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Research Article

**Formulation of green tea polyphenols as solid food
supplement**

R.W.N Tanuja, R.P Perera and G.H.C.M.Hettiarachchi*

Department of Chemistry, University of Colombo, Colombo 3 Sri Lanka.

ABSTRACT

Green tea is a beverage, associated with numerous health benefits due to antioxidant properties of polyphenol; which is the major component of it. The study was conducted to develop a solid dosage form of green tea polyphenols that can be used as a food supplement to improve lifestyle instead of the crude green tea extract. The total polyphenols were extracted from green tea leaves and freeze dried. The freeze dried sample was checked for many parameters before formulating into solid dosage form. The total polyphenol content in the freeze dried sample was evaluated using folin ciocalteu reagent and the results showed the presence of 58 % of total polyphenols in the sample. The hygroscopicity was determined using constant relative humidity solutions; potassium chromate 98% RH, sodium chloride 75% RH and magnesium nitrate 53% RH at 25°C. The lowest percentage of moisture absorbed was recorded at 53%RH. Based on pre formulation studies (organoleptic properties, hygroscopicity, percentage of moisture content etc) two hard gelatin capsule formulations were developed. Disintegration times for capsule formula 1 and 2 were found as 1.53 min and 1.37 min respectively. An antioxidant activity was tested in the sample, capsule formula 1 and 2 using DPPH assay and it was recorded as 81%, 76% and 78% respectively at a concentration of 250µg/ml. Stability tests were done only giving humidity challenges to the capsule formula 1 and 2 for 19 days and disintegration times after 19 days were compared with initial disintegration times of two formulae. The results showed that the both formula 1 and 2 were stable at 33% RH and formula 1 had a slightly higher stability than formula 2 at 33% RH. In conclusion, preliminary data of the study indicated that the total polyphenol can be formulated into solid dosage form and it can be used as a food supplement.

Key words: Green tea, Polyphenols, Anti oxidant activity and Solid dosage form.

INTRODUCTION

Tea is the major product produced from fresh tea leaves of *Camellia sinensis* that is grown in Sri Lanka, India, China, Japan, and Java.^{1,2} It is a famous non alcoholic beverage in the world. Based on the degree of fermentation, processed tea leaves are categorized as unfermented, semi fermented and fully fermented are called green tea, oo-long tea and black tea respectively.³

Green tea manufacturing process, fresh tea leaves do not undergo fermentation which can lead to degradation of chemical compounds such as vitamins and polyphenols. Hence green tea shows aroma and flavors of the original untreated tea leaves.³ In addition to carbohydrates and proteins, green tea contains polyphenol (24%-36%), lignin (7%), amino acids (3%-4%), caffeine (2%-4%), organic acid (2%)

and chlorophyll (0.5%).¹ It contains mixture of polyphenolic compounds such as flavonols, flavandiol, flavanoids, and phenolic acid. Tea flavanols are also known as catechins.^{2,4} Green tea mainly consists of six kinds of catechins. (-)-Epigallocatechin gallate (9-12%), (-)-Epicatechin gallate (3-6%), (-)-Epigallocatechin(3-6%), (-)-Epicatechin(1-2%) are the major polyphenols in green tea.⁴ In addition (+)-Gallocatechin and (+)-Catechin are present in minor amounts.^{4,5}

Many researchers have showed that tea polyphenols have antioxidant activity/ free radical scavenging activity.^{2,4,6,7} It leads to unique combination of health benefits such as anticancer effect, prevention of cardiovascular diseases, prevention of inflammatory diseases(multiple sclerosis, rheumatoid arthritis)

reduction of body weight, anti aging activity, strengthen the immune system, anti bacterial and anti viral effect etc.^{2,6-16}

At present, Sri Lanka exports bulk tea (green tea, black tea and oo-long tea) to value added tea products, such as instant tea, tea bags, iced tea, ready to drink bottles, flavored tea, and organic tea etc.¹⁷ Among them green tea is preferred by worldwide consumers, due to its health benefits. Green tea has health benefits over the other tea products such as black and oo-long tea due to its non fermented manufacturing process. The polyphenol content in green tea is higher than the black and oo-long tea.¹

