Fermentation and Antioxidant Activity of Catechins From Hibiscus Sabdariffa

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Abstract- *Hibiscus sabdariffa belonging to Malvaceae family and commonly called as Roselle. It has antioxidant activities because of the presence Catechins and other biological components. Catechins are a group of polyphenolic compounds classified as "flavanols" which comes under flavonoids. The present work is aimed to perform the fermentative and antioxidant activity of Catechins which are extracted from Hibiscus sabdariffa L. The result of antioxidant activity was "IC50 of Hibiscus sabdariffa-1770.58µg/mL" and the result for fermentation was 42mg/l on second day.*

Keywords- Hibiscus sabdariffa, Catechins, Antioxidant activity, Fermentation.

I. INTRODUCTION

In this present research Hibiscus sabdariffa is used which is commonly 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[1]. Roselle is used as a traditional medicine. Roselle is considered to have fermentative and antioxidant activity and has shown in-vitro antimicrobial activity against E.coli bacteria [2]. Few extracts of the H.sabdariffa exhibit activities against atherosclerosis[3], liver disease, cancer, diabetes and other metabolic syndromes [4].The role of herbal medicines in traditional healing, the pharmacological treatment of disease began long ago with the use of herbs.Leaves, calyx, stems, seeds, roots [5], all parts of Roselle have lot of significance and are used for different purpose in industrial and pharmacological sector [6].

In the present work fermentation and antioxidant activity of Catechins from Hibiscus sabdariffa were performed.

II. MATERIALS AND METHODS

Chemicals:

Ethanol, Methanol, Distilled Water, DPPH, Ascorbic acid as a standard.

Microorganism: Bacillus subtilis

I. Extract preparation for fermentation of Catechins:

a) Non- fermented extract: One gram of dried seed powder (85 micron size) was mixed with 50 ml of distilled water and the mixture was soaked for 1hour [7]. Then the mixture was filtered and used as an aqueous extract. That extract is tested for the presence of Catechins by quantitative determination.

b) Fermented extract: One gram of dried seed powder (85 micron size) was mixed with 50 ml of distilled water.The mixture was sterilized at 121^0C temperature and 15lb pressure for 15min[8].

1ml of Bacillus subtilis is inoculated in each conical flask under laminar air flow conditions and kept in incubation under sterilized conditions as shown in fig 1. The fermented samples were taken at intervals of time (1day, 2days and 3days) and the samples were filtered with Whatman filter paper no.1 and the filtrate was tested for concentration of Catechins.

Fig1: Conical flasks containing Bacillus subtilis organism

II. Extract preparation for Anti-oxidant activity of Catechins:

0.1mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of Catechin extract in ethanol at different concentrations [9]. 30 min later, the absorbance was measured at 517 nm [10]. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity [11].

The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH Scavenged (%) = (A control – A test / A control) × 100

Where, "A control" is the absorbance of the control reaction and "A test/std" is the absorbance in the presence of the extracts. The antioxidant activity of the extracts was expressed as IC50. The IC50 value was defined as the concentration in µg/ml of extracts that inhibits the formation of DPPH radicals by 50%.

III. RESULTS AND DISCUSSIONS

I. Fermentation of Catechins:

Non- fermented Aqueous Extract: The concentration of Catechins in non fermented aqueous extract of seed powder is 20mg/l.

Fermented: The concentrations of catechins in fermented aqueous extract of seed powder in three days time period is observed and the fermented filtrate is extracted with 100% ethanol in 1:1 ratio and following values were observed as in Table 1 and Fig 2.

Table1 : Catechins concentrations of fermented extract and ethanolic extract of fermented sample.

