

SCREENING OF ANTI-CANCEROUS COMPOUND KOJIC ACID BY A NOVEL FUNGAL ISOLATES FROM ECONOMICALLY INEXPENSIVE NUTRITIVE SOURCES

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Abstract

Novel soil fungal isolates were studied for the kojic acid production by utilizing four different inexpensive raw materials through surface fermentation under laboratory-scale conditions. Traditional one-factor-at-a-time method was employed for the optimization of process parameters, the results revealed that maximum accumulation of kojic acid crystals 16.8g/L takes place in the conical flask containing liquid substrate Coconut water. The yield obtained was 47g/L with colorimetric method by *Aspergillus flavus* FJ537130 at optimized physicochemical conditions Initial pH 7.0, Time 16d, Temperature 28°C, Substrate concentration 100ml, Peptone concentration 4g/L, MgSO₄ concentration 0.1g/L and KH₂PO₄ concentration 1.0g/L. The recovery steps included solvent extraction and crystallization methods and the product purity confirmation was done with ¹H-NMR spectroscopy. Antibacterial activity and anti-cancerous activity of kojic acid was tested on Gram positive bacteria, Gram negative bacteria and cancerous cell lines.

Introduction

During centuries ago, it was found that divergent types of natural sources like animals, microorganisms, insects and some plants were capable of producing numerous active metabolites. Out of these sources, the microorganisms producing metabolites, occupy promptly the utmost industrial applications because they are chemoorganotropic in nature, possess a maximum growth rate within a short period of life cycle so produces high amounts of biomass in short time. Hence generation of fungal metabolites industrially claims minor complex operational control process¹. These isolated natural products, one such compound, kojic acid covers a wide range of applications in various fields like food, pharmaceutical, cosmetics, medical, chemical industries etc., Numerous fungal strains have been used for the production of kojic acid which include 19 different *Aspergillus* species and five *Penicillium* species and few bacteria like *Bacterium xylinoides*, *Glucono-acetobacter opacus var. mobilis* and *Gyrinium roseum*². Nearly 14 genes A0090113000132 to A0090113000145 including a transcription factor gene KojR, an enzyme gene KojA and a transporter gene KojJ which form a cluster were involved in the biosynthesis of kojic acid³. A search on kojic acid biosynthesis mechanism utilizing different

carbon sources by various researchers have unveiled that the kojic acid synthesis was occurred from phosphorylated intermediates. The change in the C/N ratio in the production medium highly effects the metabolite production in numerous types of fermentation processes⁴. An alternative and economically feasible carbon sources which were not used for the kojic acid production till now were used in the current study. The other objective of the study was to determine the effect of enhancers on the kojic acid production.

Materials and methods

Micro-organisms

Paddy soil, peanut soil and garden soil were choosed for the isolation of various fungal organisms like *Aspergillus flavus*, *Aspergillus sojae*, *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus fumigatus*, *A.terrus*, *Mucor*, *Fusarium* and *Pencillium* species using serial dilution technique. The isolated cultures were identified using Lactophenol cotton blue staining method and maintained on CZA slants at 4°C.

Screening of kojic acid producers

Initially 50ml of seed medium was added to 250 conical flasks. The medium contains (g/L) glucose -50, yeast extract-5.0, KH₂PO₄-1.0 and MgSO₄.7H₂O-0.5 (Ariff's medium). From all isolated fungal cultures, spore suspension was prepared using 0.1% Tween 80. 5ml of spore suspension was added to seed medium and incubated at 28°C for 12d. Production was performed under surface and submerged conditions. Each experiment was done in duplicates. When the fermentation was terminated, the broth was filtered. In supernatant the concentration of kojic acid was determined by Bentley's colorimetric method⁵. Whereas the collected mycelia mat was dried and weighed. The kojic acid positive producers screened from negative cultures were now subjected to optimization studies using the Ariff's nutritional medium⁶ by replacing the source of carbon with the liquid and fruit substrates.

