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Natural Colorants from *Cosmos Sulphureus Cav.* and *Tagetes Erecta L.*: Extraction And Characterization

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The objective of this research is to determine the optimum conditions in the extraction process of carotenoid from the flower *Tagetes erecta L*, which are found in Yogyakarta and to use these optimum conditions for the extraction and identification of carotenoid content in *Tagetes erecta L.* and *Cosmos sulphurous Cav*., that are found in the highlands and lowlands in the area of Yogyakarta.

A laboratory-scale extraction was done in a three-neck flask with a heating mantle, which was equipped with cooler, mixer, and thermometer. The temperature of the extraction process in the entire study was fixed at 50°C to avoid the colorant degradation. The carotenoid content in the extract solutions was determined using gravimetric, chromatography, and spectrometry methods. Determination of the optimum conditions was done by varying the type of solvent, extraction time (10-120 minutes), dry solid mass to solvent volume ratio (0.05 - 1.0 g/mL), and agitation speed (600 - 900 rpm). The optimum conditions for the carotenoid extraction were used for extraction of the carotenoid content of *Tagetes erecta L*. and *Cosmos sulphureus Cav.*, cultivated in lowlands and highlands in Yogyakarta.

Using ethanol (96%) as a solvent resulted in a higher yield compared to the use of acetone. Optimization of the extraction process and evaluation of interaction effects of different operating variables (time, dry solid mass to solvent volume ratio, and agitation speed) was obtained using the Response Surface Method (RSM) of DOE Software Minitab. The optimum conditions for extraction of carotenoid compounds from *Tagetes erecta L.* were found to be 81 minutes, with a solid to solvent ratio of 0.05 g/mL, and agitation speed of 800 rpm. The yield of solute (g colorant in extract solution/g dry solid sample) of extraction under these optimum conditions was found to be 10.16%. Furthermore, it was found that the carotenoid content in *Cosmos sulphureus Cav*. was higher than that in *Tagetes erecta L*., and the plants from the lowlands have a higher carotenoid content than those from the highlands.

Keywords : Carotenoid, Extraction, *Cosmos sulphureus Cav*., *Tagetes erecta L*., Optimization

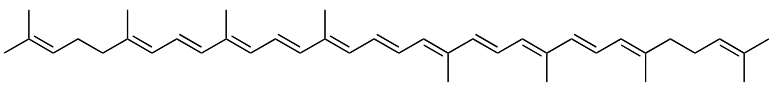
INTRODUCTION

The use of natural colorants for food, cosmetics, and textile coloring is a culture in Indonesia that has been handed down throughout the years by our ancestors (Heyne, 1987). This is due to the abundance of natural resources and great biodiversity that Indonesia has, which are the source of raw materials to produce the natural colorants. In spite of those facts, the use of synthetic colorants is still dominant. Nowadays, due to the worldwide rise of environmental sustainability issues, eco-friendly products have received increasing attention from governments, industries, academia, and researchers. Many attempts have been made in a wide range of areas, to find substitute materials to replace synthetic colorants and materials that are harmful to the population and the environment. The genotoxicity of food, drug, and cosmetic colorants has been reviewed previously (Combes and Hveland, 1982), while Shahid *et al*. (2013) stated that the market of natural food colorants is going to grow on a global scale at a faster rate than synthetic colorants.

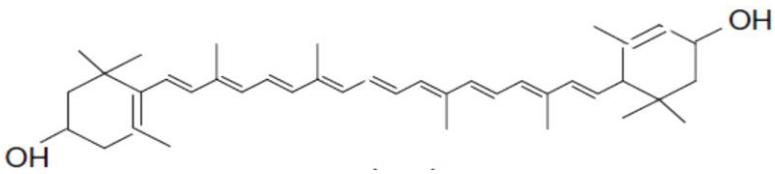
Lutein is a yellow pigment that exists in higher plants and other photoautotrophic organisms like algae (Beck, 2010). According to INS-Number E161b lutein can be used as food colorant (Delgado and Paradez, 2003). Moreover, lutein is an effective functional nutrient that can benefit human health, ameliorating cardiovascular diseases, cancers, and age-related macular degeneration. The worldwide market for lutein was worth roughly US$233 million in 2010 and it is expected to grow to US$308 million by 2018, an annual growth rate of 3.6% (Lin et al. 2014).

Lutein is a member of the carotenoid compound. There are two types of carotenoid, namely hydrocarbon carotenoid, and lutein. The former do not contain oxygen molecules, such as in β-carotene and α-carotene, while the latter contain oxygen molecules, such as in lutein and zeaxanthin. The chemical structures of the hydrocarbon carotenoid and lutein are depicted in **Figure 1** (Bechtold and Mussak, 2009).

The long non-polar hydrocarbon chain combined with the polar hydroxyl groups at both ends causes the lutein molecule to



(a) Hydrocarbon carotenoid



(b) Lutein

Fig. 1: The chemical structure of carotenoid compound

have a semi-polar characteristic so that it can be dissolved in both non-polar and polar solvents (Britton et al. 2008; Davies, 2004). For example, lutein can dissolve well in non-polar n-hexane, as well as in polar acetone and ethanol. Lutein is stable in pH range 3 to 9. Lutein structure consists of conjugated bonds, which when reacting with the oxygen present in air, causes oxidation to take place and lead to a color loss. At extreme pH and in the presence of light, lutein undergoes isomerization resulting in the loss of color. For better retention of lutein, the extraction temperature needs to be maintained between 40°C and 45°C and removal of the solvent from micelle is carried out in a vacuum at a temperature of between 50°C and 55°C (Sowbhagya et al. 2004).

