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ISOLATION AND PURIFICATION OF LUTEIN FROM ALTERNANTHERA FICOIDEA

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ABSTRACT

Lutein is a pharmacologically vital phytochemical belonging to the class of carotenoids widely distributed in green vegetables and marigold flowers. It is industrially significant for its biological activities such as antioxidant, macular protective and immune stimulatory properties. In this study, isolation and purification of lutein from *Alternanthera ficoidea* was carried out. For the extraction of lutein, petroleum ether- acetone extract of *Alternanthera ficoidea* leaves was prepared which yielded 2.1mg/100g of lutein. Preparative Thin Layer Chromatography of the extract yielded lutein with a Rf value of 0.12. Lutein isolated was subjected to spectral data characterization by Ultra Violet (UV) -Visible and FT-IR spectroscopy. The spectroscopical results obtained corresponded well with those reported for standard lutein. Results of the study indicated that *Alternanthera ficoidea* leaves can be used as a source for obtaining lutein.

KEYWORDS

Alternanthera ficoidea, Lutein, Solvent extraction and Spectral characterization.

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INTRODUCTON

Plants yield secondary metabolites termed in general as 'phytochemicals' having the potential to combat diseases such as cancer, heart stroke or metabolic syndrome. They are non-essential nutrients and have certain properties like antimicrobial, antioxidant and immune modulating properties[1]. Carotenoids, constituting a vital class of phytochemicals, are a group of red, yellow and orange pigments that have extended conjugated double bond system[2] and belong to the class of tetraterpenoid group[3]. Carotenoids are derived from isoprenoid precursors and are basically classified into two groups: The carotenes are cyclic or acyclic

hydrocarbons and the xanthophylls are oxygenated derivatives of carotenes[4]. The widely distributed antioxidant property of the carotenoids is due to its double bond which reacts with Reactive Oxygen Species (ROS) to scavenge the radicals[5].

Among carotenoids, there has been a special importance in lutein and zeaxanthin, due to its enormous health benefits[6]. Lutein is found in vegetables, such as kale, broccoli, and spinach. Currently, marigold flowers are used for commercial scale of isolation of lutein[7]. It has physiologically important role in improving vision and in eye protection from harmful UV (Ultra Violet) light[8] and to quench singlet oxygen, a highly reactive free radical that can impair or damage DNA[9]. During inflammation, lutein in immune tissues is depleted and the level of reduction is dependent on dietary lutein intake[10]. There are various applications of lutein which include drugs and cosmetics, pigmentation of poultry products, prevention of age related macular degeneration and coloration of food[11].

Alternanthera ficoidea is monoecious herb and is around 100-300cm height. It probably cultivated in India and China and found throughout India in waste places. It's widely spread in tropical and subtropical regions of the world. The common name of this plant known as green amaranth in English. This plant is growing under a wide range of climatic conditions, and they can produce leafy edible vegetables. Current industrial and public use of Amaranth plant has not only been linked to its recognized nutritional properties but also to its potential beneficial use as the therapeutic adjunct in diets for hypercholesterolemia susceptible individuals[12].

In recent decades metabolites derived from the extract of medicinal plants are frequently used by allopathic physicians as cardio-vascular, immunomodulatory, anticancer and antibiotic medicines. One of the Indian medicinal plant, *Alternanthera ficoidea* (L.) (Synonyms: *Alternanthera tenella Colla* and Gomphera ficoidea) belongs to family Amaranthaceae. The plant is traditionally used as diuretic, antiseptic, anti-pyretic, anti-inflammatory and anti- diarrheal agent etc. The secondary metabolites like tannins, saponins, phytols, carotenoids and polyphenols are major classes of compounds are identified for the plant. Some phytomolecules are isolated from the extract as vitexin, quercetin, kaempferol, amarantin, betaine, isoamarantin and sterol etc. Pharmacological activities like hypolipidemic, anti-inflammatory, antidiabetic, antibacterial and immunomodulatory activity are also investigated on this plant[13]. At present, lutein extraction is limited to a few sources like marigold flowers and microalgae, wherein the process is tedious and expensive[14-15]. *Alternanthera ficoidea* leaves are known to contain significant amounts of lutein[16]. However, no efforts were made towards its exploitation for isolating lutein. Hence, in this study, we report the isolation and purification of lutein from the leaves of *Alternanthera ficoidea*.

MATERIAL AND METHODS

Materials

Chemicals

Iodine crystals, potassium iodide, copper acetate, picric acid, α-naphthol, copper sulphate, sodium hydroxide, sodium potassium tartarate, ninhydrin, ferric chloride, hydrochloric acid, sulphuric acid, acetic anhydride, petroleum ether, diethyl ether, acetone, acetic acid and silica gel R254 were purchased from Technico (India).

