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Secondary Metabolites and Bioactivity of the Monascus Pigments Review Article

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Abstract: *Monascus* pigments have many applications such as coloring agents in foodstuffs, texture industries, pharmacology, medicine and cosmetics. Globule production and annual consumption of the pigment around the world were reported. Thirteen *Monascus* species were reported as pigment producers. All the recent information's about *Monascus* bioactive metabolites were reported and the pigments also are classified into four categories including: yellow, orange, red and colorless metabolites. Also, bioactivities of their metabolites were classified into: antibacterial, antioxidant and anticancer, anti-cholesterols and anti-cardiovascular disease, human health supporting agents and immune enhancer metabolites. Pigment production are maximizing and improving their productivity by selection of the good producing strains, genetic and metabolic engineering.

Key words: Monascus pigments · Bioactive metabolites · Regulation of pigment production

INTRODUCTION

Monascus pigments have many applications such as coloring agents in foodstuffs and texture industries[1], pharmacology, medicine [1-7] and cosmetics [8]. Globule production and annual consumption of *Monascus* pigments in Japan increased from 100 tons in 1981 to 600 tons in 1992 and was valued at $12 million according to a survey published in 1992 [7,8]. Numerous investigators recorded that the *Monascus* pigments were produced on commercial scales by many *Monascus* species specially *M. anka* [7-10], *M. pilosus* [11,12], *M. purpureus* [4,12]. and *M. ruber* [3].

In ancient times, *Monascus* pigments were produced by primitive, rudimentary, simple methods and identified visually. Recently, there are sophisticated and more advanced methods for production, detection, identification and determination of the bioactive metabolites in the pigments for good quality, high yield, color, stability and safety [1,7,13].

Monascus azaphilones polyketide pigmented metabolites were classified on two bases including the color and their bioactivity. I: They were classified into four categories based on color including yellow, orange, red and colorless metabolites [1,14-16]. II: They were classified into eight categories based on their bioactivity including aroma & flavor compounds, antibacterial, anticancer, anti-cardiovascular disease agents and anti-cholesterols specially moncolin K which also acts as an anti-(colon cancer, microbial and oxidant), immune enhancer, liver protector: spleen strengthener, digestion promoter, eliminator of dampness and phlegm, removes blood stasis and increases insulin production [17-25].

To improve the pigment yield as well as to achieve highest standards of quality, health and safety regulation of the methods of production must be done on three levels. Selection of the *Monascus* pigments producers strains [26]. Improvement of strain productivity could be achieved by genetic engineering[27] and metabolic engineering [27,28].

This review is designed to summarize all the available and recent publication on the pigmented bioactive *Monascus* metabolites formed by fermentation on different substrates. It also explains how to improve pigment productivity with high standard of quality and bioactivity.

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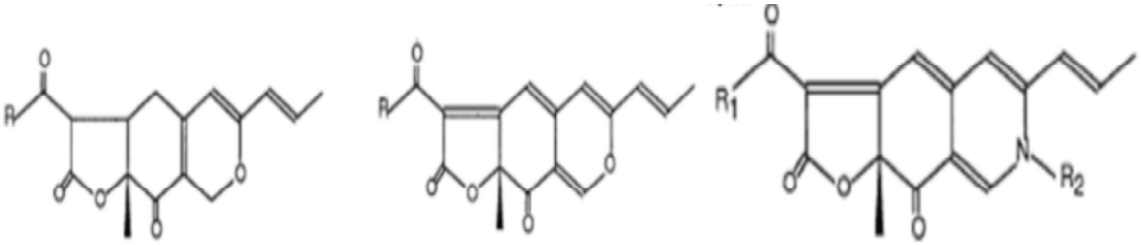


Yellow (reduced form) Orange (oxidized form) Red (oxidized form containes N)

Fig. 1: Relationship between major azaphilones pigments from Monascus (7).

Application of *Monascus* Pigments: *Monascus* natural fermented pigments have high economic value around the world and attracted worldwide attention as a coloring agent, they have many advantages such as easily production on non expensive substrates, good solubility in water and ethanol, numerous bioactive metabolites and completely safe when produced under specific conditions. Researchers try to replace synthetic food colorant by *Monascus* natural pigments [1,7,17]. They are applied as a natural coloring agent in foodstuffs and texture industries [1], they also have aroma & flavor enhancer and used as condiment [2,4,20,29]. In pharmacology and medicine *Monascus* pigments have wide uses in prevention and treatment of numerous human diseases [5,6] and also they are used in cosmetics [7].

Pigment-producing *Monascus* Species: Many *Monascus* species were used for pigment production with several bioactive metabolites including *M. anka* [7,9], *M. bakeri* [9], *M. floridanus* [15], *M. kaoliang* [17,29], *M. lunisporas* [30], *M. major* [9] *M. pilosus* [11,12], *M. pubigerus* [12], *M. purpureus* [4,14], *M. ruber* [3]. *M. rubiginosus* [9], *M. rubropunctatus* [9] and *M. vitreus* [12].

Detection and Identification of *Monascus* Pigments: The pigment composition was identified, determined and confirmed by many recent advanced methods and instruments such as Thin Layer Chromatography (TLC), Spectrophotometer by using the absorption maxima exhibited around 400, 470 and 500 nm by the yellow, orange and red metabolites, respectively [14]. High Performance Liquid Chromatography (HPLC), Gas chromatography/Mass Spectrophotometer (GC/MS), Dionex methods, Nuclear Magnetic Resonance (NMR), Spectroscopy Mass Spectrometry (SMS), Ultra Violet Spectroscopy (UVS) and Polarography and Thin-Layer Voltammetry (PTLV) [1,7,13,16,25]. Irradiation of wild *Monascus* strains by UV light, neutron or X-rays, using mutations induced by chemical and physical mutagens especially by further UV irradiation [27].

