Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Review on Kojic Acid - A Secondary Metabolite from *Aspergillus* Fungi

Soujanya N*, Suresha B S, Balasubramanian T, Rakshitha S, Meghana B P

Department of Pharmacology, Bharathi College of Pharmacy, Bharathinagar-571422, Maddur Taluk, Mandya District, Karnataka, India.

ARTICLE HISTORY

Received: 13.07.2022

Accepted: 07.08.2022

Available online: 30.09.2022

DOI:

10.5530/ajphs.2022.12.20

Keywords:

Secondary fungal metabolite, Aspergillus species, Kojic acid, Cosmetic, Skin-lightening agent, Production and applications.

*Corresponding author:

Email : soujanyan719@gmail.com
Phone : +91-7019228831

ABSTRACT

Fungi are an understudied, biotechnologically valuable group of organisms. Fungi have provided the world with penicillin, lovastatin, and other globally significant medicines, and they remain an untapped resource with enormous industrial potential. This review article reveals the properties, production and various uses of Kojic acid which is a secondary metabolite mainly isolated from various species of *Aspergillus* fungi. Kojic acid is a heterocyclic crystalline compound. It can be produced from different strains of microbes by the process of fermentation. In recent times Kojic acid plays a crucial role in cosmetics, especially in skin care products because of its UV protectant and skin lightening properties. This article is aimed to review its applications in medical, food and agricultural fields including cosmetics. Hence Kojic acid continues to attract attention of researchers because of its economic potential in various fields.

INTRODUCTION

The Kojic acid (KA) is a chemical product that is obtained from various types of fungi.[1] In 1907 Saito discovered KA from mycelium of the fungus *Aspergillus oryzae* grown on steamed rice. Kojic acid originally named as "Koji" which is the name of fungus from which kojic acid is derived. Then the name kojic acid was given by Yabuta in 1913. Yabuta also elucidated the structure of kojic acid.[2] The term koji means steamed rice in Japanese.[3]

CHEMISTRY OF KOJIC ACID

A. Structure of kojic acid

Kojic acid is classified in the group of organic acids, which is obtained from different types of fungi during aerobic fermentation process.[1] It is a heterocyclic crystalline compound. The Kojic acid structure was determined to be 5-hydroxy-2-hydroxymethyl-δ-pyrone.[4] According to a review article by Beelik, the enolic hydroxyl group at C5 gives kojic acid its weakly acidic nature and allows it to form salts with a number of metals.[3]

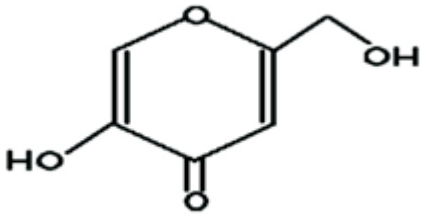

Fig. 1 : Structure of kojic acid

B. Physical properties of kojic acid

- Kojic acid has melting point between 151 ^{0}C and 154^{0}C.[5]
- Molecular formula of KA is $C_6H_6O_4$ and molecular weight is 142.11g/mol.[6]
- Kojic acid crystallizes in form of colorless, prismatic needles that sublime under vacuum conditions without any changes.[7]

C. Chemical properties of kojic acid

- Kojic acid is soluble in polar substances like water, ethanol, acetone, ethyl acetate etc. It is very less soluble or sparingly soluble in chloroform, ether, and pyridine; insoluble in benzene.
- KA is classified as having a weekly acidic poly-functional active quinone-pyrone.
- Kojic acid molecule is reactive at every position on a ring.
- At carbon 5 position hydroxyl (OH-) acts as weak acid, which is capable to form salts with few metals such as Sodium, Zinc, Copper etc., which make it more reactive.[8]
- Kojic acid and its derivatives with saccharin molecule are soluble in water.
- Structure of kojic acid can be modified by glycosylation.[9]
- The side chains of C5 behaves as a primary alcohol whose reactivity can be enhanced by the adjacent oxygen atom in the nucleus.[2]

