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Compatibility studies between Paracetamol and *Spirulina*

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ABSTRACT

Most of the synthetic drugs which induce side effect during overdose. The natural compounds are playing important role to reducing side effects of the synthetic drugs and also increase the immunity of recipients. The objective of the present study is to investigate the compatibility between Paracetamol as a synthetic compound with *Spirulina* as a natural compound. The Paracetamol and *Spirulina* mixture were prepared in different ratio 1:1 (w/w) (Paracetamol: *Spirulina*), 1:2 (w/w) (Paracetamol: *Spirulina*) and 2: 1 (w/w) (Paracetamol: *Spirulina*). These samples were stored at different temperature (5 ˚C, 15 ˚ C, 30 ˚ C and 40 ˚ C) for one month. For every week samples were taken in the mixtures and the quantification of Paracetamol by using reversed phase liquid chromatography and estimation of chlorophyll A, B, total chlorophyll and total carotenoids in the mixture by using UV spectrophotometer. There was no significant loss of Paracetamol, chlorophyll, and carotenoids in their mixture. Both Paracetamol and *Spirulina* were stable under different temperature. Therefore it conclude that Paracetamol and *Spirulina* were compatible when mixed in a ratio of 1:1(w/w), 1:2 (w/w) and 2:1 w/w)

Keywords: Paracetamol, *Spirulina*, RP-HPLC, UV spectrophotometer

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INTRODUCTION

Paracetamol (N-acetyl-*p*-aminophenol) is a widely used analgesic and antipyretic that is safe and effective, with few adverse effects, when taken at therapeutic doses [1]. The toxicity depends on the metabolic activation of acetyl- para -aminophenol via cytochrome P-450, which results in the formation of an electrophilic reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI) [2]. NAPQI is rapidly conjugated with glutathione (GSH) and the GSH-APAP adduct is excreted mainly into bile [2]. *Spirulina* is blue green algae of the Oscillateriaceae family which grows naturally in countries which have a warm climate and has been considered as supplement in human and animal food [3]. *Spirulina* contain protein, lipids, carbohydrates, minerals, vitamins including β-carotene and a pigmented protein, C-phycocyanin [4]. The antioxidant properties of *Spirulina* and its capacity to scavenge hydroxyl radicals [5] and to inhibit lipid peroxidation [6] have attracted the attention of many researches. *Spirulina* species exhibited various biological activities such as antihypertensive and antihyperlipemic[7], Chemopreventive of cancer [8] and hepatoprotective against cadmium toxicity [9]. There were number of method had been reported compatibility the Paracetamol with active pharmaceutical ingredient [10, 11] and excipients [12, 13]. There were no method reported compatibility studies between Paracetamol with *Spirulina*. The objective of the present study was to evaluate the compatibility of Paracetamol with *Spirulina*.

MATERIALS AND METHOD

Chemical and Reagents

Pure Paracetamol, HPLC grade methanol, HPLC water and acetone were purchased from Coimbatore Scientific Suppliers, Coimbatore, Tamil Nadu. *Spirulina* material was obtained from Sanet products limited, Kodai Road, Tamil Nadu.

Preparation of Paracetamol and *Spirulina* mixture [17]

1: 1 ratio w/w (Paracetamol: *Spirulina*)

Weight accurately 10 g of Paracetamol and 10 g of *Spirulina* were mixed together. The sample was spread in petridish and stored at different temperature such as 5 ˚C, 15˚C, 30˚C and 40 ˚ C

1: 2 ratio w/w (Paracetamol: *Spirulina*)

Weight accurately 10 g of Paracetamol and 20 g of *Spirulina* were mixed together. The sample was spread in petridish and stored at different temperature such as 5 ˚C, 15 ˚ C, 30˚C and 40 ˚ C

2: 1 ratio w/w (Paracetamol: *Spirulina*)

20 g of Paracetamol and 10 g of *Spirulina* were taken and mixed together. The sample was spread in petridish and stored at different temperature such as 5 ˚C, 15 ˚ C, 30 ˚ C and 40 ˚ C

Instruments and condition

The Paracetamol was analysed in Agilent technologies 1200 liquid chromatography. A 20 µl of rheodyne injector was used for injection the samples. The column was used was C-18 Zorbax column (4.6 mm X 250 mm, 5 µm particle size). The mobile phase consists of methanol and water in the ratio of 70:30 v/v [14]. The flow rate of the mobile phase was kept at 1 ml/ minute. UV detection was carried out at 275 nm [14] and column temperature was maintained at ambient condition. Data was analyzed by using Chem Station software.