Bioactive *Monascus* Metabolites: The colour and the bioactivity of the azaphilones polyketide *Monascus* metabolites are are classified into four categories [31] including yellow, orange, red pigment and colorless metabolites [1,7,14-16] (Figure 1).

Yellow Pigment: All the reduced forms of the azaphilones hexaketides pigment including ankaflavin; monascin C; monascidin A (citrinin); monascorubrin; rubropunctatin; yellow II and xanthomonascin A [27,32,33] (Figure 2). Cheng *et al* [34] investigated that the 95% ethanol extract of red mold rice fermented with the yellow mutant of the *M. pilosus* strain produced many bioactive metabolites such as monascuspyrone, 6-(2-hydroxydodecan-2-yl)-3-(hydroxyl methyl)-4-methoxy-2*H*-pyran-2-one, monascin, ankaflavin, monasfluore B, 3-*epi*-betulinic acid, 3-*epi*-betulinic acid acetate, á-tocospiro B, methyl isovanillate, *p*-dihydro coumaric acid, methylparaben and one new pyran-2-one derivative also isolated (Figure 2 & 3). Monascidin A (citrinin) is a yellow pigment produced by *Monascus* under anaerobic condition. Pigment production must be occurred under specific nutritional and environmental condition for prevention the mycotoxins yield or occurs by non toxigenic *Monascus* strains [6,26,34-38] (Table 1). Recently Radu *et al* [13] recorded that the novel yellow pigments have been discovered and including monasnicotinate A-D (Figure 4 & 5).

Orange Pigment: Color intensity of the orange pigment is detected by spectrophotometer at 470 nm [14]. It produced by *M. purpureus* and *M. ruber*. Only rubropunctatin is also produced by *M. rubropunctatus & M. kaoliang*. Bioactivity of the orange pigments of phosphoindositides comprises cold

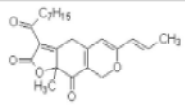
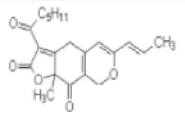
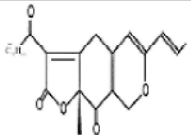
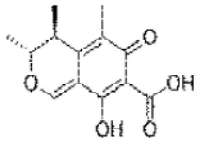
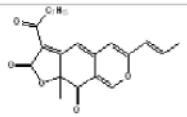
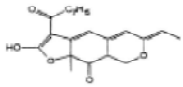
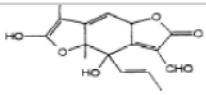
	Ankaflavin (E)-9a-methyl-3-octanoyl -6-(prop-1-enyl)-3a,4-di hydro-3H-furo [3,2-9]isochromene-2,9 (8H, 9aH)-dione $C_{21}H_{26}O_5$ R=C_7H_{15}
	Monascin C (Benzopyran, 2,9-(3H)–dione,(3S)-3axX-4, 8,9a-tetrahydro-9aB-methyl-3-(1-oxohyexyl)-6-[(E)-1-propentyl] -2H-faro[3,2-9][2]) $C_{21}H_{26}O_5$ R=C_5H_{11}
	Monascorubrin (E)-3-hexanoyl-9a-methyl-6-(prop-1-enyl)-9aH-furo [3,2-9] isochromene-2,9-dione $C_{21}H_{26}O_5$ R=C_5H_{11}
	Monascidin A (citrinin or antimycin) toxic (3R,4S)-8-hydroxy-3,4,5-trimethyl-6-oxo-4,6-dihydro-3H-isochromene-7-carboxylic acid (3H-2-benzo-pyran-7-carboxylic acid -4,6,-dihydro-8-hydroxy-3, 4,5-trimethyl-6-oxo- $C_{13}H_{14}O_5$
	Rubropunctatin (E)-9a-methyl-3-octanoyl-6-(prop -1-enyl)-9aH-furo[3,2-9]isochromene-2,9-dione $C_{21}H_{26}O_5$ R=C_7H_{15}
	Yellow II (E)-9a-methyl-3-octanoyl-6-(prop-1-enyl)-9aH-furo[3,2-9]iso chromene-2,9-dione $C_{21}H_{26}O_5$ R=C_7H_5
	Xanthomonascin A (E)-9a-methyl-3-octanoyl-6-(prop-1-enyl)-9aH-furo[3,2-9] isochromene-2,9-dione $C_{21}H_{26}O_5$ R=C_5H_{11}

Fig. 2: Chemical structure, common and IUPAC names, and molecular formula, of yellow pigmented metabolites (27,32-34).

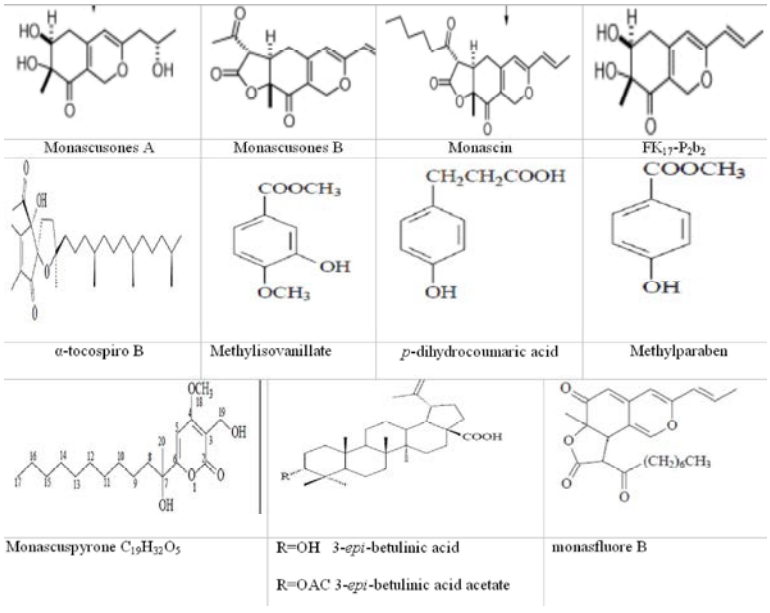


Fig. 3: Secondary metabolites isolated from M. pilosus yellow mutant strain (32).