Regarding the exploration of natural resources, *Tagetes erecta L.*, also known as Marigold flowers are an excellent source of lutein because they contain high levels of lutein; the petal of Marigold flower contains 9000 mg/kg lutein (Bechtold and Mussak, 2009). The yield of Marigold flowers is 11,000 to 30,000 kg/hectare, depending on the varieties, cultivar, and horticulture practices. Approximately 40% to 50% of the flower consists of petals. The yield of carotenoid compound or oleoresin is about 8% to 10% of the petals, with a lutein content of 8 to 12 g per 100 g oleoresin (Sowbhagya, et al. 2004). The extract of Marigold petals is an oleoresin compound or often called micelle. The concentration of lutein fatty acid esters in Marigold extracts can be enhanced by purification steps using solvents like isopropanol followed by precipitation. Enrichment of lutein can be achieved by phase partition between 70% and 90% of methanol, ethanol, acetone, and hexane.

Another interesting plant that can be used as raw material to produce natural yellow colorants is *Cosmos sulphureus Cav.*, also known as Kenikir Jawa flowers. Arini *et al.* (2015) studied the effect of SP36 fertilizer on the rate of growth and the period of flowering of both *Cosmos sulphureus Cav.* and *Tagetes erecta L.* in the lowlands. The authors reported that the use of 150 kg/ha of SP36 on *Cosmos sulphureus Cav.* resulted in the best plant growth, the total weight of flowers, the amount of flowers, and flowering period. While the use of SP36 on *Tagetes erecta L.* resulted in the fastest rate of flower growth and the biggest flower diameter. Pratiwi (2015) investigated the effect of SP36 fertilizer on the growth and flowering of *Cosmos sulphurous Cav.* and *Tagetes erecta L.* in the highlands. The authors reported that the use of 75 kg/ha of SP36 to fertilize *Cosmos sulphurous Cav.* resulted in the best plant growth, the total weight of flowers, the amount of flowers, and flowering period. Pratiwi (2015) found that fertilizing *Tagetes erecta L.* with 150 kg/ha of SP36 in highlands resulted in the fastest rate of flower growth and the biggest flower diameter. This is in good agreement with Arini et al. (2015).

Because of the great potential use and benefit of lutein a lot of research on lutein has been carried out e.g. Sowbhagya et al. (2004) studied chemistry, processing, and stability of the pigment and its applications as natural colorant from Marigold. Lin et al. (2014) have compared the production of lutein from Marigold

with the production from microalgae. They have even patented the isolation and purification of carotenoid from Marigold flower and the process of isolation, purification, and recrystallization of lutein from saponified Marigold oleoresin (European patent application No: 95300273.0, 17.01.95).

The objective of this research is to determine the optimum conditions in the extraction process of carotenoid from the flower *Tagetes erecta L.*, which are found in Yogyakarta and to use these optimum conditions for the extraction and identification of carotenoid content in *Tagetes erecta L*. and *Cosmos sulphurous Cav*. that are found in the highlands and lowlands. We expect that this study will, in turn, contribute to minimizing the use of hazardous synthetic colorants.

Optimization of Extraction Conditions Using the Response Surface Methodology (RSM)

The present work involves optimization of different variables governing the extraction process. Determining these optimum values is carried out by varying one parameter while keeping the other parameters at an unspecified constant level. The major disadvantage of this single variable optimization is that it does not take into consideration the interactive effects among the variables; thus it does not depict the net effects of various variables on the extraction rate. In order to overcome this problem, optimization studies have been done using Response Surface Methodology (RSM) (Keka et al. 2012). RSM is an effective statistical technique for optimizing complex processes. RSM reduces the number of experimental trials required to evaluate multiple parameters and their interactions. It is less laborious and less time-consuming than other approaches.

MATERIALS AND EXTRACTION METHODS

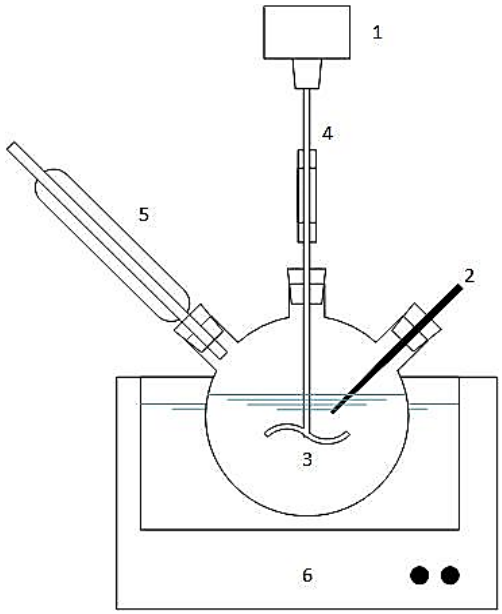
Materials

Tagetes erecta L. flowers can be purchased at flower shops in Yogyakarta. *Tagetes erecta L*. and *Cosmos sulphureus Cav*. both are cultivated in Kalitirto, Berbah (lowlands) and in Ngipiksari Pakembinangun, Sleman, Indonesia which is in the highlands. Ethanol (96%, technical grade) and acetone (99.5%, technical grade) were used as the solvents.

