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Biotransformation of Flavonol Rut in to Quercetin from Citrus Medica Peel by Using Bacillus Cereus

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Abstract:

Quercetin and rutin are a kind of flavonoid drugs which belongs to group Flavonols. Quercetin which is produced mainly by direct extracting from Vegetables, fruits, citrus, red grapes, onions etc, or acid hydrolysing from rutin. In this experiment Bacillus cereus strain was used to transform rutin to Quercetin. Phytochemical analysis of Citrus medica peel shows that they are rich source of flavonoids. Initially Quercetin and rutin were extracted from the Citrus medica peel extract by using soxhlet extractor for 180min and methanol as solvent and later they were purified by using solvent-solvent extraction. After inoculation of Bacillus strain into the soxhlet extracted solution results in the transformation of rutin to quercetin. Before transformation the concentration of rutin and Quercetin were 18.72mg/l and 8.36mg/l and after biotransformation the concentration of quercetin was increased to 16.14mg/l. Purification of fermented extract with the n-hexane solvent by liquid-liquid extraction showed the concentration of quercetin increase from 16.14mg/l to 19.38mg/l. These findings support the literature, showing that bioconversion is a useful strategy for production of biological active metabolites.

Keywords: Quercetin; Rutin; Bacillus Cereus; Biotransformation

Introduction:

Rutaceae family includes many aromatic and medicinal plants, which are used in traditional medicine. Citrus medica L is commonly known as citron, grown in Assam, central India and Western Ghats of India. It is more commonly present in the Mediterranean region [1], central and southern parts of America. In ancient times, the citron was used mainly for medical purposes: to combat sea sickness, pulmonary troubles, intestinal ailments, and other disorders. The peel of *Citrus medica* fruit is a rich source of flavonoids [5] and many polymethoxylated flavones which are very rare in other plants. Quercetin and Rutin have many biological activities such as antioxidant, antimutagenic effect, analgesic, anti-inflammatory etc [9]. The major effective components of Citrus medica peel are flavonoids composed of large quantity of rutin (24.46%, w/w) and little amount of quercetin(1.41%,w/w) [2]. Both rutin and quercetin are medicines and have many physiological activities. They have similar activities in eliminating free-radicals, antioxidative activities and protective effect in the hypoxia/hypoglycemia model of bacteria precipitation, antioxidative activities in vitro and anti-lipid peroxidation, but quercetin act preponderant function than rutin[8]. At present, only little part of Citrus medica are used for rutin extraction, most of them are wasted.

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Rutin is often hydrolyzed by acid to obtain the more active quercetin in modern industry production, and large quantity of acidic waste water is produced[7]. Hence, it is necessary to develop a simple, economical, environment friendly and efficient method for quercetin production. Microbial transformation of organic compounds is currently an important research pursuit. Although this approach has several advantages over chemical transformation methods [10](e.g.milder reaction conditions, regio- and stereoselectivity, and absence of protection-deprotection requirements), the biotransformation as they are usually carried out have an important drawback, which is lack of control on the course of the reaction and the products formed. Also, competing degradative reactions lead to a somewhat lower yield [6]. This study aimed to find the transformation yields of quercetin from rutin by using *Bacillus cereus* bacterial strain and purified the product by using solvent-solvent extraction. If this procedure comes to reality in industry, it will reduce the wastage of Citrus medica peel and improves its additional value, but also can reduce the environmental pollution caused by the quercetin acid hydrolysis technology.

Materials and Methods:

Sample collection and Preparation:

The fruits were collected either from the fruits stalls, neighbours, relatives or friends and kept it in the refrigerator at 4^{o}C to maintain their freshness and later their peels were removed. Separated peels were dried under the shade up to 48 hours. By using the kitchen blender the dried plant material was grounded to a fine powder form and it placed in small plastic bags and stored at 4◦C until the use.

Microorganism: The microorganism used for the biotransformation of rutin to quercetin was *Bacillus cereus* MCC 2236. This strain was purchased from the Microbial culture collection of NCCS, Pune.