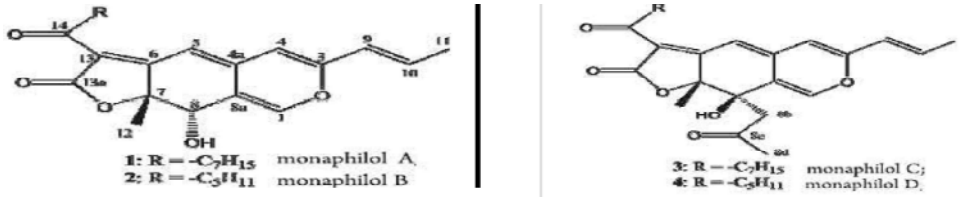
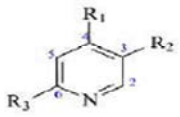


Fig. 4: Chemical structure, chemical formula and common name of monaphiloles A-D (13).



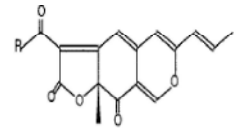
1 R_1 = CH=C($COCH_3$)$CH_2CO(CH_2)_4CH_3$, R_2 = $COOCH_3$, R_3 = (E)CH=$CHCH_3$

2 R_1 = CH=C($COCH_3$)$CH_2CO(CH_2)_4CH_3$, R_2 = $COOC_2H_5$, R_3 = (E)CH=$CHCH_3$

3 R_1 = CH=C($COCH_3$)$CH_2CO(CH_2)_6CH_3$, R_2 = $COOCH_3$, R_3 = (E)CH=$CHCH_3$

4 R_1 = CH=C($COCH_3$)$CH_2CO(CH_2)_4CH_3$, R_2 = $COOCH_3$, R_3 = *n*-C_3H_7

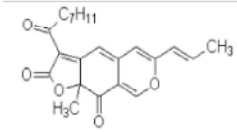
Fig. 5: Chemical structure of monaphiloles A-D (13).



Common name: Main nucleus

IUPAC name: (E)-3-hexanoyl-9a-methyl -6-(prop-1-enyl)-9aH-furo [3,2-9] isochromene-2,9 dione

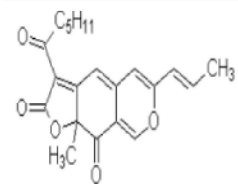
$C_{23}H_{26}O_5$ (R)



Monascorubrin

(E)-3-hexanoyl-9a-methyl -6-(prop-1-enyl)-9aH-furo [3,2-9]-isochromene-2,9 dione

$C_{23}H_{26}O_5$ R=C_7H_{11}



Rubropunctatin

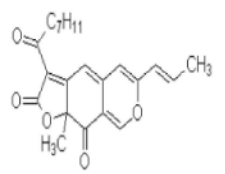
(R)-9a-Methyl-3-(1-oxohexyl)-6-[(E)-1-propenyl]-2H-furo[3,2-9][2]benzopyran-2,9(9aH)-dione

$C_{21}H_{22}O_5$ R=C_7H_{15}

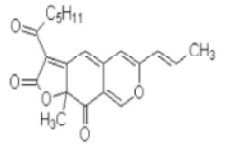
Fig. 6: Chemical structure, common and IUPAC names, and molecular formula, of orange pigmented metabolites (14,39-41).

prevention toothpaste, sand prevention and plugging agent. Monascorubin and rubropunctatin are produced in the cell-bound state; insoluble in water and synthesized in the cytosol from ACoA by the multi-enzyme complex of polyketide synthase I and possess aminophiles structure responsible for the color. They are sensitive to heat, unstable at pH 2-10 and fade with exposure to light. When orange pigments react with amino group containing compounds in the medium (proteins, amino acids and nucleic acids) they are converted to water-soluble red pigments [39-41] (Figure 6). Another four orange azaphylones compounds, named monaphilol A-D (Figure 5), which inhibit against human laryngeal carcinoma and human colon adenocarinoma, they also have antioxidant activity, they are soluble in ethanol and give two absorption bands at 374 and 508 nm. The antioxidant activity found around 90% is attributed to a good stability to the oxidative processes. Also concanavalin A is recorded as an orange pigment having a stimulatory effect for proliferation of mouse splenocytes and human peripheral blood cells and exhibit toxic and teratogenic effects on chicken embryos [13].

