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Evaluation of Xanthohumol as a potent drug from nature: Synthesis, isolation and anticancer activity

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Abstract

In this paper we are reviewing the potent anticancer activity of xanthohumol (XN) molecule and its analogues. The anticancer activity is explained according to the mechanisms of action of the molecule on tumour cells. We also comparing the isolation techniques and the synthetic routes that have been developed as far as now, indicating the most reasonable way of taking this molecule for preliminary studies. Finally, we are giving our opinion of future research that it is preferable to been followed based on these findings.

Keywords : xantohumol, synthesis, isolation, anticancer

1. Introduction

During the last two decades the xanthohumol molecule and some of the xanthohumol analogue molecules have been studied extensively by the researches as a potential drug, especially because of their anticancer (Sławińska-Brych *et al.*, 2016; Jiang *et al.*, 2018),

antimicrobial (Cetin-Karaca and Newman, 2015) and antioxidant (Jacob, Jamier and Ba, 2011; Leonida *et al.*, 2018) activity. Moreover, several studies indicated apoptotic activity against different cancer cell lines (Radović, Schmutzler and Köhrle, 2005; Kunnimalaiyaan *et al.*, 2015; Sławińska-Brych *et al.*, 2015).

The antimicrobial and antioxidant activity of XN, revealed because of the antimicrobial and antioxidant capacity of beer that has been discussed earlier (Nozawa, 2005; Ferk *et al.*, 2010). XN is a prenylated chalcone which can be found extensively in high amounts in hops, and eventually into the beer as one of the main flavonoids (Stevens and Page, 2004). Hops are responsible for the bitter taste of the beer and they contribute a lot to its quality and nutritional value (Leonida *et al.*, 2018). Because of this fact several studies followed to indicate the antioxidant activity of beer (Stevens and Page, 2004; Gerhäuser, 2005a; Venturelli *et al.*, 2016).

The next step for the researchers was to examine thoroughly the structure and chemical activity of this molecule following similar paths of other important molecules that can be found in food matrixes such as tocopherol (Castan *et al.*, 2005; Nava *et al.*, 2006; Gliszczyńska-świgło *et al.*, 2007), resveratrol (Acharya, Sanguansri and Augustin, 2013) etc. Because of the molecule's high importance several ways of isolation from hops were developed (Chadwick *et al.*, 2005; Anioł, Szymańska and Zołnierczyk, 2008; Kac *et al.*, 2008; Magalhães *et al.*, 2010; Chen *et al.*, 2012). In addition to this, some chemists developed synthetic routes of total or semi-synthesis (Yong *et al.*, 2008; Ferk *et al.*, 2010; Zhang *et al.*, 2015; Ellinwood *et al.*, 2017) of this valuable molecule. We are discussing extensively isolation techniques and synthetic procedures of XN molecules and XN analogues few paragraphs below in this work.

In medicinal chemistry XN, is playing a serious role with many researchers from chemical and biological backgrounds trying to test the chalcone against obesity (Miyata *et al.*, 2015), menopause (Keiler, Zierau and Kretzschmar, 2013), cholesterol levels (Miyata *et al.*, 2015), infections (Ferk *et al.*, 2016) and cancer (Cassidy and Setzer, 2010; Sung *et al.*, 2012; Jiang *et al.*, 2015; Kunnimalaiyaan *et al.*, 2015; Sławińska-Brych *et al.*, 2015). The results seem very promising and the evaluation of the action of these molecules in the prospect studies are the pylons that we were based in this review.

2. Prenylated Chalcones

Prenylated chalcones gained attention because of their multiple benefits in nutrition and in prevention of cancer as well. In addition to this, prenylated chalcones were studied because they modulate metabolism of carcinogens by inhibition of distinct phase 1 metabolic enzymes and activation of phase 2 detoxifying enzymes. Prenylated chalcones possess one or more prenyl groups covalently bound to the flavonoid backbone (see Fig 1). The highest concentrations of prenylflavonoids are detected in *Humulus lupulus* (Cannabinaceae), better known as hops and can be found in high amounts in certain types of beers and they are responsible for the beer's bitter taste. Increasing the number of prenyl groups in that molecules it can increase their lipophilicity, cell membrane attachment and transmembrane transport(Ferk *et al.*, 2010; Passalacqua *et al.*, 2015; Popło´ nski *et al.*, 2018).