Raw materials

Coconut water

It is the liquid which is present in the endosperm of coconut taken as a healthy and nutritional drink by human beings worldwide. The water is clear, sterile and contains unique compound like sugars, vitamins, electrolytes, amino acids, enzymes, minerals, phytoharmones and cytokines. Its scientific name is *Cocos nucifera* belonging to *Arecaceae* family. The tree grows commonly in coastal tropical areas. The liquid is rich with sugars and amino acid and consists of very less amounts of sodium and chlorides. The composition of coconut water per 100g is energy-19Kcal, carbohydrates-3.71g, protein-0.72g, total fat-0.2g and dietary fiber-1.1g. The waste coconut water in huge amounts was collected from temples and filtered, purified and used as one of the raw material in the research.

Paneer whey

Whey is the by-product from dairy products since has no added value, it is dumped in to the environment. It was determined that paneer production in India was high and is 1,50,000 tonnes⁷. This may result in the production of 2 million tonnes of whey which might contain 1,30,000 tonnes/annum of desired milk nutrients. The amount of nutrients present in the whey depends on the ingredient composition of milk from where the whey was derived and also based on the milk processing methods. The major sugar present in whey was lactose within the range of 70%. The nutritional composition of whey was Na (mg/L)-350, K (mg/L)-1300, Ca (mg/L)-480, Mg (mg/L)-59, Cl (mg/L)-1349, Citrate (mg/L)-6750, Zn (mg/L)-280, total proteins (%)-0.41, fat (%)-0.01, lactose (%)-4.5, total solids (%)-5.8.

Sugarcane baggase

In the form of feed stock, sugarcane was utilized worldwide for the production of sugar and bio-ethanol. Through the milling process, the juice was extracted from the sugarcane and left out of the baggase as a by-product which comprises 60 – 80% of carbohydrates. Though it is a good source of carbohydrates, it is often treated as an agricultural waste and discharged in the environment which makes pollution. Now-a-days this by-product baggase was used as a renewable feed stock for the production of various industrial compounds like ethanol, citric acid, bio-fuel production and in pulp industry etc. The carbohydrates cellulose and hemicelluloses embedded in the matrix of lignin were present in the baggase. The baggase contains 35.2% cellulose, 24.5% hemicelluloses, 22.2% lignin and 20.9% ash.

Cashew apple

It is scientifically called as *Anacardium occidentale* L from *Anacardiaceae* family and also called pseudo fruit treated as a by-product in cashew nut industry. Though the plant was first identified in North Eastern Brazil but now it is pan tropical in distribution. The shape of the fruit nut is kidney shaped and during the maturation phase, the stalk above it becomes swollen, fleshy and becomes a pear shaped accessory fruit which is 2-4 inches contain yellow or red coloured juice. The mature fruit was often characterized by a good aroma, more sugar content and acidity and less astringent. The fruit contains 84-88% H₂O, 0.1-0.16g protein, 0.05-0.5g fat, 9.1-9.8g carbohydrates, 0.4-1.0g dietary fibre, 0.9-5.4mg Ca, 0.2-0.7mg Fe, 0.02-0.03mg Vit-B₁, 0.1-0.4mg Vit-B₂, 0.1-0.5mg Vit-B₃, 147-372mg Vit-C. Carotenoids, quercetin, anacardic acid, tannins, organic acids, antioxidants were also present. All the raw materials were collected from local areas of Visakhapatnam, India.

Kojic acid fermentation and optimization

The fermentation medium contains 50g of solid substrates sugarcane baggase or Cashew apple pulp or 50ml of liquid substrates Coconut water or Paneer whey and contains the nitrogen source peptone: 0.05g, Mineral salts MgSO₄.7H₂O: 0.025g, KH₂PO₄: 0.05g. 500ml of distilled water was added to the conical flasks contain solid substrates. For optimization studies, different parameters were taken into consideration to get very higher yields of kojic acid. The parameters tested were Substrate concentration (10g/L-100g/L) or (10ml-100ml), Peptone Concentration (1-5g/L), KH₂PO₄ (0.5-2.5g/L), MgSO₄.7H₂O (0.1-0.9g/L), pH (4.0-8.0), Time (11d-37d) and Temperature (20-35^oC). 1N HCl or 1N NaOH was used to adjust the pH ranges with pH meter. Static fermentation was conducted with the kojic acid positive producers *A.flavus* and *A.sojae* with all the four different substrates. When once the optimized conditions were found out, the final production was performed to determine the optimum kojic acid yields with both the cultures and the dry cell weight of the mycelium was determined.

