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PHYTOCHEMICAL EVALUATION OF HYDROXY CITRIC ACID, CATECHINS AND CALCIUM PANTOTHENATE PRESENT IN HERBAL FORMULATION

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ABSTRACT

The analytical method for the estimation of phytochemical active ingredients present in Garginia combogia extract, Green tea extract with calcium pantothenate for herbal formulations are evaluated for it's Assay content and dissolution release. This herbal formulation with slim formula consists of several active ingredients such as Hydroxy Citric acid, catechins and calcium pantothenate. The HPLC method for the estimation of active content and the in-vitro dissolution release is developed for the study.

INTRODUCTION: The active ingredients present in *Garcinia combogia* and green tea extracts in herbal formulations contains hydroxy citric acid and catechins respectively which is act as an appetizer and antioxidant.

- ***Garcinia combogia* Extract**: The Garcinia combogia is extracted from *Garcinia combogia* fruits. It is a pale brown colored powder, odorless or slightly smoky odor. It is soluble in dilute mineral acids like Hydrochloric acid, sulphuric acid, partially soluble in water, slightly soluble in hot water and insoluble in organic solvents.

- **Green Tea Extract**: The Green tea is extracted from *Camella sinensis* leaves. It is brown colored powder having characteristic odor which soluble in dilute mineral acids, organic solvents and practically insoluble in water. The alkaloids contents of green tea are as follows Catechins, Caffeine, triterpenes, Saponins, flavonoids, poly phenols and caffeic acid derivatives (**fig. 1-5**).

Catechins are classified as follows;

1. Catechin
2. Epi catechin
3. Gallocatechin
4. Gallo catechin gallate
5. Catechin gallate.

These catechins act as anti oxidants.

Calcium Pantothenate: It is a white powder slightly hygroscopic in nature. It is freely soluble in water, slightly soluble in alcohol and insoluble in ether. It is a component of Vitamin B and it contains 98% to 101% of bis [3-(2R)-2, 4-dihydroxy-3, 3-dimethylbutanoyl] amino propanoate]

Structure:

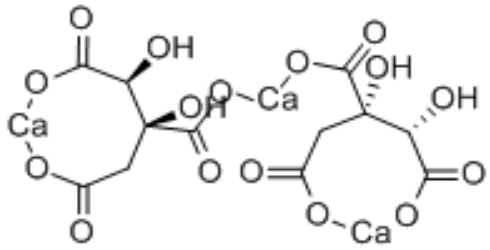

FIG. 1: CALCIUM SALT OF HYDROXY CITRIC ACID [1]

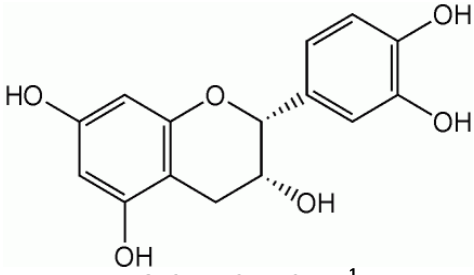

FIG. 2: EPICATECHIN [1]

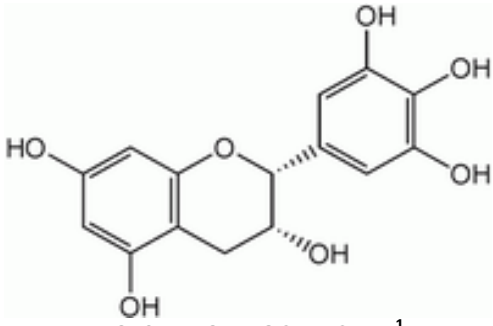

FIG. 3: EPIGALLOCATECHIN [1]