Extraction Methods

A Total Solute that can be Extracted from Tagetes erecta L.

The size of *Tagetes erecta L*. sample was reduced using a blender with sharp blades. A certain weight of the sample ($W_{solid\ sample}$) was then wrapped with a filter paper and put into a soxhlet apparatus. Ethanol was used as the solvent. To ensure that the total amount of solute in the samples have been extracted completely, the experiment was stopped when a solvent that bathes the solid does not change their color. The yield of total solute (g total colorant in extract solution/g dry solid sample) was determined using the gravimetric method. The extraction temperature in the entire study was fixed at 50°C. This temperature was chosen based on the results of a research carried out by Hojnik et al. (2008) which stated that at a temperature of



1. Motor
2. Thermometer
3. Impeller
4. Stirrer
5. Reflux condenser
6. Waterbath

Fig. 2: The experimental set-up used for carotenoid extraction

between 20° to 40°C the degradation of lutein in the temperature range investigated was not observed. Sowbhagya et al. (2004) found that the lutein compound degrades at a temperature of 60-65°C.

Optimization of Extraction Conditions of Solute from Dry Tagetes erecta L.

In finding the optimum extraction conditions, the extraction process was performed using experimental equipment as illustrated in **Fig. 2**. The size of *Tagetes erecta L*. sample was reduced by means of a blender with sharp blades. After the solvent temperature reached 50°C, a certain amount of *Tagetes erecta L*. was added into the three-necked flask. Samples were taken periodically at 10, 20, 35, 50, 65, 80, 100, and 120 minutes, samples were taken as soon as the last batch of the sample was put into the flask. Two types of solvent were used, *i.e.* technical grade ethanol, and technical grade acetone. The variations of solid *Tagetes erecta L*. to solvent ratio (w/v) were set at 0.05 ; 0.10 ; 0.20 ; 0.33; and 1.0 g/mL. The agitation speeds were set at 600, 700, 800, and 900 rpm. The yield of solute (g colorant in extract solution/g dry solid sample) was determined using the gravimetric method. The optimization of extraction conditions was done using the Response Surface Methodology (RSM) DOE Software Minitab.

Characterization Methods: gravimetric, chromatography, and spectrophotometry

The gravimetric method was performed to determine the % yield of solute at a certain time or % yield of total solute. The volume total of the extract solution in the flask was measured (V_1) at a certain time or at the end of the extraction time, 5 mL (V_2) extract solution was pipetted from the flask, and put into a clean and previously weighed porcelain crucible (W_1). The

crucible was then put in an oven and heated to a temperature above the solvent boiling point, in order to evaporate the solvent. At each interval of 30 minutes, the crucible was put in an exicator for 5-10 minutes, weighed, and put in an oven again. If the weight of the crucible and the sample extract does not change in the next weightings, the complete evaporation of the solvent has been reached. The weight of crucible and sample extract (W2). The % yield was calculated using Eq. (1).

$$\%yield = \left(\frac{W_2 - W_1}{W_{dry\ solid\ sample}}\right) x \left(\frac{V_1}{V_2}\right) x\ 100\% \qquad (1)$$

Characteristics related to the carotenoid content was measured with High-Performance Liquid Chromatography (HPLC) and were performed in the laboratory of Instrumental Analysis, Chemical Engineering Department, Gadjah Mada University, Yogyakarta, Indonesia, using a Shimadzu LC-2010HT apparatus with serial dual plunger pump and a 250 mm length x 2.0 diameter 5 µm Shim-pack VP ODS column. The mobile phase with a flow-rate of 1 mL/min consisted of Acetonitrile-water (9:1, in volume) + 0.5% EPA and Ethyl Acetate + 0.5% EPA.

Characteristics related to the organoleptic or visual test was measured with UV-Vis. The UV-Vis spectrophotometry was performed using a Shimadzu UVmini-1240, at 570 nm in the Instrumental Analysis Laboratory, at the Chemical Engineering Department, Gadjah Mada University, Yogyakarta, Indonesia.

RESULTS AND DISCUSSION

Total Solute from Dry *Tagetes Erecta L.*

The % yield of total carotenoid compounds contained in *Tagetes erecta* L. was extracted using a soxhlet, the result obtained is 18.76%. The total carotenoid compounds contained in *Tagetes erecta* L. is also known as oleoresin (Sowbhagya et al. 2004; Verghese, 1998). Sowbhagya et al. (2004) stated that the % yield of oleoresin in *Tagetes erecta* L. is about 8% to 10% with a lutein content of 8 to 12 g per 100 g oleoresin. Compared to the result obtained by Sowbhagya et al. (2004) it can be concluded that the content of oleoresin in *Tagetes erecta L*. in this study has a higher % yield. Therefore it has a better potential to be used as raw material to make natural food colorant.

Optimization of Extraction Conditions of Carotenoid from *Tagetes erecta L.*

The Effect of Using Different Types of Solvents and Different Extraction Times

Figure 3 shows the % yield of carotenoid of *Tagetes erecta L*., as a function of extraction time and type of solvent.

