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EXTRACTION, MODELING AND PURIFICATION OF CATECHINS FROM HIBISCUS SABDARIFFA

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ABSTRACT

Hibiscus sabdariffa commonly called as Roselle belongs to Malvaceae family. It has many pharmacological activities because of catechins and other biological components. The present work is aimed to study the group of phenolic compounds called catechins from Hibiscus sabdariffa L.Catechins are a group of polyphenolic compounds classified as "flavanols" which comes under flavonoids. The objective of present work is to develop a modelling equation and purification of catechins by Soxhlet extraction and Column chromatography. The present studies were targeted on Soxhlet extraction and the purification of catechins by column chromatography. The final form of the proposed model equation is $E(t)= -0.077t^2 + 12.98t - 24.75$ *with* $R^2= 0.954$ *where* E_s*= yield extract (mg/L of catechins) and t= extraction time (min).and the highest concentration has shown as 517mg/l. And the highest Catechins concentration was observed after purification from column chromatography is 535.37mg/l.*

Keywords: Hibiscus Sabdariffa, Catechins, Soxhlet Extraction, Column Chromatography, Solvent Extraction, Modelling Equation.

I. INTRODUCTION

The use of plants as medicines dates as far back as the origin of human kind. Even carnivorous animals are known to consume plants when ill. In this present research *Hibiscus sabdariffa*is used which iscommonly called as Roselle. It is an annual herbaceous shrub i.e mainly cultivated in warm countries like India, Indonesia, Philippines, Malaysia, Tropical Africa and also in Brazil, Australia, Hawaii and Florida. Roselle is used as a traditional medicine. Roselle is considered to have antihypertensive activity, has shown in-vitro antimicrobial activity against E.coli bacteria. Few extracts of the *H.sabdariffa* exhibit activities against atherosclerosis, liver disease, cancer, diabetes and other metabolic syndromes[1].The roleof herbal medicines in traditional healing, the pharmacological treatment of disease began long ago with the use of herbs[2].Leaves,calyx,stems,seeds,roots,all parts of Roselle have lot of significance and are used for different purpose in industrial and pharmacological sectors[3].

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II. MATERIALS AND METHODS

2.1 Collection and Processing

The seeds of *Hibiscus sabdariffa* were collected from local market, Rajahmundry, Andhra Pradesh, India. Some seeds (Fig-1) were dried under sunlight for 10-16 hrs & some were roasted in a microwave oven for 3-4 min at 180^0C & powdered [4]. Powder was sieved with different particle sizes from 354-20µ & stored in air tight container.

Fig-1: Hibiscus sabdariffa seeds

Soxhlet extractor–An experimental and modelling study:

Extraction of Catechins from *Hibiscus sabdariffa* species and to develop a modelling equation to quantitatively describe in the extraction phenomena. Ethanol was found to be the best solvent for the extraction of Catechins from *Hibiscus sabdariffa* species.

Solvent extraction using Soxhlet Extractor:

Prior to the solvent extraction study, 200ml of 100% ethanol is poured in the still of the extractor. 4 grams of dry seed powder was placed in thimble and fix it to the condenser. Now the total apparatus were placed in the heater. Using the Soxhlet apparatus continuous extraction was done for $2^{1/2}$ hrs. The entire system was shown in fig-2.Readings were noted down for every half an hour by taking1ml of solvent extract taken in a test tube and 1.725ml of Buffer C is added and to this add 375µl of Ferric chloride reagent 510nm using UV spectrophotometer in order to note the exact time when maximum extraction was observed.

Modelling of extraction of Catechins[5] using Soxhlet extractor apparatus was studied in order to describe the catechins from the seeds of *Hibiscus sabdariffa* to the bulk of the solvent. The mass transfer coefficient is constant. The solvent in the extractor is perfectly mixed, while the transfer resistance in the liquid phase is negligible and the catechins concentration in the solvent depends only on time. The transfer of the catechins was a diffusion phenomenon and independent of time. By this hypothesis an equation can be developed.

$$E(t) = At^2 + Bt + C$$

Where A, B & C are constants, E_s= yield extract (mg/L of catechins) and t= extraction time (min).

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Fig-2: Soxhlet extractor

Purification of catechins by column chromatography:

Chemicals required:

- Buffer C (5% triethanolamine (v/v), 5% SDS (w/v) and pH adjusted to 9.4 with HCl),
- Ferric Chloride Reagent (0.01 N HCl, 10M $FeCl_3$),
- Ethanol, Methanol, Distilled Water, silica gel

Preparation of the extract:

Weight the amount of 85 micron particle size of *Hibiscus sabdariffa* seed powder (4g) was added with ethanol (100% v/v) in conical flask and the volume was made 200 ml. Set the pH at 4. By optimizing the conditions Soxhlet was carried out for about $2^{1/2}$hours. The optimized result obtained at 1hr run of Soxhlet operation and that extract solution was used as a sample in the Column chromatography.