Effect of enhancers on kojic acid production

Different types of enhancers were generally used to increase the yields of kojic acid by the fungal cultures and they include Copper-monovalent-nicotinic acid complex and Copper (I)-B₂ complex⁸, Cycasin or Methyl-azoxymethyl-β-D-glucose⁹, Methanol¹⁰. In the current research, methanol at a concentration of 4% v/v Copper (I) B₂ complex and Copper (I) B₃ complex at a concen-

tration of 0.1mg/ml were tested as enhancers for the kojic acid production.

Table 3. Effect of enhancers on Kojic acid production

Substrate	Before addition of enhancer (g/L)	After addition of methanol (g/L)	After addition of Cu-Vitamin B2 complex (g/L)	After addition of Cu-Vitamin B3 complex (g/L)
Sugarcane baggase	3.95	4.85	4.01	4.16
Cashew apple	15.9	16.87	16.2	16.39
Paneer whey	14.2	14.5	14.31	14.35
Coconut water	16.8	17.9	17.1	17.05

Effect of peptone concentration on kojic acid production with the substrate Coconut water

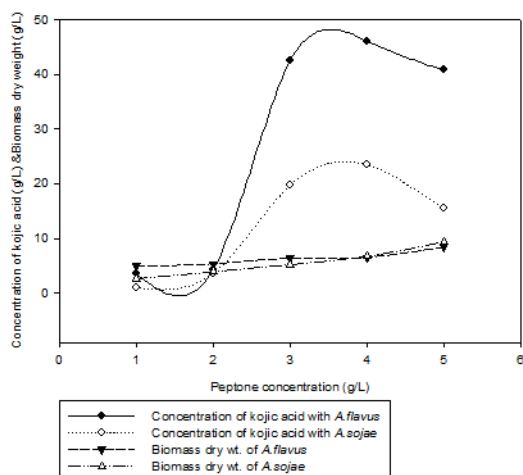


Figure 1. Effect of peptone concentration on Kojic acid production with the substrate coconut water

Effect of Time on kojic acid production with the substrate Paneer whey

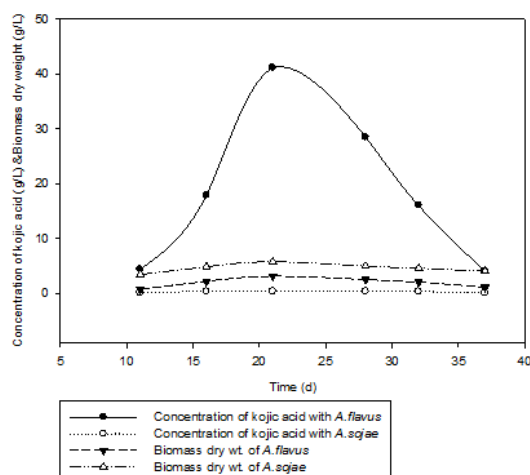


Figure 2. Effect of kojic acid production with the substrate paneer whey

Effect of Substrate concentration on kojic acid production with the substrate Sugar cane baggase

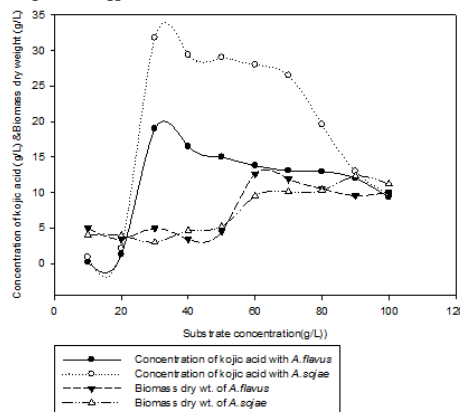


Figure 3. Effect of substrate concentration on Kojic acid production with the substrate Sugarcane baggase