It is seen in Fig. 3 that extraction with ethanol resulted in a higher % yield, compared to that with acetone. This phenomenon is due to the difference of chemical structure between ethanol and acetone, in which the former contains hydroxyl (-OH) groups while the latter does not. It is expected that the solute is mostly carotenoid that contains 80% lutein

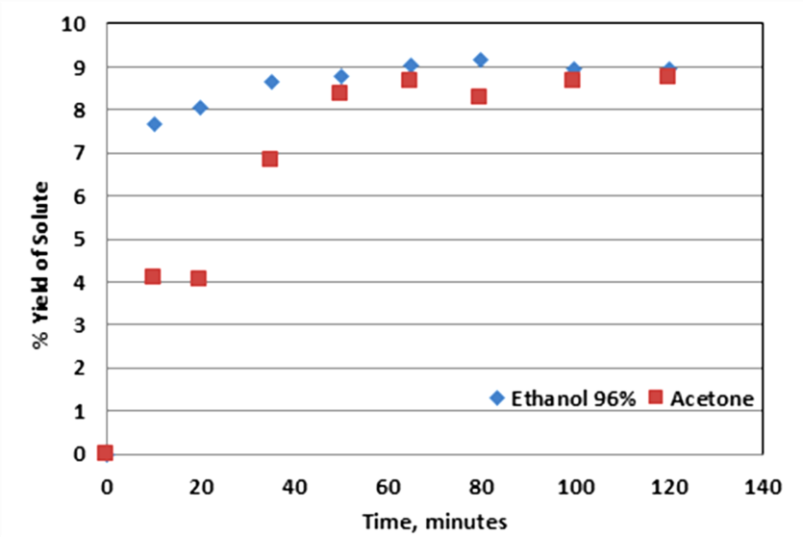


Fig. 3: The % yield of carotenoid compound in *Tagetes erecta L.* versus extraction time, in ethanol and acetone. The extraction temperature, agitation speed, and solid to the solvent ratio (w/v) were fixed at 50°C, 800 rpm and 0.1 g/mL, respectively.

molecules. The presence of hydroxyl groups in such polar-protic solvent like ethanol thus enhances the interaction between the solvent and the lutein molecules that also carry hydroxyl groups, via hydrogen bonds (Keka et al. 2012; Surendranath et al. 2016).

The results of this research are compatible with the results of the research carried out by Sari et al. (2013), they did an extraction process of curcuminoid from *Curcuma xanthorrhiz Roxb*. The yield of crude curcuminoid from *Curcuma xanthorrhiz Roxb*., without defatization in extraction using the following solvents, ethanol, acetone, and ethyl acetate producing 5.96%, 7.77%, and 7.06%. The crude curcuminoid yield after defatization on the crude curcuminoid in extract using the solvents ethanol, acetone and ethyl acetate are respectively 5.27%, 4.16%, and 3.78%. Based on these results it can be stated that ethanol as a solvent dissolves the lowest fat content in the micelle if compared to acetone and ethyl acetate. Based on the research of Sari et al. (2013) and the result in Fig. 3 ethanol is chosen as the solvent for extraction of carotenoid from Marigold.

The Influence of Dry Solid to Solvent Ratio (Solid/Solvent)

Figure 4 shows the % yield of the solute of *Tagetes erecta L.*, as a function of solid to solvent ratio while other parameters were fixed at constant values.

Generally, during conventional extraction processes, the percentage of lutein in the liquid phase will increase until equilibrium is reached. In order to determine the optimal volume of extracting solvent used per kg of raw material the effects of ratios, the volume of solvent per kg of raw material on the extraction rate was examined. It is seen in Fig. 4 that the % yield of solute tends to decrease insignificantly with increasing solid to solvent ratio values between 0.05-0.20 g/mL. Above 0.20 gram per mL, the % yield of solute decreases linearly with

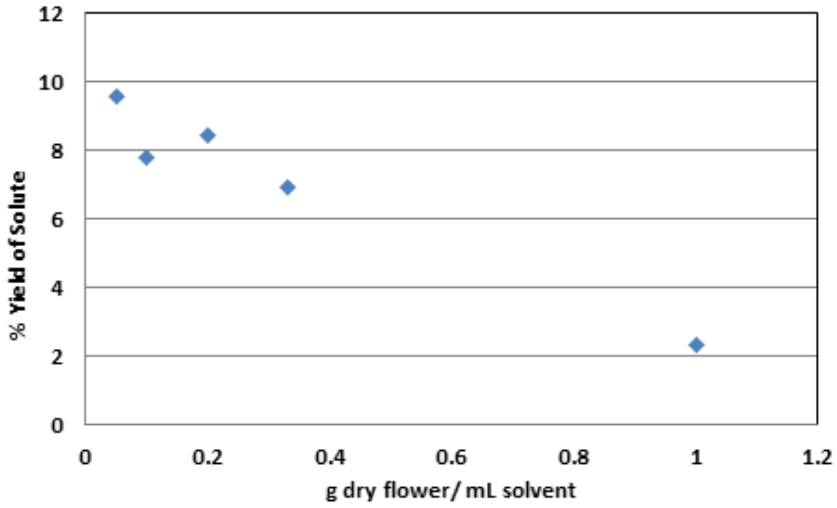


Fig. 4: The % yield of the solute of *Tagetes erecta L*. versus solid/solvent ratio, in ethanol. The temperature, agitation speed, and extraction time were fixed at 50°C, 800 rpm, and 65 minutes, respectively.

