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Research Article

STUDIES ON THE ENHANCEMENT OF CHEBULINIC ACID USING THE COMPOSITION OF MEDICINAL HERBS BY BAKER'S YEAST

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Abstract

Chebulinic acid is a phenolic compound, commonly found in the *Terminalia chebula*, *Phyllanthus emblica*, *Dimocarpus longan* species etc. The enhancement of Chebulinic acid was obtained from the composition of medicinal herbs by using Baker's yeast (*Saccharomyces cerevisiae*) under fermentation process. The optimum results were observed for the effect of % inoculum, substrate wt, incubation period, temperature, pH, carbon sources and nitrogen sources were 2.0ml, 6g, 48hr, 30°C, 4.0, sucrose and yeast extract respectively. The Chebulinic acid concentration enhanced from 3.4 to 6.8mg/ml for the optimised conditions.

Key words: Chebulinic acid; *Terminalia chebula*; *Phyllanthus emblica*; *Dimocarpus longan*; Yeast; incubation time.

Introduction

Chebulinic acid is a phenolic compound (Manosroi et al., 2010) commonly found in the fruits of *Terminalia chebula*, leaves and fruits of *Phyllanthus emblica* and seeds of *Dimocarpus longan* species, which has many potential uses in medicine. It is also found in the leaves of *Dendrophthoe falcata*, *Lumnttzera racemosa*, *Terminalia macroptera* species. It is a Faint yellowish crystalline powder and sparingly soluble in water, soluble in ethanol, methanol and ethyl acetate. Chebulinic acid (Karel et al., 2004) was helps to remove toxins and unwanted fat from the body, improves skin glow and complexion also. It showed many pharmacological activities (Surya prakash and Meena, 2014) including inhibition of cancer cell growth like human leukemia K562 cells (Yi *et al.,* 2004) colon adenocarcinoma HT-29 cell lines (Meena *et al.,* 2013), anti-neisseria gonorrhoeae activity, anti-hypertensive (Ta-Chen Lin *et al.,* 1993), inhibiting the contractile responses of cardiovascular muscles(Guan *et al.,* 1996), anti-oxidant, anti-bacterial activities etc. The dried fruits of *Terminalia chebula* is used to produced the dye. The appearance of dye powder is brown and the main colouring component is chebulinic acid and this fruit contains an astringent matter. The astringency is because of the characteristic principle of chebulinic acid. Mainly the structural and conformational analysis of chebulinic acid component by using high pressure liquid chromatography (Pawar *et al.,* 2009) and Reverse Phase HPLC (Anil and Nandini, 2011). In the fermentation process, the enhancement of Chebulinic acid was obtained from the Composition of Medicinal herbs by Baker's yeast (*Saccharomyces cerevisiae*). The extractability of active compound like Chebulinic acid depends on factors like % inoculum, substrate wt, incubation period, temperature, pH, carbon sources and nitrogen sources. For the optimal conditions, the Chebulinic acid concentrations were obtained from the Composition of Medicinal herbs.

Material and methods

Microorganism and Inoculum Preparation

Baker's yeast (*Saccharomyces cerevisiae*) used in this fermentation process for the enhancement of chebulinic acid by using the composition of Medicinal herbs like *Terminalia chebula*, Amla fruit and Longan seeds. Mainly Yeast mixed with deionized water for a ratio of 1:10 (w/v). Then the mixture was manually swirled to dissolve it and then halted for 15 min. A deionized water suspension of the activated yeast was used to ferment.

Fermentartion Process

The pulverized powders of the *Terminalia chebula* fruit, the *Phyllanthus emblica* fruit and the *Dimocarpus longan* seed are mixed in the ratio of 3:1:2. This substrate composition was prepared up to 21g. Take 4g of substrate was mixed with 50 ml of distilled water, and the mixture was boiled for 1hr. Then the mixture was filtered to obtain the water extract prior to fermentation. Add 0.5g carbon source, 0.5g nitrogen source, and the contents are maintained at pH 5 and sterilized at 15 lbs/in at 121°C for 15 min. Afterwards the flask was inoculated with 0.5ml of yeast suspension. It was placed in the orbital shaker incubator at room temperature for 24h.

Optimization Studies

The fundamental factors influencing the enhancement of chebulinic acid studied were inoculum(%), pH, incubation time (h), amount of substrate (g) and incubation temperature (°C). Effect of supplementary carbon and nitrogen sources was also investigated. At the end of fermentation, the sample was centrifuged at 5000 rpm for 10min. The obtained supernatant was used for the estimation of chebulinic acid.