Effect of Time on kojic acid production with the substrate Cashew apple

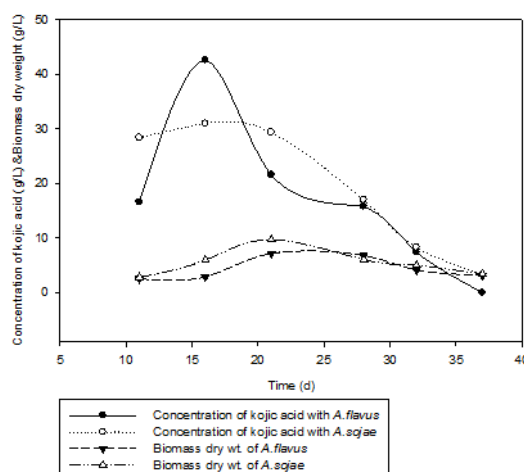


Figure 4. Effect of time on kojic acid production with the substrate cashew apple

Recovery of kojic acid

The fermented broth samples were filtered and kept in a refrigerator at 5°C for 24 h. For further extractions of kojic acid, the soluble kojic acid in the fermented broth was extracted using ethyl acetate. Upon evaporation, the extractant yields kojic acid crystals in the form of needles. The crystals were collected and dried at 80°C for 24 h. For purification, repeated crystallization was performed with a mixture of water and acetone. Later the dry weight of the crystals from each sample was determined¹¹. The purity of the crystals was confirmed by Proton Nuclear magnetic resonance spectroscopy (¹HNMR).

Antimicrobial activity of kojic acid

Bacteria of dissimilar genera including *Proteus*, *Staphylococcus*, *Streptococcus*, *Pseudomonas*, *Bacillus*, *Corynebacterium*, *Clostridium*, *Aerobacter*, *Escherichia*, *Klebsiella*, *Salmonella* etc were inhibited by kojic acid. The agar-slants of pathogenic bacteria suspensions were collected from Doctor's Diagnostic Centre, Gajuwaka, Visakhapatnam. Individual bacterial suspensions were prepared using a sterile distilled water so as to contain 10⁸CFU/ml. By using spread plate technique 0.5ml of bacterial inoculum was spread uniformly on a petridish containing Muller Hinton agar. The isolated kojic acid crystals were made to 0.1ml suspension with sterile distilled water at a concentration of 250µg/ml was inoculated into one of the well created in the petridish. Into other two wells negative control was maintained with distilled water and a positive control with a standard drug Cefrazidime prepared like test sample. The plates were incubated at 37°C for 16-24 hrs. After 24 h, zone of inhibitions surrounding the wells were identified and measured in millimetres¹².

Antiproliferative activity of kojic acid

Cell culture and MTT assay

Human cancer cell lines used in this study were obtained from National Centre for Cell Science, Pune. They were grown in a Minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO₂ incubator. The standard MTT assay was used to determine the inhibitory effects of test compound kojic acid on cell growth in vitro. The trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of 5x10³ cells/well in growth medium and cultured at 37°C in 5% CO₂ to adhere. After 48h incubation, the supernatant was removed and the cells were pre-treated with growth medium and afterward mixed with

different concentrations of kojic acid (12.5, 25, 50, 100 and 200 µg/ml) to achieve a final volume of 100µl and then cultivated for 48h. The compound was prepared as 1.0mg/ml concentration stock solutions in PBS. Culture medium and solvent are used as controls. Each well then received 20µl of fresh MTT (0.5mg/ml in PBS) followed by incubation for 4hr at 37°C. The supernatant growth medium was removed from the wells and replaced with 200µl of DMSO to solubilize the colored formazan product. After 30 min incubation, the absorbance (O.D) of the culture plate was read at a wavelength of 492nm on an ELISA reader and Anthos 2020 spectrophotometer.

Table 1. Effect of kojic acid on MDAMB435S cell lines

Conc. in µg/ml	OD at 492nm	% cell survival	% of inhibition activity
12.5	0.613	87.93	12.6
25	0.61	87.48	12.51
50	0.599	85.82	14.17
100	0.545	77.67	22.32
200	0.565	80.69	19.3

IC50=863.029471 µg/mL Control 0.693, Blank-0.030

Table 2. Effect of kojic acid on K562 cell lines (Leukemia)

Conc. in µg/ml	OD at 492nm	% cell survival	% of inhibition activity
12.5	0.717	83.79	16.21
25	0.507	58.17	41.82
50	0.506	58.04	41.95
100	0.466	53.17	46.82
200	0.3	32.92	67.07

