Performance Thin Layer Chromatography (HPTLC) with their respective authentic compounds, to find out the best source for these natural products.

Kaempferol extraction and its Quantification by High Performance Liquid Chromatography (HPTLC) analysis

The 6 varieties of Cow pea were powdered and subjected for Kampferol extraction by using the methanol cold extraction method at room temperature. The extracts were dried in vacuo and subjected for its Kaempferol content analysis by using HPTLC spectral studies separately with standard reference compound of Kaempferol. HPTLC analysis was carried out in Anchrom Test Lab Pvt. Ltd using silica gel plates (60F254 Manufacturer E. MERCK KGaA). Sample application was carried out on CAMAG Linomat 5 Instrument (CAMAG Linomat 5 "Linomat5_080222" S/N 080222). Inert gas was used as spray gas. Sample solvent type was methanol. Dosage speed was 150nl/s and syringe size was 100µl and the analysis wave length was 430 nm. Toluene: Ethyl acetate: Diethyl amine (14:2:2) was used as mobile phase.

Stigmasterol extraction and Quantification by High Performance Thin Layer Chromatography (HPTLC)

HPTLC analysis was carried out in the Anchrome Pvt. Ltd, Mulund, Mumbai .All the prepared samples were subjected for HPTLC analysis with that standard reference compound of Stigmasterol. Merck Silica gel 60 F_{254} TLC was used as a stationary phase while Toluene: Ethyl acetate: Methanol (7:1:0.5) was used as mobile phase. Camag Scanner IV equipped with win CATS Planar Chromatography manager software version 1.4.7 equipment was used for HPTLC spectral studies at 366 nm wavelength. 10 % Methanolic sulphuric acid was used as derivatizing agent while nitrogen was used as carrier gas.

Results and Discussion

Legumes including cow pea have been widely grown and their seeds are used as human and animal food to provide calories and protein. As food, cowpea seeds are consumed in different forms as they provide important vitamins, phyto-nutrients including antioxidants besides carbohydrates, minerals and trace elements. In addition, it is a cheap source of high quality protein in the diets of millions.

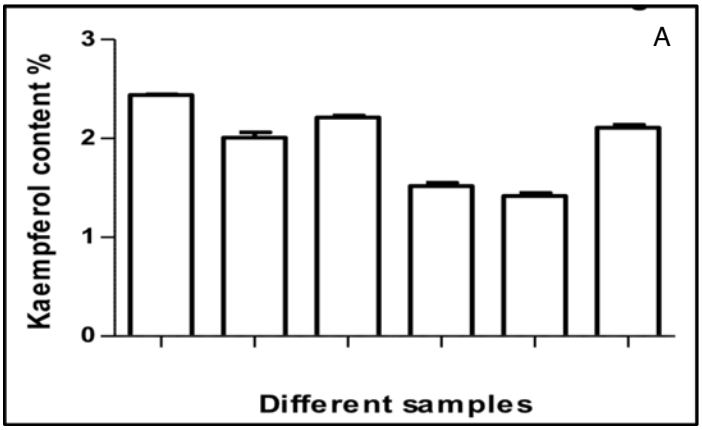
The experimental plant is reported as medicinally important to combat nuerodiseases. Kaempferol [12, 13] and Stigmasterol [1] both are known to conflict with Alzheimer disease, therefore it is recommended to find the better source of these compounds, which have already been reported in Vigna *unguiculata*. Many phenolic compounds have bioactive properties [14, 15], on the other hand Phytosterol has a role as precursors of many hormones as also many therapeutic actions. Hence we have selected six different varieties of *Vigna unguiculata* seeds collected from Dapoli Agricultural University to examine their Kaempferol as well as Stigmasterol content. Biosynthetically, the mevalonic acid pathway produces terpenes, sterols, and carotenoids, while flavonoidal metabolites are produced by the shikimic acid [16] pathway. Their production is also governed by plant's genetic makeup [17]; their content varies intra as well as interspecies. *Ramalina americana* was split into two different species (*R. culbersoniorum* and *R. americana*) based on secondary metabolite and nucleotide sequence divergence [18]. Patterns of intra-species variation in composition and concentration of secondary metabolites among geographic regions or habitats have been well documented for terrestrial plants [19]. These

Table1: Showing % Kaempferol and % Stigmasterol variations in different varieties of *Vigna unguiculata*

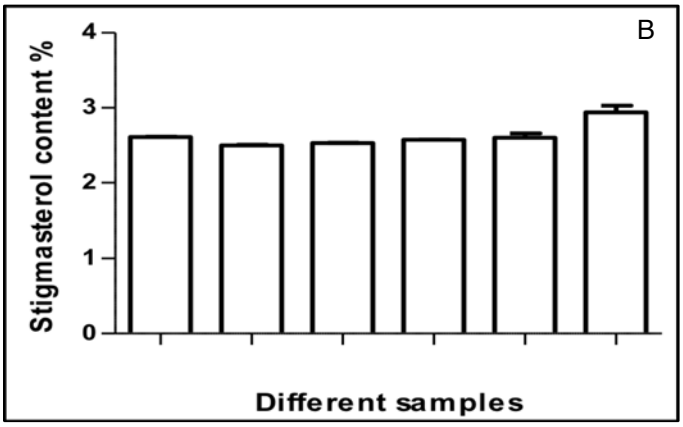
Sno	Variety type of Cow pea	% Kaempferol	% Stigmasterol
1.	Pusa Kolum	2.42±0.015	2.619±0.01
2.	Konkan Sadabahar	1.99±0.091	2.503 ±0.015
3.	PCP-97021	2.21±0.04	2.535 ±0.0104
4.	DCP-2	1.53±0.055	2.572 ±0.05
5.	CP-20116	1.42±0.050	2.611 ±0.10
6.	CH-9863	2.11±0.050	2.922±0.15

±Mean Value SD of three replicates. p value<0.05 significant (Statistically analysis was done by Annova test)

Figure 1: Showing the variation in percentages of Kaempferol (Fig 1A) and Stigmasterol (Fig 1B) content in different varieties of *Vigna unguiculata*. Statistical analysis was done by using Annova test.