PRODUCTION OF KOJIC ACID

Kojic acid is produced industrially by Aspergillus species in aerobic fermentation. The production of kojic acid is increasing because of its commercial value in industry.[10] Kojic acid is naturally produced as a secondary metabolite in the following *Aspergillus* strains: *Albus, alliaceus, awamori, arachidicola, bombycis, caelatus, candidus, clavatus, effusus, flavus, fumigatus, giganteus, glaucus, gymnosardae, leporis, luteovirescens, lutescens, minisclerotigenes, nidulans, nomius, parasiticus, parvisclerotigenus, pseudotamarii, tamarii* and *wentii.*[3,11] It is also the secondary metabolite of several strains of *Penicillium* and *Acetobacter* fungi and several species of acetic acid bacilli.[3,12,13]

There have been 58 different strains used for production of kojic acid are *Penicillium, Mucor, Aspergillus* etc.[14] It is also produced from the fermentation of some Asian foods (e.g soy sauce and rice wine), which acts as a primer for fungus or inoculum.

Kojic Acid was first marketed in 1955. The Charles Pfizer and Company, USA, was the first company to try to build this product. In recent years, kojic acid producing companies include two in China and three companies in Japan, Switzerland, and the USA. Rapid growth of industries and discovery of the potential uses of kojic acid and its derivatives generated great demands for this product.[1]

Kojic acid is produced industrially by *Aspergillus* species in aerobic fermentation. Industrial kojic acid production includes following main three stages:

1) Inoculum development.

2) Bio-production of kojic acid.

3) Extraction & Purification of kojic acid.

There are some factors that affect fermentation of kojic acid include use of high kojic acid yielding microorganisms, production media, type of fermentation operation, physiological conditions, aeration & agitation and minerals.[14]

PHARMACOLOGICAL POTENTIALS OF KOJIC ACID

Kojic acid (KA) is a natural metabolite produced by fungi that has the ability to inhibit tyrosinase activity in synthesis of melanin. The major applications of KA and its derivatives in medicine are based on their biocompatibility, antimicrobial and

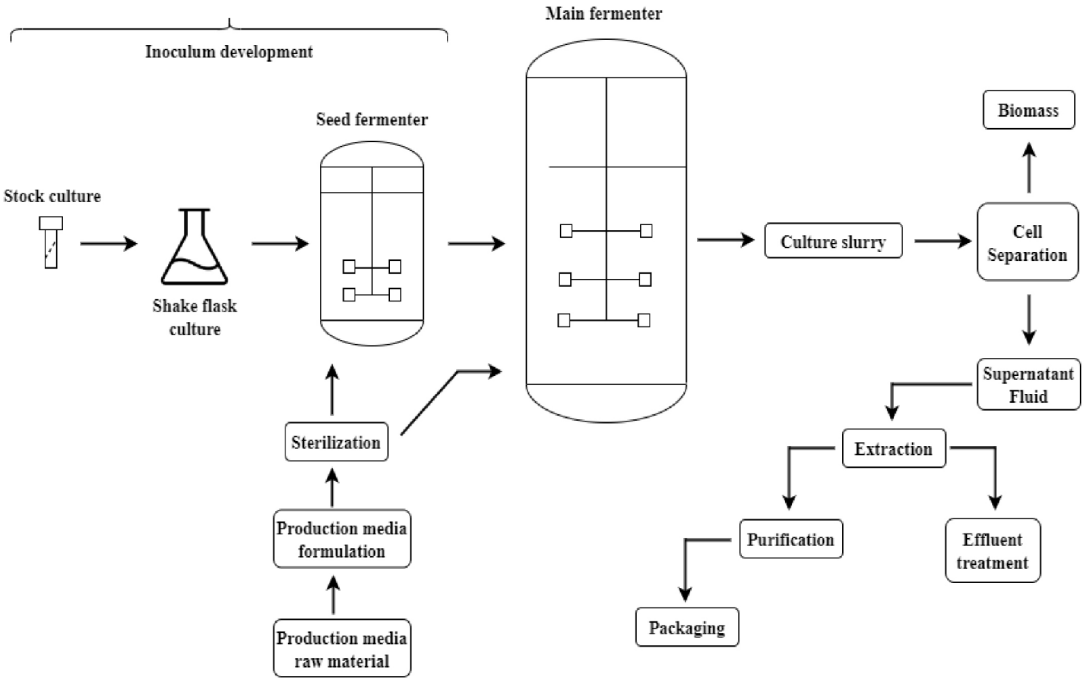

Fig. 2 : Schematic representation of fermentative Kojic acid production.[14]

antiviral, antitumor, antidiabetic, anticancer, anti-speck, anti-parasitic, and pesticidal and insecticidal properties.[1]