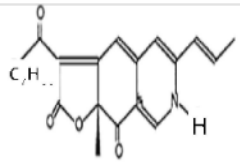
Red Pigment: Monascorubramine and rubropunctamine are formed from the oxidation of orange pigment, they react with amino acids, amino polysaccharides and amino



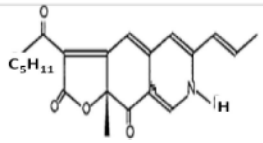
Monascorubrin insoluble in water
(E)-3-hexanoyl-9a-methyl -6-(prop-1-enyl)-9aH-furo [3,2-9]-isochromene-2,9 dione
$C_{23}H_{26}O_5$ R=C_7H_{11}



Rubropunctatin insoluble in water
(R)-9a-Methyl-3-(1-oxohexyl)-6-[(E)-1-propenyl]-2H-furo[3,2-9][2]benzopyran-2,9(9aH)-dione
$C_{21}H_{22}O_5$ R=C_7H_{15}



Monascorubramine soluble in water
(E)-3-hexanoyl-9a-methyl-6-(prop-1-enyl)furo[3,2-g]isoquinoline-2,9(7H,9aH)-dione
$C_{23}H_{27}NO_4$ R_1=C_7H_{11} R_2=H



Rubropunctamine soluble in water
(E)-9a-methyl-3-octanoyl-6-(prop-1-enyl)-9aH-furo[3,2-9]isoquinoline-2,9-dione
$C_{23}H_{27}NO_4$ R_1=C_5H_{11} R_2=H

Fig. 7: Chemical structure, common and IUPAC names, and molecular formula, of red pigmented metabolites (9,10).

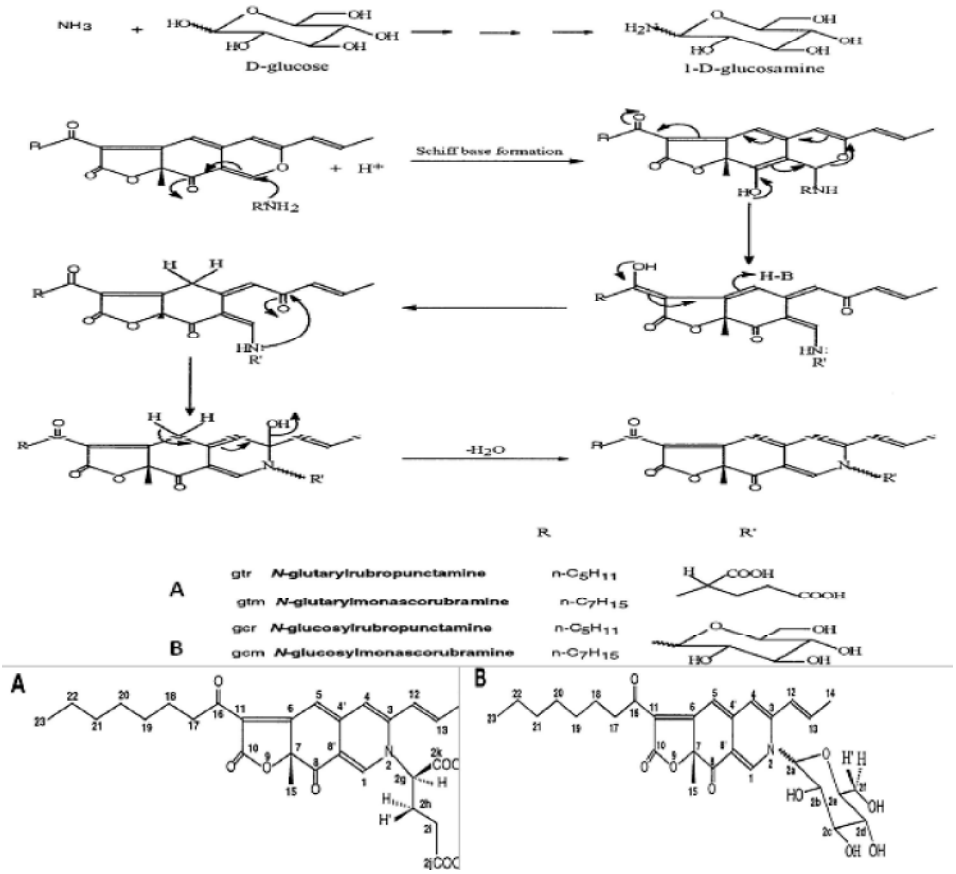


Fig. 8: Biosynthesis of the red pigments and chemical structure, chemical formula, common and IUPAC names (45-48).

alcohols [9-10]; converted to a deep red color formed as the *Monascus* culture ages; they are extracellular; safe (non toxic); with high stability against pH; light, high temperature over 70°C [17,18,22], while they are converted to a blackish color when exposed to 100°C for 15 min, they are highly soluble in water and alcohol and have good stability in Ca, Mg, Fe, Cu and other metal ions[4]. Submerged fermentation medium with Na-glutamate promotes better production of extracellular red pigments [39-44] most of them being a complex of pigments and glutamic acid[44]. Aqueous red extract is degraded fastly in a few days but extract with organic solvent especially *N*-butanol is stable against daylight for several months [44,45] (Figure 1 & 7). *Monascus ruber* produced water-soluble red pigments in a submerged culture when grown on glucose and monosodium glutamate includes N-glutarylrubropunctamine, N-glutarylmon ascorubramine; N-glucosylrubropunctamine and N-glucosylmonascorubramine [45] (Figure 8). Monacolins, γ-aminobutyric acid and dimerumic acid act as hydroxymethylglutaryl CoA (HMG-CoA) reductase inhibitors [46]. Monascorublysine is a pigment-protein complex formed during fungal digestion [47]. Monacolin K and their derivatives have different names includes lovastatin, mevinolin, mevacor, $MB_{530}B$, MK_{803} or MSD_{803} [1]. Monacolin K derivatives vary in composition of the C_4 side chain and includes monacolins J, X and M or lack this chain (monacolin L, dihydromonacolin L and compacting derivative ML-236C. Monacolin L is the precursor of monacolin J which, can be converted to monacolin K [1,47-55]. Compactin is referred to 6-demethylmevinolin, mevastatin, $ML_{236}B$, CS_{500}). All mevinolin-producing strains were inferior in red pigment formation [1] (Figure 9). *Monascus pilosus* grow on rice in the presence of 1% of collagen gave powerful antioxidant effect, in which the quenching ratio is 95% in comparison with luminol, used as witness. It also has a potential cicatrisation effect, probably due to presence of glucosamine compound which acts as a cicatrisation factor. Monascusone A was obtained by their reaction with amino acids. Monascotinates are other types of compounds identified from ethyl acetate extracts [13] (Figure 3-5).