IC50 =112.0212 ug/mL Control 0.850, Blank - 0.030

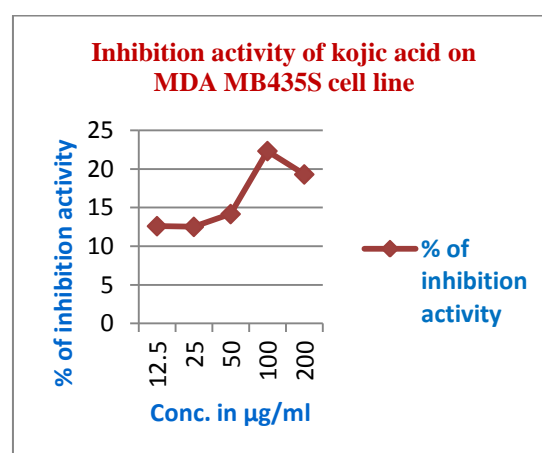


Figure 5. Inhibition activity of kojic acid on MDA MB435S cell lines (Breast cancer)

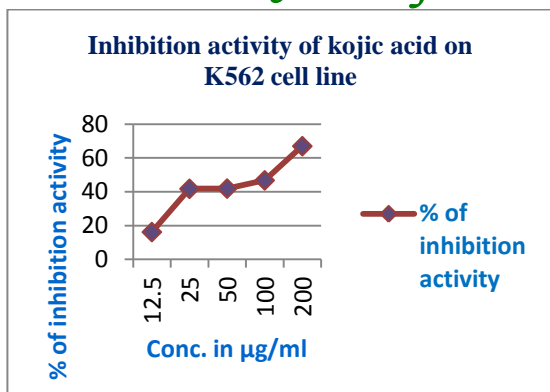


Figure 6. Inhibition activity of kojic acid on K562 cell lines (Leukemia)

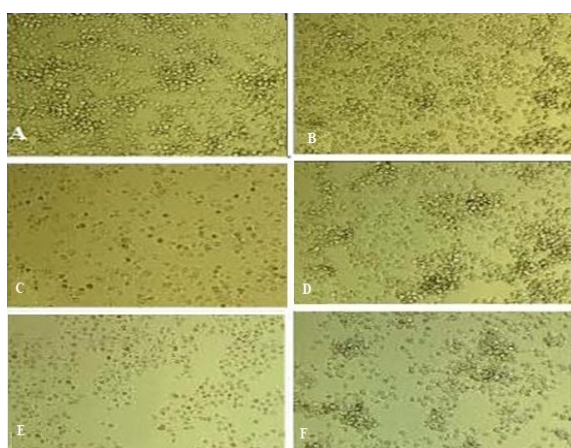


Figure 7. Activity of kojic acid on MDA-MB435S (Breast cancer) cell lines before and after addition

A- Control B- 12.5 µg/ml
C- 25 µg/ml D- 50 µg/ml
E- 100 µg/ml F- 200 µg/ml

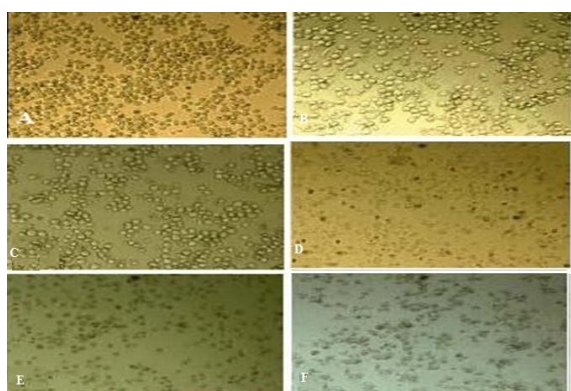


Figure 8. Activity of kojic acid on K562 (Leukemia) cell lines before and after addition