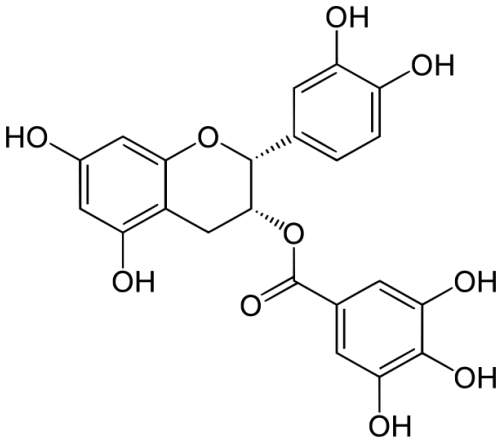

FIG. 4: EPICATECHIN GALLATE [1]

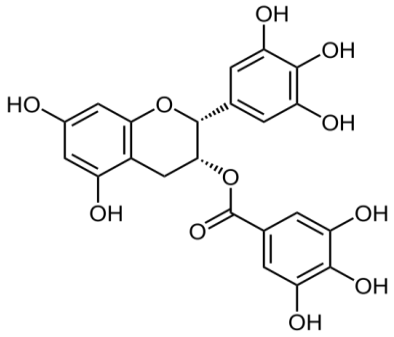

FIG. 5: EPI GALLO CATECHIN GALLATE [1]

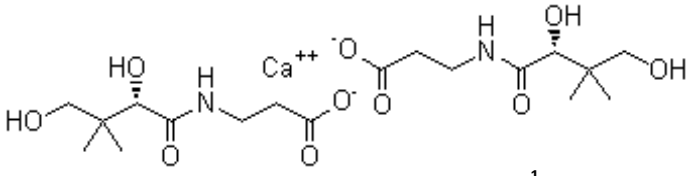

FIG. 6: CALCIUM PANTOTHENATE [1]

The High Performance Liquid Chromatography method has developed for this herbal combination by suitable analytical procedures to separate all the active ingredients present in the formulation.

MATERIALS AND METHODS:

Estimation of content of hydroxy citric acid by HPLC method [3]:

I. **Chromatographic Conditions:**

- HPLC system : Agilent 1100 series
- Mobile phase : Filtered and degassed mixture of 0.1% orthophosphoric acid in water
- Flow rate : 0.5 ml/minute
- Column Temperature : 25°C
- Wavelength : 210nm
- Injection volume : 20µl
- Chromatographic column: 250 X 4.6 mm, C_{18}, 5µm (Rp18e is suitable)
- Run Time : 30.0 min

II. Method of analysis

A) **Reference Standard Preparation** [4]: Transfer an accurately weighed quantity of hydroxy citric acid reference standard or *Garninia combogia* working standard about 50 mg in to 50 ml volumetric flask. Dissolve the substance by adding 2 ml of 3M hydrochloric acid and dilute the volume to the mark with water. Filter the solution through 0.45 µm membrane filter.

B) **Test Solution Preparation** [4]**:** Transfer an accurately weighed quantity of sample equivalent to 50 mg hydroxy citric acid into a 50 ml volumetric flask and add 2ml of 3M hydrochloric acid, sonicate the flask for 10 minutes with intermittent shaking to dissolve the contents. Then, add 30 ml of water further sonicate for 5 minutes and make up the volume with water. Filter the solution through 0.45 µm membrane filter.

C) **Procedure** [4]: Inject 20µl each of blank, reference standard preparation and test preparation into the chromatograph. From the peak area responses of the major peaks calculate the content of active ingredients present in the sample.

a. **System Suitability** [5, 6, 7]: The relative standard deviation for five replicate injections of standard is not more than 2.0%and the asymmetry as tailing factor the peak is not more than 2.0.The column efficiency as theoretical plates is not less than 2000

b. **Calculation for Content Estimation:**

$$\frac{AT \times WS \times 50 \times P \times AV}{AS \times 50 \times WT \times 100}$$

Where 'AT is the average peak area response of test preparation; 'AS' is the average peak area response of reference preparation; 'WS' is the weight of reference standard taken for standard preparation; 'WT' is the weight of sample taken for test preparation; 'P' is the percent potency of reference standard and 'AV' is the average weight of tablets or average fill weight of capsules.