Extraction of flavonoids:

Flavonoids in the citrus medica peel were extracted by using soxhlet extractor. 5gm of peel powder was placed in thimble and fix it to the condenser. Prior to that, 250ml of 80% methanol was poured into the condensation flask of the extractor. Now the total apparatus were placed on the heating mantel. Using the soxhlet apparatus continuous extraction was done for 3 hrs. After extraction the flavonoids were purified by using liquid-liquid extraction using n-hexane as solvent. The concentration of rutin and quercetin in the extract were determined by using spectrophotometer.

Quantitative determination of flavonoids:

Estimation of Quercetin:

Aluminum chloride method was used for quercetin determination [3**]**. In this method Quercetin was determined by using a standard graph. For this purpose, the calibration curve of quercetin was prepared by taking 1ml of standard or extract solution of different concentrations (2, 4, 6, 8,10 µg/ml) into 10ml test tubes, containing 4ml of distilled water. 0.3ml of 5%$NaNO_2$ was added to the test tube. After 5min, 0.3ml 10%$AlCl_3$ was added to the mixture. At the 6th min add 2ml of 1M NaOH was added and volume made up to 10ml with distilled water. The absorbance was noted at 510nm using UV-Visible spectrophotometer.

Estimation of rutin:

Rutin concentration was determined by preparing a standard graph from working stock solution of rutin which is prepared by dissolving 50mg of rutin in 50ml of methanol (1000 µg/ml) [4]. 50mg quantity of *Citrus medica* powder was transferred to 50 mL volumetric flask and dissolved in methanol and final volume was made up with methanol. The sample solution was then filtered through Whatman filter paper

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No.1. From the above solution 0.373ml of solution was taken and diluted to 10 mL with methanol to get final concentration. The concentration of rutin was determined by taking absorbance value at 359nm using UV-Visible spectrophotometer.

Biotransformation:

Prepare Nutrient broth medium of (100ml), autoclaved and distributed the medium into four Erlenmeyer flasks each of capacity 250ml. 1ml of *Bacillus subtilis* was taken from the overnight grown culture and inoculated into three conical flasks under Laminar air flow hood and the 4th flask taken as control without inoculums. Incubate the cultures in the orbital shaker incubator at 32^{0}C temperature at 120rpm for 24hrs.When an evidence of growth was observed, transfer 50ml of sterile citron fruit peel methanol extract, which is produced by soxhlet extractor and also purified it by liquid-liquid extraction, into the above four conical flasks individually.

The fermented samples were taken out at a time intervals of (1day, 2days, 3days) and the samples were filtered with Whatman filter paper no.1 and the filtrate was centrifuged at 10,000rpm for 15 min at 4°C to sediment the bacterial cells and unwanted particles as pellet. Concentration of quercetin and rutin in the supernatant was determined by using UV-visible spectrophotometer.

Extraction: This supernatant solution was extracted with 100 % nhexane solvent in the ratio of 1:1. And further the concentration of Quercetin and rutin in the resulted extract was determined using calibration curve.

Results and discussion:

The concentration of rutin and quercetin in non-fermented methanolic extract of *Citrus medica* peel powder is 18.72 mg/l and 8.36 mg/l respectively.

The concentrations of rutin and quercetin in fermented methanolic extract of peel powder in three days' time period was observed and the fermented filtrate is extracted with 100% n-hexane in 1:1 ratio and following values were observed.

Table-1: Flavonoids concentrations of fermented sample and their concentration after extraction with n-hexane solvent.

S.no	Time period(days)	Concentration of flavonoids of fermented solution(mg/l)		Concentration of flavonoids after extraction with n-hexane (mg/l)	
		Rutin	Quercetin	Rutin	Quercetin
1	1	17.34	11.32	17.9	12.43
2	2	14.63	15.28	15.2	17.26
3	3	12.58	16.14	12.72	19.38