The total chlorophyll and carotenoids were analysed in Chemito Spectrascan UV2600 double spectrophotometer and SPECTRUM™ as software.

Preparation of Paracetamol standard solution

An accurately weigh quantity of Paracetamol about 50 mg was transfer into 50 ml volumetric flask, add 25 ml of mobile phase (methanol 70: water 30 v/v) and sonicated for 2 minute and make up to volume with mobile phase (1000 µg/ml) used as stock solution. Further 2 ml of the stock solution was transfer into 50 ml volumetric flask and diluted with mobile phase (methanol 70: water 30 v/v) and got the final concentration 40 µg/ml used as working standard.

System Suitability test

The system suitability test was performed by six replicate injection of 40 µg/ml of Paracetamol to the chromatographic system and chromatographic parameters calculated such as number of theoretical plates, percentage RSD of peak area, capacity factor, tailing factor and asymmetry factor[14].

Instrument Linearity

The linearity of Paracetamol was studied by prepare standard solution of different concentration in range from 10 – 50 µg/ml. The solutions were injected into HPLC system and constructed linearity curve by plotting peak area (Y) against concentration (X) and calculate the correlation coefficient [14].

Quantification of Paracetamol by RP-HPLC

Weight accurately 50 mg of sample into each mixture and transfer into 50 ml volumetric flask. Add 25 ml of mobile phase (methanol 70: water 30 v/v) and sonicate for 5 minutes then make up to volume with mobile phase. The solution was filter through what man No 1 filter paper, from the filtrate 2 ml was transfer into the 50 ml volumetric flask and make up to volume with mobile phase (methanol 70: water 30 v/v) and 20 µl was injected in HPLC system.

Estimation of total Chlorophyll and Carotenoids by UV spectrophotometer [15]

In each mixture, 20 mg of sample were taken and grinded with 80% acetone using pestle and

mortar. The crude extracts were centrifuge at 5000 rpm for 5 minutes. The supernatant were measured at 663.6, 646.6 and 440.5 nm. Calculate the content using following equation and the value was expressed as µg/ml.

Chlorophyll a = 12.25 $A_{663.3}$ - 2.55 $A_{646.6}$

Chlorophyll b = 20.31 $A_{646.6}$ - 4.91 $A_{663.6}$

Chlorophyll a+b = 17.76 $A_{646.6}$ + 7.34 $A_{663.6}$

Carotenoids = 4.69 $A_{440.5}$ - 0.267 Chlorophyll a+b

RESULTS AND DISCUSSION

For system suitability test, six replicate standard solutions were injected and capacity, tailing factor, % RSD of peak area was found to be less than 2 and theoretical plates was found > 2000. Table 1 shows the system suitability parameters and figure 1 standard chromatogram of Paracetamol.

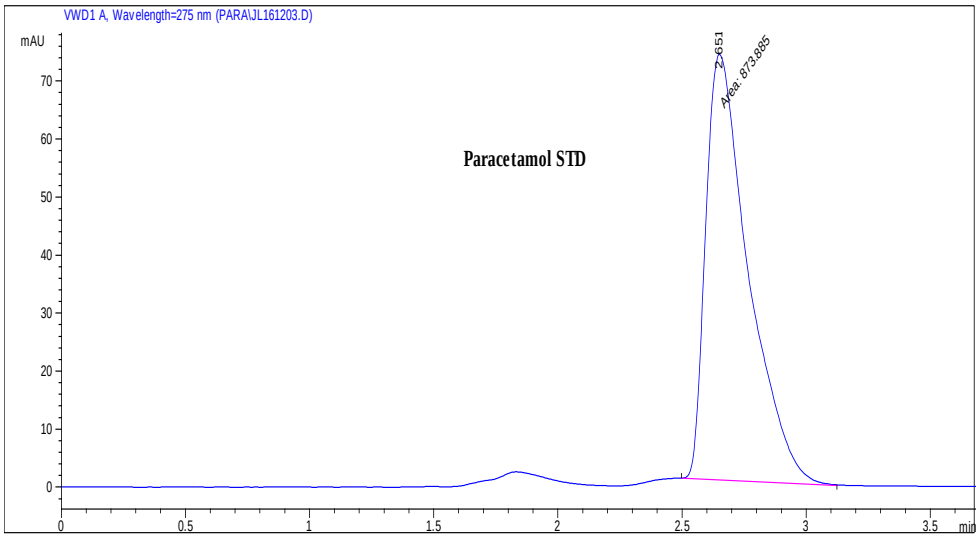

Figure 1: Standard Chromatogram of Paracetamol

Table 1: System suitability parameters for HPLC method

Parameters	Paracetamol
Capacity factor (K)	0.36
Tailing factor	1.59
Number of theoretical plate	2600
% RSD of peak area	0.61
Asymmetry factor	1.7