A- Control B- 12.5 µg/ml
C- 25 µg/ml D- 50 µg/ml
E- 100 µg/ml F- 200 µg/ml

Result and Discussion

Among the 10 isolated fungal organisms *A.flavus* and *A.sojae* were considered to be the highly active organisms for the production of kojic acid as they produce a deep intense red colour to the Bentley's colorimetric method under static conditions. However traces of kojic acid concentration were noticed with shake flask experiments. The concentration of kojic acid produced by *A.flavus* and *A.sojae* were 20.16g/L and 14.7g/L with the glucose media. These results agreed well with the results of earlier reports that maximum production of kojic acid was obtained under static conditions than shake-flask conditions^{11,13,14,15}. From the previous studies, it was came to know that, different species of *Aspergillus* like *A.flavus*, *A.oryzae*, *A.fumigatus*, *A.candidus*, *A.awamori*, *A.clavatus*, *A.ustus*, *A.wentii*, *A.nidulans*, *A.parasiticus*, *A.tamarii*, *A.terrus*, *A.versicolor*, *A.phoenicis*, *A.sclerotium* and *Eurotium* sps. were known to produce kojic acid. The present study had reported for the first time in using *A.sojae* for the kojic acid production. It is still uncertain for kojic acid production about the efficiency and production economics because the nutritional media plays a more significant role in the betterment of such fermentation process. Now-a-days, research trials were done on to identify the novel and potential nutritive sources and most advanced fermentative methods to achieve very higher yields of product and at the same time high conversion rate of the substrate molecules. Usage of pure sugars resulted in the production of pure product subsequently the cost of the purification process reduced. But economically this was not feasible because of the high cost of the pure sugars. Hence these expensive sources were substituted by the usage of low cost agricultural resources. Though literature was available on kojic acid production by using various industrial and agro-waste by products like pea, kidney bean, vegetable waste, fruit waste of apricot, orange and peach, carrot waste, turnip waste, cornsteep liquor, molasses, cheese whey, wheat bran, rice husk, rice fragments by El-kady the production yield was very low 21.2g/L medium. It was reported that, the kojic acid production from gelatinized sago starch and revealed 4.51g/L with *A.flavus* 44-1 strain under submerged conditions¹⁶. This report presents exclusively in utilizing a four different high-yielding substrates- two agricultural by-products Cashew apples and Sugarcane baggase and one industrial by-product Paneer whey and waste Coconut water collected from temples for kojic acid production in order to bring down the final cost of the production process. Based on the evaluation of results when Coconut water was used as a carbon source, the fungal organism *A.flavus* produces highest kojic acid yield of 47g/L at optimal conditions of pH 7.0, Time 16d, Temperature 28°C, Substrate concentration 100ml, Peptone concentration 4g/L (Figure 1) MgSO₄ concentration 0.1g/L and KH₂PO₄ concentration 1.0g/L. Later the evaporation of broth and further

crystallization produces 16.8g/L of pure kojic acid crystals. The organism was confirmed as *A.flavus* FJ537130 strain using 18Sr RNA based molecular analysis. It showed 99% maximum identity using BLAST method. Whereas *A.sojae* produce 35.1g/L of kojic acid to colorimetric method and 12g/L of dry crystals were produced significantly at optimum experimental parameters pH 7.0, Time 28d, Temperature 28°C, Substrate concentration 100ml, Peptone concentration 4g/L, MgSO₄ concentration 0.1g/L and KH₂PO₄ concentration 1.0g/L. The maximum dry cell mycelia mat obtained was 11.92g/L. For the substrate Paneer whey, *A.flavus* liberate 42.4g/L of kojic acid concentration and 14.2g/L of dry crystals significantly at optimum conditions pH 4.0, Time 21d (Figure 2), Temperature 28°C, Substrate concentration 100ml, Peptone concentration 2g/L, MgSO₄ concentration 0.3g/L and KH₂PO₄ concentration 1.0g/L. The other organism *A.sojae* produces 34.2g/L of kojic acid concentration and 11.9g/L of dry crystals at optimum parameters pH 4.0, Time 28d, Temperature 28°C, Substrate concentration 100ml, Peptone concentration 5g/L, MgSO₄ concentration 0.3g/L and KH₂PO₄ concentration 0.5g/L. The highest dry cell weight obtained was 10.3g/L. Maximum yield of 17g/L and dry crystals 3.95g/L was significantly obtained with *A.flavus* at optimal parameters pH 5.5, Time 21d, Temperature 28°C, Substrate concentration 30g/L, Peptone concentration 4g/L, MgSO₄ concentration 0.7g/L and KH₂PO₄ concentration 1.5g/L whereas *A.sojae* excrete significant production of kojic acid 33.5g/L and 11.5g/L dry crystals at favourable parameter conditions pH 6.0, Time 21d, Temperature 30°C, Substrate concentration 30g/L (Figure 3), Peptone concentration 4g/L, MgSO₄ concentration 0.7g/L and KH₂PO₄ concentration 2.0g/L by using the substrate Sugarcane baggase. The dry cell obtained was 14.2g/L. The agro waste by-product Cashew apple when used as a carbon source produce 45g/L of concentration of kojic acid and yielded 15.9g/L of dry crystals at optimized conditions pH 6.0, Time 16d (Figure 4), Temperature 28°C, Substrate concentration 50g/L, Peptone concentration 4g/L, MgSO₄ concentration 0.7g/L and KH₂PO₄ concentration 1.5g/L with the fungal organism *A.flavus* significantly. *A.sojae* at optimum conditions pH 6.0, Time 16d, Temperature 28°C, Substrate concentration 50g/L, Peptone concentration 4g/L, MgSO₄ concentration 0.7g/L and KH₂PO₄ concentration 1.5g/L produce significant yield of 34.8g/L of kojic acid concentration and upon crystallization gave 10.82g/L of dry crystals. The dry cell weight obtained was 10.7g/L. Sigma 10.0 version software was used for plotting of graphs. From the results it was found out that, the dry cell weight of the mycelia mat were not at all correlated to the production rate of kojic acid. *Aspergillus* generally during the non-growing phase, secrete very high

