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Review Article

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Anti-biofilm activities of Flavonoids: An overview

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Abstract

A functional colonisation of microbial species on biotic and abiotic surfaces embedded within extracellular polymeric substances (EPS) is known as biofilms. Formation of these sessile communities and their inherent resistance to antimicrobial agents, created major economic and health impact in significant industrial sectors including Food, Pharmaceutical and Healthcare industries. Different plant material has been used from ancient period for therapeutic purposes; the last few decades have witnessed increased investigations, which have been geared to investigate the anti-biofilm effects of natural products with a proven antimicrobial potency. Flavonoids are one among those, which has multiple therapeutic actions owing to their polyphenolic nature, with confirmed antimicrobial activities and also have an ability to inhibit biofilms. The present work is an attempt to summarise flavnoids their classification, structural properties along with their biofilm inhibition capability. The in-vitro anti-biofilm activity and its mode of actions to combat the biofilm associated problems have been discussed which may be applied to many industries for better productivity.

Keywords: Flavonoid, Classification and Structures, Antibiofilm potential

Introduction

The flavonoids are polyphenols in nature. They are essential compounds with positive health effect; their various positive effect has been repeatedly established. The different groups attached to the basic flavonoid structure, makes them more effective. Thus flavonoids have very important role in pharmacology and in human health. The flavonoid poses different activities in human and prevents many diseases. The phyto-compounds have been repeatedly reported to be the strong antioxidant agents. The high content of phenolic group in the flavonols makes them important antioxidant molecules. The hydroxyl group forms stable complexes with free radicals and thus act as antioxidant in nature (Sofna D.S. Banjarnahor ‘et al, 2014) and Also they show(low-density lipoproteins) LDL- lowering activity (kiharu I Garashi ‘et al,1995)cardio tonic activity (Mori A, Nishinoc ‘et al,1988), anti-carcinogenic activity (Morino M ‘et al,1997), hepatoprotective activity (Lorenz W, ‘et al, 1973), anti-inflammatory activity (Shahidi F ‘et al,1998),antifungal (Jindal Alka ‘et al,2012), antiviral activities (K. Raj Narayana ‘et al, 2000).

Flavonoids are one of the important secondary metabolites and are widely distributed in plant. Flavonoids are 15carbon skeleton(C6-C3-C6). (Fig.1) containing 2 phenyl ring (A and B) and one heterocyclic ring(C). They composed of three phenyl ring, with different substituents in carbon 2 and 3position in second ring.

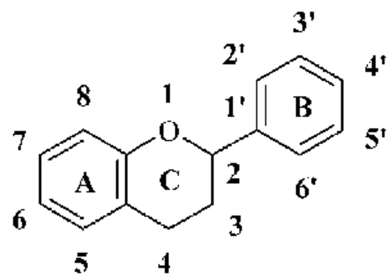

Fig.1. Basic flavonoid skeleton (Orsolya Farkas‘ et al, 2004)

Flavonoids are found as aglycones, glycosides, and methyl derivated flavonoids. The basic flavonoid structure is aglycones (Fig.1) the different substituents at c ring divides the flavonoids into different classes (K. Raj Narayana ‘et al., 2000) and according to this total 3000 varieties of flavonoids are known. Flavonoids are divided into different classesviz: Flavonol, Flavone, Isoflavone, Flavan-3-ol, Flavanone and Anthocyanidin (Shin Nishiumi‘et al, 2011)

Flavonol

The flavonols contains double bond between position 2 and 3in C ring and the flavonols structure differs from the others by the presence of the –OH group at position 3 in C ring and a carbonyl group(C=O) at C-4 of C ring (Saul Ruiz-Cruz ‘et al,2017). Flavonols are commonly found in fruits (apples and berries), in vegetables (red and white onion, spinach, lettuce broccoli), in chocolates and in some beverages (tea and wine) according to the (Marijanseruga ‘et al, 2017) also different – OH groups at different position in basic flavonol structure makes its different isomers.