TABLE 1: SYSTEM SUITABILITY PARAMETERS FOR STANDARD PREPARATION [5, 6, 7]

Name of the Peak	Retention Time	Relative retention time	Average area of Standard
Hydroxy citric acid	8.60 min	--------	1807.8 mAu
Gallocatechin	4.23 min	0.78	
Catechin	5.38 min	1.00	
Epicatechin	11.52min	2.14	16325.9 mAu
Gallocatechin Gallate	21.73 min	4.04	
Catechin Gallate	47.52min	8.83	
Calcium pantothenate	5.50 min	----------	2955.6 mAu

Estimation of Dissolution Release of hydroxy citric acid by HPLC Method [3]:

I. **Chromatographic Conditions** [9, 10]**:**

- HPLC system : Agilent 1100 series
- Mobile phase : Filtered and degassed mixture of 0.1% orthophosphoric acid in water
- Flow rate : 0.5 ml/minute
- Column Temperature : 25°C
- Wavelength : 210nm
- Injection volume : 20µl
- Chromatographic column : 250 X 4.6 mm, C18, 5µm (Rp18e is suitable)
- Run Time : 30.0 min.

II. **Dissolution Parameters**:

- Apparatus : Dissolution Tester (Dista TDT)
- Type : Paddle
- Dissolution Medium: 0.1M Hydrochloric acid
- Volume : 900 ml
- RPM : 100 rpm
- Temperature : 37°C
- Test time : 1 hr

III. **Method of Analysis:**

A) **Reference Standard Preparation** [4]: Transfer an accurately weighed quantity of hydroxy citric acid reference standard or *Garninia combogia* working standard about 50 mg in to 50 ml volumetric flask. Dissolve the substance by adding 0.1M hydrochloric acid and dilute the volume to the mark with 0.1M hydrochloric acid. Filter the solution through 0.45 µm membrane filter.

B) **Test Solution Preparation** [4]: Set the dissolution apparatus to attain the condition as per the dissolution parameters mentioned above. After reaching bowl temperature 37±2°C place one tablet or capsule in each bowl to the six dissolution bowls and run the apparatus for specified time. Withdraw 10 to 20 ml of the medium in the middle between paddle and top of the medium level. Filter the solution through 0.45 µm membrane filter. Use this filtrate for checking the percentage release.

C) **Procedure** [4]: Inject 20µl each of blank, reference standard preparation and test preparation into the chromatograph. From the peak area responses of the major peaks calculate the percentage release of active ingredients present in the sample.

a. **System Suitability** [5, 6, 7]: The relative standard deviation for five replicate injections of standard is not more than 2.0% and the asymmetry as tailing factor for the peak is not more than 2.0. The column efficiency as theoretical plates is not less than 2000.

b. **Calculation for Percentage Release** [4, 11]:

$$\frac{AT \times WS \times 900 \times P \times 100 \times 100}{AS \times 50 \times 1 \times 100 \times LC}$$

Where 'AT is the individual peak area response of test preparation; 'AS' is the average peak area response of reference preparation; 'WS' is the weight of reference standard taken for standard preparation; 'P' is the percent potency of reference standard and 'LC' is the Label claim of tablet or capsule.

Estimation of content of Catechins by HPLC method [3]:

I. **Chromatographic Conditions** [9, 10]:

- HPLC system : Agilent 1100 series
- Mobile phase : Filtered and degassed mixture of 0.05% Sulphuric acid, acetonitrile and methanol in the ratio of (840:140:20)
- Flow rate : 1.0 ml/minute
- Column Temperature : 25°C
- Wavelength : 280nm
- Injection volume : 20µl
- Chromatographic column: 250 X 4.6 mm, C_{18}, 5µm (Rp18e is suitable)
- Run Time : 60.0 min.