The different types of green tea products, green tea bags and green tea supplements are available in the market. Consumers have options to choose the green tea products which are flavored with kiwi and pear, plum and berry, orange and spice, mango and mint etc.¹⁵ Green tea capsules and tablets are also available in current market as a food supplement instead of the beverage and the consumers prefer green tea supplements over the tea bags. These capsules and tablets are consisted of crude green tea extract obtained from green tea leaves. In some of these products decaffeinated dry green tea extract or certain amount of concentrated chemically synthesized polyphenols may be present.¹⁸

The aim of this study was to develop a solid dosage form which contains freeze dried green tea polyphenols powder as an active ingredient instead of green tea extract and concentrated or chemically synthesized polyphenols to achieve maximum health benefits in an effective manner. For this purpose two formulae were developed and tested for its activity.

MATERIALS AND METHODS

Materials:

Green tea leaves were obtained from Tea Board, Colombo, Sri Lanka. All the other chemicals and reagents used in the assays were of analytical grade (Sigma, BDH).

Extraction of polyphenols from green tea leaves:

A volume of 1.0 L of 70% methanol and ascorbic acid (4g) were added to crushed green tea leaves (200.0g). Mixture was placed in the sonicator at 40°C for 45 minutes and filtrate was collected. Polyphenols were obtained using liquid- liquid extraction method with the use of methylene chloride and ethyl acetate respectively.⁴

Determination of total polyphenol content:

Total polyphenol content in green tea leaves and freeze dried polyphenols were determined using

Folin ciocalteu reagent by following ISO 14025- 1 method.¹⁹

Determination of hygroscopicity of freeze dried polyphenol powder:

Saturated solutions of potassium chromate (RH 98% at 25°C), sodium chloride (RH 75% at 25°C) and magnesium nitrate (RH 53% at 25°C) were prepared. Weight increase was calculated in each hour after placing 1.00g of polyphenol powder at each of the above relative humidity. The control was carried out in room temperature and the changes of relative humidity with the room temperature were measured.

Formulation of green tea polyphenols into solid dosage form:

Formula 1 for 250 mg of capsule

A mass of 125.0 mg of freeze dried polyphenol powder was mixed with 100.0 mg of anhydrous lactose powder and milled. The mixture was sieved through No 20 mesh and the powder mixture was mixed for 5 min. The remaining anhydrous lactose powder (22.5 mg) was mixed with 1.25 mg of aerosil and sieved through No 20 mesh. This mixture was added to the anhydrous lactose and polyphenols mixture and mixed for another 10 min. Finally 1.25 mg of magnesium stearate was added and mixed for 30 seconds. This mass was used to fill in one capsule.

Formula 2 for 250 mg of capsule

A mass of 125.0 mg of freeze dried polyphenol powder was milled with a 50.0 mg of mannitol powder and sieved through No: 20 mesh. The resultant powder was mixed with 50.0 mg of anhydrous lactose for 5 min. A mass of 22.5 mg of anhydrous lactose and 1.25 mg of aerosil were mixed and sieved through No: 20 mesh. This mixture was added to the mixture of anhydrous lactose and polyphenols and mixed for another 10 minutes. Finally 1.25mg of magnesium stearate was added and mixed for 30 seconds. This mass was used to fill in one capsule.

Determination of Disintegration time and anti oxidant properties

Disintegration time for both formula 1 and 2 capsules was determined using disintegration apparatus. Antioxidant activity of freeze dried polyphenol powder and powder blend in two capsule formulae were determined by the slightly modified method of Brand-Williams.²⁰⁻²²

Determination of effect of relative humidity on two capsule formulae:

Saturated solutions of magnesium chloride (RH 32.78% at 25⁰C), magnesium nitrate (RH 53% at 25⁰C) and sodium chloride (RH 75% at 25⁰C) were prepared. One capsule from each formula was placed in each relative humidity and weight increases of the samples were calculated for 19 days. The control was carried out in the room temperature. The disintegration times of the capsules subjected to relative humidity assay and room temperature were determined.

