



Optimization of Physico-Chemical Parameters for the Extraction of Catechins from seeds of Hibiscus sabdariffa species.

KEYWORDS

Hibiscus sabdariffa L, catechins, Flavanols, Roselle seeds.

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ABSTRACT *Hibiscus sabdariffa L, an annual herbaceous shrub commonly called as Roselle has many industrial, pharmaceutical uses in many countries all over the world. Phytochemical composition of Hibiscus sabdariffa includes alkaloids, anthocyanins, flavonoids, polyphenols, quinones, saponins, tannins, terpenes and sterols. The objectives of this work were extraction of group of phenolic compounds called catechins (Flavanols) from the seeds of Hibiscus sabdariffa. The present studies target on optimization of physico-chemical parameters like effects of different solvents, different solvent percentages, pH, particle size of the seed powder and the soaking time for the extraction of catechins from the seeds of Roselle.*

For the extraction of Catechins, the optimum results were observed for the effects of different solvents, different solvent percentages, pH, particle size of the seed powder and the soaking time as ethanol, 100%(v/v), pH 4.0, microns, and 2days respectively. The highest Catechin concentration for optimized conditions was 485mg/l.

Introduction:

Hibiscus sabdariffa is a herbaceous shrub that belongs to Malvaceae family. It has versatile applications. It is also used in Traditional medicine. Roselle is cultivated in warm countries and total tropical and subtropical species number is more than 300.^[1] Calyces are abundantly used for the production of beverages and leaves, stems, roots, seeds are also used for different purposes in food, pharmaceutical, industrial applications. The seeds of Roselle is a valuable food resource because of the high protein and calorie content (33% protein, 24% carbohydrate, 22% fat on dry weight basis) and also more amounts of fibre (14% dry weight as fibre) and also valuable micro-nutrients.^[2] The sorrel seed oil is rich in unsaturated fatty acids and is also rich of source of lipid soluble antioxidants, particularly γ -tocopherol.^[3] The presence of phytochemicals like flavonoids, steroids, phenolic compounds, tannins, alkaloids were also observed.^[4]

Catechins are a group of polyphenolic compounds classified as Flavanols which comes under flavonoids. Catechins are found in more concentrations in food sources like fresh tea leaves, red wine, broad beans, black grapes, cider, chocolates, apricots and strawberries.^[5] Flavanol group include (-)-epigallocatechin gallate (EGCG), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), (+)-gallocatechin gallate (GCG), (-)-epicatechin (EC), (+) gallocatechin(GC) and (+)-catechin (C).^[6] These catechins are reported to show antioxidant, anti cancer, anti cataract, anti inflammatory, anti arthritic, anti hypercholesteremic, anti proliferative activities.^[5]

MATERIAL AND METHODS:

Chemicals and reagents:

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, 10 mM FeCl₃),

Ethanol, Methanol, Distilled Water.

Collection of Plant material

The seeds of Hibiscus Sabdariffa collected from local market in Rajahmundry, Andhra Pradesh, India.

Processing of the Plant material

These seeds were dried, some seeds were roasted and powdered separately. The total powders done were sieved with different mesh sizes from 44 to 85. The different size powders were stored in the air tight small covers.

Extract preparation

Weigh the amount of 1g of powder and add water, ethanol(100%) and methanol(100%), in different flasks and makeup this solution up to 50 ml. Soak the solution for some period of times. After the soaking time, filter the solution by using Whatman No.1 filter paper. And the filtrate is checked for the presence of Catechins.

Determination of Catechins by UV-visible Spectrometer.

1ml of solvent extract taken in a test tube and 1.725ml of Buffer C is added and to this add 375 μ l of Ferric chloride reagent. The absorbance of the reaction mixture was measured at 510 nm using UV-Visible Spectrophotometer. Catechins were determined by using calibration curve.^[7]

Results and Discussion:

Effect of Different Solvents for Extraction of Catechins(for both dried and Roasted powder) :

Different organic solvents such as methanol, ethanol and water were used to extract the optimum yield of catechins from Hibiscus sabdariffa^[5] from both Dried and Roasted powders. The higher concentration of Catechin was observed with the solvent 'Ethanol' than methanol and water. And its concentration was 266.5mg/l for dried powder and 206.13mg/l for roasted powder. The results were shown in table 1 and fig (1):

The results showed the higher concentrations of catechins 266.5mg/l at 100% of the ethanol

S.no	Type of the solvent	Concentration (mg/l) dried	Concentration(mg/l) Roasted
1	Water	20.6	10
2	Methanol	183.5	130.84
3	Ethanol	266.5	206.13

Table 1:Type of solvents vs. Concentration of Catechins

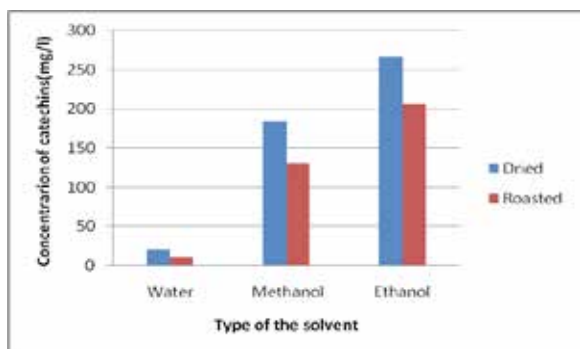


Fig 1: Effect of different solvents and type of powders in extraction of catechins.