➢ KA in cosmetic industry:

The most significant benefits of kojic acid are in the cosmetics and healthcare industry. It is mainly used as an essential material for the skin whitening creams production, skin care lotions, whitening soaps & dental care products. It acts as UV protectant & inhibits the melanin production by inhibiting the tyrosinase formation, the enzyme which is responsible for skin pigmentation so thereby inhibiting hyper pigmentation of human skin.[5,15] KA is an antioxidant used by the cosmetics industry and has been described as an alternative to hydroquinone in skin lightening.[12]

➢ Anti-Inflammatory properties of KA derivatives:

It has been shown that KA can apply a minor anti-inflammatory effect which can be favorably improved by the successive derivatization of the selected derivative of kojic acid. In a recent study to develop a safe anti-inflammatory compound, a derivative of KA and p-coumaric acid were synthesized, as they are known to have anti-inflammatory properties. The study suggested that the anti-inflammatory action of KA was enhanced by the addition of cinnamate moiety in p-coumaric acid as a hydrophobic part.[16] A study assessed the anti-inflammatory activity of KA and p-coumaric acid and revealed that both possessed anti-inflammatory properties.[17]

In another study, KA and its two novel derivatives were isolated from the fungus *Aspergillus versicolor* and evaluated for their anti-inflammatory effects, showing that KA has a moderate anti-inflammatory effect, while the derivatives were found to have improved effects.[18]

➢ Antineoplastic properties of KA derivatives:

While looking for new compounds with antitumor activity the impact of kojic acid halogen derivatives on the propagation of leukemia L-1210 cells & pituitary GH4C1 tumor cells was examined. It was stated for the first time that a group of numerous halogen derivatives of 5-hydroxy-2-hydroxymethyl-4-pyrone may be capable medications with anti-leukemia activity.[19] Two halogen derivatives of kojic acid 5-hydroxy-2-chloromethyl-4-pyrone and 5-hydroxy-2-hydroxymethyl-4-pyrone were found to inhibit DNA, RNA & protein synthesis.[20]

➢ KA in food industries:

The Aspergillus flavus group is traditionally used to produce many foods, including miso (fermented soya bean paste), soy sauce & sake. KA is extensively utilized as food additive to prevent enzymatic browning in the food industry, & KA is used as an agent to prevent undesired blackening of agricultural products such as vegetables, fruits & crustaceans during storage. KA has the ability to inhibit the action of polyphenol oxidase enzymes when these products are exposed to oxygen.[21] In addition; it is utilized as anti-seed agent in raw noodles during the production process. This is to avoid color change & black dot formation on the noodles by tyrosinase inhibition.[6, 22]

➢ Radio protective effects of KA:

Kojic acid stimulates lymphocyte proliferation, enhances neutrophil function and removes increased reactive oxygen species from leukocytes in the blood. Recent studies have shown that taking KA twenty four hours before gamma irradiation can provide and radiation protection in recent studies. This study evaluated the lethal dose of gamma. The mice were irradiated with KA for 1 hour before irradiation and compared with Amifostine as a radio protective drug and an antioxidant. A single administration of a natural compound KA prior to gamma irradiation reduces the mortality caused by irradiation. Maximum protection was observed at a dose of 350mg/kg KA one hour before irradiation, and it had similar protective efficacy as the amifostine one hour before irradiation at a dose of 200mg/kg.[23]

➢ KA as anti-oxidant and Scavenges free radicals:

KA significantly reduces the level of ROS produced by neutrophils and cellular ROS production systems. It significantly enhances the phagocytosis of neutrophils. Furthermore, the concentration of calcium in human neutrophils increases in KA presence. These consequences indicate that KA is advantageous in terms of host defense because it improves many activities of leukocytes but eliminates ROS that are extremely released from cells or formed in tissues or blood vessels that may be harmful to tissues of host.[24]

The correlation between anti-melanogenic activity with oxidative effects of KA and KA esters was investigated by Lajis et al., 2012. The results of the study showed that both KA and its esters had mild free radical scavenging activities at concentrations ranging from 1.95 to 1000 µg/mL.[25]

➢ KA as anti-fungal agent:

