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Spectrophotometric Analysis of different flowers of *Tagetus erecta* Colourant at different pH Values

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ABSTRACT

Spectrophotometric analysis of the natural colourant from different flowers of Tagetus erecta were carried out at different pH levels. The result shows that Tagetus erecta extract (colourant) was stable at the experimental pH range of 2.6 – 9.4(light yellow colored flowers) and 2.5- 8.4 (orange colored flowers). This was indicated by the similar peaks of absorption maxima at the different pH values. Beer's law is valid over the concentration range 50 - 400 ppm. The complex has absorption maximum at 470 and 480 nm respectively. Tagetus erecta extract, with further studies could be used as a colourant in pharmaceutical formulations.

Keywords: Spectrophotometric analysis, *Tagetus erecta*, colourant, pH effect, Colourant.

INTRODUCTION

The use of colourants in cosmetics, foods and drugs dates back to antiquity. Colors are so common in our environment that we are not always aware just how much we depend on them. Colors could be used as a means of identification, improvement of aesthetics and protection from various factors of degradation, warning and concealment, to mention but a few [1]. Natural dyes of plant origin are present in plants as a glycones or glycosides which are free or bound to sugars [2]. Natural dyes seem to be less harmful on humans and environment and nowadays they could be considered as a better alternative to synthetic dyes [3-8]. Marigold is a plant of the genus *Tagetes* of the family *Asteraceae*, mostly cultivated as garden flower that is one of the natural sources for achieving yellow color. It was used to demarcate special spaces like pavilions and to line sacred fire-pits or kunds in which ceremonies were performed [3, 4]. Currently, marigold plants are grown for pigment production in Mexico, Peru, and India. For decoration to dyes, brightly colored yellow and orange marigolds provide familiar splashes of color in South Asia. In Britain, marigold planted by gardeners to discourage pests. The essential oil from the leaves of this flower has been reported to have some anti-fungal and insecticidal properties [3, 4]. The petals are used in South Asia to dye fabrics and wool yarns [10]. Marigold plants are stout and branching and can grow up to 60 cm tall. Leaves of marigold are dark green in color and are strongly scented. Flowers vary in color from yellow and gold to orange, red and mahogany.

The taller and larger-flowered *Tagetes erecta* was often called African marigold and the smaller *Tagetes patula* was known as French marigold. But marigolds have been cultivated all over the World and lots of

similar hybrid varieties have been developed from the two species [11,12]. Marigold flowers contain compounds called carotenoids. Lutein ($C_{40}H_{56}O_2$) is a natural pigment of the carotenoid family. Lutein and its isomer zeaxanthin also known as oxycarotenoids. They are the main xanthophylls in marigold flowers and also in vegetables such as spinach and kale. It is also present in foods such as corn and egg yolks and can be found in the eye, skin, cervix and the breast. It is a powerful antioxidant and helps in maintaining healthy eyes [5-15]. The effect of pH changes on the colourant is the main focus of this study.

MATERIALS AND METHODS

All materials/chemicals used were of AR grade (Merck).

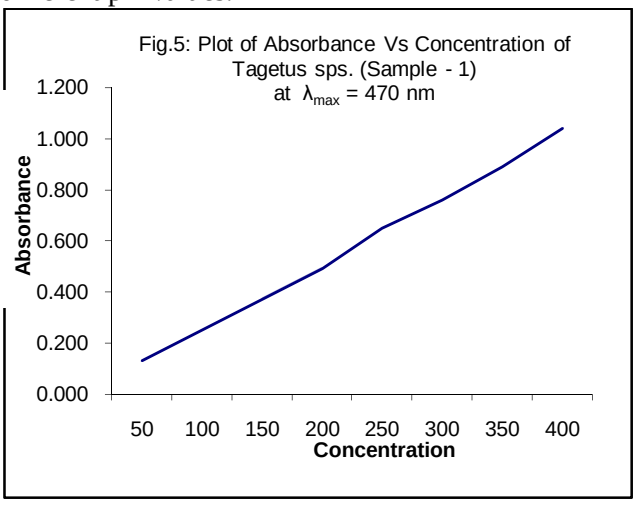
Preparation of *Tagetus* Extract: 20 g of dry flowers of *Tagetus* were dispersed in 1 dm^3 ethanol and heated to above 50 to 60 ^oC in soxhelt extractor for five hours. All the color was extracted from flowers in 5h. After extraction the extract was vacuum distilled and recovered to dryness. 100 ml of distilled water was added to this extract and subsequent aliquots were prepared using this stock solution and stored in amber-colored bottles to prevent darkening which often occurs when the material is exposed to light.