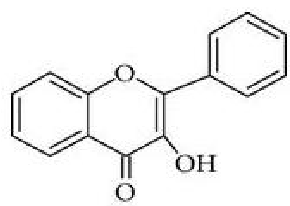

Fig.2. Basic structure of flavonol (Shashank Kumar et al, 2013)

Some of the examples of the flavonols are quercetin, kaempferol, galangin, myericetin, fiesetin, quercitrin, rutin, Morin they differ from each other by the presence of different –OH group at different positions and thus they also have different activities. The different positions of the –OH in flavonols are given in Table 1 (K. Rai Narayana ‘et al, 2000).

Table 1. Different positions of –OH in flavonols

POSITIONS	3	5	7	2’	3’	4’	5’
FISETIN	OH	H	OH	H	OH	OH	H
GALANGIN	OH	OH	OH	H	H	H	H
KAEMPFEROL	OH	OH	OH	H	H	OH	H
MORIN	OH	OH	OH	OH	H	OH	H
MYRICETIN	OH	OH	OH	H	OH	OH	OH
MYRICITRIN	O-Rh	OH	OH	H	OH	OH	OH
QUERCETIN	OH	OH	OH	H	OH	OH	H
QUERCITRIN	O-Rh	OH	OH	H	OH	OH	H
ROBININ	O-R^1	OH	OH	H	H	OH	H
RUTIN	O-R^1	OH	OH	H	OH	OH	H

O-R^1 – Alkoxy   OH – Hydroxyl   O-Glu – Glucosyl   O-Me – Methoxy

Flavone

The flavone also contains double bond between position 2 and 3 in C ring they also have carobnyl group(C=O) at position 4 of C ring. They are differ from flavonols by the absens of the –OH group at position 3 of C ring. The flavones have diferent derivatives based on their different structure(isomers) and different glycoxyl and other groups at various positions in flavone structure.

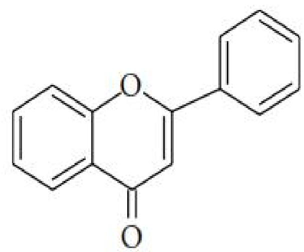

Fig.3 .Basic structure of flavone (Shashank Kumar et al, 2013)

The flavones and certain flavonols are widely called as plant pigments generally white and yellow coloured pigments(Gregory L Hostetler ‘et al,2017) some of the common examples of flavones are luteolin,apigenin,chrysin.the different derivatives of flavones are given in Table.2 (K. Rai Narayana ‘et al, 2000).

Table 2. Different derivatives of flavones

POSITIONS	3	5	7	2’	3’	4’	5’
APIGENIN	H	OH	OH	H	H	OH	H
BAICALEIN	H	OH	OH	H	H	H	H
CHRYSIN	H	OH	OH	H	H	H	H
DIOSMETIN	H	OH	OH	H	OH	O-Me	H
DIOSMIN	H	OH	$O\text{-}R^1$	H	OH	O-Me	H
LUTEOLIN	H	OH	OH	H	OH	OH	H
RPOIFOLIN	H	OH	O-R	H	H	OH	H
TANGERETIN	H	O-Me	O-Me	H	H	O-Me	H
TECHTOCHRYSIN	H	OH	O-Me	H	H	H	H

(OH- Hydroxyl ; O-Me – Methoxy; $O\text{-}R^1$ – Alkoxy)

Isoflavone

Isoflavone have 3-phenyl chromone skeleton with 3rings A B C BUT IN SOME of their derivatives they have extra ring in their structure i.e. Ring D (Saul Ruiz-Cruz ‘et al, 2017). The isoflavones are widely distributed in the leguminous plants. They are estrogenic compounds in plants. The soybean is the richest source of the isoflavone. isoflavonoids structure changes according to the molecules bound to different positions. The basic structure of an isoflavone is given in the Fig.4.