Determination of Chebulinic acid

Collect the 1ml of supernatant was added 0.5ml FD reagent (Avani *et al*., 2010) and 1ml Na_2CO_3. Make up this solution up to 10ml with distilled water. After 30min read the absorbance at 700nm for Chebulinic acid concentration.

Results and Discussion

Effect of Inoculum level

The nature of Inoculum (Baker's yeast) as well as its size may affect the microbial process (Chia-Lin Chang and Che-San Lin, 2010). Different inoculum levels of 0.5, 1.0, 1.5, 2.0 and 2.5 ml were tried to investigate their effect on chebulinic acid production so as to achieve an optimum inoculum level. The highest Chebulinic acid production at 2.0% w/v for Baker yeast was found to be 3.4 mg/ml. The results were shown in Fig. 1.

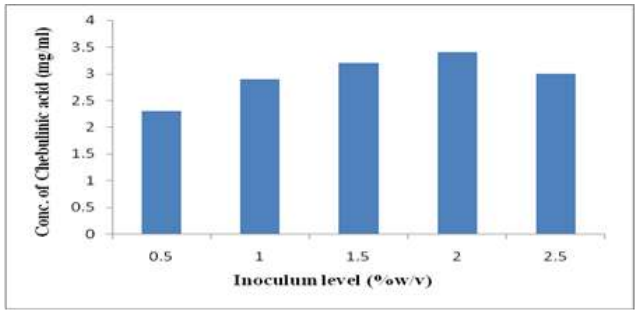

Fig. 1: Effect of Inoculum level on Chebulinic acid

Effect of Substrate level

Optimization of substrate level was carried out by varying the amount of substrate (2-10 g) in the fermentation process. The results showed that 6g of substrate yielded maximum chebulinic acid concentration was found to be 4.2 mg/ml. The results were shown in Fig. 2.

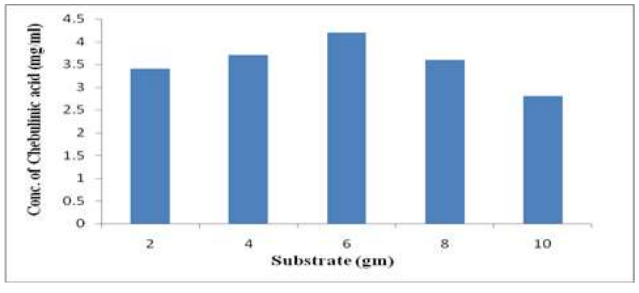

Fig. 2: Effect of Substrate level on Chebulinic acid

Effect of Incubation Time

To determine the effect of incubation time on chebulinic acid production (Surya prakash *et al*., 2012), the inoculated flasks were incubated for 24hr, 48hr, 72hr, 96hr and 120hrs duration. After incubation period, the maximum chebulinic acid concentration was found to be 4.9mg/ml at 48hr. The results were shown in Fig. 3.

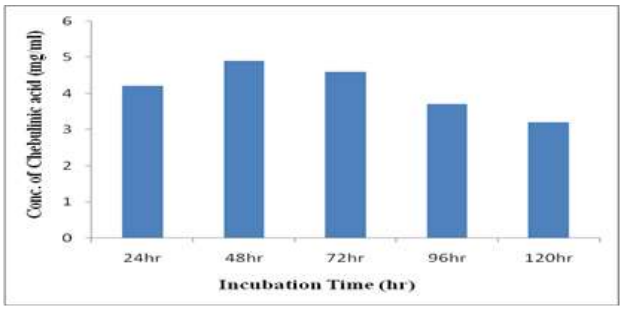

Fig. 3: Effect of Incubation Time on Chebulinic acid

Effect of Incubation Temperature

Incubation temperature is a significant parameter which plays a key role in the biochemical activities of microorganism. To examine the effect of temperature on chebulinic acid concentration, the temperature of incubating chamber was varied from 20 to 40°C. The maximum chebulinic acid was found to be 5.2mg/ml at 30°C. The results were shown in Fig. 4.

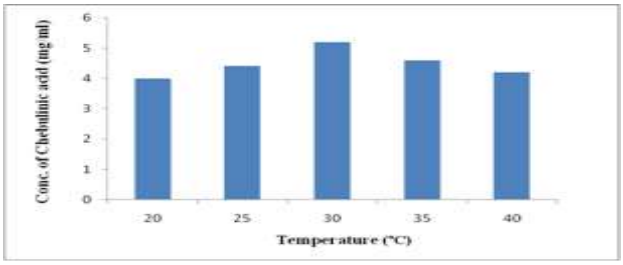

Fig. 4: Effect of Incubation Temperature.