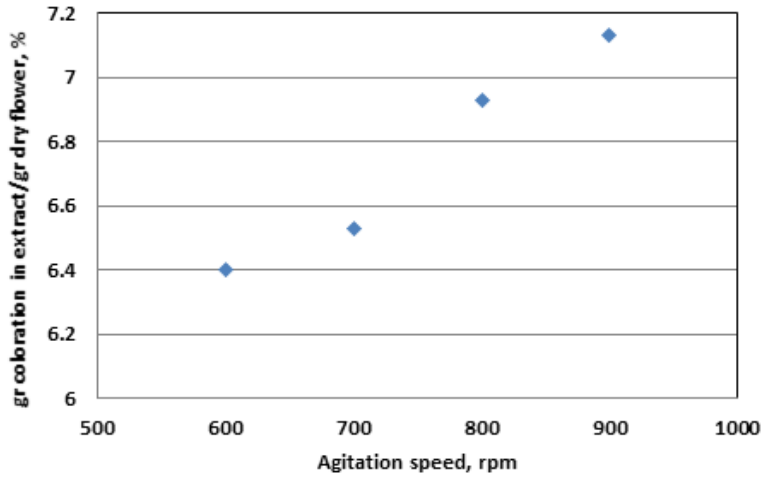


Fig. 5: The % yield of the solute of *Tagetes erecta L*. versus agitation speed, in ethanol. The temperature, solid to solvent ratio, and extraction time were fixed at 50°C, 0.33 g/mL, and 65 minutes, respectively.

increasing solid to solvent ratio. As the solid to solvent ratio increases, the leaching capacity of solvent decreases thus decreasing the amount of solute in the final extract. This explains the observed phenomenon in Fig. 4. Based on this observed data, the optimum ratio of solid to solvent occurs at a range ratio between 0.05 and 0.20 gram per mL. This result is compatible with the research results of Hojnik et al. (2008) which stated that the final efficiency of the extraction of lutein esters increases with the increase of the ratio of raw material to solvent volume of 0.06 to 0.40 kg/L

The Effect of Agitation (Stirrer) Speed

Figure 5 shows the % yield of the solute of *Tagetes erecta L*., as a function of stirrer speed while other parameters were

Table 1. The ranges and levels of the investigated variables in the research.

Time (minutes)	Solid to solvent ratio (g/mL)	Agitation speed (rpm)	Yield of solute (%, mass)
0	0.10	800	0.00
10	0.10	800	7.66
20	0.10	800	8.04
35	0.10	800	8.64
50	0.10	800	8.78
65	0.10	800	9.02
80	0.10	800	9.18
100	0.10	800	8.94
120	0.10	800	8.94
65	0.05	800	9.60
65	0.10	800	7.82
65	0.20	800	8.47
65	0.33	800	6.93
65	1.00	800	2.33
65	0.20	600	6.40
65	0.20	700	6.53
65	0.20	800	6.93
65	0.20	900	7.13

kept at constant values.

Fig. 5 shows that the % yield of solute increases with increasing agitation speed. The increase of agitation speed increases the mixing velocity and creates more turbulence and causes more contact between the solid and the solvent, which enhances the extraction process.

Optimization of Extraction Conditions Using RSM

In this study, optimization was obtained using the RSM DOE Software Minitab (Keka et al. 2012). The optimization of the % yield of carotenoid of *Tagetes erecta L.* extraction process was carried out by optimizing three chosen independent process variables which are the extraction time, dry solid mass to solvent volume ratio, and agitation speed. The ranges and levels of the variables investigated in the research are given in **Table 1**.

The effect of single parameter toward % yield of solute had been investigated trough **Fig. 6**. Main effect plots have shown that solid to solvent ratio give the highest range of % yield of solute. The increase of % yield of solute is observed as solid to solvent ratio is decreased from 1.00 to 0.50 g/mL. The rate of solute transfer is higher at low solid to solvent ratio, thus higher % yield could be obtained at the lower level of solid to solvent ratio. A similar trend also was observed in the extraction of alizarin from roots of *Rubia cordifolia* (Vedaraman et al. 2017). On the other hand, higher % yield of solute could be achieved using a higher level of extraction time and agitation speed. Higher levels of extraction time and