II. Method of Analysis:

A. **Reference Standard Preparation** [4]**:** Transfer an accurately weighed quantity of green tea extract working standard or catechins reference standard about 50 mg in to 50 ml volumetric flask. Dissolve the substance by adding 1 ml of acetonitrile and dilute the volume to the mark with mobile phase. Filter the solution through 0.45 µm membrane filter.

B. **Test Solution Preparation** [4]: Transfer an accurately weighed quantity of sample equivalent to 50 mg of catechins into a 50 ml volumetric flask and add 1ml of acetonitrile, sonicate the flask for 10 minutes with intermittent shaking to dissolve the contents. Then add 30 ml of mobile phase further sonicate for 5 minutes and make up the volume with mobile phase. Filter the solution through 0.45 µm membrane filter.

III. **Procedure**: Inject 20µl each of blank preparation, five replicate injections of reference standard preparation and two replicate injections of test preparation into the chromatograph. From the peak area responses of the major peaks calculate the content of Gallo catechin, catechin, Epicatechin, Gallo catechins gallate and catechins gallate present in the sample.

a. **System Suitability** [5, 6, 7]: The relative standard deviation for five replicate injections of standard is not more than 2.0% and the asymmetry as tailing factor the peak is not more than 2.0. The column efficiency as theoretical plates is not less than 2000 and the resolution between closely eluting peak is not less than 2.0.

b. **Calculation for Content Estimation**:

$$\frac{AT \times WS \times 50 \times P \times AV}{AS \times 50 \times WT \times 100}$$

Where 'AT is the average peak area response of test preparation; 'AS' is the average peak area response of reference preparation; 'WS' is the weight of reference

standard taken for standard preparation; 'WT' is the weight of sample taken for test preparation; 'P' is the percent potency of reference standard and 'AV' is the average weight of tablets or average fill weight of capsules.

Estimation of Dissolution Release of Catechins by HPLC method [3]:

I. **Chromatographic Conditions** [9, 10]:

- HPLC system : Agilent 1100 series
- Mobile phase : Filtered and degassed mixture of 0.05% Sulphuric acid, acetonitrile and methanol in the ratio of (840:140:20)
- Flow rate : 1.0 ml/minute
- Column Temperature : 25°C
- Wavelength : 280nm
- Injection volume : 20µl
- Chromatographic column: 250 X 4.6 mm, C_{18}, 5µm (Rp18e is suitable)
- Run Time : 60.0 min.

II. **Dissolution Parameters:**

- Apparatus : Dissolution Tester (Dista TDT)
- Type : Paddle
- Dissolution Medium: 0.1M Hydrochloric acid
- Volume : 900 ml
- RPM : 100rpm
- Temperature : 37°C
- Test time : 1 hr.

III. **Method of Analysis [4]:**

A. **Reference Standard Preparation** [4]: Transfer an accurately weighed quantity of green tea extract working standard or catechins reference standard about 50 mg in to 50 ml volumetric flask. Dissolve the substance by adding 0.1M hydrochloric acid and dilute the volume to the mark with 0.1M hydrochloric acid. Filter the solution through 0.45 µm membrane filter.

B. **Test Solution Preparation** [4]: Set the dissolution apparatus to attain the condition as per the dissolution parameters mentioned above. After reaching bowl temperature 37±2°C place one tablet or capsule in each bowl to the six dissolution bowls and run the apparatus for specified time. Withdraw 10 ml of the medium in the middle between paddle and top of the medium level. Filter the solution through 0.45 µm membrane filter. Transfer 10 ml of the solution into 25 ml volumetric flask and the volume make up with dissolution medium Use this filtrate for checking the percentage release.