The standard solutions in the range of 10 – 50 µg/ml were injected in the HPLC system and the regression analysis of Paracetamol was found to be 0.999. Linearity curve of Paracetamol shows in figure 2

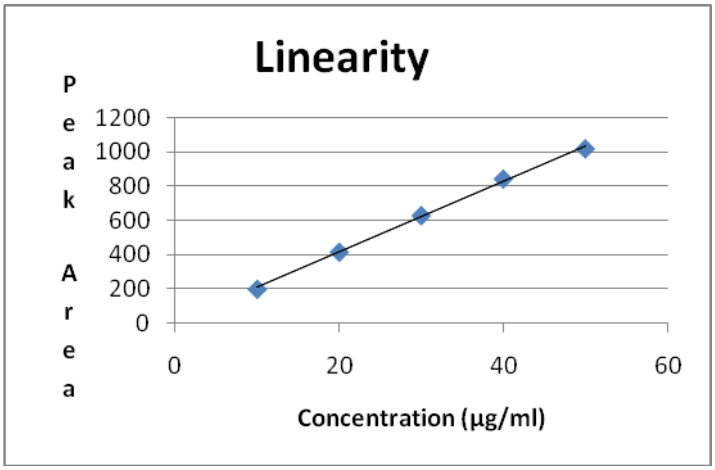

Figure 2: Linearity of Paracetamol

The initial quantification of Paracetamol in 1:1, 1:2, 2:1 mixture were found 49.1%, 31.2% and 65.7% respectively and estimation of total chlorophyll in the 1:1, 1:2, 2:1 mixture were found 12.18, 18.29 and 7.44 µg/ml. The total carotenoids in the mixture 1:1, 1:2 and 2:1 were 1.56, 1.99 and 0.98 µg/ml respectively. Figure 3, 4, 5 shows HPLC chromatogram of Paracetamol 1:1, 1:2, 2:1

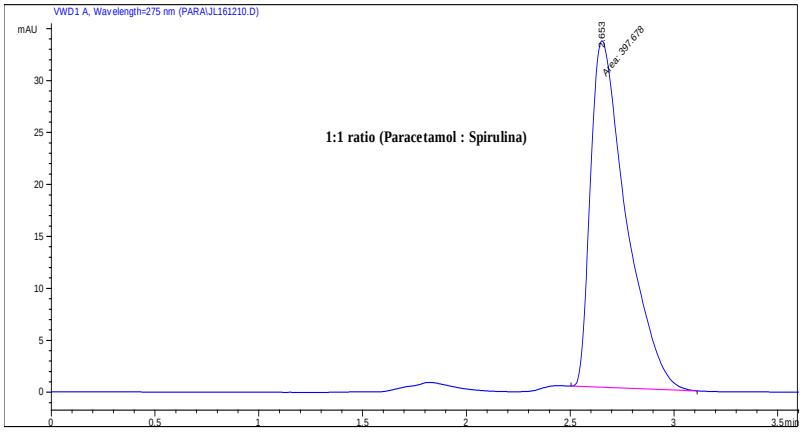

Figure 3: HPLC chromatogram of Paracetamol 1:1 ratio (Paracetamol: *Spirulina*)

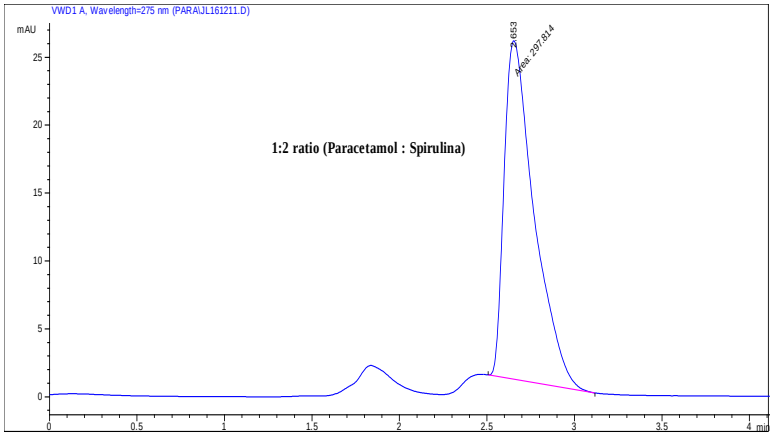

Figure 4: HPLC chromatogram of Paracetamol 1:2 ratio (Paracetamol: *Spirulina*)