Absorption spectra of *Tagetus* extract at different pH values: 50-100 ppm concentration of the colourant was made in aqueous solution and pH values were adjusted to 2.6, 6.95, 8.20 and 9.30 for sample no.1 and pH values 4, 6.15, 8 and 12 for sample no. 2 respectively. These solutions were scanned in turn at intervals of 1 nm using UV – Vis spectrophotometer (Equiptronics). Their peak absorbances were all determined.

Beer's plot of *Tagetus* colourant solution: A stock solution of *Tagetus* colourant (1000 ppm) was made in distilled water. A serial dilution of the stock was made to obtain concentrations of 50-400 ppm. The maximum absorbances of these solutions were determined using the UV –VIS spectrophotometer at 470 nm for sample no. 1 and 410 nm for sample no. 2 and plotted against the concentration to obtain a standard plot.

RESULTS AND DISCUSSION

A straight line passing through the origin was obtained for the Beer's plot (fig.5 & fig. 10). This show that light absorbance of standard solution of Tagetus colourant extract can be used in quantitative analysis of the crude extract. Tables 1 and 2 shows the absorption peaks of the *Tagetus* extract solution prepared at different pH values.



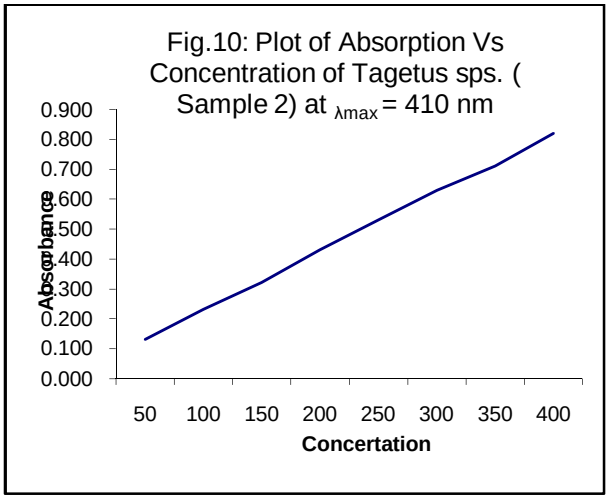


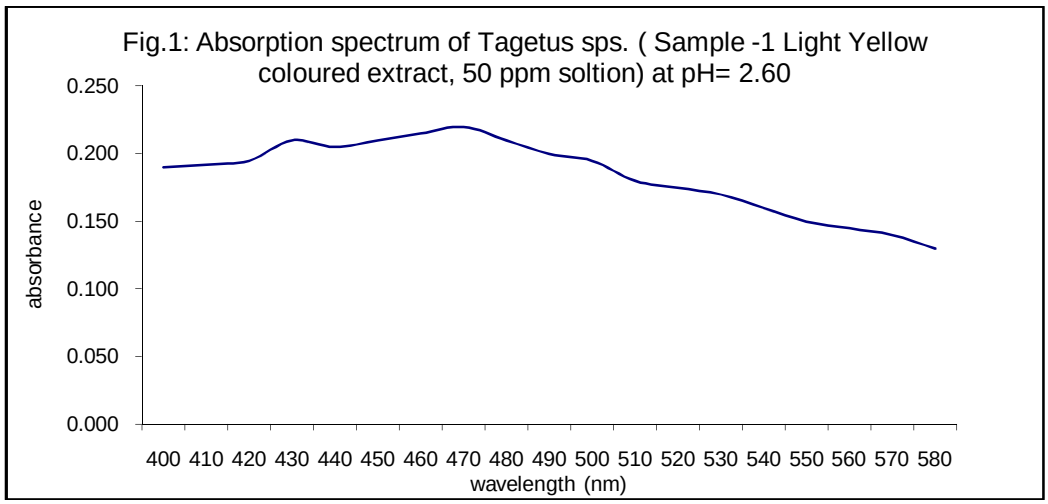
Table 1: Effect of pH on the peak absorption values