Monascus polyketides metabolites are derived from acetate malonate pathway [1,31], it begins with the condensation of one acetyl-CoA and three malonyl-CoA molecules, followed by a series of reactions producing the orange-colored molecules in the cytosol and oxidation of orange-coloured molecules yields the yellow-colored pigments [3,36,37] (Figure 10). Monacolin have 14 kinds include monacolins K, J, L, M and X with their corresponding hydroxyl acid forms, as well as dehydromonacolin K, dihydromonacolin L, compactin and 3-hydroxy-3,5-dihydromonacolin L [1,4,30,42]. They are used in the treatment of cardiovascular diseases and anti hypertensive [51,52]. Monacolin K is produced by the same metabolic pathway as a mixture of a lactone and a free hydroxy acid (Figure 9).

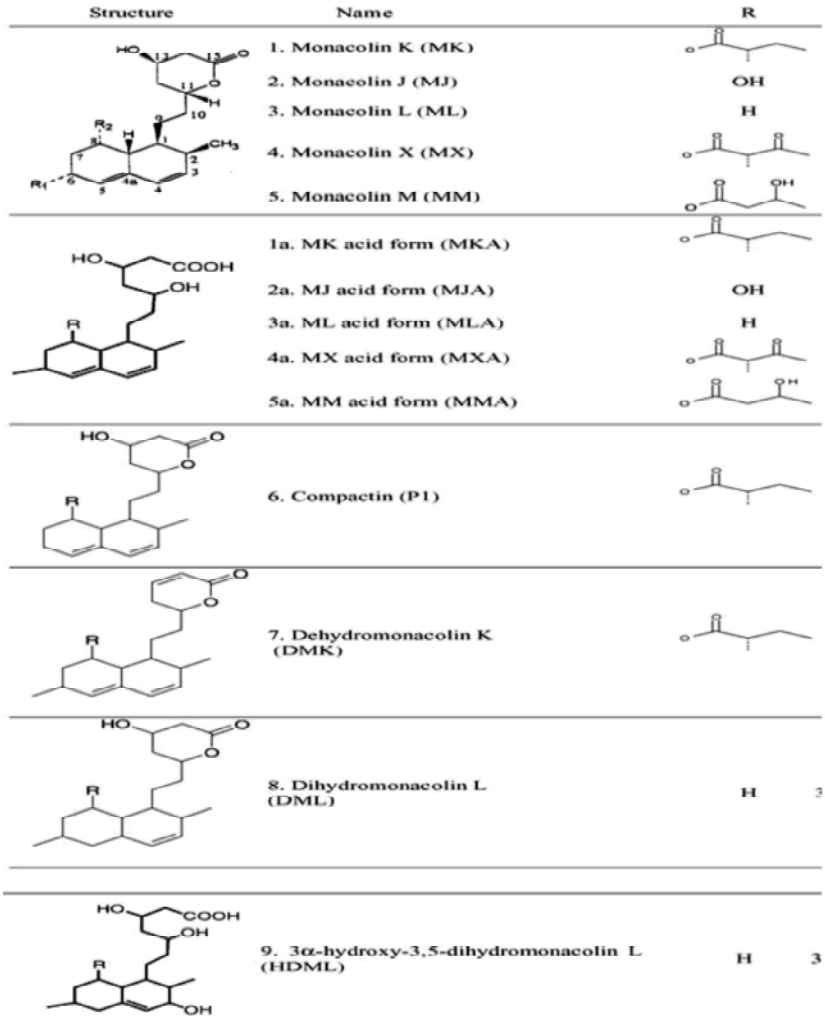
Colorless metabolites: Ankalactone (3-unsaturated-7-1actone derivative) is produced on glucose-peptone medium for 7 days [1]. Monascopyridine A-D are produced by *M. purpureus* and *M. ruber* [56].

Therapeutic activity of *Monascus* red pigment depends on the presence of some bioactive metabolites like monascopyridines, xanthomonasin, monascumic acid, ascorbic acid and polyphenol [13]. Also moncolin K acts as anti- (cardiovascular diseases, cholesterol, colon cancer, microbial & oxidant), immune enhancer, liver protector, spleen strengthener, promoter of digestion, eliminator of dampness and phlegm, remove blood stasis and increases insulin production against high level of blood glucose [17-22].

Classification of the *Monascus* Metabolites: it Classified into Eight Categories Including:

Anticancer Agents: *Monascus* pigments have numerous metabolites used in the treatment and prevention of many kinds of human cancer such as ergosterol, ergothioneine, essential fatty acids, eicosanoids, â-glucan, glycoproteins, lectins, monacolin K, pyran derivatives, phenols and triterpenoids. Essential fatty acids act against aromatase an enzyme, used in estrogen production which leads to the development of breast cancer [24]. Eighty-eight metabolites including 25 derivatives of butyric acid, 19 other fatty acids & their derivatives, 22 pyran & their derivatives and other 22 metabolites were detected. Pyran, fatty acids specially butyric, linoleic acids and their derivatives are act as anti-breast, anti-colon and anti-prostate cancer[25]. Flavonoid acts as anti-lung cancer agent [4]. Monacolin K can suppress tumor growth *in vivo* owing to its capability in inhibiting the synthesis of isoprenoid compounds such as dolichol, ubiquinone and isopentenyl-tRNA [1,6].