RESULTS AND DISCUSSION

Total polyphenol content in green tea leaves and extracted polyphenol powder:

The total polyphenol percentage of the extracted polyphenol powder and green tea leaves were determined using folin ciocalteu reagent and gallic acid standard calibration curve.¹⁹ (Figure 1A) The total polyphenol percentage in aqueous green tea extract and freeze dried polyphenols were calculated as 15% and 58% respectively. The result showed that total polyphenol in extracted sample is 3 fold higher than the green leaves. (Figure 1B)

Hygroscopicity of total polyphenol powder:

The hygroscopicity of freeze dried polyphenol powder was checked under three different constant relative humidity environments, covering the possible humidity conditions at the normal room temperature in Sri Lanka. The results were obtained for 30 hours. At 53% RH showed the lowest moisture absorbed percentage when compared with other two relative humidity environments (Figure 2). The control was done at the room temperature and the percentage of moisture absorbed for 30 hrs was varied significantly with the time (Figure 3). At room temperature percentage of moisture absorbed was always higher than at 53% RH during the 30 hours. According to results it is necessary to formulate the capsules at low relative humidity environments to minimize the moisture absorption. Figure 4 showed that relative humidity decreases as temperature increases, but it was not in a linear relationship. Hence it is necessary to consider the relative humidity and temperature in the determination of the water absorption capacity of solid dosage form.

Formulation of solid dosage form using green tea polyphenols:

Excipients were selected for formulations to improve the stability of polyphenol during the storage and two formulas were developed. In formula 1, anhydrous lactose was used as the capsule filler.²³ Magnesium stearate and colloidal silicon dioxide were used as a lubricant and glidant respectively.^{24,25} In formula 2

both mannitol and lactose anhydrous were added as the capsule filler. Purposes of using other excipients in formula 2 are same as that of formula 1. Initial disintegration time for capsule formula 1 and 2 were determined as 1.53 min and 1.37 min respectively.

Anti oxidant properties of polyphenols powder blend in the capsules:

Antioxidant activity of polyphenols were tested according to the slightly modified method of Brand-Williams.²⁰⁻²² An antioxidant activity of freeze dried polyphenol powder, formula 1 and 2 are 81%, 76% and 78% respectively (Figure 5). Total dietary intake of polyphenols is approximately 1g/day.²⁶ The results revealed that consumption of 4 tablets per day will fulfill the required amount of antioxidant per day.

Effect of relative humidity during the storage:

The effect of relative humidity on capsule formula 1 and 2 were checked at 33%, 53% and 75% RHs for 20 days. Capsule formula 1 showed the highest percentage of moisture absorption at 75% RH and lowest at 33% RH (Figure 6). Disintegration times for formula 1 after 19 days at 33%, 53% and 75% were 1.44 min, 1.21 min and 49 sec respectively. The disintegration time obtained just after formulation of formula 1 was 1.53 min. That value was much closer to disintegration time obtained after 19 days at 33% RH (1.44min). Thus, formula 1 was more stable at 33% relative humidity.

Compared to formula 1, Capsule formula 2 also showed the similar results. The percentage of moisture absorption was highest at the 75% RH and lowest at the 33% RH (Figure 7). Disintegration times for formula 2 after 19 days at 33%, 53% and 75% were 1.21 min, 1.14 min and 38 sec respectively. The disintegration time obtained just after formulation of formula 2 was 1.37 min. That value was much closer to disintegration time obtained after 19 days at 33% RH (1.21min). Taken together, these results revealed both formula 1 & 2 are most stable at 33% RH.

Also this study has proved the disintegration times of capsule formula 1 and 2 at 33% RH just after formulation and after 19 days were more or less same. However Formula 1 capsules were more stable than formula 2. Because the difference between disintegration times after 19 days and just after formulation was much less in formula 1 than of formula 2. Altogether these results revealed that the formula 1 of polyphenol solid dosage form developed in the study can be used as a food supplement to improve lifestyle instead of crude green tea extract.

