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LUTEIN ISOLATION FROM PERENNIAL FLOWERS

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ABSTRACT

Lutein is an antioxidant which is a fat-soluble xanthophyll and could be found in a wide range of natural sources. A variety of separation and analytical methods have been developed for the determination of lutein, including solvent extraction, supercritical fluid extraction, and high performance liquid chromatography. There are many sources for lutein extraction but the search for a more economical source still exists. Moreover, the cost of production of lutein depends on the availability of the source and also the quantity of lutein produced from each source. The cost of lutein is very high and it also depends on the availability of the source and the cost incurred in its production. The objective of the present study is to extract and estimate the lutein present in a variety of flowers available in the vicinity of the institute in a view to identity a cheaper source and to reduce the overall production cost. The plants selected for the study were *Senna bicapsularis*, *Tecoma stans, Caesalpinia pulcherrima* (red), *Caesalpinia pulcherrima* (yellow) and *Tagetus erecta*. Solvent extraction was carried out using petroleum ether, benzene, diethyl ether and chloroform. Among the solvents used, chloroform was found to be the best solvent. The extracted lutein was estimated using UV-Vis spectrophotometer at 445 nm. The lutein content was found to be the highest in *Senna bicapsularis* when chloroform was used as the solvent (1217.2 µg / g) and the lowest is *Tecoma stans* when benzene was used as the solvent (30.74 µg / g). *T. erectus* and *Tecoma stans* preformed well with respect to lutien extraction when diethyl ether was used as solvent. When chloroform was used, *S. bicapsularis* and *T. stans* showed good result. Similarly, in benzene, *T. erectus* and *S. bicapsularis* showed good amounts of lutein. Finally in petroleum ether all plants showed significant amounts of lutein.

Keywords: Lutein, *Senna bicapsularis, Tecoma stans,* Carotenoid, *Caesalpinia pulcherrima, Tagetes erecta*

INTRODUCTION

Lutein is a xanthophyll and is among the group of naturally occurring carotenoids [1]. It is synthesized by photosynthetic organisms. The presence of the long chromophore of conjugated

double bonds provides the distinctive light-absorbing properties. Its polyene chain is susceptible to oxidative degradation by light or heat and is chemically unstable in acids [2, 3]. Lutein cannot be synthesized by human so it must be obtained through dietary sources. Lutein (β-3-carotene-3,30-diol) belongs to a class of oxygenated carotenoids (Xanthophylls). It is used as a natural colorant like zeaxanthin which is closely related to it. It absorbs blue light and therefore appears yellow at low concentrations and orange-red at high concentrations. It is considered as an "eye vitamin" as it serves as a photo protectant for the retina from the damaging effects of free radicals produced by blue light, thereby protecting the eye tissues from sunlight damage [2-5]. They protect lipid membranes against oxidative damage [6-9]. They use it to prevent eye diseases including age-related macular degeneration (AMD). It also protects the lungs and arteries from free radical damage. It is closely related to β carotene and vitamin A [10]. They are found in algae and also used as preservatives [11, 12]. Foods rich in lutein include broccoli, spinach, kale, corn, orange pepper, kiwi fruit, grapes, orange juice, zucchini, and squash [13-18]. Carotenoids are isolated from different sources using different solvents [18-24]. It has also been implicated in imparting a protective role in age related maculopathy [25-27]. Lutein is found to be good for the skin as well as the eyesight [28-30]. The present study was carried out to isolate lutein from six flowers namely, *Tagetes erecta*-marigold, *Caesalpinia pulcherrima (red)*-peacock red, *Caesalpinia pulcherrima(yellow)*- peacock yellow, *Senna bicapsularies*- Cassia, *Crotalaria retusa*-Rattle weed, and *Tecoma stans*-Yellow bells.

MATERIALS AND METHODS

The six flowers used for this investigation namely, *Senna bicapsularis, Tecoma stans, Caesalpinia pulcherrima* (red), *Caesalpinia pulcherrima* (yellow) and *Tagetus erecta* is shown in Fig 1.

A

B

C



D **E** **F**

Fig1: A: *Tagetes erecta*-marigold, **B:** *Caesalpinia pulcherrima* (red)-peacock red, **C:** *Caesalpinia pulcherrima* (yellow) - peacock yellow, **D:** *Senna bicapsularies*-Cassia, **E:** *Crotalaria retusa*-Rattle weed, **F:** *Tecoma stans*-Yellow bells

Sample Preparation

The flowers used were bought fresh from the market and streets (wild flowers) washed under tap water, cleaned with sterile water and air dried at room temperature. The raw materials were chosen based on their availability and also on the basis of information gathered during literature studies.