IV. **Procedure** [4]: Inject 20µl each of blank, reference standard preparation and test preparation into the chromatograph. From the peak area responses of the major peaks calculate the percentage release of catechins present in the sample.

a) **System Suitability** [5, 6, 7]: The relative standard deviation for five replicate injections of standard is not more than 2.0% and the asymmetry as tailing factor for the peak is not more than 2.0. The column efficiency as theoretical plates is not less than 2000 and the resolution between two closely eluting peaks is not less than 2.0.

b) **Calculation for Percentage Release** [4, 11]:

$$\frac{AT \times WS \times 900 \times 25 \times P \times 100 \times 100}{AS \times 50 \times 1 \times 10 \times 100 \times LC}$$

Where 'AT is the individual peak area response of test preparation; 'AS' is the average peak area response of reference preparation; 'WS' is the weight of reference standard taken for standard preparation; 'P' is the percent potency of reference standard and 'LC' is the Label claim of tablet or capsule.

Estimation of content of Calcium Pantothenate by HPLC method [3]:

I. Chromatographic conditions [9, 10]:

- HPLC system : Agilent 1100 series
- Mobile phase : Filtered and degassed mixture of Acetonitrile, water, sodiumperchlorate and orthophosphoric acid in the ratio of (85:915:1:1)
- Flow rate : 1.5 ml/minute
- Column Temperature : 25°C
- Wavelength : 200nm
- Injection volume : 50µl

- Chromatographic column: 250 X 4.6 mm, C_8, 5µm (supelco discovery C_8 is suitable)
- Run Time : 30.0 min.
- Diluent : Filtered and degassed mixture 250 ml of water, 40 ml of acetonitrile and 5 ml of glacial acetic acid.

II. Method of analysis:

a) **Reference Standard Preparation** [4]: Transfer an accurately weighed quantity of working standard or reference standard about 25 mg in to 50 ml volumetric flask. Dissolve the substance by adding 30 ml of diluent and dilute the volume to the mark with diluent. Filter the solution through 0.45 µm membrane filter. Dilute 5 ml of the above solution to 50 ml with diluents.

b) **Test Solution Preparation** [4]: Transfer an accurately weighed quantity of sample equivalent to 25 mg of calcium pantothenate into a 50 ml volumetric flask and add 30ml of diluent, sonicate the flask for 10 minutes with intermittent shaking to dissolve the contents and make up the volume with diluent. Filter the solution through 0.45 µm membrane filter. Dilute 5 ml of the above solution to 50 ml with diluent.

III. Procedure [4]: Inject 50µl each of blank preparation, five replicate injections of reference standard preparation and two replicate injections of test preparation into the chromatograph. From the peak area response of the major peak calculate the content of calcium pantothenate present in the sample.

a. **System Suitability** [5, 6, 7]: The relative standard deviation for five replicate injections of standard is not more than 2.0% and the asymmetry as tailing factor the peak is not more than 2.0. The column efficiency as theoretical plates is not less than 2000.

b. **Calculation for Content Estimation**: [4,11]

$$\frac{AT \times WS \times 5 \times 50 \times 50 \times P \times AV}{AS \times 50 \times 50 \times WT \times 5X \times 100}$$

Where 'AT is the average peak area response of test preparation; 'AS' is the average peak area response of reference preparation; 'WS' is the weight of reference standard taken for standard preparation; 'WT' is the weight of sample taken for test preparation; 'P' is the percent potency of reference standard and 'AV' is the average weight of tablets or average fill weight of capsules.

Estimation of Dissolution Release of Catechins by HPLC Method [3]:

I. Chromatographic Conditions [9, 10]:

- HPLC system : Agilent 1100 series
- Mobile phase : Filtered and degassed mixture of Acetonitrile, water, sodiumperchlorate and orthophosphoric acid in the ratio of (85:915:1:1)
- Flow rate : 1.5 ml/minute
- Column Temperature : 25°C
- Wavelength : 200nm
- Injection volume : 50µl
- Chromatographic column: 250 X 4.6 mm, C_8, 5µm (supelco discovery C_8 is suitable)
- Run Time : 30.0 min.
- Diluent : Filtered and degassed mixture 250 ml of water, 40 ml of acetonitrile and 5 ml of glacial acetic acid.