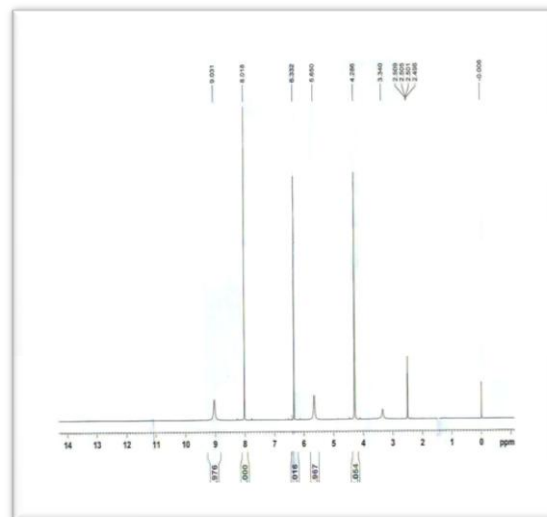


Figure 9. ¹H-NMR of kojic acid crystals

concentrations of kojic acid where the glucose molecules were rapidly consumed by the culture to synthesize kojic acid by the cell bound enzymes¹⁷. The cessation of kojic acid production was takes place when once all the glucose molecules were consumed by the culture. When the antimicrobial activity was examined for the isolated kojic acid crystals, kojic acid strongly inhibited the growth of *E.coli* and *Staphylococcus aureus* and the observed zone of inhibition was 12mm. The compound kojic acid exhibits antiproliferative activity on the breast cancer cell lines MDAMB435S (Table 1), (Figure 5, Figure 7) and Leukemia cell lines K562 (Table 2), (Figure 6, Figure 8). The percentage of inhibitory activity for the Breast cancer cell line was 22.32% at 100 µg/ml concentration and for the Leukemia it was 67.07% at 200 µg/ml concentration. By the addition of 4% v/v methanol, maximum enhancement of yield was obtained with the substrate sugarcane baggase whereas 1 -12% increase in yield was observed with other 3 substrates. The ¹H NMR of kojic acid crystals extracted from (Figure 9) showed 4 characteristic protonic signals of varying size intensity and few smaller signals. The four peaks obtained at 8.018 (s, 1H), 6.332 (s, 1H), 4.286 (s, 2H), 3.340 (s, OH). The result was similar to standard sample.

Conclusion

The possibility of using waste carbon sources as profitable substrates for kojic acid production using *Aspergillus* sps. were studied for the first time and can used commercially for large-scale production of value added products like kojic acid. High production was noticed with Coconut water and Paneer whey. The study also revealed that the production was enhanced from Sugarcane baggase, a waste obtained in huge quantities with the chemical enhancer like methanol.