The antifungal action of KA on therapeutically vital fungi was assessed. *Trichophyton rubrum* & *Candida albicans* are significant human pathogenic fungi that cause numerous human fungal diseases. *Cryptococcus neoformans* is a yeast-like fungal pathogen which causes infection in immune compromised individuals, especially fatal meningitis in AIDS patients.[26] In *Cryptococcus neoformans*, melanin producing bacteria are known to have melanin associated with their virulence. It has been suggested that melanin may help protect *Cryptococcus neoformans* from the host immune system by removing leukocyte antibacterial oxidants. Therefore, these facts indicate that melanin synthesis inhibitors in melanin-producing bacteria may be probable targets for antifungal agents. Some investigations have revealed the possibility of KA inhibiting melanin production and have the opportunity to diminish the virulence of *Cryptococcus neoformans* and thus acting as an effective antifungal agent.[27] Also, KA could be developed as a chemo sensitizer to enhance efficacy of commercial antifungal drugs or fungicides.[1]

➢ KA as anti-bacterial and anti-microbial agent:

Preceding antimicrobial activity assays showed that KA was more active against Gram-negative bacteria than against Gram-positive bacteria.[28] The antimicrobial activity of the ethyl acetate extract of *Colletotrichum gloeosporioides* and its major compound KA were evaluated, and the results showed considerable antimicrobial activity against all tested strains. When tested against various microorganisms, KA was most active against *Micrococcus luteus* and least active against *Pseudomonas aeruginosa.*[29]

➢ Kojic acid in agriculture:

KA is widely utilized as a chelating agent & insecticide activator for pesticide production in agriculture. The newly designed two ligands consisting of vanillin and o-vanillin molecules, each containing two kojic acid molecules linked to methylene groups have been shown to be potent iron and

aluminum chelators.[30]

➢ Kojic acid as anti-diabetic agent:

The combination of KA and vanadium enhances its efficacy and safety as an anti-diabetic agent. The effect of KA derivative on streptozocin induced diabetic rats effectively reduced blood glucose levels indicating that it can be used as an effective and safe anti diabetic agent.[31]

➢ Kojic acid as anti-leishmanial agent:

It is well known that chemotherapy is the only effective treatment for Leishmaniasis infections, however, the anti-leishmanial drugs available are, in general, toxic expensive and require long-term treatment.

KA acted as an anti-leishmanial agent without affecting the host cell. KA treatment decreased the growth of *L. amazonensis promastigotes* by 62% at 50 mg/mL (IC50: 34 mg/mL). Moreover, KA was more effective against intracellular amastigotes, with a growth inhibition of 79% for 50 mg/mL of KA after 72 h of treatment (IC50: 27.84 mg/mL).[32]

➢ Kojic acid as a pancreatic lipase inhibitor:

Sarah Mohamed El-Korany, et al., reported that the kojic acid is a potent inhibitor of pancreatic lipase enzyme.[33] Pancreatic lipase (PL) is the key enzyme in lipid metabolism. Its inhibition alters the absorption of the ingested triglycerides and is thus considered one of the major targets in obesity management.[34]

Docking studies revealed the ability of kojic acid to interact with the key amino acids in the binding site of PL similarly to the well-known standard inhibitor (orlistat) and this supports its activity. Ruilin and coworkers used the same docking approach to suggest the PL inhibitory effect of the decapeptide PP1 (Leu-Leu-ValVal-Try-Pro-Trp-Thr-Gln-Arg) using orlistat and simvastatin as positive controls.[35] Hence there is an increasing need to document scientific data by conducting various animal experimentation on emerging new molecule kojic acid to discover its new pharmacological potentials.

The applications of Kojic acid in various fields are summarized in the below table.

Table 1 : Applications of Kojic acid

Field	Applications	References
In the field of medicine	• Anti-inflammatory • Anti-neoplastic • Anti-diabetic • Anti-oxidant • Anti-fungal • Anti-microbial • Anti-bacterial • Anti-leishmanial • PL inhibitor	[16, 17, 18] [19, 20] [31] [24, 25] [26, 27, 1] [28, 29] [28,29] [32] [33, 34, 35]
In the field of Cosmetics	• Skin whitening agent • UV protectant • Tyrosinase inhibitor • Radio-protective • Anti-oxidant	[5, 15] [5, 15] [5,15] [23] [12]
In food industries	• Anti-oxidant • Preservative • Anti-seed agent	[21] [6, 22]
In the field of agriculture	• Chelating agent • Insecticide activator	[30]