Extraction

The samples (5 g each) were ground with a mortar and pestle and mixed with solvent. Four different solvents 20 ml each of petroleum ether, diethyl ether, benzene and chloroform were used. The solutions were filtered using Whatman No. 1 filter paper. The filtrate was centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was collected and stored at 4˚C.

Measurement of Absorbance

Absorbance was measured using UV-Vis spectrophotometer at a wavelength of 445 nm. Concentration of lutein was calculated using the following formula:

$$\text{Concentration of lutein (µg/g of sample)} = \frac{A \times V\ (ml)}{E^{1\%}\ cm \times w\ (g)} \times \text{dilution factor}$$

Where,

A =Absorbance,

V = Volume (ml)

$E^{1\%}$ cm = Extinction coefficient of solvents

W= Weight in (g)

Test for Carotenoids

The colour of sample containing pigments in solvent disappears after the addition of 5% solution of sodium nitrite and 0.5M H_2SO_4. This test was performed for samples in order to confirm the presence of carotenoids.

RESULTS AND DISCUSSION

The experiment was carried out to look for the most suitable raw material for extraction of lutein. The lutein extraction was carried out in all the sources mentioned in Fig 1 with different solvents such as ethanol, diethyl ether and petroleum ether the most preferred solvents for extracting lutein and the results were shown. The colour of the residue was found to be yellow which indicates the complete extraction of lutein from these sources. The extracted lutein was estimated in UV-Vis spectrophotometer at 445 nm. The concentration of lutein in extracts of diethyl ether and chloroform is shown in Fig 2 and Fig 3. In this set of experiments the maximum extraction was found to be higher in chloroform extracts from flowers of *Senna bicapsularies* among the flowers investigated and estimated to be 1217.2µg/g. The least was found in extracts of Benzene from *Tecoma stans* and estimated to be 30.74µg/g. However, Rajashree Hajare, 2013[24] has reported that the ethanolic extracts from marigold was found to have the highest content of lutein (1.163 µg /g) among the various sources employed for the study.