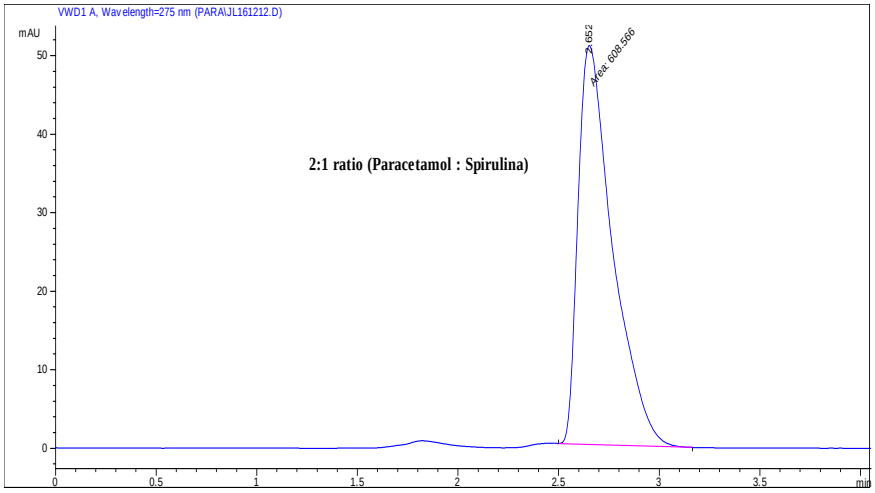

Figure 5: HPLC chromatogram of Paracetamol 2:1 (Paracetamol: *Spirulina*)

All the mixtures were stored in different temperature (5^0C, 15 ^{0}C, 30 ^{0}C and 40^0C) for one month and every week samples were taken for quantification of Paracetamol and estimation of total chlorophyll and carotenoids.

In 1:1 ratio, the Paracetamol concentration of I, II, III and IV week were found 52.02 % , 49.6% , 49.54% and 48.13 % at 5^0C and 46.5 %, 48.4 % , 50.9% , 48 % at 15^0C and 50.1 %, 46.91 %, 47.27 % ,46.08% at 30^0C and 45.8%, 49.08 %, 44.5 %, 53.35 % at 40^0C temperature respectively (Figure 6).

In 1:2 ratio, I, II, III and IV week of Paracetamol concentration were obtained 31.12%, 33.2%, 34.12%, 31.98 % at 5^0C and 34.09 %, 31.81 %, 34.13 %, 34.44 % at 15^0C and 34.51 %, 31.86 %, 32.02 %, 31.35 % at 30^0C and 30.60 %, 35.91 %, 32.85 %, 35.1 % at 40^0C temperature respectively (Figure 6).

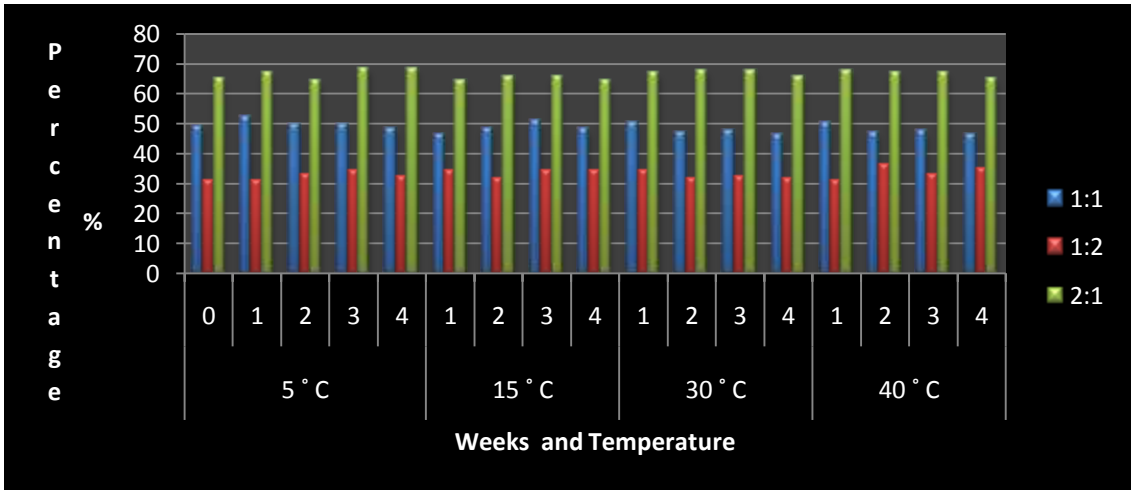

Figure 6: Figure 6 shows comparison of Paracetamol quantification in the mixture at 5˚ C, 15˚ C, 30˚ C and 40˚ C temperature for four weeks.