S.no	Time period(days)	Concentration of Catechins of fermented solution(mg/l)	Concentration of Catechins of ethanolic extract of fermented filtrate (mg/l)
1	1	25	34
2	2	42	50
3	3	20	23

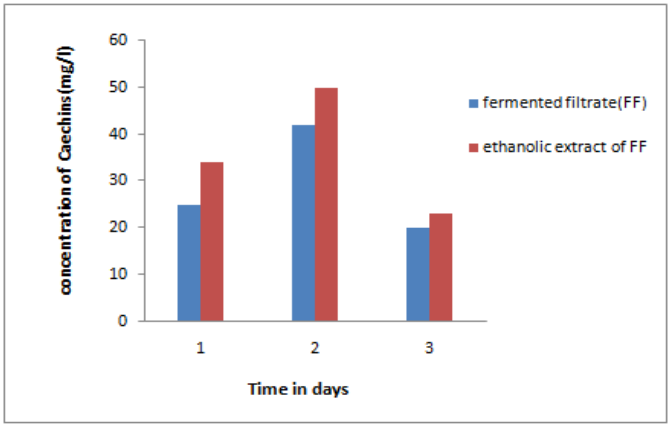

Fig 2: Catechins concentration in fermented sample and ethanolic extract of FF

The concentration of Catechins in aqueous extract is 20mg/l and from the fermentation process it had increased to 42mg/l on second day which refers the stationary phase of Bacillus subtilis, as the Catechins are the secondary metabolites this increase had occurred. And the ethanolic extract of fermented filtrate showed concentration increase from 42mg/l to 50mg/l.Fermented extracts showed better concentrations than the non fermented aqueous extract.

II. Anti oxidant activity of Catechins:

DPPH free radical scavenging activity:

The extracts are able to reduce the stable radical DPPH to the yellow coloured diphenylpicrylhydrazine. The present results are shown in Table 2 and Fig 3. It may be postulated that Hibiscus sabdariffa extract reduces the radicals to the corresponding hydrazine when it reacts with the hydrogen donor in the antioxidant principles

DPPH Assay of sample (Hibiscus sabdariffa seed extract):

Table 2: Sample Concentration vs inhibition of DPPH activity

% of Inhibition activity	
Conc. in µg /mL	*Hibiscus sabdariffa seed* extract
32.25	9.52
62.5	11.23
125	16.29
250	21.82
500	25.73
1000	31.28

IC_{50} value for *Hibiscus sabdariffa* extract - 1770.58 µg /mL.

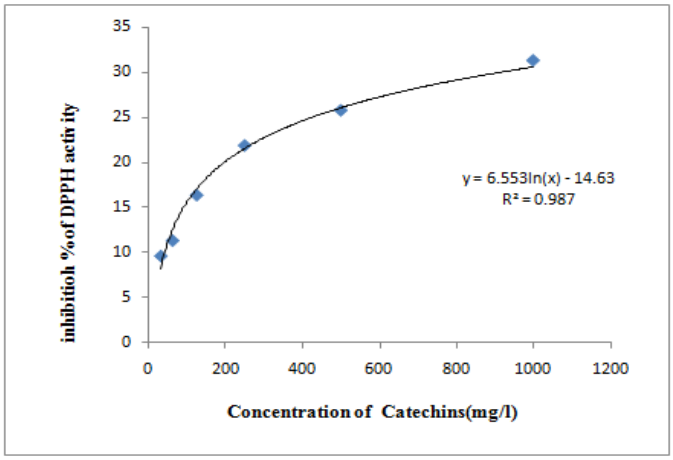

Fig 3: Effect of Concentration of Roselle seed extract on DPPH inhibition

IV. CONCLUSION

The concentration of Catechins in aqueous extract is 20mg/l and in the fermentation process the concentration is

42mg/l. And the ethanolic extraction of fermented filtrate resulted in increased concentration of 50mg/l. Fermented seed extracts showed more concentration than the non fermented aqueous seed extract.5.Anti oxidant activity of the extract from *Hibiscus sabdariffa observed as* IC_{50} of *Hibiscus sabdariffa* extract - 1770.58 µg /ml.

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