In Column Chromatography, 200 micron particle size silica gel was used as stationary phase. A piece of cotton was inserted into the column towards outlet. Column was tightly fixed to the clamp.Pour the sea sand of 1cm bed in the column. Silica gel powder wasadded in the column up to 10cm length from the neck of the column[10]. Solvent methanol was run in the column until the bed was entirely wet. The column was gently tapped with hand or soft materials. After tapping gentle pressure was applied. Before loading the sample in the column, little silica gel was added to the sample. 20ml of sample was poured along the side walls of the column. Now sand was added on top of the sample. The entire system was shown in fig-3. After collecting the samples for every 5 minutes from the column, 1ml of sample was taken from each test tube and 1.725ml of Buffer C was added, and finally 375µl of Ferric chloride reagent was added. The absorbance of the reaction mixture was measured at 510 nm using UV-Visible Spectrophotometer for the determination of catechins.

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Fig-3: Column chromatography

III. RESULTS AND DISCUSSIONS

3.1 Soxhlet Extraction and Modelling

Soxhlet extractor was run for $2^{1/2}$hrs at 60-80^{0}C with 100 % ethanol as a solvent. From the obtained data it was observed that maximum extraction of catechin is 517mg/l at 1 hr. The results were shown in table with figure.

Table-1: Extraction time vs. extraction yield of Catechins by Soxhlet extraction

Time (min)	Concentration of Catechins (mg/l)
30	275.37
60	517
90	512
120	385.4
150	200.7

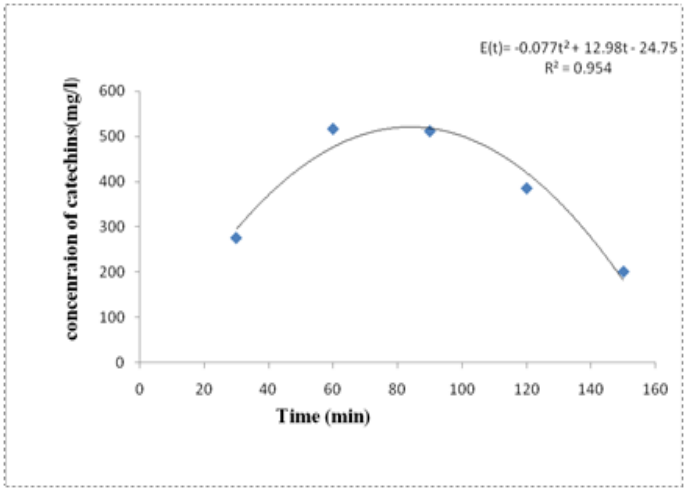

Fig-4: Effect of Extraction yield with Extraction time of catechins

Modelling of extraction of Catechins using Soxhlet extractor apparatus:

From the results obtained and the above graph to describe the catechins from the seeds of *Hibiscus sabdariffa* to the bulk of the solvent following hypothesis was used. The mass transfer coefficient is constant. The solvent in the extractor is perfectly mixed, while the transfer resistance in the liquid phase is negligible and the catechins concentration in the solvent depends only on time. The transfer of the catechins was a diffusion phenomenon and independent of time. The results satisfy the following polynomial equation:

The final form of the proposed model equation is

$$E(t) = -0.077t^2 + 12.98t - 24.75 \text{ with } R^2 = 0.954$$

E_s= yield extract (mg/L of catechins) and t= extraction time (min).

Purification of catechins by column chromatography:

From the Soxhlet extraction studies, with ethanol (100% v/v) as the best solvent for Catechins extraction, the extract with a concentration of 517mg/l catechins was subjected to column chromatography for purification. The highest Catechins concentration was observed after 10 min of purification as 535.37mg/l. The purity of Catechins was improved by Column chromatography. The results were shown in table and graphs.

Table-2: Extraction time vs Catechins concentrations by Column chromatography

S.NO	Time (min)	Catechins concentration(mg/l)
1	5	224.24
2	10	535.37
3	15	439.5
4	20	121.7
5	25	84.09
6	30	30.84

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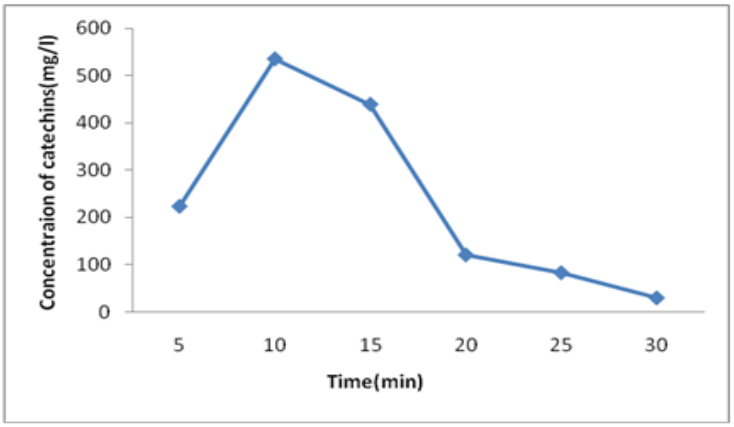