II. **Dissolution Parameters**:

- Apparatus : Dissolution Teste (Dista TDT)
- Type : Paddle
- Dissolution Medium: 0.1M Hydrochloric acid
- Volume : 900 ml
- RPM : 100rpm
- Temperature : 37°C
- Test time : 45 minutes

III. **Method of Analysis**:

A. **Reference Standard Preparation** [4]: Transfer an accurately weighed quantity of Calcium pantothenate working standard or reference standard about 30 mg in to 100 ml volumetric flask. Dissolve the substance by adding diluent and dilute the volume to the mark with diluent. Filter the solution through 0.45 µm membrane filter. Dilute 5 ml of the above solution to 100 ml with 0.1M hydrochloric acid.

B. **Test Solution Preparation**: Set the dissolution apparatus to attain the condition as per the dissolution parameters mentioned above. After reaching bowl temperature 37±2°C place one tablet or capsule in each bowl to the six dissolution bowls and run the apparatus for specified time. Withdraw 10 ml of the medium in the middle between paddle and top of the medium level. Filter the solution through 0.45 µm membrane filter. Use this filtrate for checking the percentage release.

IV. **Procedure** [4]: Inject 20µl each of blank, reference standard preparation and test preparation into the chromatograph. From the peak area response of the major peak calculate the percentage release of calcium pantothenate present in the sample.

a. **System Suitability** [5, 6, 7]: The relative standard deviation for five replicate injections of standard is not more than 2.0% and the asymmetry as tailing factor for the peak is not more than 2.0. The column efficiency as theoretical plates is not less than 2000 and the resolution between two closely eluting peaks is not less than 2.0.

b. **Calculation for Percentage Release** [4, 11]:

$$\frac{AT \times WS \times 900 \times P \times 100 \times 100}{AS \times 100 \times 1 \times 100 \times LC}$$

Where 'AT is the individual peak area response of test preparation; 'AS' is the average peak area response of reference preparation; 'WS' is the weight of reference standard taken for standard preparation; 'P' is the percent potency of reference standard and 'LC' is the Label claim of tablet or capsule.

RESULTS AND DISCUSSIONS: The content estimation and the percentage release for hydroxyl citric acid, catechins and Calcium pantothenate are as follows.

i) Hydroxy citric acid : Assay : 142.7 mg/Capsules Dissolution: 92.6% to 99.4%

ii) Catechins (Total) : Assay : 107.6 mg/capsules. Dissolution: 75.6% to 88.5%

iii) Calcium pantothenate : Assay : 14.4 mg/Capsules Dissolution : 90.1% to 99.2%

TABLE 2: FINAL RESULTS OBTAINED FOR ASSAY AND DISSSOLUTION [4, 11]

Name of the Active	Assay in mg	Dissolution release in %
Hydroxy citric acid	142.7 mg	92.6%to 99.1%
Total Catechins	107.6 mg	75.6%to 88.5%
Calcium pantothenate	14.4 mg	90.1%to 99.2%

The amount of drug estimated in the herbal formulation is similar to the one which added for making the formulation. The limits for assay value is 90% to 110% of label claim and the limits for Dissolution release is not less than 70% of the active dissolved within specified period of time. In this study by the evaluation for actives present in herbal formulation the results obtained by HPLC methods and the amount added for formulating the drug product are almost similar.

CONCLUSION: Any herbal formulation contains plant extracts, minerals and excipients and hence it is very difficult estimate the trace content of active ingredients present in that. The study has used to evaluate the content of Hydroxy citric acid present in *Garcinia combogia*, Catechins present in green tea and the added vitamins like calcium pantothenate which is widely used in the ultra slim capsules formula to reduce the body weight. The HPLC method developed for this estimation of active ingredients and *in-vitro* dissolution release is very much useful in Pharmaceutical analysis.

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