The result explains the more concentration of catechins in 'dried powder' than roasted one observed in extraction with solvent 'ethanol'

2. Effect of Different Solvent percentages for the Extraction of Catechins

From the results of more concentration of catechins observed for the dried powder of Roselle seeds extracted with ethanol, various percentages of the ethanol (0,20%,40%,60%,80%,100%) for the extraction of catechins were checked and the results are as follows:

S.no	% of Ethanol	Concentration of catechins(mg/l)
1	0	20.66
2	20	7.83
3	40	4
4	60	5.3
5	80	13.86
6	100	266.5

Table 2: % of solvents vs. Concentration of Catechins

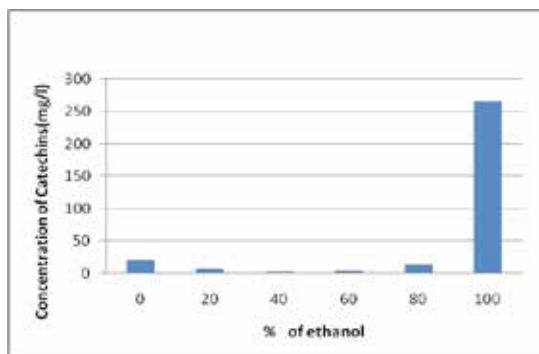


Fig 2: Effect of % of solvent in extraction of catechins

3. Effect of pH for the Extraction of Catechins

Different pHs were checked with the ethanol for the extraction of catechins from dried powder of Hibiscus sabdariffa. The different pHs were set as 3,4,5,6,8 and the initial pH observed for the solvent was 7. And the results are as follows:

S.no	pH	Concentration of catechins(mg/l)
1	3	209.5
2	4	291.6
3	5	263.67
4	6	225
5	7	243
6	8	219

Table 3: pH vs. Concentration of Catechins

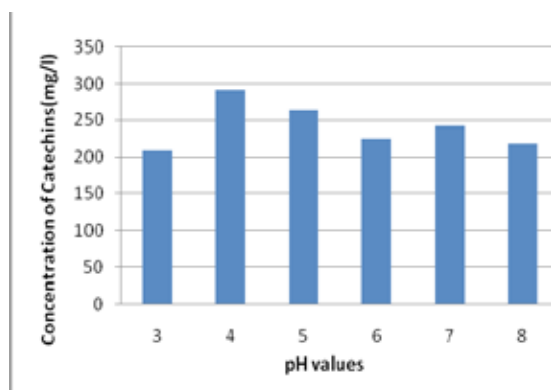


Fig 3: Effect of pH in extraction of catechins

The results showed more concentrations of Catechins 291.6mg/l at the pH value of 4.

4. Effect of particle size in extraction of catechins:

The powder sieved at different mesh sizes 44,52,60,85 resulted in different particle sizes 354

328,250, 205 respectively and the different particle sizes showed the results as follows:

S.no	Paricle size(microns)	Concentration of Catechins(mg/l)
1	354	264.24
2	328	285.37
3	250	325
4	205	372.16

Table 4: Particle size vs. Concentration of Catechins

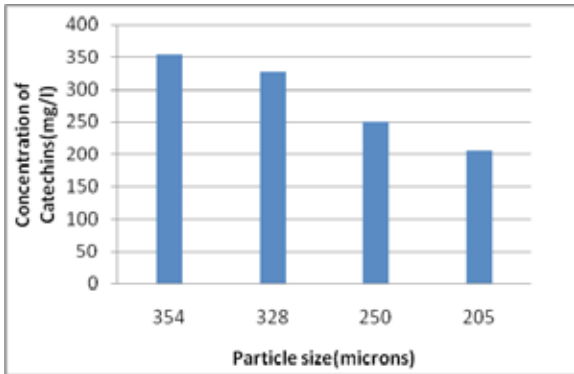


Fig 4: Effect of particle size in extraction of catechins
The results showed more concentration of catechins 372.16 mg/l with the particle size 205 microns.

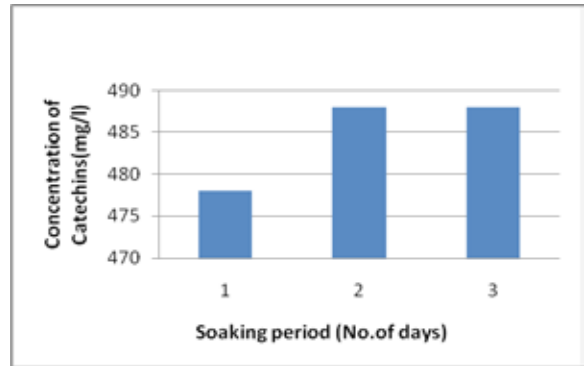


Fig 5: Effect of soaking period in extraction of catechins
The results showed more concentration of catechins 488mg/ml after 2nd day of soaking and the soaking more than 2 days showed the standard value up to 4 days.

5. Effect of Soaking time for the extraction of catechins:

The powder was soaked in the solvent for different time periods like 1day, 2days, 3days and observed the following result:

S.no	No. of Days	Concentration of catechins(mg/l)
1	1	478
2	2	488
3	3	488

Table 5: Time of Soaking vs. conc of catechins

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