Fig-5: Effect of Extraction yield with extraction time for Catechins

IV. CONCLUSION

Hibiscus sabdariffa L is an annual herbaceous shrub that has many industrial, pharmaceutical uses in many countries all over the world. It has been used in traditional medicine. The work has aimed at the processing,extraction and quantitative determination of catechins in the herb Hibiscus sabdariffa and then the plant extracts were subjected to investigate and evaluate the invitro anti-oxidant effects. Soxhlet extraction was carried out for 3hr and an optimum concentration obtained at 1hr with a concentration of 517mg/l. And the observed values fitted the following modelling equation: $E(t)= -0.077t^2 + 12.98t - 24.75$ for Catechins where E_s= yield extract (mg/l of catechin) and t= extraction time (min). Column chromatography was also carried out for the purification of the Soxhlet extract and the concentration was improved to 535mg/l.

REFERENCES

[1]. A. Herrera-Arellanoa, S. Flores-Romerob, M.A. Chlavez-Sotoc, J. Tortorielloa. Effectiveness and tolerability of a standardized extract from Hibiscus sabdariffa in patients with mild to moderate hypertension: a controlled and randomized clinical trial, Phytomedicine, 2004, 11 (2004): 375–382.

[2]. Badreldin H. Ali, Naser Al Wabel and Gerald Blunden. Phytochemical, Pharmacological and Toxicological Aspects of Hibiscus sabdariffa L.: A Review, Phytother. Res. 2005, 19: 369–375.

[3]. DeependraSoni. Anshita Gupta, RashmiSolanki and Goutam Kumar Jana. Pharmacognostical, phytochemical and physiochemical findings over the root extract of Hibiscus rosasinesis, J. Nat. Prod. Plant Resour., 2011, 1 (4): 73-79.

[4]. Diane L. McKay, C-Y. Oliver Chen, Edward Saltzman and Jeffrey B. Blumberg, Hibiscus Sabdariffa L. Tea (Tisane) Lowers Blood Pressure in Prehypertensive and Mildly Hypertensive Adults, The Journal of Nutrition, 2009, 2(3): 298-303.

[5]. E. Betiku1 and T. F. Adepoju. Sorrel (Hibiscus sabdariffa) Seed Oil Extraction Optimization and Quality Characterization, American Chemical Science Journal 2013, 3(4): 449-458.

[6]. Enrico Prenesti, Silvia Berto, Pier G. Daniele and SimonaToso. Antioxidant power quantification of decoction and cold infusions of Hibiscus sabdariffa flowers, Food Chemistry, 2007, 100: 433–438.

ISSN: 2454-9665

[7]. Fatoumata Tounkara. Extraction, characterization, nutritional and functional properties of Roselle (Hibiscus sabdariffa Linn) seed proteins, Songklanakarin J. Sci. Technol. 2013, 35(2):159-166.

[8]. Geetha T., Garg A., Chopra K and Kaur I.P. Delineation of antimutagenic activity of catechin, epicatechin and green tea extract. Mutat. Res., 2004, 556: 65–74.

[9]. I. O. Omotuyi, A. Ologundudu, V. O. Onwubiko, M. D. Wogu and F. O. Obi, "Hibiscus sabdariffa Linn anthocyanins alter circulating reproductive hormones in rabbits(Oryctolaguscuniculus), Journal of Diabetes and Endocrinology, 2010, 1(3): 36-45.

[10]. Joseph J. Dalluge, Bryant C. Nelson, Jeanice Brown Thomas and Lane C. Sander. Selection of column and gradient elution system for the separation of catechins in green tea using high-performance liquid chromatography, Journal of Chromatography A, 1998, 793 (1998): 265–274.

[11]. M. Osman, K.K.Y. Wong and A. Fernyhough. The laccase/ABTS system oxidizes (+)-catechin to oligomeric products, Enzyme and Microbial Technology, 2007, 40 (2007) 1272–127.

[12]. Mahadevan, Shivali and Pradeepkamboj, Hibiscusssabdariffa.Linn- an overview, Natural product Radiance, 2009, 8(1): 77-83.

[13]. Obouayeba Abba Pacôme, DjyhNazaire Bernard, DiabateSékou, Djaman Allico Joseph, N'guessan Jean David, Kone Mongomaké and Kouakou Tanoh Hilaire. Phytochemical and Antioxidant Activity of Roselle (Hibiscus Sabdariffa L.) Petal Extracts, Research Journal of Pharmaceutical, Biological and Chemical Sciences 2014, 2(4): 1453-1465.

[14]. Olaleye and Mary Tolulope. Cytotoxicity and antibacterial activity of Methanolic extract of Hibiscus sabdariffa, Journal of Medicinal Plants Research, 2007, 1(1), 9-13.