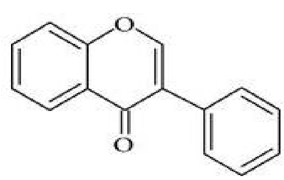

Fig.4. Basic structure of isoflavone (Shashank Kumar et al, 2013)

Like in isoflavonoids if the sugar is bound to the flavonoid aglycones through –OH group then they are called as O-glycosylflavonoids and if the sugar is bound to the flavonoid aglycones through carbon bond then they are called as C-glycosylflavonoids (Seyyed Reza Riyazi ‘et al, 2011). The common examples of the isoflavonoids are daizein, genistein, glycitein. The different derivatives of the isoflavonoids are given in the Table 3 (K. Rai Narayana ‘et al, 2000).

Table 3. Different derivatives of isoflavones

POSITIONS	3	5	7	2'	3'	4'	5'
DAIDZIN	-	H	O-Glu	H	H	OH	H
GENISTEIN	-	OH	OH	H	H	OH	H
TECTORIGENIN	-	OH	OH	H	H	OH	H

(OH - Hydroxyl ; O-Glu –Glucosyl)

Flavan- 3-ol

They are also called as flavanol(Flavan-3-ol). They have hydroxyl group in C3ring of C. They donot contain carbonyl group(C=O) at position 4of C ring thus they are differing from other flavonoid. They have different derivative, like monomeric and oligomeric. The flavanol are colourless compounds and some shows yellow coloration (Saul Ruiz-Cruz et al, 2017).The basic structure of the Flavan-3-ol is given in the Fig.5

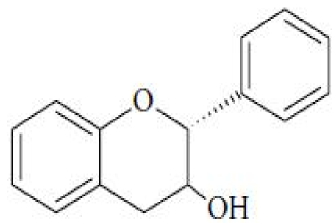

Fig. 5. Basic structure of Flavan-3-ol (Shashank Kumar et al, 2013)

The natural sources of flava-3-ol are the cocoa, beans, berries, apples. The richest source of the flavanol is the chocolate. The different flavanol like monomeric flavanol arecatechin, epicatechin and different oligomers are procyanidin (dimeric), cinnamatanin (tetramer) (Naomi Osakabest al, 2013) The common structural details of flavanol is given in the Table 4(K. Rai Narayana 'et al, 2000).

Table 4. The derivatives of flavanol

POSITIONS	3	5	7	2'	3'	4'	5'
(+) CATECHIN	OH	OH	OH	H	OH	OH	H
(-) EPICATECHIN	OH	OH	OH	H	OH	OH	H
(-) EPIGALLOCATECHIN	OH	OH	OH	H	OH	OH	OH

(OH-Hydroxyl)

Flavanone

The basic structure of the flavanone has the Oxo group; they don't have 2 -3 double bond. And have 2-phenylbenzopiran-4-one-skeleton.the play important role in metabolic pathways. The flavanones are colourless ketones, some are pale and other are yellow (Saul Ruiz-Cruz 'et al, 2017).The basic structure of the flavanone is given in the Fig.6.

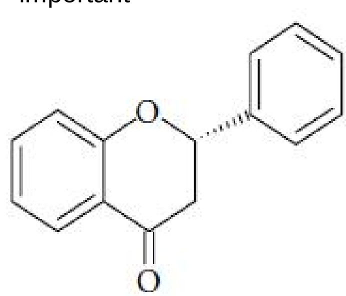

Fig.6. Basic structure of the flavanone (Shashank Kumar 'et al, 2013)

The examples of flavanone are naringenin, hesperidin, likvirtinetc. The different subgroups of flavanone are given in the Table 5 (K. Rai Narayana 'et al, 2000).

Table 5. Subgroups in different flavanones.