Anti-cardiovascular Diseases: *Monascus* pigment metabolites including flavonoid [4], monacolins, fatty acids, trepenoids and pyran derivative are reported to have anti-cardiovascular disease [1,13].



Monacoline L= 2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl)-4-hydroxy-tetrahydropyran-2-one
Monacoline K=4-hydroxy-6-oxo-tetrahydro-2H-pyran-2-yl)ethyl)-3,7-dimethyl-1,2,3, 7,8,8a-hexahydronaphthalen-1-yl) 2-methylbutanoate
Monacoline J= 4-hydroxy-6-(2-((1S,2S,6R,8S-8aR)-8-hydroxy-1-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl) -4-hydroxy-tetrahydropyran-2-one
Compactin ML-236B= 2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl)-4-hydroxy-tetrahydropyran-2-one

Fig. 9: Monacolin K, compactin and their derivatives (1,49-52)

Human Health Supporting Agents: Fatty acids act as supporter of dermal integration, anti-inflammatory and skin moisturizing agent, enhance the renal function and therefore used in the treatment of arthritis, lupus and asthma. They prevent many diseases such as rheumatoid arthritis. ?-aminobutyric acid or carbamate act as antihypertensive, muscle relaxant, immunity system activator and anti-lipid per-oxidation and supports healthy liver function. Dimerumic acid prevent lipid per oxidation. In general fatty acids support healthy liver function [1-5,24,25,34]. Ergosterol terpenoids: Acts as a precursor of vitamin D_2 which act as antifungal, antitumor, an irritant to the skin, eyes and the respiratory tract[25]. Flavonoid: acts as anti-allergic, anti-inflammatory and anti-diarrhea agent [4].

Immune Enhancer Metabolites: Glycoproteins, polysaccharides, sterols and fatty acids (specially oleic, palmitic, stearic, γ-linolenic and arachidonic acid) are recorded as immune enhancer agents [24].

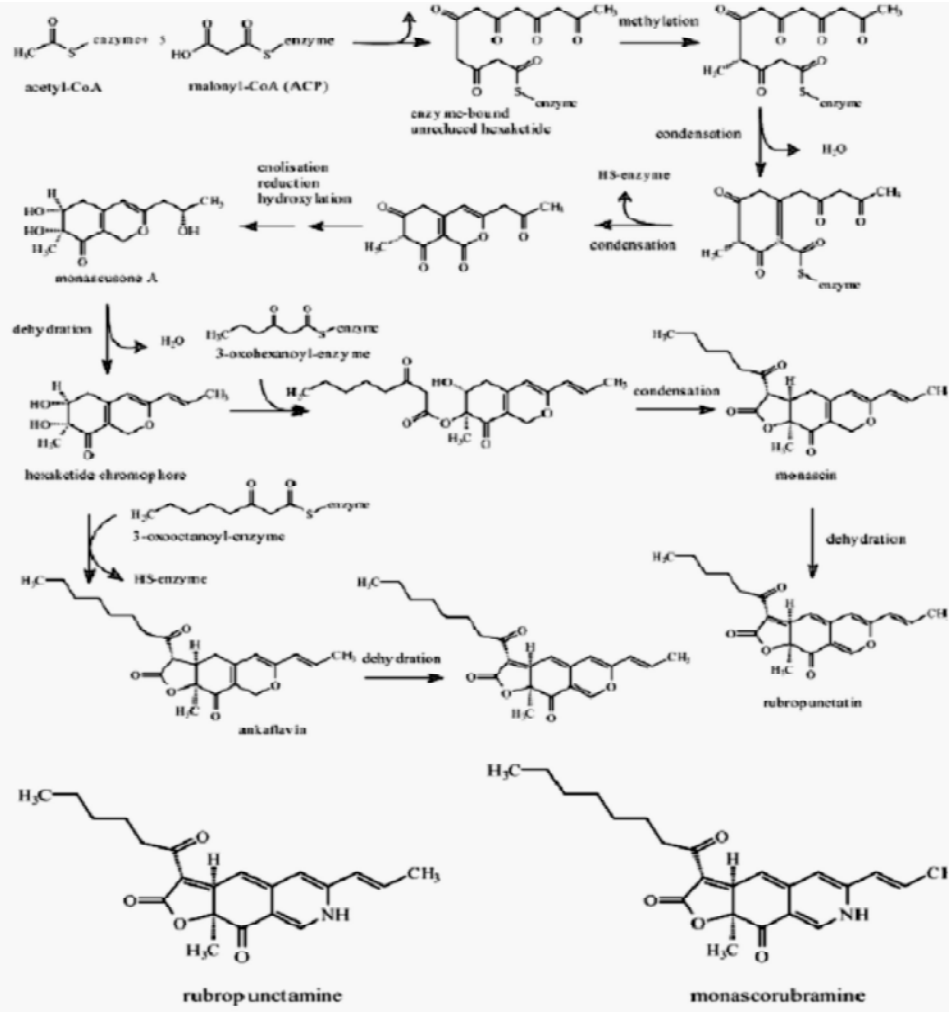


Fig. 10: Monascus azaphylone pigment biosynthetic pathway (3,36,37).

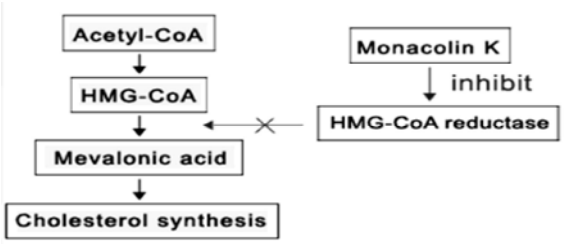


Fig. 11: Anticholesterols activity of Monascus metabolites occurs by inhibition mechanism of the hydroxy methylglutaryl CoA (HMG CoA) reductase enzyme and prevent the biosynthesis of the cholesterols (17-22).