Plant Material and Extraction:

The leaves of *Alternanthera ficoidea* were collected from local farms of sulur, Coimbatore, Tamilnadu (India). The plant was identified by local farmers and authenticated (BSI/SRC/5/23/2021/Tech) by Dr. M.U. Sharief, Scientist 'E' and Head of office, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. *Alternanthera ficoidea* were washed, shade dried and powdered using an analytical mill. The powder was stored in air-tight container at 4˚C until further analyses. Two methods were followed for extraction. In the first method, 100g of *Alternanthera ficoidea* powder was suspended in 1000ml of diethyl ether and methanol mixture (2:1) and was agitated on a shaker for 24 h. The filtrate was collected and concentrated to obtain the extract. In the second extraction method, 100g of leaf powder was extracted with 1000ml of petroleum ether and acetone mixture (1:1) using soxhlet

apparatus for 8 hours. The extract was concentrated to yield thick paste dark green colored.

Methods

Evaluation of phytoconstituents in the extract

Alternanthera ficoidea extract was subjected to preliminary phytochemical screening to identify the presence of various phytoconstituents in the extract[17].

Preparative Thin Layer Chromatography (TLC)

The extract (5mg) was dissolved in 50µl dichloromethane and spotted on silica gel plates. Compounds were eluted using petroleum ether: diethyl ether: acetic acid ratio 80:20:1 as a mobile phase. From the developed TLC plates, Rf values were noted and compared to that reported in the literature. The spot corresponding to lutein was recovered from TLC plate, dissolved in dichloromethane and concentrated to obtain lutein.

Spectral Analysis of the Pigment

For UV spectral analysis, 1mg of isolated lutein was dissolved in 1ml of dichloromethane. Taking dichloromethane as reference, the sample was screened for absorbance maxima by scanning in the wavelength range of 400-600nm. Spectral characterization of the sample was carried out using UV-visible spectrophotometer (UV-3000+, Lab India).

Fourier Transform Infrared Spectrometric (FT-IR) analysis

The lutein recovered from the TLC plates was analysis with FT-IR. FT-IR spectrum of lutein was recorded in the range of 4000-400cm-1 using FTIR Shimadzu IR 8400S spectrophotometer. The peaks obtained form isolated lutein were compared with the peaks reported for lutein in the literature.

Purification of lutein using column chromatography

For this, 2g of the *Alternanthera ficoidea* extract was chromatographed using was silica gel column (60-120 mesh) and eluted using the solvent system petroleum ether – diethyl ether mixture (80:20). The elute was concentrated to give yellow colour semisolid, which upon TLC, yielded lutein with a similar Rf value reported for standard lutein.

RESULTS AND DISCUSSION

The first method employed for extraction, upon phytochemical and phytoconstituents screening was found to be unsuitable as it did not show the presence of phenolics and flavonoids in abundance. Screening of *Alternanthera ficoidea* petroleum ether – acetone extract for phytochemicals indicated the presence of saponins, phytosterols, phenolics and flavonoids in large quantities. In this investigation, lutein was separated using TLC at Rf value of 0.12 and it matched with the results obtained by Ashwini Prabhu[18] Petroleum ether, Diethyl ether and Acetic acid in the ratio 80:20:1 solvent system used (Figure No.1).

The UV spectral analysis for lutein revealed a peak at 447.5nm in dichloromethane which was matching with the results obtained by Craft and Soares[19]. The spectral characteristic of lutein in dichloromethane is represented in Figure No.2.

The FT-IR spectral analysis for lutein shown a broad peak at 3394 cm-1 indicating O–H stretch. The peaks at 2916, 2848 cm-1 indicated C–H stretch; 1662 cm-1indicated –C=C– stretch; 1579, 1528cm-1 indicated C–C stretch for aromatics; 1452cm-1 indicated C–H bend; 1152, 1103, 1080, 1045cm-1 indicated C–O stretch for alcohols; 847cm-1 indicated C–H for aromatics; 761, 704cm-1 indicated C–H rocking (Figure No.3). Similar results were reported in a study carried out by Joseph[20].

From the results of this study, it is evident that *Alternanthera ficoidea* is a rich source of lutein that can be isolated easily using petroleum ether – diethyl ether solvent system. The method used for extraction in this research study is relatively economical with high yield. Extraction of lutein from *Alternanthera ficoidea* a may have advantage over extraction using marigold flower petals[21] or microalgae like Murielopsis sp. And Scenesdesmus almeriensis. Extraction of lutein from marigold flowers is tedious process involving broad column chromatography techniques while, that from microalgae is an expensive method because of its specific growth requirements. The study carried out on the isolation and purification of lutein has enormous importance. This study focuses on extraction of lutein from easily available plant material *Alternanthera ficoidea* to offer high yield and high affordability. Lutein being an antioxidant, protects the cells from damage or impair caused by free radicals. Besides, lutein has a several biological

actions including inhibition of cell transformation, inhibition of the monocyte mediated inflammatory response, intercellular communication, immune enhancement, inhibition of LDL resistance to oxidation and macula protection[22].