S. No.	pH values	Peak absorption (nm)
1	2.60	470
2	6.95	470
3	8.20	470
4	9.30	470

Table2 : Effect of pH on the peak absorption values

S.No.	pH values	Peak absorption (nm)
1	4.00	410
2	6.15	410
3	8.00	410
4	12.00	410

Tagetus colourant solution, within the limits of pH range chose, showed consistency in its wavelength of the peak of absorption of light spectrum (figs 1, 2, 3, 4 & fig. 6, 7, 8, 9). This shows that a single chromophore is contained in the *Tagetus* extract (sample- 1). Had it been composed of several chromophores, it would have shown different wavelengths of peak absorption. As in the case of sample – 2 which shows two peaks at 410 and 480 nm respectively. Results also indicate the stability of the colourant within the stipulated pH range. Different colors have their different and characteristic wavelength of peak absorption. It was found that *Tagetus* colourant extract was stable at the experimental pH-range of 2.6 – 9.36 (sample – 1) and 4.0 to 12.0 for sample – 2 respectively. The spectrophotometric analysis did not show significant instability as a result of the pH modifications. Due to the adverse effects of some synthetic colourants, which include carcinogenicity, tetratogenicity and various other toxic effects [15] natural colourant such as *Tagetus erecta* can be used. Natural colourants have wider margin of safety than synthetic colourants.



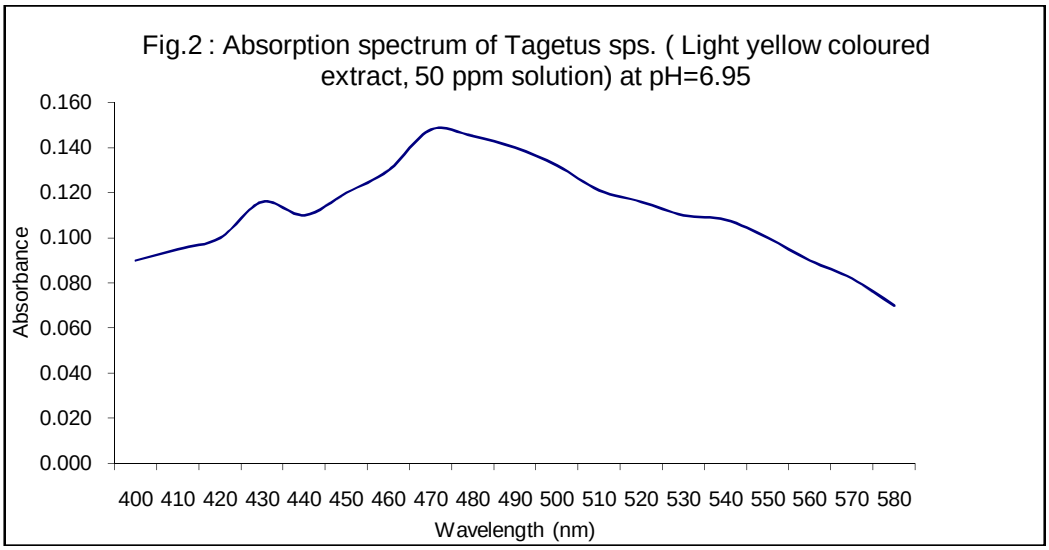


Fig.2 : Absorption spectrum of Tagetus sps. (Light yellow coloured extract, 50 ppm solution) at pH=6.95
Absorbance
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
400 410 420 430 440 450 460 470 480 490 500 510 520 530 540 550 560 570 580
Wavelength (nm)