LIMITATIONS OF KA

Though Kojic acid is a trending molecule due to most of its tremendous applications in the various fields, it also contains few of the drawbacks like Cytotoxicity and storage instability, which can be overcome by producing safer derivatives of KA by conducting various studies and adopting vigorous screening methods to test the efficacy and safety of newly derived moieties.[36]

CONCLUSION

Kojic acid is extracted from over 58 different strains of microbes and is mainly used as a skin-lightening agent in cosmetics. This article reveals that KA and it's derivatives are having widespread applications in various fields including medical, food, agriculture, cosmetics etc. The molecule is having certain drawbacks which can be overcome by subsequent derivatization to produce new drugs with high efficacy and safety. This article is presented in a view to open a new window for the researchers in the field of research to carry out further research regarding new derivatives of kojic acid and to present the world with new safer and effective drugs.

REFERENCES

1. Saeedia M, Masoumeh E, Khadijeh K. Kojic acid applications in cosmetic and pharmaceutical preparations. Biomed Pharmacother. 2019;110:582 593.
2. Beélik, A. Advances in carbohydrate chemistry. Elsevier. 1956;11:145-183.
3. Brtko J, Rondahl L, Fickova M, Hudecova D, Eybl V, Uher M. Kojic acid and its derivatives: history and present state of art. Cent Eur J Public Health. 2004;12:16-7.
4. Yabuta T. The constitution of kojic acid: A d-pyrone derivative formed by Aspergillus flavus from carbohydrates. J Chem Soc Trans. 1924;125:575- 587.
5. Ohyama, Y. Melanogenesis-inhibitory effect of kojic acid and its action mechanism. Fragrance J. 1990;6:53-58.
6. Uchino K, Nagawa M, Tonosaki Y, Oda M, Fukuchi A. Kojic acid as an anti-spec agent. Agric Biol Chem. 1988;52(10):2609-2670.
7. Saito, K. Über die Säurebildung bei Aspergillus Oryzœ Shokubutsugaku Zasshi. Bot Mag.1907;21:7-11.
8. Crueger W, Crueger A. A Textbook of Industrial Microbiology. 2nd ed. London: Sinauer Associates Inc; 1984.
9. Nakajima N, Ishihara K, Hamada H. Functional glucosylation of kojic acid and daidzein with the Eucalyptus membrane-associated UDP-glucosyltransferase reaction system. J Biosci Bioeng. 2001;92:469471.
10. Chaudhary J, Pathak A. N, & Lakhawat, S. Production Technology and Applications of Kojic Acid. Annu Res Rev Bio. 2014;4(21):3165-3196.
11. Pildain MB, Frisvad JC, Vaamonde G, Cabral D, Varga J, Samson RA. Two novel aflatoxin-producing Aspergillus species from Argentinean peanuts. Int J Syst Evol Microbiol. 2008;58(3):725-35.
12. Masse MO, Duvallet V, Borremans M, Goeyens L. Identification and quantitative analysis of kojic acid and arbutine in skin-whitening cosmetics. Int J Cosmet Sci. 2001;23(4):219-32.
13. Agha N, Betina V, Kutkova M, Nemec P. Kojic acid from Penicillium simplicissimum (Oud.) Thom Biologia. 1968;23(6):466-7.
14. El-Aziz ABA. Improvement of kojic acid production by a mutant strain of Aspergillus Flavus. J Nat Sci Res. 2013;3(4):31-41.
15. Noh, J.-M. et al. Kojic acidamino acid conjugates as tyrosinase inhibitors. Bioorganic Med Chem Lett. 2009;19:5586-5589.
16. Lee M, Rho HS, Choi K. Anti-inflammatory effects of a P-coumaric acid and kojic acid derivative in LPS-stimulated RAW2647 macrophage cells. Biotechnol Bioprocess Eng. 2019;24(4):653-7.
17. Zhang J, Chen H, Chen M, Wang H, Wang Q, Song X, et al. Kojic acid-mediated damage responses induce mycelial regeneration in the basidiomycete Hypsizygus marmoreus. PloS one. 2017;12(11):187-351.
18. Li TX, Liang JX, Liu LL, Shi FC, Jia XW, Li MH, et al. Novel kojic acid derivatives with anti-inflammatory effects from Aspergillus versicolor. Fitoterapia. 2021;154:105027.
19. Bransová, J., Brtko, J., Uher, M., Novotný, L. Antileukemic activity of 4- pyranone derivatives. Int J Biochem Cell Biol. 1995;27:701-706.
20. . Bransova, J., Uher, M. & Brtko, J. Regulation of selected biological processes in neoplastic cell lines by halogen derivatives of 5- hydroxy-2-hydroxymethyl-4- pyranone. Anticancer Res. 1998;18:4423-4428.
21. Son, S., Moon, K. & Lee, C. Inhibitory effects of various antibrowning agents on apple slices. Food Chem. 2001;73:23-30.
22. Kaatz, H., Streffer, K., Wollenberger, U. & Peter, M. G. Inhibition of mushroom tyrosinase by kojic acid octanoates. Zeitschrift für Naturforschung. 1999;54:70-74
23. Hosseinimehr, S. J., Emami, S., Zakaryaee, V . , Ahmadi , A . & Moslemi, D . Radioprotective effects of kojic acid against mortality induced by gamma irradiation in mice. Saudi Med J. 2009;30:490-493.
24. Niwa, Y. & Akamatsu, H. Kojic acid scavenges free radicals while potentiating leukocyte functions including free radical generation. Inflammation. 1991;15:303-315.
25. Lajis AF, Hamid M, Ariff AB. Depigmenting effect of kojic acid esters in hyperpigmented B16F1 melanoma cells. J Biomed Biotech. 2012;21:56-97.
26. Jimbow, K. & Minamitsuji, Y. Topical therapies for melasma and disorders of hyperpigmentation. Dermatol Ther. 2001;14:35-45.
27. Emami, S. et al. Mannich bases of 7- piperazinylquinolones and kojic acid derivatives: Synthesis, in vitro antibacterial activity and in silico study. Eur J Med Chem. 2013;68:185-191.
28. Aytemir, D.M.; Karakay, G. Kojic Acid Derivatives. Med. Chem. Drug Des. 2012;36:127.
29. Nurunnabi TR, Al-Majmaie S, Nakouti I, Nahar L, Rahman