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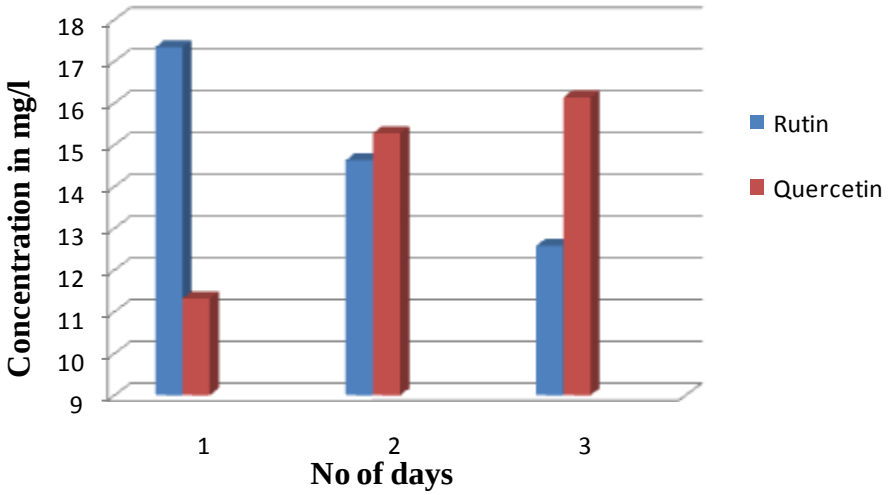

Figure-1: Concentration of quercetin and rutin in fermented sample.

The concentration of rutin and quercetin in methanolic extract is 18.72mg/l and 8.36 mg/l respectively in the control sample. After the fermentation process the quercetin concentration had increased to 16.14mg/l on third day which refers the stationary phase of *Bacillus cereus*, and the concentration of rutin had decreased to 12.58 mg/l. And the extraction with n-hexane of fermented filtrate showed concentration increase from 16.14mg/l to 19.38mg/l.

Conclusion:

In the present study, quercetin and their glycosidic derivatives were obtained fromfermentation of *Citrus medica* peel extract produced by soxhlet extraction, using *Bacillus cereus*. Furthermore, the fermentation using the various standard compounds revealed that rutin could be deglycosylated to form quercetin and quercetin-3- glucoside by *Bacillus cereus*. Meanwhile, rutin could also be biotransformed to kaempferol and kaempferol-3-glucoside. Based on the observations the rutin was converted into quercetin by *Bacillus cereus* and it was proposed that rutin was C-30 dehydroxylated to produce quercetin and quercetin-3-glucoside.

References:

[1] Beatriz AA, Luis RL. Pharmacological properties of *Citrus* and their ancient and medieval uses in the Mediterranean region, J.Ethnopharmacology,97,89.2005.

[2] Kalpesh Panara* Krutika Joshi and K. Nishteswar. A Review on Phytochemical and Pharmacological Properties of *Citrus medica Linn*. International Journal of Pharmaceutical & Biological Archives, 3(6):1292-1297, 2012.

[3] Rajeshwari Sahu and Jyoti Saxena, Screening of Total Phenolic and Flavonoid Content in Conventional and Non-Conventional Species of Curcuma, Journal of Pharmacognosy and Phytochemistry,. Vol. 2 No. 1,176-179, 2013.

[4] N P Pawar, V R Salunkhe, Development And Validation Of UV Spectrophotometric Method For Simultaneous Estimation Of Rutin And Gallic Acid In Hydroalcoholic Extract Of Triphala churna, International Journal of Pharm Tech Research, Vol.5, No.2, pp 724-729, 2013.

[5] M. N. I. Bhuiyan, J. Begum, Constituents of Peel and Leaf Essential Oils of *Citrus Medica* L. Journal of Scientific Research 1(2):387-92, 2009.

[6] Haluk,J.P.,and M. Metche. Microbiological transformation of quercetin by Aspergillus niger. Bull.Soc. Chim. Biol. 52:667-677, 1970.

[7] Krishnamurty, H. C., and F. J. Simpson. Degradationof rutin by Aspergillus flavus. J. Biol. Chem. 245:1467-1471, 1970.

[8] Barber, C. A. Enzymic glycosidation of quercetin to rutin. Biochemistry 1:463-468. 1962.

[9] Jiang, P. Burczynski, F. Campbell, C. *et al*.. Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum*, *F. tataricum*, and their protective effects against lipid peroxidation. *Food Research International*, 40(3), 356-364, 2007.

[10] Jia Dongdong, Li Shufen, Yang Honglin.. Determined the contents of rutin and isoquercetin by RP-HPLC. *Food Science*, 29(8), 499-501. 2008.