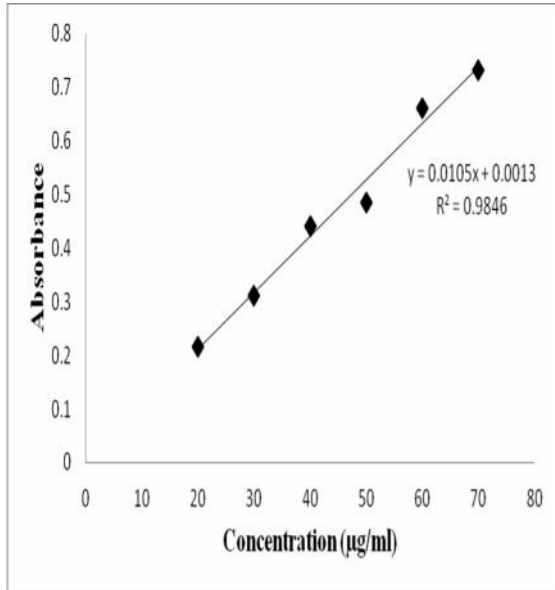


Figure 1A

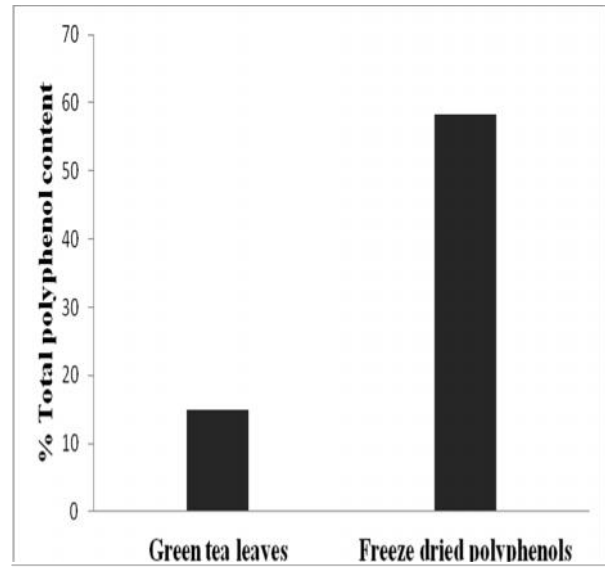


Figure 1B

Figure 1A
Variation of the absorbance with Gallic acid concentration
Figure 1B
% polyphenol content in freeze dried polyphenol powder and dried tea leaves