Aroma and Flavor Compounds: Including fatty acids (specially butanoic acid), alcohols, benzaldehydes, esters, lactones, phenol, terpenoid, thiols and mercapto compounds. Forty two aroma compounds were recorded including 4 alcohols, 2 benzaldehydes, 27 esters, 3 lactones, 1 phenol, 1 terpenoid, 2 thiols and 1 mercapto compounds[25]. Esters give different fruity fragrance compounds such as methyl butyrate (apple and pineapple fragrance), ethyl butyrate (orange and pineapple), pentyl butyrate (pear) and pentyl butanoate (apricot). The application of these metabolites gives desirable fruit fragrances in food, perfume, cosmetic and pharmaceutical industries. They are used as defoaming agents to improve shelf-life and safety of minimally processed fruits and also in folk medicine [53,56].

Antimicrobial Agents: Ankalactone, crude pigment and yellow pigment (rubropunctatin & monascorubrin) have antibacterial activity, ergosterol [25], flavonoid

Table 1: Optimum nutritional and environmental factors used for safe, high level and quality of the Monascus pigment

Carbon source g / L	Nitrogen Source	Substrates	O2 rpm	Metabolites	pH	°C	Days	References
Lactose 5; Yeast 0.5; Glucose 5	Na-glutamate	Rice powder	+	High yield of monacolin K	2-46.5	30	12	(1,2,22) (61)
		Malt Agar	+	Monascorubramine red pigment	6	30	7-10	(14)
Glycerol	Peptone	1% rice + $KHPO_4$	480	Yellow 4.132 ppm; red pigment 8.480	9			(26,38)
Glycerol	Peptone	1% rice + $MgSO_4$		Orange pigment 4.573 ppm	9			
Olive oil	Gelatin	1% wheat flour + NaCl		Citrinin reduced to 0.055 ppb	3			
Lactose 1%		Yeast extract 0.5% or MEA		Red pigment			3	(22,42)
PDA medium	$NaNO_3$	PDA + $KH_2 PO_4$		High pigment yield	6	25	7-12	
Glucose 20g/L	Na-glutamate 5g/L		+	Water-soluble red pigment				
				without citrinin		27-37		(35-38)
Glucose	Na-glutamate		300	Red pigment	6	30	7	(11)
Glucose; glycerol	Peptone, $NaNO_3$ & $ZnNO_3$	Soy bean powder, olive oil		Monacolin				(50)
Glucose 10g/L	Na-glutamate 10g/L	$MgSO_4.7H_2O$ 0.5, KH_2PO_4 5, K_2HPO_4 5, $ZnSO_4.7H_2O$ 0.01, $FeSO_4.7H_2O$ 0.01, CaCl2 0.1, $MnSO_4.H_2O$ 0.03	250	Water-soluble red pigment	6.5	30	7	(62)

[4,54] and orange pigments and have antibacterial and antifungal. Red pigment with amino-acid complexes probably lacking toxic effects [1].

Antioxidant Agents: Xanthomonasin A,B, glycylrubropunctatin, glycylmonascorubrin, laccaia acid A-C, curcumin and dimerumic acid have antioxidant and hepatoprotecter agents [13].

Anticholesterols: Monacolins, γ-aminobutyric acid (GABA), dimerumic acid are inhibited the hydroxymethylglutaryl CoA (HMG-CoA) reductase enzyme which synthesized the human cholesterols [46-57] (Figure 11).

Regulation: The regulation of the *Monascus* pigment production must occurred in three levels:- First level: Selection of the non-toxigenic producing strains. The producing strains must non toxigenic. The gene responsible for citrinin synthesis *(pksCT*, *ctnA* and *orf3)* is absent or significantly different in *M. pilosus*, *M. ruber, M. barkeri*, *M. floridanus, M. lunisporas* and *M. pallens)*. But the distribution of mycotoxin citrinin biosynthesis-related genes (acyltransferase and ketosynthase domains of the *pksCT* gene encoding citrinin polyketide synthase) were found in *M. purpureus*, *M. kaoliang* and *M. sanguineus*. Furthermore, the *ctnA* gene, a major activator of citrinin biosynthesis, is found in *M. purpureus* and *M. kaoliang*, but is absent in *M. sanguineus*. The *orf3* gene encoding oxygenase (located between *pksCT* and *ctnA)* it was also present in *M. purpureus* and *M. kaoliang*. The *pksCT* gene was highly conserved in *M. purpureus*, *M. kaoliang* and *M. sanguineus,* while the *ctnA* and *orf3* genes were shown to be highly homologous in *M. purpureus* and *M. kaoliang*. The highly conserved *citrinin* gene cluster in *M. purpureus* and *M. kaoliang* carries out citrinin biosynthesis. Phylogenetic subgroups established with the *â-tubulin* gene, the *citrinin* gene cluster can group the species of *Monascus*. A citrinin-producing phenotype was detected only in *M. purpureus* and *M. kaoliang* using HPLC [53]. The results recorded by [53] were confirmed by Moharram *et al*. [25] which showed that no citrinin produced on all the ten tested media used for the growth of the two *M. ruber* strains.

Second level: Improvement of the pigment production by genetic engineering [13].