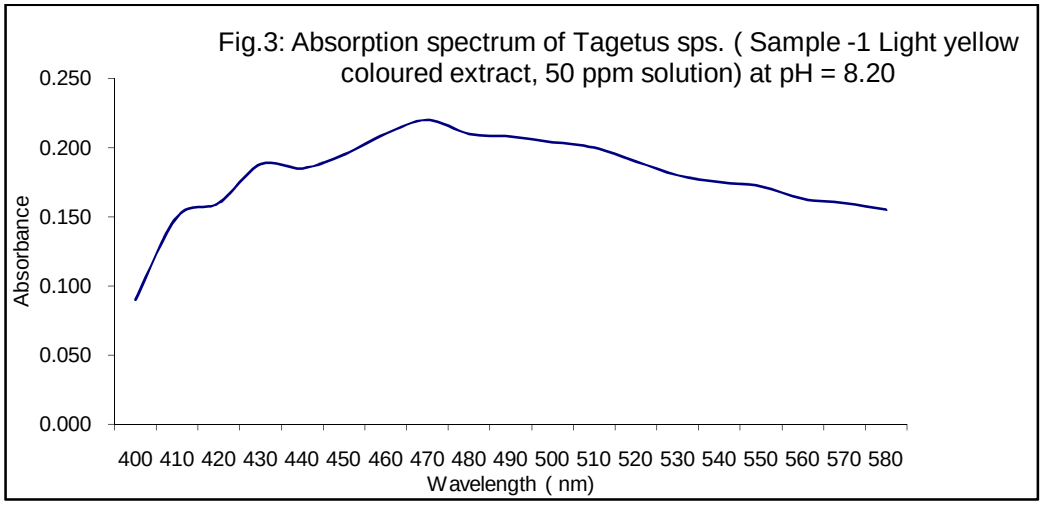


Fig.3: Absorption spectrum of Tagetus sps. (Sample -1 Light yellow coloured extract, 50 ppm solution) at pH = 8.20
Absorbance
0.250
0.200
0.150
0.100
0.050
0.000
400 410 420 430 440 450 460 470 480 490 500 510 520 530 540 550 560 570 580
Wavelength (nm)

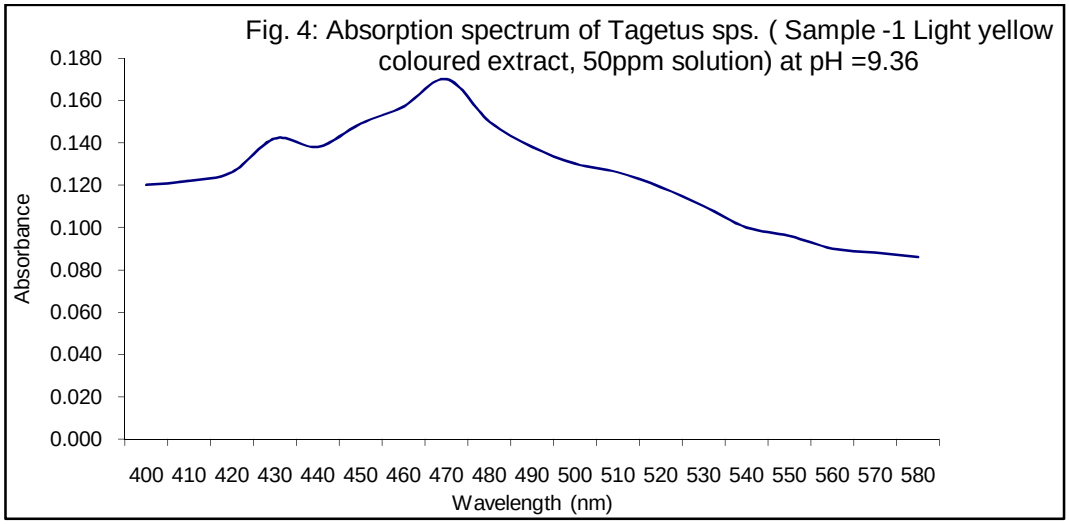


Fig. 4: Absorption spectrum of Tagetus sps. (Sample -1 Light yellow coloured extract, 50ppm solution) at pH =9.36
Absorbance
0.180
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
400 410 420 430 440 450 460 470 480 490 500 510 520 530 540 550 560 570 580
Wavelength (nm)