References

- [1] Adriana A P Bracarense & Jacqueline A Takahashi, Modulation of antimicrobial metabolites production by the fungus *Aspergillus parasiticus*, *Brazilian J microbial*, 45 (2014) 313-321.
- [2] Arnstein H RV & Bentley, The biosynthesis of kojic acid-The occurrence of Aldolase and Triosephosphate isomerase in *Aspergillus* species and the relationship to kojic acid biosynthesis, *National institute for medical research* (Mill Hill, London, N.W.7), 1952.
- [3] Terabayashi Y, Sano M, Yamane N, Marui J, Tamano K, Sagara J, Dohmoto M, Oda K, Ohshima E, Tachibana K, Higa Y, Ohashi S, Koike H & Machida M, Identification and characterization of genes responsible for biosynthesis of kojic acid, an industrially important compound from *Aspergillus oryzae*, *Fungal Genet Biol*, 47 (2010) 953-961.
- [4] Rosfarizan M & Ariff A B, Kinetics of kojic acid fermentation by *Aspergillus flavus* Link S44-1 using sucrose as a carbon source under different pH conditions, *Biotechnol Bioprocess eng*, 11 (2006) 72-79.
- [5] Bentley R. Preparation and analysis of kojic acid. *Methods Enzymol*. 1957; 3:238-249.
- [6] Ariff A B, Salleh M S, Gani B, Hassan M A & Rusul G, Aeration and yeast extract requirements for kojic acid production by *Aspergillus flavus* Link, *Enz Microbiol Technol*, 19 (1996) 545-550.
- [7] Aneja R P, Mathur B N, Chandan R C & Banerjee A K, Heat-acid coagulated products, in *Technology of Indian milk products* (New Delhi, India), 2002.
- [8] Megalla S E, Nassar A Y & Gohar M A S, The role of copper (I) nicotinic acid complex on kojic acid biosynthesis by *Aspergillus flavus*, *J Basic Microbiol*, 27 (1987) 29-33.
- [9] Tadera K, Yagi F & Kobayashi A, Effects of cycasin on kojic acid producing molds, *Agric Biol Chem*, 49 (1985) 203-205.
- [10] Madihah M S, Ariff A B, Hassan M A Rusul G & Karim M I A, Enhanced kojic acid production by *Aspergillus flavus* Link in Growth medium containing methanol, *Asean food J*, 11 (1996) 158-162.
- [11] Hazzaa M M, Saad A E N M, Hassan H M, Ibrahim E I, High production of kojic acid crystals by isolated *Aspergillus oryzae* var. *effusus* NRC14, *J Appl sci Res*, 9 (2013) 1714-1723.
- [12] Saleh R M, Kabli S A, Al-Garni S M, Mohamed S A, Screening and production of antibacterial compound from *Trichoderma* species against human pathogenic bacteria, *Afr J Microbiol res*, 5 (2011)1619- 1628.
- [13] Wei C I, Huang I S, Chen J S, Marshall M R & Chung K I, Production of kojic acid by *Aspergillus candidus* in three culture media, *J food prot*, 54 (1991) 546-548.
- [14] Saad A M, Hamed H A & Saad M M, Kojic acid production by a toxigenic strain of *Aspergillus parasiticus* and a non-toxicogenic strain of *Aspergillus flavus*, *African J of Mycol and Biotechnol*, 4 (1996) 19-27.
- [15] Ogawa A, Wakisaka Y, Tanaka T, Sakiyama T, Nakanishi K, Production of kojic acid by membrane-surface liquid culture of *Aspergillus oryzae* NRRL484, *J.Ferment.Bioeng*, 80 (1995) 41-45.
- [16] Rosfarizan M & Ariff A, Importance of carbon source feeding and pH control strategies for maximum kojic acid production from sago starch by *Aspergillus flavus*, *J Biosci Biotech*, 94 (2002) 99-105.
- [17] Bajpai P, Agrawala P K & Vishwanathan L, Enzymes relevant to kojic acid biosynthesis in *Aspergillus flavus*, *J Gen Microbiol*, 127 (1981) 131-136.

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