Third Level: Metabolic engineering by controlling in the environmental and nutritional growth factors. Pigment quality with good yield, high colour degree & stability, safety and kind of bioactive metabolites depend upon the *Monascus* species; strains, nutritional and environmental conditions (Table 1). Improvement of all the environmental factors (temperature, visible light, radiations, pH, type & concentrations of media, type & size of culture vessels, agitation, internal factors, inhibitors and producing *Monascus* species [4,28]. Optimal cultivation temperature for individual *Monascus* strains varies from 25-30 °C. High temperature more than 35°C inhibited the monacolins production[1]. Good aeration in submerged cultivation at 300-480 rpm gives good yield of pigment without citrinin production [9,34]. Pigment extracted from submerged fermentation are more resistant [1,4,22]. Pigment formation is independent on visible light [1-10]. The pH of the fermentation media completely controlled the produced pigments and their amounts. Good yield of monacolin k was recorded at pH 2-4 [1,22]. pH 6 gives good yield of red pigment [14] but the pH 9 enhances the orange and yellow pigment [25].

Improvement of the Nutritional Conditions: By controlling macro-elements (C, N, H_2,O, S, Ph, K, Mg), micro-elements, Co-factors and other chemicals [59-65].

Glucose was found to be a superior substrate for ketones but cannot be utilized as a sole carbon source. Also glucose at 20g/L concentration was superior for good yield of red pigment by aerobic submerged cultures. On the other hand starch, maltose, sucrose and galactose were suitable carbon sources for pigment production, whereas lactose, fructose and xylose were inferior substrates. Nevertheless, fructose gave low pigment yields comparable to glucose. Cellulose gave negligible level of pigment but ethanol stimulated pigment production. Maltitol and glycerol are superior sources which gave poor pigment production. Fatty acids cannot be utilized as sole carbon sources [1].

Effect of the Nitrogen Source [63-65]: The best nitrogen source used for good pigment yields is NH_4Cl and peptone [9-11] but yeast extract-stimulated biomass production. It was proposed that the orange pigments entered reactions with amino acids because the pH (above 5) in cultures assimilating organic nitrogen source was favorable for this interaction. Sodium nitrate supported the spoors production, limited growth and gave intermediate pigment yields; the use of ammonium chloride resulted in a repression of condition and the sexual cycle and led to the best pigment yields. In this medium the dramatic pH decrease impaired the pigment-amine interactions giving the origin to red pigments. Monascorubtamine is produced by Na-glutamate but ammonium glutamate gaves high red pigment yields [1]. Glucose and Na-glutamate stimulate pigment production by *M. pilosus* by batch cultivation at pH 6, 30°C for 7 days [10,11-14].

Effect of Other Medium Components: Methanproline and azetidinecarboxylic acid (nonprotein amino acids) increased pigment production. Addition of a crystallization inducer, poly(oxyethylene) sorbitane esters of palmitic acid (Tween), to the cultivation medium resulted in the production of extracellular pigments. The only trace element which was reported to support growth and pigment production by *Monascus* species was zinc. This effect could be due to the participation of zinc in the uptake and utilization of carbon sources. Addition of individual amino acids influenced neither growth nor pigment production. All protein amino acids except lysine stimulated growth. Pigment production was also increased by the addition of nonprotein amino acids, especially methanproline and azetidinecarboxylic acid. Leucine, valine, lysine and methionine amino acids had strong negative effects on the formation of hydrophilic red pigments, pigments containing an amino acid side-chain [1].

Effect of Substrates and Cultivation Methods on the Pigment Production: Moharram *et al* [25]. recorded that the change in substrate gives high variation in colour intensity and the metabolic profile of the pigments. Starchy grains were good substrates for pigment production. Heamano and Kilikian [3] evaluated the production of red pigments by *M. ruber* using complex culture medium (glucose or sucrose, corn steep liquor and monosodium glutamate) and found that the complex medium gave higher pigment yield than the semi-synthetic medium. Oat, wheat, barley and corn [58], rice, bread, bran meats, soy bean and several cereal substrates were used as a *M. purpureus* cultures [2,3,36,37,42,46].

Solid State Cultivation: Traditional manufacture of red rice could be achieved by the following steps the rice is washed, soaked in water for 24 h, drained, steamed, sterilized, fermented and dried. Manufacturing process must occur at substrate water humidity of 25-30%, but some substrates need 50% w/w, O_2(0.5 atm) and CO_2 (0.02 atm) and solid state cultivation resulted in a higher pigment yield than cultivation in shaken flasks. In solid state culture, pigments were released into grains while during submerged cultivation were accumulated in the mycelium. Better pigment yields by solid state cultures were probably not caused by the extractive effect of rice grains because addition of sorbent particles into submerged cultures did not result in an increase of pigment production [55]. Industrial by-products (sugar cane molasses, corn steep liquor and cheese whey) are rich by amino acids, ash and vitamins [3]. Moharram *et al* [25] studied the production of the *M. ruber* strains red pigments on ten kinds of media [liquid & solid Malt, grains & seeds (barley, corn, rice, sorghum, wheat, broad bean), whey and molasses, all were supplemented with kH_2PO_4 1 g and $NaNO_3$ 2 g / L distilled water, at 26 °C for 10 days. They found that Malt Yeast medium was the best medium for pigment production.

CONCLUSINS

Monascus natural fermented pigments have many advantages in using it as coloring agent, in pharmacology and medicine. Furanoisophthalides, azaphilone, amino acid, fatty acids and polyketides are the major secondary

metabolites of *Monascus* species. *Monascus* azaphilones polyketide pigmented metabolites were classified into four categories based on colour including yellow, orange, red pigment and colorless metabolites. Also these metabolites were classified into eight categories based on their bioactivity including: aroma & flavor compound, antibacterial, anticancer, anti-cardiovascular disease, anticholesterols & antioxidant, human health supporting health care and immune enhancer metabolites. It may be possible to find more new bioactive natural products by cultivating *Monascus* under different conditions. Bioactivity of *Monascus* metabolites remain to be investigated. In this respect, additional studies on the metabolites, medicinal, pharmacological bioactivity of the pigments merits further research.

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