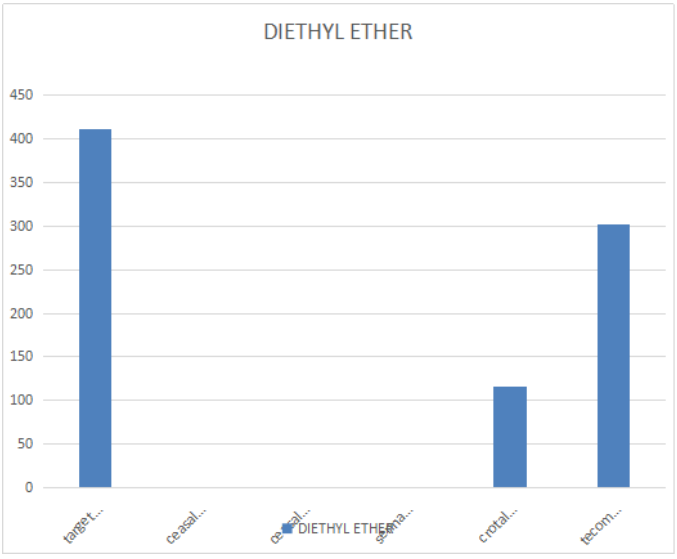

Fig 2: Extraction of lutein in diethyl ether

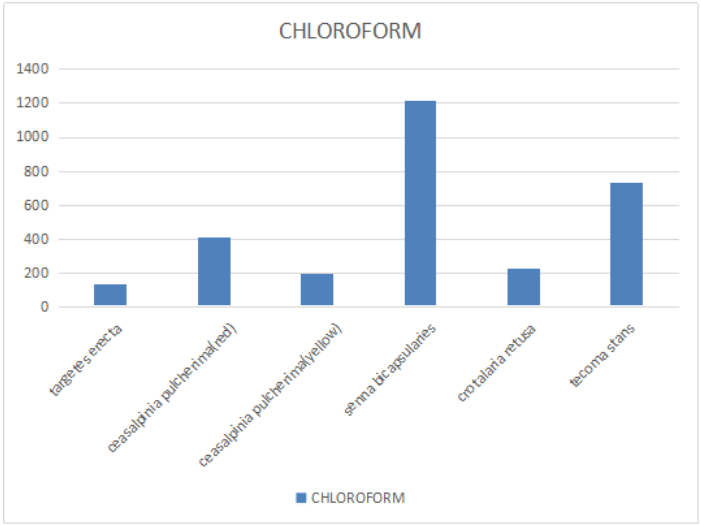

Fig 3: Extraction of lutein in chloroform

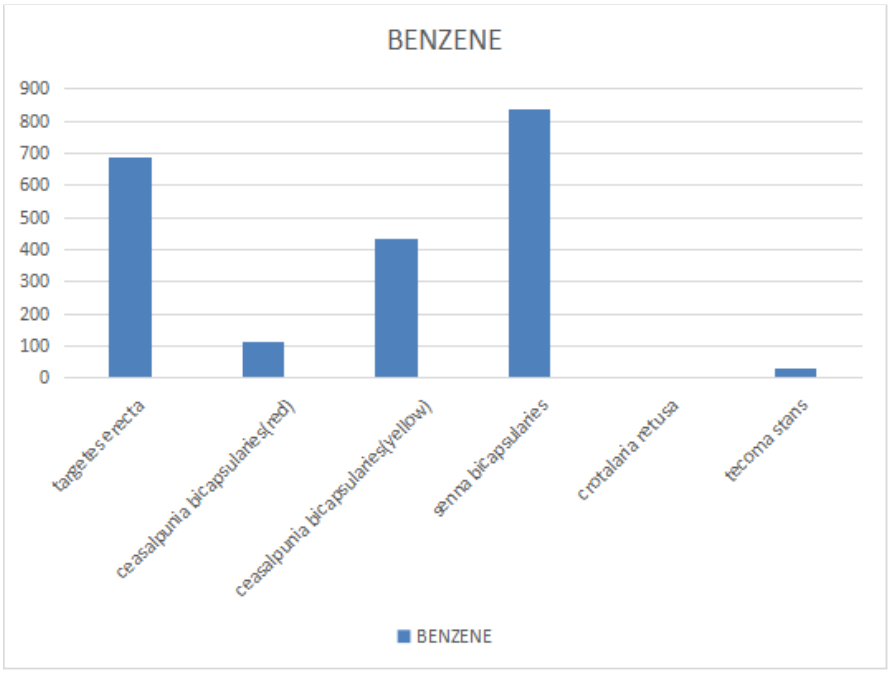

Fig 4: Extraction of lutein in benzene

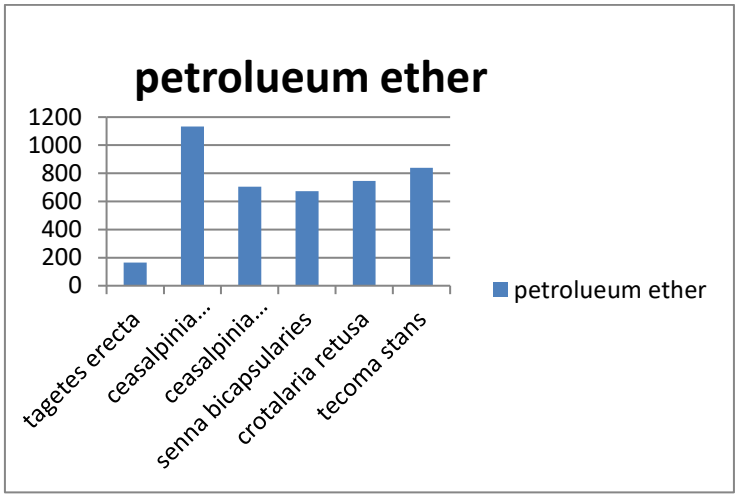

Fig 5: Extraction of lutein in petroleum ether

CONCLUSION

Lutein is of major commercial interest because of its use in functional food and cosmetics, as well as in pharmaceuticals. The production yield is very important for the large-scale extraction of lutein, in terms of cost efficiency. Moreover, it is observed that the yield from the extraction depends on the solvent used also. However ease of purification of this antioxidant needs further study. Moreover this study reveals that the lutein is often present in the highly pigmented flowers.

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