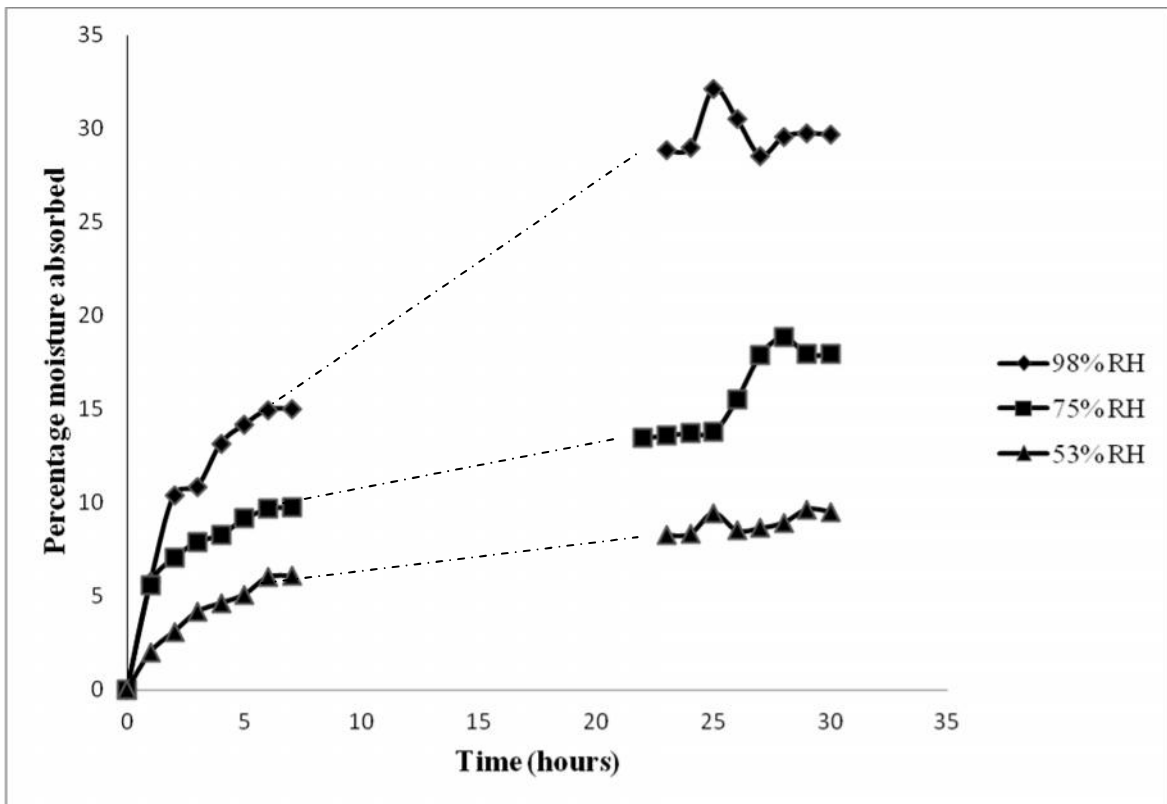


Figure 2
Variation of percentage moisture absorbed with time

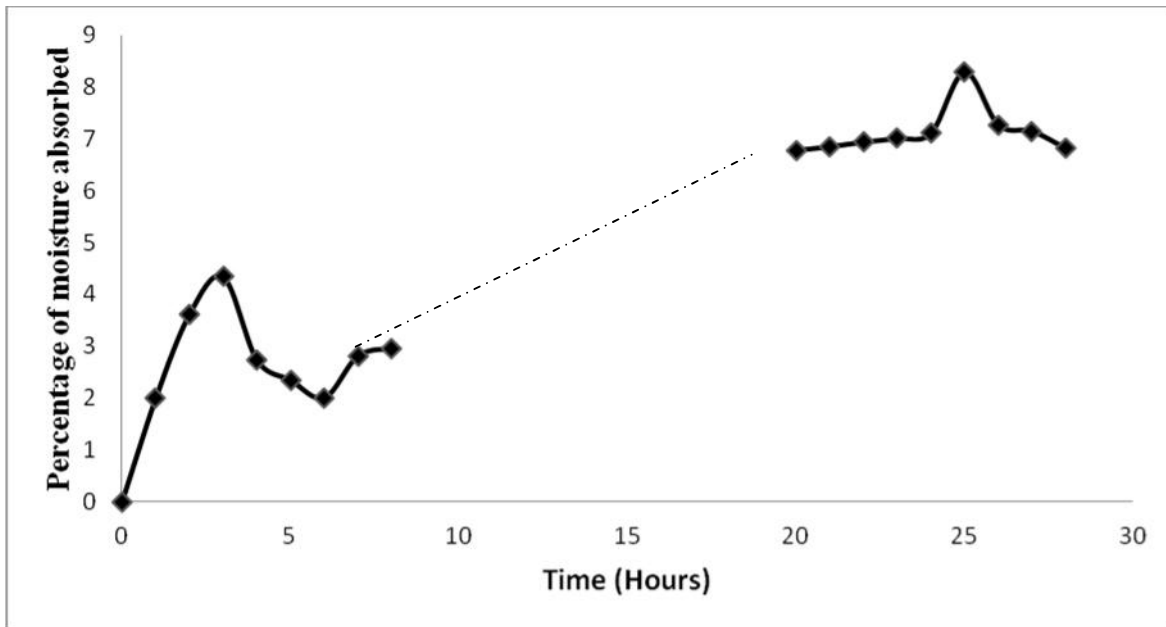


Figure 3
Variation of the relative humidity with room temperature

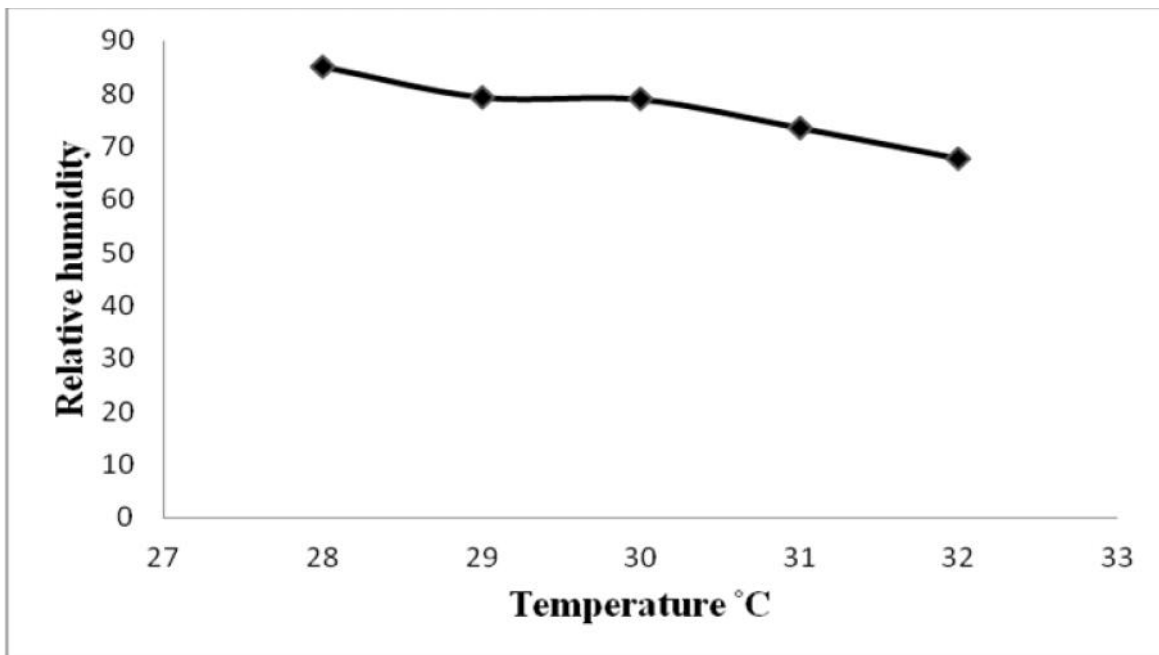