In 2:1 ratio, 67.20%, 64.20 %, 68.29 %, 68.32 % at 5^0C and 62.44 %, 65.78 %, 65.53 %, 64.37 % at 15^0C and 66.7 %, 67.7 %, 67.8 %, 65.56 % at 30^0C and 67.67 %, 67.07 %, 67.13 %, 64.87 % at 40^0C of Paracetamol was found in I, II, III and IV week respectively (Figure 6).

In 1:1 ratio, the total chlorophyll content in the I, II, III, and IV week were found 11.64, 11.8, 12.2, 12.2 µg/ml at 5^0C, 12.57, 12.05, 12.03, 12.42 µg/ml at 15^0C and 12.57, 12.7, 12.1, 12.08 µg/ml at 30^0C and 12.1, 12.7, 12.11, 12.2 µg/ml at 40^0C temperature respectively (Figure 7).

In 1:2 ratio, I, II, III, and IV week of total chlorophyll content were obtained 18.39, 17.55, 17.9, 17.96 µg/ml at 5^0C and 17.9, 18.1, 18.06, 17.96 µg/ml at 15^0C and 18.05, 18.4, 17.8, 18.1 µg/ml at 30^0C and 18.14, 17.91, 18.09, 18.1 at 40^0C temperature respectively (Figure 7).

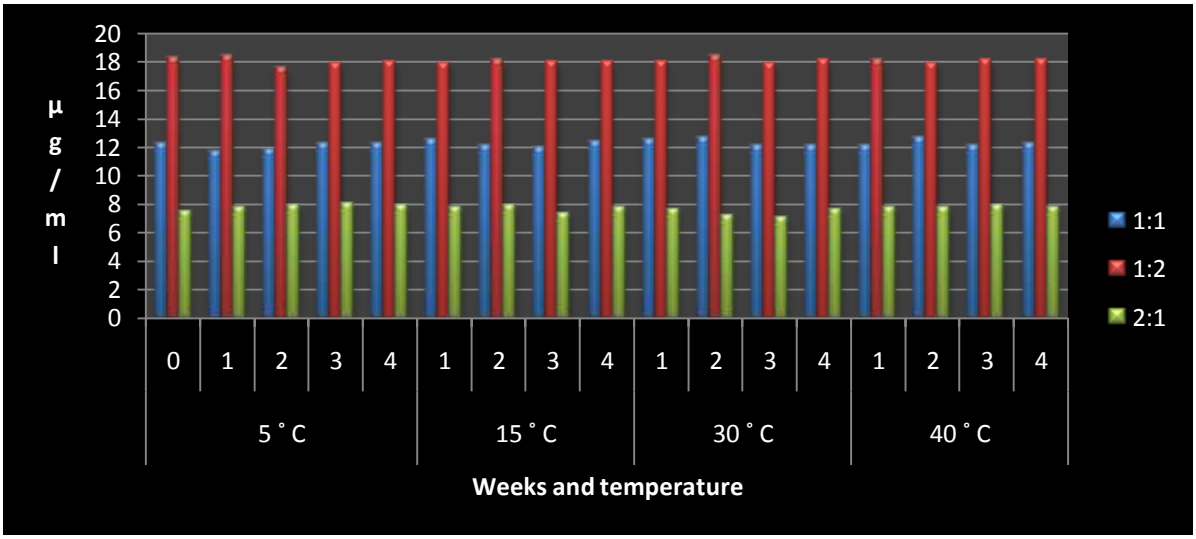

Figure 7: Comparison of Chlorophyll estimation in the mixture at 5˚ C, 15˚ C, 30˚ C and 40˚ C temperature for four weeks

I, II, III, and IV week of total chlorophyll content in 2:1 mixture were found 7.75, 7.91, 7.99, 7.84 µg/ml at 5^0C and 7.75, 7.88, 7.39, 7.79 µg/ml at 15^0C and 7.64, 7.24, 7.07, 7.58 µg/ml at 30^0C and 7.78, 7.7, 7.93, 7.71 µg/ml at 40^0C temperature respectively (Figure 7).