POSITIONS	3	5	7	2'	3'	4'	5'
ERIODICTYOL	H	OH	OH	H	OH	OH	H
HESPERIDIN	H	OH	O-Me	H	OH	O-Me	H
HESPERITIN	H	OH	OH	H	OH	O-Me	H
LIKVIRTIN	H	H	OH	H	H	O-Glu	H
NARINGIN	H	OH	O-R	H	H	OH	H
NARINGENIN	H	OH	OH	H	H	OH	H
PINOCEMBNIN	H	OH	OH	H	H	H	H

(OH-Hydroxyl; O-R-Alkoxy; O-Me –Methyl; O-Glu- Glucosyl)

Anthocyanidines

The Anthocyanidines contains hydroxyl group at 3 position and double between position 3 and 4 of c ring (saulruiz-cruz 'et al, 2017). The Anthocyanins are the plant pigments. They are purple pigments. The basic structure of Anthocyanidines is given in the Fig.7.

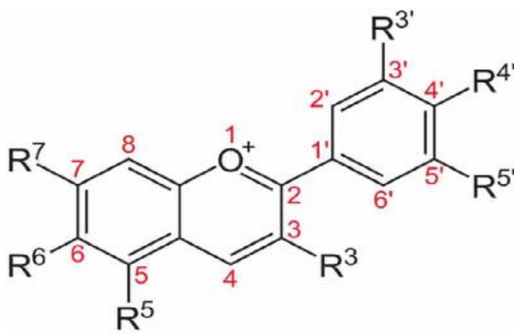

Fig.7. Basic structure of Anthocyanidines (saulruiz-cruz, et al, 2017)

They are commonly found in flowers(hibiscus, rose, red pineapple, corn flowr, blue rosemary mint lavender) and in fruits(black carrot, red cabbage).the major Anthocyanidines found in plants are petunidin, malvidin, peonidin, cyaniding (Hock Engkhoo 'et al, 2017).

Anti-biofilm properties associated with Flavonoids

As biofilm is the stable structure. These biofilms are highly toxic and also they can be fatal. Many industries face this problem and this biofilm as they show resistant to the different antimicrobial agents (Shriti Singh 'et al, 2017). Biofilms also can form on the food product surfaces, medical devices and may contribute to different life threatening diseases which are found to be fatal. Different medical devices include urinary catheters, pacemakers, heart valves and many more (Donald C Vinh 'et al, 2005). As the biofilm is the major problem occurs in major industries, there are different preventing measures have been employed to control these harmful biofilm from medical devices (Maria Esperanza Cortés et al,). Apart from different strategies being employed to manage biofilm associated healthcare problems, certain novel strategies using plants based material have been reported to receive significant importance (M. Sadekuzzaman 'et al, 2015) like different oils and other plant materials due to their minimal toxicity attributes. Several plant derived secondary metabolites like flavonoids, tannins, lignins (David M. Pereira 'et al, 2009), polyphenolic compounds confirmed with anti-biofilm activity (Lívia Slobodníková' et al, 2016).

Flavonoids obtained from red wine has been reported to contain quercetin, fisetin, apigenin, leutolin, chrysin, kaempferol, these flavonoids has found to be inhibitors of the *Staphylococus aureus* biofilm formation. These flavonoids also found to cause decreased blood haemolysis induced by *Staphylococus aureus* (Cho HS 'et al, 2015). Earlier studies confirmed that the quercetin also inhibits the *Listeria monocytogenes* biofilm by interfering with the proteins that requires for the biofilm formation (F.J. Vazquez-Armenta 'et al,,2018). In presence of quercetin the amount of extracellular proteins formed has found to be reduced substantially and thus quercetin acts as protein inhibitor in *Listeria monocytogenes* biofilm. Quercetin also found to act as a quorum sensing inhibitor in different food borne pathogens biofilm (Venkadesaperumal Gopu 'et al, 2015).