Figure 4
Variation of the relative humidity with room temperature

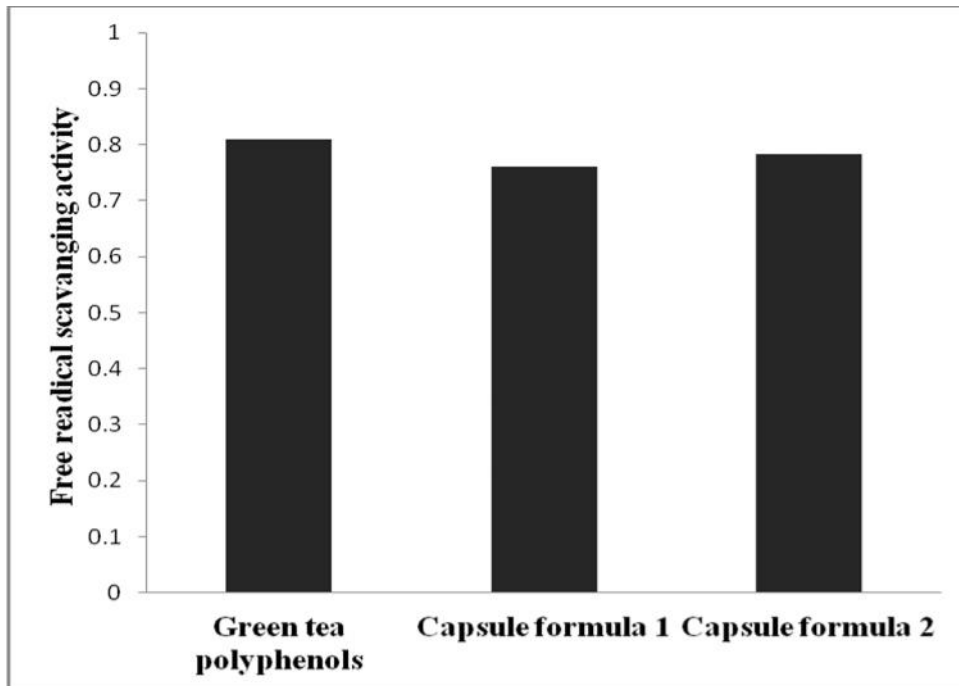


Figure 5

Column chart which shows antioxidant activities of capsule formulations and extracted polyphenols