The carotenoids content in 1:1 mixture were found 1.55, 1.6, 1.58, 1.59 µg/ml at 5^0C and 1.54, 1.51, 1.58, 1.53 µg/ml at 15^0C and 1.58, 1.62, 1.52, 1.58 µg/ml at 30^0C and 1.54, 1.55, 1.62, 1.5 µg/ml at 40^0C for I, II, III and IV week (Figure 8).

In 1:2 ratio, the carotenoids content for I, II, III and IV week were found 2.02, 1.91, 2.15, 2.1 µg/ml at 5^0C and 2.06, 1.96, 1.92, 1.97 µg/ml at 15^0C and 1.95, 2.02, 1.94, 2.07 µg/ml at 30^0C and 1.93, 2.01, 2.06, 1.98 µg/ml at 40^0C temperature respectively (Figure 8).

The carotenoids content in 2:1 mixture were obtained 1.06, 1.14, 0.99, 0.95 µg/ml at 5^0C and 1.01, 0.97, 1.19, 0.99 µg/ml at 15^0C and 0.94, 1.1, 1.08, 0.97 µg/ml at 30^0C and 1.03, 0.96, 1.01, 1.13 µg/ml at 40^0C for I, II, III and IV weeks (Figure 8).

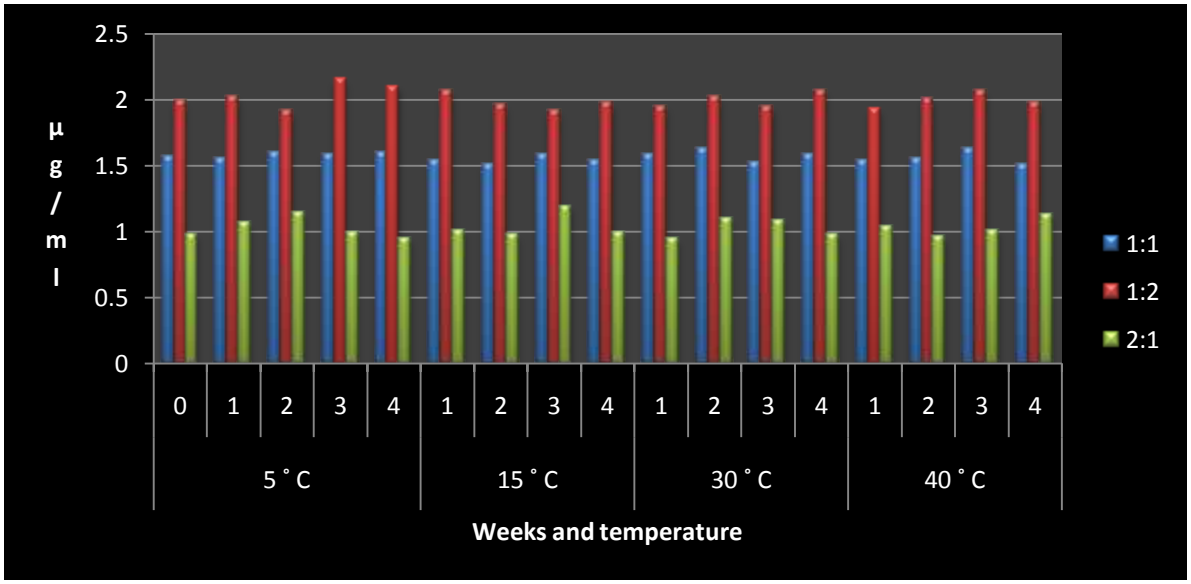

Figure 8: Shows comparison of Carotenoid estimation in the mixture at 5˚ C, 15˚ C, 30˚ C and 40˚ C temperature for four weeks

The initial, I, II, III, and IV week of Paracetamol concentration was remain. There was no found degradation of Paracetamol content in all mixtures and does not react with *Spirulina* in 1:1, 1:2, 2:1 ratios. Thus the Paracetamol was stable with *Spirulina.*

There was no major degradation of total chlorophyll and carotenoids of *Spirulina* were found in the mixtures. The natural compound of *Spirulina* was stable with Paracetamol.

CONCLUSION

From the present investigation, it can be concluded that the Paracetamol and *Spirulina* are compatible when mixed in a ratio of 1:1(w/w), 1:2 (w/w) and 2:1 w/w) and it can be pharmaceutical formulated together.

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