The Chalcones and flavones i.e. apigenin, luteolin showed anti-biofilm activity against *Porphyromonas gingivalis.* Dietary flavonoids, present in citrus, such as kaempferol and naringenin have been proven to act as quorum sensing (QS) inhibitors. By interfering with the interaction between acyl-homoserine lactones (AHLs, the signal molecules of Gram-negative bacteria) and their receptors, leading to inhibition of biofilm formation by *Escherichia coli* O157:H7 and *Vibrio harveyi* BB120 (Vikram A 'et al, 2010; Vikram, A 'et al, 2013)

Studies performed by (Marcin Rozalski 'et al, 2013) with from the extract of the hop *Humulus lupulus* after performing ultra-performance liquid chromatography – mass spectroscopy total 10 flavan-3-ols were identified which were two monomers((+)catechin and (-) epicatechin),four dimers and four trimers were identified. Also flavonols also identified (quercetin and kaempferol) and xanthohumol (a flavonoid from humulus hop) was found to be active against *Staphylococus aureus* biofilm, the anti-biofilm mode of action was observed by damaging the stability of biofilm. flavonoid xanthohumol from same plant possess anti-biofilm activity which was found to be associated with membrane damaging component

The extract from ficus Plant also showed anti-biofilm activity against gram positive bacteria specifically *Staphylococus aureus* biofilm, and the extract found to be contained epicatechin, 5,7,4′, -trihydroxyflavan-3-ol which showed anti-biofilm activity (Gbonjubola V. A. 'et al, 2014) it has reported that the mostly the phytochemical (polyphenols) are active against biofilm. The phloretin common flavonoid found in the apples was active against *Escherichia coli* O157:H7 biofilm by inhibiting fimbria formation, it has been reported that the phloretin represses the gene activity for fimbria (Jin-Hyung Lee 'et al, 2011). Luteolin treatment prevents the urinary tract infection caused by biofilm of uropathogenic *Escherichia coli* by reducing the motility of organisms (Shen XF 'et al, 2014)

Dental biofilms mainly observed in gum diseases and periodontal diseases. Theglucosyl transferase is the exoenzyme play important role in dental biofilms. The problem with oral biofilm is that they are very difficult to remove and only by means of mechanical remover is the option available. However, recently, phyto-ingreadients have been found to be active against such problems, inIt has been observed that many polyphenols are having inhibitory effect on dental biofilms. Many catechin based polyphenols, proanthocyanidins and flavonoids inhibits *Streptococcus mutans* glucosyl transferase which is important insynthesis ofthe polysaccharide which is the major component in the biofilm extracellular matrix (Zhi Ren 'et al, 2016). It has been reported that the novel anti-biofilm agent that prevents biofilm in periodontal diseases includes the tea catechin and epigallocatechin gallate destroys the established biofilm by *Porphyromonas gingivalis.* (Asahi Y. 'et al, 2014) Flavonoid, proanthocyanidins from cranberry are potent anti-cariogenic and inhibitory to the biofilm formation. Thecranberries extract is rich in flavonoids majorityflavonols is confirmed as quercetin glycosides which shows activity against *Streptococcus mutans* biofilm by acting on glucosyltransferase activity and F-ATPase activity (Simone Duarte 'et al, 2006).

Conclusion

Flavonoids the polyphenols are ubiquitously present in plant kingdom and are predominantly found inmajorityof plant products like fruits, flower, stem. Many flavonoids have been used from ancient period for the medicinal purposes. Theflavonoid is the important component of diet and possesses different biological activities like antioxidant and anti-inflammatory which play quite significant role in maintaining human health. The present review we have summarised the structural aspects of flavonoids

and their different properties which contribute important role in the therapeutics more precisely their ability to combat biofilms associated problems faced by many industries at present. Their antibiofilm mode of action reveals that some flavonoid potent individually, whilst others can more actively deal the biofilms in synergistic mode. The flavonoids are the attractive class of phyto-ingreadients for combating biofilms in a natural way without any significant threat tohuman health and environment. The ubiquitousness of flavonoids and their different actions from simply protectant in plants from different sources to the life threatening diseases make them novel class for novel agents. As compared with the synthetic and other therapeutics they stand out because of their advantages that they are naturally present, their easy absorption ability less with lesstoxicity and side effect make them quite unique and foresee a significant role in different industries including pharmaceutical, dairy and food industries in products or during processing activities with economic value.

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