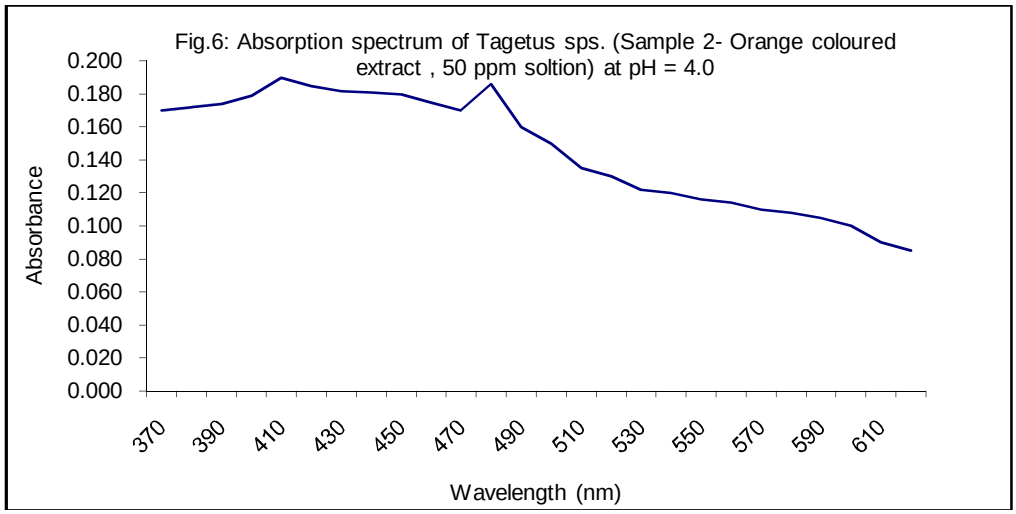


Fig.6: Absorption spectrum of Tagetus sps. (Sample 2- Orange coloured extract , 50 ppm soltion) at pH = 4.0
Absorbance
0.200
0.180
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
370 390 410 430 450 470 490 510 530 550 570 590 610
Wavelength (nm)

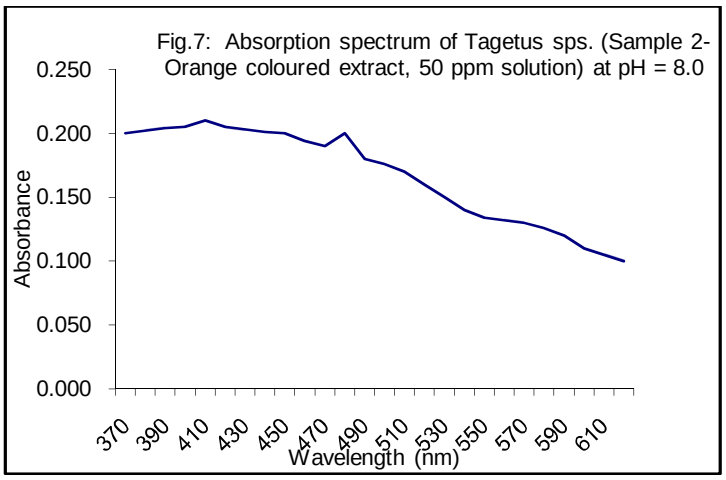


Fig.7: Absorption spectrum of Tagetus sps. (Sample 2- Orange coloured extract, 50 ppm solution) at pH = 8.0
Absorbance
0.250
0.200
0.150
0.100
0.050
0.000
370 390 410 430 450 470 490 510 530 550 570 590 610
Wavelength (nm)