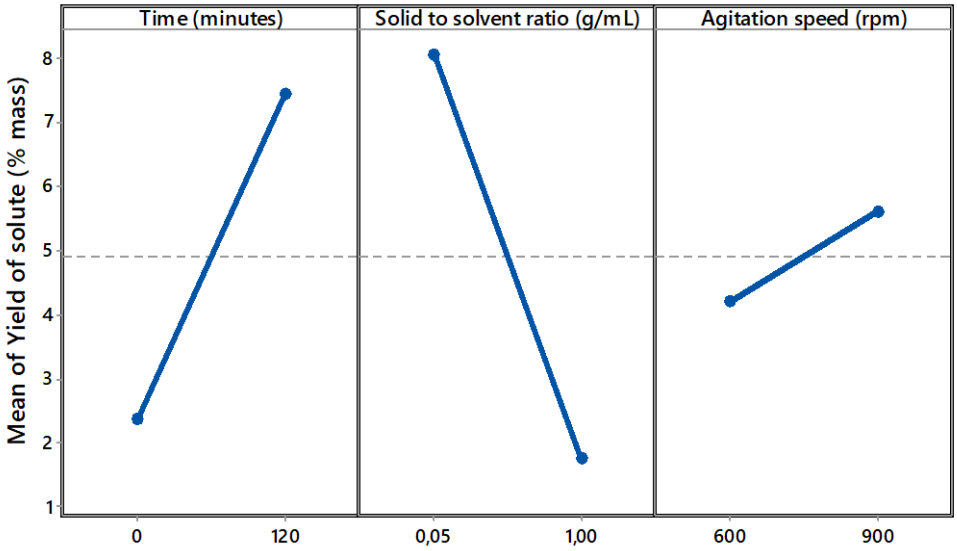


Fig. 6: Main effect plot of the investigated variables.

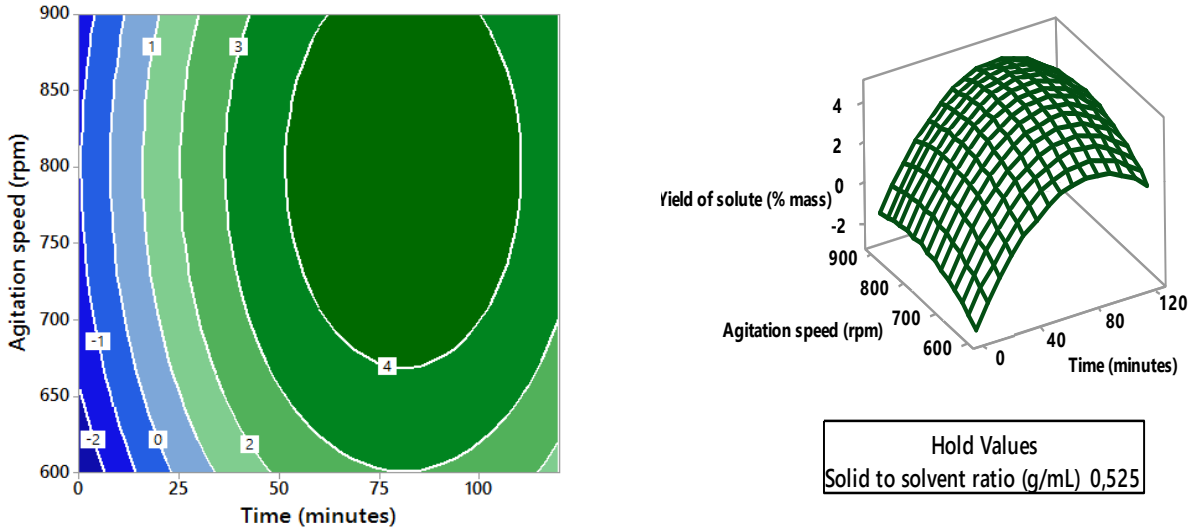


Fig. 7: Response surface plot showing the effect of agitation speed and extraction time

agitation speed give longer time for the solute to enter to the liquid phase and give turbulence condition, which enhances the rate of solute transfer. The study of Maran and Manikadan (2012) and Yin et al. (2017) have the same tendency with this research in the effect of extraction time.

Figure 7-9 give valuable information to analyze the relationships between responses and variables. Each contour and surface plot represents a number of combinations of two test variables with the other variable maintained at different levels. Contour and surface plots show how a response variable relates to two factors based on the model equation. These graphs are useful for determining the desired response values and operating conditions (Elksibi et al. 2014).

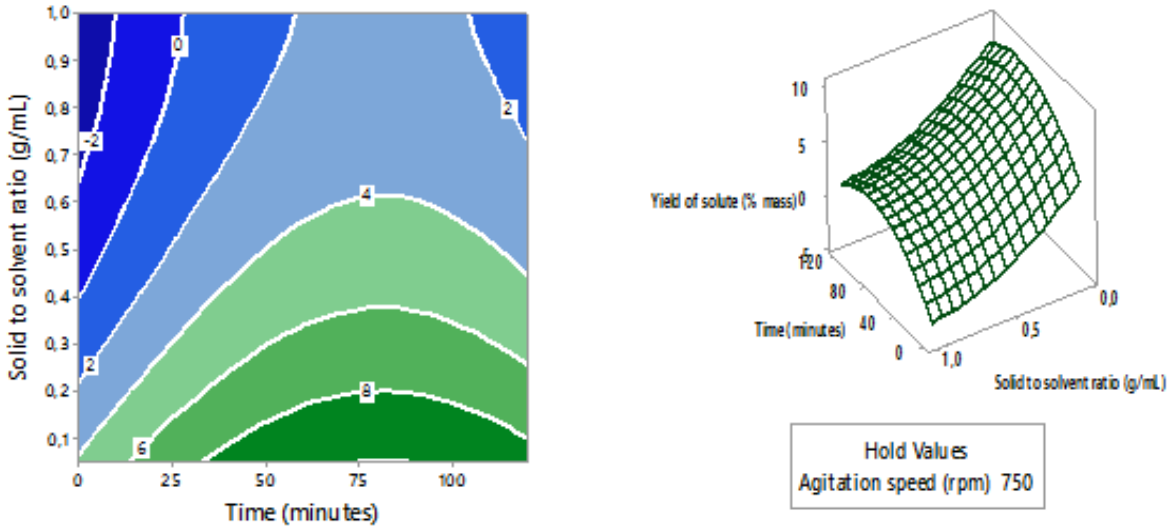


Fig. 8: Response surface plot showing the effect of solid to solvent ratio and extraction time