Studies reported that higher intake of lutein can reduce the risk of lung cancer[23]. Daily intake of 6 mg lutein is required to prevent the onset of age-related macular degeneration according to a research by El-Sayed *et al*[24]. The individual or single carotenoid content is considered as high and very high if the yields are 0.5-2mg/100g and >2 mg/100g respectively[25]. Extraction of lutein from *Alternanthera ficoidea* yielded 2.1mg/100 g of the carotenoid, which is considered as relatively higher yield. *Alternanthera ficoidea* can be a reliable source for lutein extraction as it can be cultivated throughout the season with no much nutrient inputs. This creates it an interesting candidate for extracting lutein.

Figure No.1: Identification of lutein from *Alternanthera ficoidea* extract using TLC

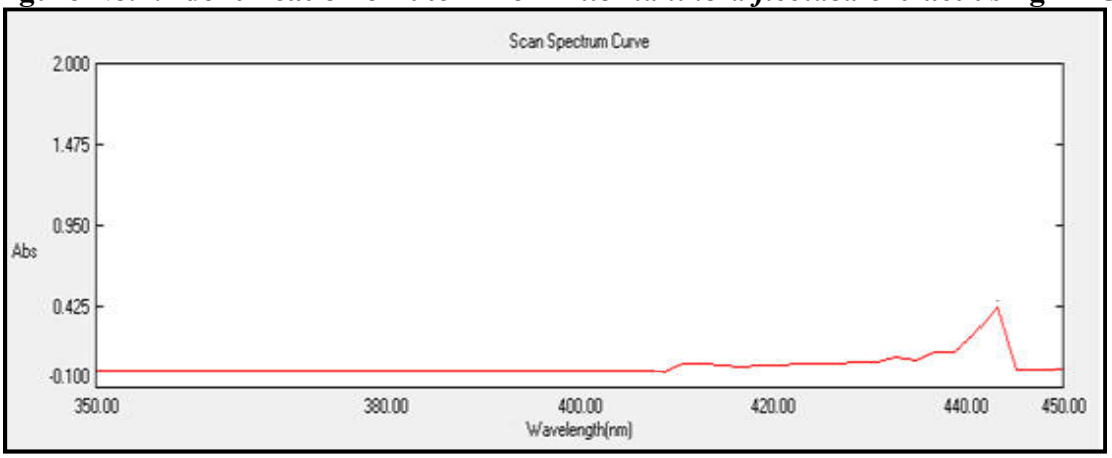

Figure No.2: Photometric spectrum of lutein isolated from *Alternanthera ficoidea*

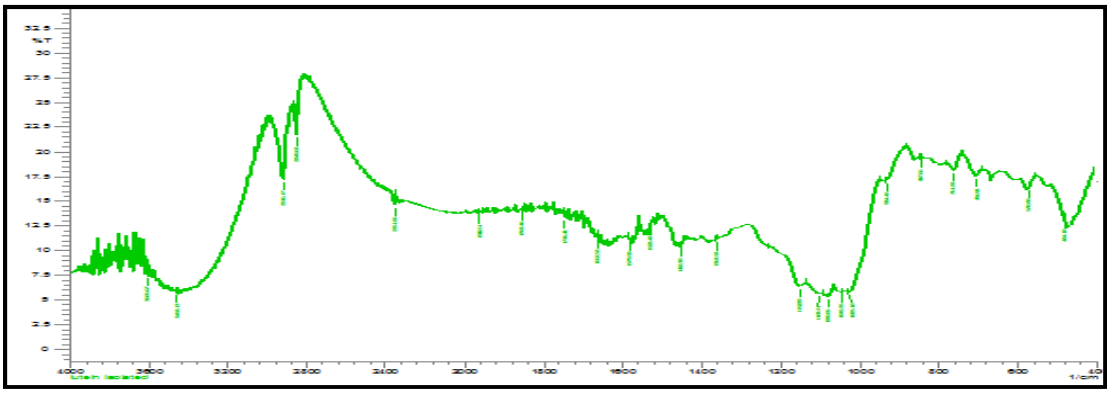

Figure No.3: FT-IR spectra of lutein isolated from *Alternanthera ficoidea*

CONCLUSION

Lutein is a neutraceutically vital natural product with enormous health benefits. This study explores into the possibility of exploiting *Alternanthera ficoidea* for the isolation of lutein and shows promising findings. Hence, we conclude that *Alternanthera ficoidea* can be used as an alternate source for lutein extraction. Also, *Alternanthera ficoidea*, being used in the regular diet, can provide this carotenoid in significant amounts meeting the physiological requirements.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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