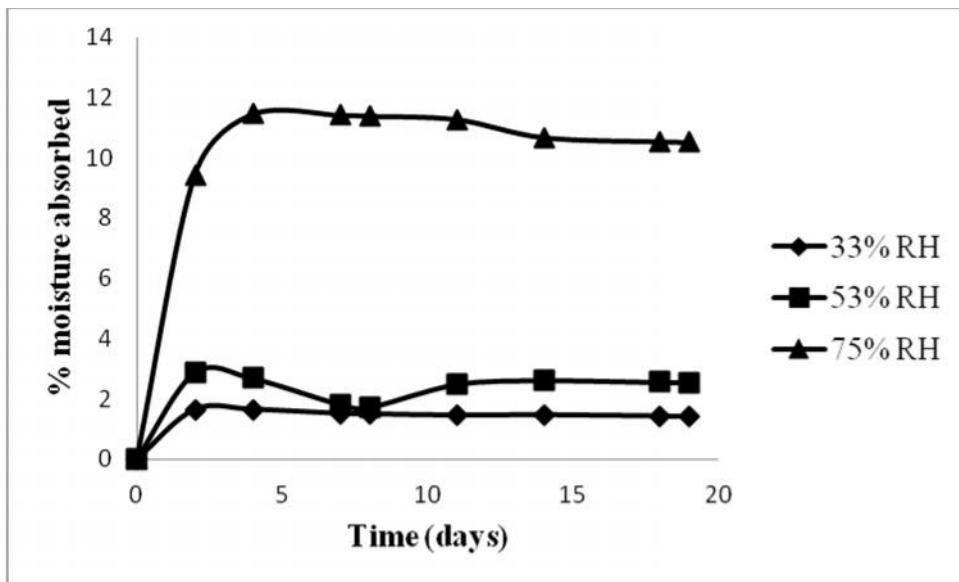


Figure 6

Variation of percentage of moisture absorbed with time for formula 1 in different relative humidity environments

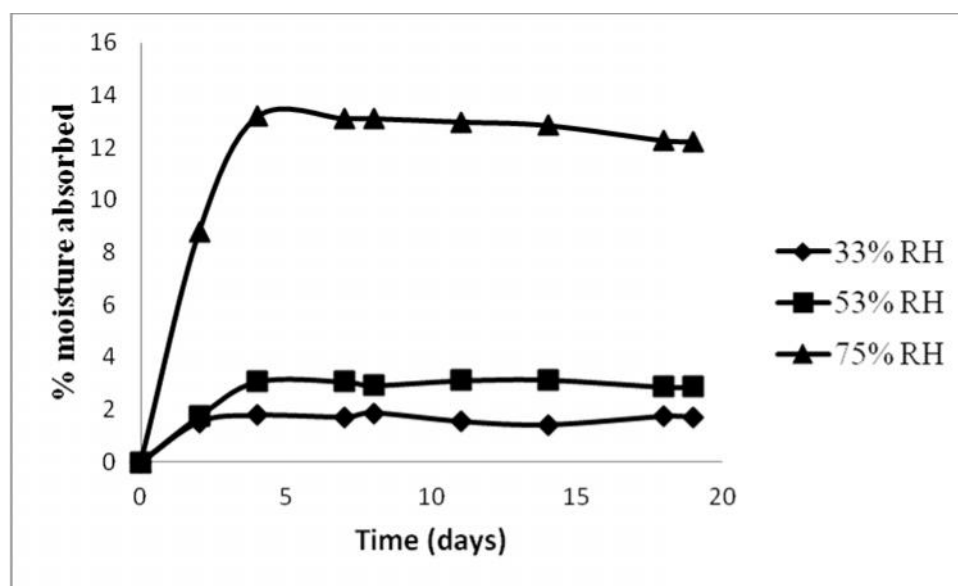


Figure 7

Variation of percentage of moisture absorbed with time for formula 2 in different relative humidity environments

CONCLUSION

The bitter taste and brown color of the freeze dried polyphenol powder result in poor acceptance of dosage forms mainly as tablets. To overcome this problem extracted freeze dried polyphenol powder was successfully incorporated in to a capsule. Formulation of hard gelatin capsules was done at low relative humidity at 25°C to minimize the effect of hygroscopicity. It should be stored in well closed containers under low humidity (at 33% RH) to minimize moisture absorption and deterioration.

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