Kaempferol content in different varities of *Vigna unguiculata*



Stigmasterol content in different varities of *Vigna unguiculata*

variations can be attributed to genetic polymorphism that is present between the various samples [20]. Stress combined with polymorphism within species is considered to be the main reason, Hence different varieties of cow pea was collected to find out the better source of Kaempferol as also of Stigmasterol. The described study revealed the presence of maximum content of Kaempferol in Pusa Kolum (2.42%) whereas Stigmasterol was highest in CH-9863 (2.922%) variety among all samples used. These varieties can be used by pharmaceutical industries. The quantitative estimation was done by using High Performance Thin Layer Chromatography with standard compounds of Kaempferol and Stigmasterol (Table1, Figure 1 and 2).

Conclusion

The study reveals that Pusa Kolum is the best source for Kaempferol whereas CH-9863 is for Stigmasterol. These sources can be used by Pharmaceutical industries.

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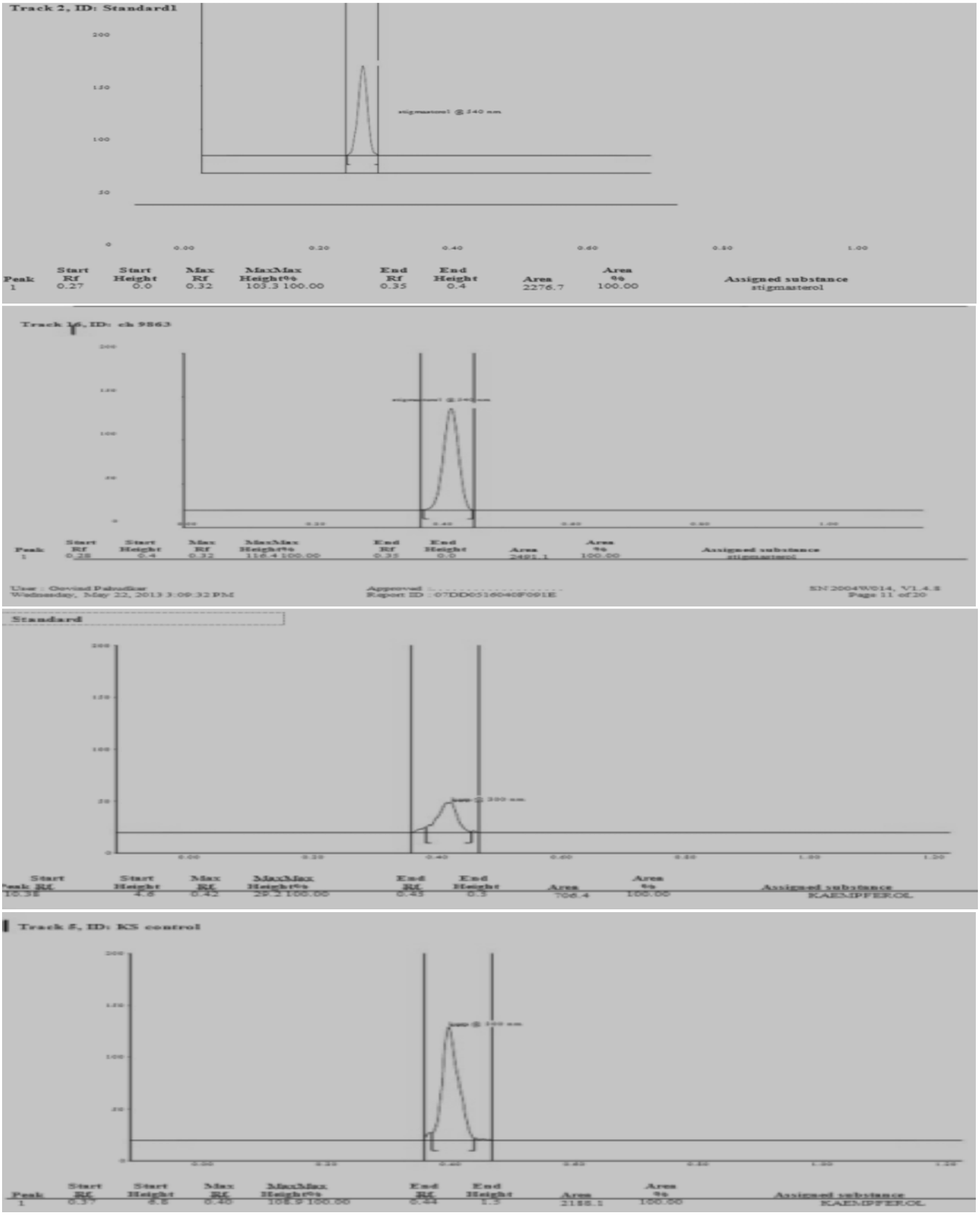
Figure 2: Showing the High Performance Thin Layer Chromatograms (HPTLC) for different varieties of *Vigna unguiculata*



A-Stigmasterol standard HPTLC chromatogram; B-Stigma sterol HPTLC chromatogram in CH-9863; C-Kaempferol standard HPTLC chromatogram; D- Kaempferol HPTLC chromatogram in Pusa kolum.

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