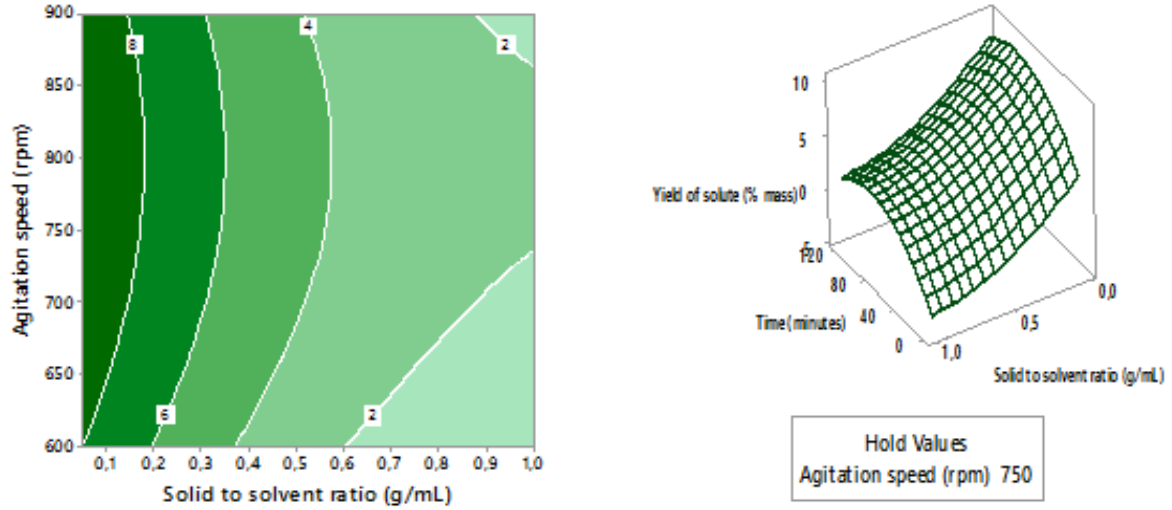
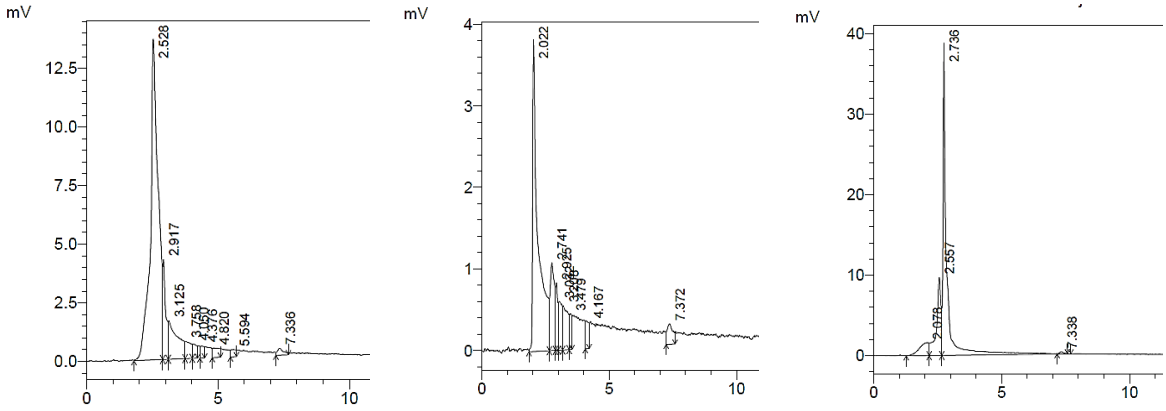


Fig. 9: Response surface plot showing the effect of agitation speed and solid to solvent ratio.

The optimum conditions for carotenoid compounds from *Tagetes erecta L* were found to be, extraction time of 81 minutes, solid to solvent ratio of 0.05 g/mL, and agitation speed of 800 rpm. The % yield of solute (g colorant in extract solution/g dry solid sample) of extraction under these optimum conditions was found 10.16%. Based on this calculation it can be stated that the RSM provides more accurate values compared to determining the optima by varying one parameter while keeping the others at an unspecified constant level. The major disadvantage of this single variable optimization is that it does not take into consideration the interactive effects among the variables; thus it does not depict the net effects of various parameters on the extraction rate (Keka et al. 2012).



a). *Tagetes erecta L.* cultivated in lowlands.

b).*Tagetes erecta L.* cultivated in highlands.

c). *Cosmos sulphureus Cav.* cultivated in lowlands.