Effect of pH

pH strongly influences the fermentation process and transport of various components across the cell membrane, which in turn supports the cell growth (Lokeswari and Jayaraju, 2006) and product production. The effect of pH on Chebulinic acid production for different pH values of 4, 5, 6, 7 and 8 were observed. The highest Chebulinic acid concentration was found to be 5.5mg/ ml at pH 4. The results were shown in Fig. 5.

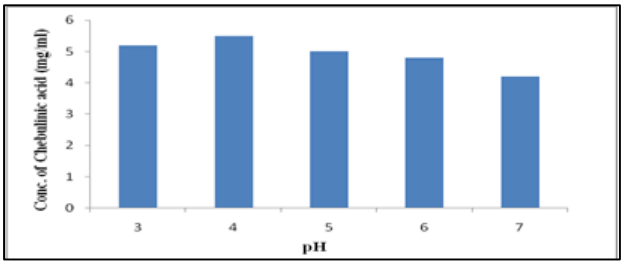

Fig. 5: Effect of pH on Chebulinic acid

Effect of Carbon source

To determine the effect of different carbon sources used which includes Glucose, Sucrose, Fructose, Maltose and Tannic acid were employed to study their effect on chebulinic acid production. Results revealed that sucrose was the most effective supplement for chebulinic acid concentration was found to be 5.7mg/ ml. The results were shown in Fig. 6.

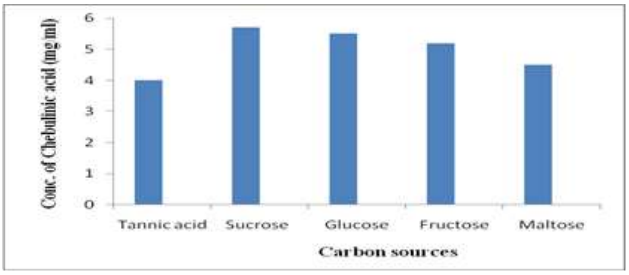

Fig. 6: Effect of Carbon source on Chebulinic acid

Effect of different Sucrose concentrations

To determine the effect of carbon supplement concentration on chebulinic acid production medium was prepared using 0.5, 1.0, 1.5, 2.0 and 2.5 %w/w of glucose were added to the substrate extract. After the fermentation process, the results indicated that the maximum chebulinic acid was found to be 6.4mg/ ml at 2.0%w/w. The results were shown in Fig. 7.

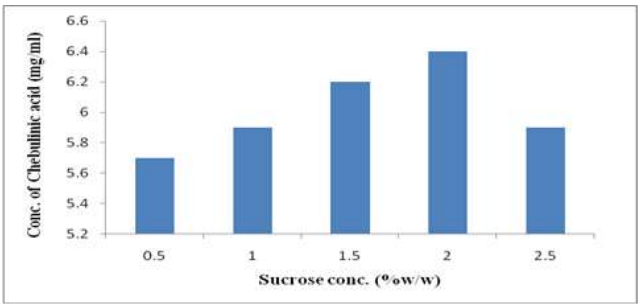

Fig. 7: Effect of different Sucrose concentrations on Chebulinic acid

Effect of Nitrogen source

To determine the effect of different nitrogen sources used which includes Sodium nitrate, yeast extract, beef extract, ammonium sulphate and ammonium nitrate were employed to study their effect on chebulinic acid production. Results revealed that yeast extract was the most effective supplement for chebulinic acid concentration was found to be 6.6mg/ ml. The results were shown in Fig. 8.

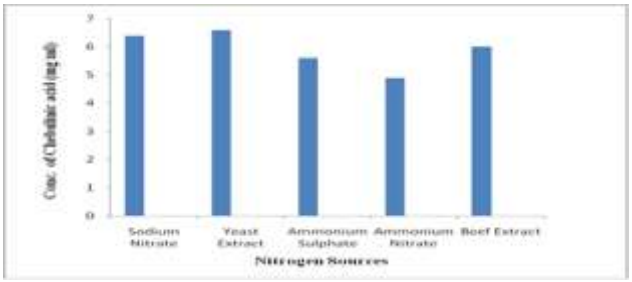

Fig. 8: Effect of Nitrogen source on Chebulinic acid

Effect of different Yeast extract concentrations

To determine the effect of nitrogen supplement concentration on chebulinic acid production medium was prepared using 0.5, 1.0, 1.5, 2.0 and 2.5 %w/w of yeast extract were added to the substrate extract. After the fermentation process, the results indicated that the maximum chebulinic acid was found to be 6.8mg/ ml at 1.5%w/w. The results were shown in Fig. 9.