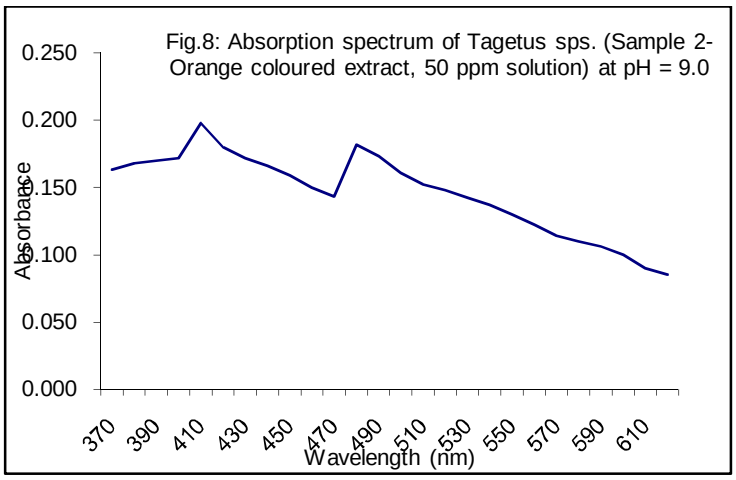
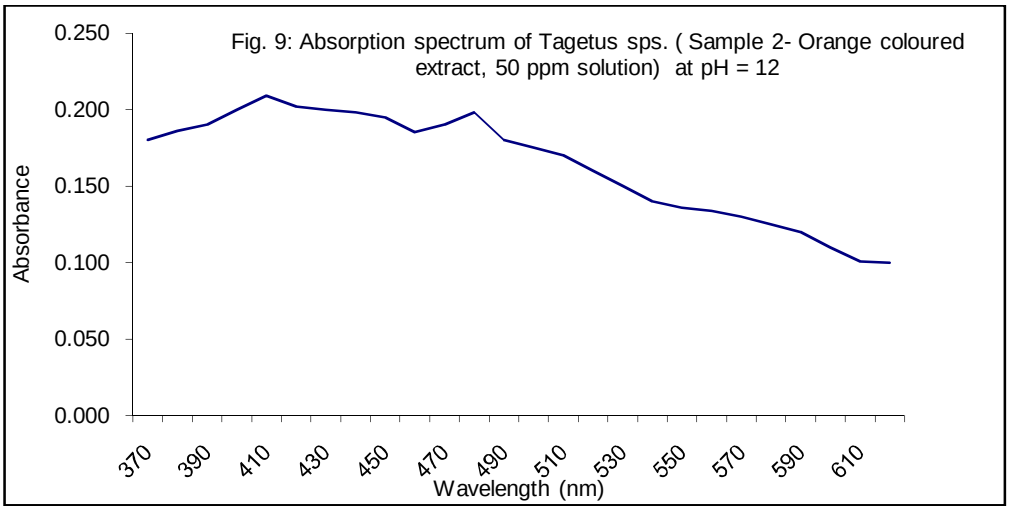


Fig.8: Absorption spectrum of Tagetus sps. (Sample 2- Orange coloured extract, 50 ppm solution) at pH = 9.0
Absorbance
0.250
0.200
0.150
0.100
0.050
0.000
370 390 410 430 450 470 490 510 530 550 570 590 610
Wavelength (nm)



APPLICATIONS

Tagetus erecta extract, could be used as a colourant in pharmaceutical formulations since the pH effect is not there like other colourants.

CONCLUSIONS

It has been seen that the natural pigments showed the sensitivity to different pH values and the structural changes in chromophore system of dyes caused by changes in pH but in the present study observed that there is no change in the properties of the extracts of *Tagetus erecta*. In conclusion it can be stated that *Tagetus erecta* colourant, with further studies, could be used as a colourant in pharmaceutical formulations.

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