Fig. 10: The HPLC Chromatograms

Verification of Carotenoid Content in the Solute and Comparison Between that in *Tagetes erecta L.* and *Cosmos sulphureus Cav.*

The % yield values obtained from the gravimetric method gives the amount of total solute that is extracted per amount of dry solid sample. The solute that is extracted contains the substance of interest, in this case carotenoid group (natural yellow colorant), in the presence of other substances. The compositions of carotenoid compound and other substances at oleoresin cannot, however, be determined by means of the gravimetric method. Thus, to verify that the carotenoid contain mainly the substance of interest, high-performance liquid chromatography (HPLC) was performed. Furthermore, the UV-Vis spectrophotometry methods were used to compare the carotenoid content between *Tagetes erecta L.* and *Cosmos sulphureus Cav.*, both cultivated in lowlands and highlands.

Each chromatogram in **Fig. 10** shows a dominant peak of a carotenoid compound that appears at a retention time around 2-2.8 min, in which the extract solution of *Cosmos sulphureus Cav.* cultivated in lowlands shows the highest intensity of carotenoid peak signal (around 39 mV) and *Tagetes erecta L.* cultivated in highlands shows the lowest intensity (around 3.9 mV). Quantitative analysis based on the peak areas resulted in carotenoid content of 90%, 75.7%, and 99% (g carotenoid per g dry solid in the extract) for *Tagetes erecta L.* cultivated in lowlands, *Tageteserecta L.* cultivated in highlands, and *Cosmos sulphureus Cav.* cultivated in highlands, respectively.

Finally, **Fig. 11** shows the average of UV-Vis absorbance values of *Tagetes erecta L.* cultivated in highlands, *Cosmos sulphureus Cav.* cultivated in highlands, and *Cosmos sulphureus Cav.* cultivated in lowlands.

It is seen in Fig. 11 that the highest UV-Vis absorbance value was obtained from

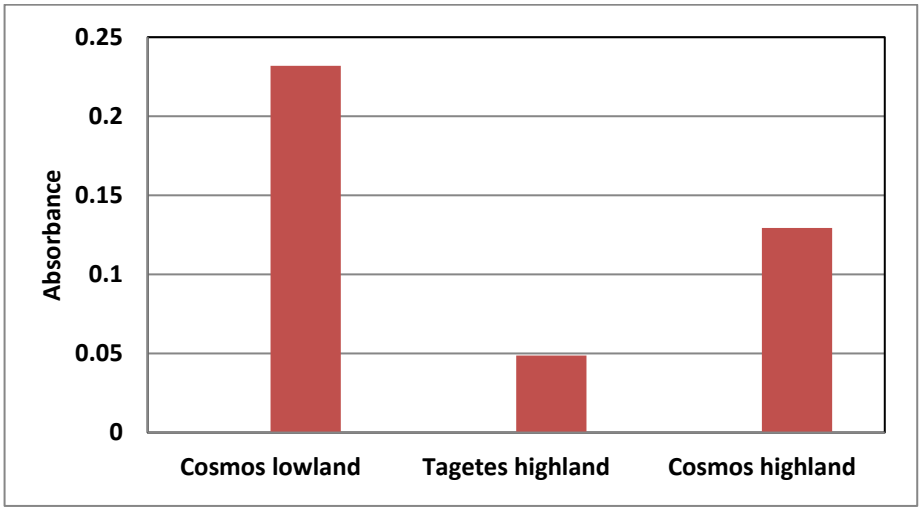


Fig. 11: The average of absorbance values of *Cosmos sulphureus Cav*. cultivated in lowlands, *Tagetes erecta L*. cultivated in highlands, and *Cosmos sulphureus Cav*. cultivated in highlands

the extract solution of *Cosmos sulphureus Cav*. cultivated in the lowland, while the lowest value was obtained from that of *Tagetes erecta L*. cultivated in highlands. These data are in agreement with the observed data from both organoleptic test and HPLC methods, in which the carotenoid content in *Cosmos sulphureus Cav*. is higher than that in *Tagetes erecta L*., and cultivation in lowlands results in higher carotenoid content compared to cultivation in highlands.

CONCLUSIONS

A systematic investigation has been made for the optimum extraction parameters of carotenoid from *Tagetes erecta L.* flower. This is done by analyzing the effects of various solvent type, extraction time, solid/solvent ratio, and agitation speed during the extraction process. Optimization of the extraction process and evaluation of interaction effects of different operating variables (time, dry solid mass to solvent volume ratio, and agitation speed) was carried out using the Response Surface Methodology (RSM) of DOE Software Minitab. The optimum parameters were then used for the extraction of carotenoid from *Tagetes erecta L.*and *Cosmos sulphureus Cav*., both cultivated in lowlands and highlands. The carotenoid contents of the plants were verified and compared using HPLC, organoleptic (visual) test, and UV-Vis methods. Final observations from the investigation are given below.

1. Ethanol 96% as a solvent resulted in higher % yield of the carotenoid compound compared to that with acetone.
2. The optimum conditions for extraction of carotenoid compounds from *Tagetes erecta L* were found to be 81 minutes, solid to solvent ratio of 0.05 g/mL, and agitation speed of 800 rpm. The yield of solute (g colorant in extract solution/g dry solid sample) of extraction under these optimum conditions was found to be 10.16%.

3. The content of carotenoid in *Cosmos* sulphureus *Cav*.is higher than that in *Tagetes erecta L*.
4. For both *Cosmos sulphureus Cav*. and Tagetes *erecta L*., cultivation in lowlands results in higher carotenoid content compared to cultivation in highlands.

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