SM, Sohrab MH, et al. Antimicrobial activity of kojic acid from endophytic fungus Colletotrichum gloeosporioides isolated from Sonneratia apetala, a mangrove plant of the Sundarbans. Asian Pac J Trop Med. 2018;11(5):350.

30. Nurchi, V. M. et al. Kojic acid derivatives as powerful chelators for iron (III) and aluminium (III). Dalton Trans. 2011;40:5984-5998.

31. Wei, Y., Zhang, C., Zhao, P., Yang, X. & Wang, K. A new salicylic acid-derivatized kojic acid vanadyl complex: synthesis, characterization and anti-diabetic therapeutic potential. J Inorg Biochem. 2011; 105: 1081-1085.

32. Rodrigues APD, Farias LHS, Carvalho ASC, Santos AS, do Nascimento JLM, et al. (2014) A Novel Function for Kojic Acid, a Secondary Metabolite from Aspergillus Fungi, as Antileishmanial Agent. PLOS ONE. 2014;9(3):91-259.

33. El-Korany SM, Helmy OM, El-Halawany AM, Ragab YE, Zedan HH. Kojic acid repurposing as a pancreatic lipase inhibitor and the optimization of its production from a local Aspergillus oryzae soil isolate. BMC 11 Biotechnol. 2020;20(1):1-6.

34. Gupta M, Saxena S, Goyal D. Lipase inhibitory activity of endophytic fungal species of Aegle marmelos: a bioresource for potential pancreatic lipase inhibitors. Symbiosis. 2014;64(3):149-57.

35. Zhang R, Chen J, Mao X, Qi P, Zhang X. Separation and lipid inhibition effects of a novel Decapeptide from Chlorella pyenoidose. Molecules. 2019;24(19):3527.

36. Bashir F, Sultana K, Khalid M, Rabia H. Kojic acid: a comprehensive review. Asian J Allied Health Sci. 2021;6(1):17.

Cite this article : Soujanya N, Suresha B S, Balasubramanian T, Rakshitha S, Meghana B P
Review on Kojic Acid - a secondary Metabolite from *Aspergillus* Fungi
Asian J. Pharm. Hea. Sci.. 2022;12(1):2707-2712. DOI : 10.5530/ajphs.2022.12.20