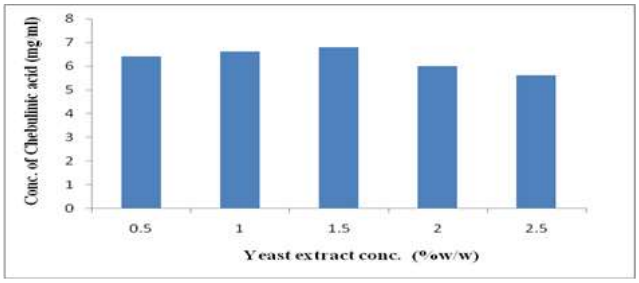

Fig. 9: Effect of different Yeast extract concentrations on Chebulinic acid

Conclusion

In the fermentation process, the Chebulinic acid was obtained from the Composition of Medicinal herbs by using Baker's yeast (*Saccharomyces cerevisiae*). The optimum results were observed for the effect of % inoculum, substrate wt, incubation period, temperature, pH, carbon sources and nitrogen sources were 2.0ml, 6g, 48hr, 30°C, 4.0, sucrose and yeast extract respectively. Hence the fermentation process enhances the Chebulinic acid content from 3.4 to 6.8mg/ml for optimised conditions.

References

Anil DM and Nandini RP (2011) Development and Validation of HPLC Method for Quantification of Phytoconstituents in Haritaki Churna. *International Journal of ChemTech Research*. **3**(1): 329-336.

Avani P, Amit P and Patel N. M (2010) Estimation of Flavonoid, Polyphenolic Content and In-vitro Antioxidant Capacity of leaves of *Tephrosia purpurea* Linn. (Leguminosae). *International Journal of Pharma Sciences and Research)*. **1**(1): 66-77.

Chia-Lin Chang and Che-San Lin (2010) Development of antioxidant activity and pattern recognition of *Terminalia chebula* retzius extracts and its fermented products. Research Institute of Biotechnology, Hungkuang University. 115-129.

Guan YY, Kwan CY , Hsu FL and Cheng JT (1996) In vitro Inhibitory effects of chebulinic acid on the contractile responses of cardiovascular muscles. Journal of *Clinical and Experimental Pharmacology and Physiology*. **23**: 745-50.

Karel D K, Ammar S, Jari S, Marja K, Jyrki L, Petri T and Kalevi P (2004) The structural and conformational analyses and antioxidant activities of chebulinic acid and its thrice-hydrolyzed derivative, 2,4-chebuloyl-β-D-

glucopyranoside, isolated from the fruit of *Terminalia chebula*. *Journal of ARKIVOC*. **7**: 83-105.

Lokeswari N and Jayaraju K (2006) Optimization of gallic acid production from *Terminalia chebula* by *Aspergillusniger*. *E-Journal of Chemistry*. **4**(2): 287-293.

Manosroi A, Jantrawut P, Akazawa H, Akihisa T and Manosroi J (2010) Biological activities of phenolic compounds isolated from galls of *Terminalia chebula* Retz. (Combretaceae). *Natural products research Journal*. **24**(20): 1915-26. DOI: 10.1080/14786419.2010.488631

Meena V, Surya prakash DV, Sree Satya N (2013) *In - vitro* anti - cancer studies of chebulinic acid on colon adenocarcinoma HT-29 cell lines. *International journal of pharmacu and pharmaceutical science*. **5**(2): 582-583.

Pawar V, Lahorkar P and Anantha Narayana DB (2009) Development of a RP-HPLC Method for Analysis of Triphala Curna and its Applicability to Test Variations in Triphala Curna Preparations. *Indian J Pharm Sci.* **71**(4): 382–386. DOI: 10.4103/0250-474X.57286

Surya Prakash D.V, Sree Satya N and Meena V (2012) Biotechnological production of chebilinic acid from *Terminalia chebula* species by Baker's yeast (*Saccharomyces cerevisiae*). *Biotechnology an Indian Journal*. **6**(11), 337-340.

Surya Prakash DV and Meena V (2014) A Review on Chebulinic acid from medicinal herbs. *World Journal of Pharmaceutical Research*. **3**(6): 2127-2139.

Ta-Chen Lin, Fengun Hsu and Juei-Tang Cheng (1993) Antihypertensive activity of corilagin and cheb ulinic acid, tannins from *lumnitzera racemosa*. *Journal of Natural Products*. **56**(4): 629-632. DOI: 10.1021/np50094a030

Yi ZC, Wang Z, Li HX, Liu MJ, Wu RC and Wang XH (2004) Effects of chebulinic acid on differentiation of humanleukemia K562 cells. *Journal of Acta Pharmacological Sinica*. **25**(2): 231-238.