The predominant prenylated chalcones in hops are xanthohumol (XN) and desmethyl xanthohumol (DMX) (Gil-Ramírez *et al.*, 2012) (Fig.2), and they are non-enzymatically and thermally isomerized during the brewing process.

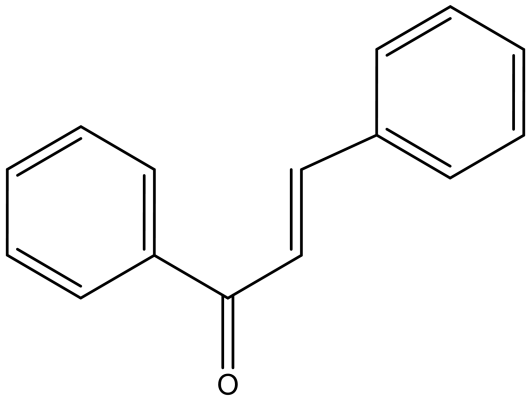

Fig 1: Chalcones backbone.

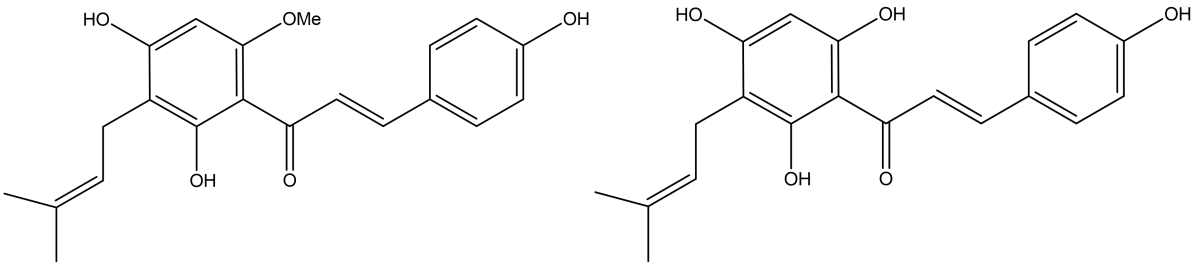

Fig 2: Xanthohumol and desmethylxanthohumol molecules.

3. Xanthohumol

XN is the main prenylated chalcone in hops and during the brewing process is converted to the prenylated flavonoid isoxanthohumol (IX) (Zołnierczyk *et al.*, 2015; Seliger *et al.*, 2018) (see Fig.3).

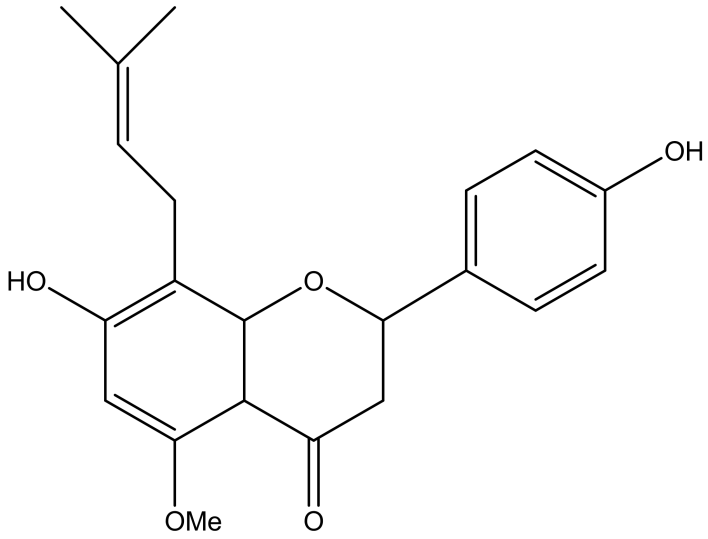

Fig 3: Isoxanthohumol.

It is well known that XN has many anticancer activities, but its low bioavailability due its low solubility is limiting its gastrointestinal absorption. On the other hand, many studies indicated valuable properties of XN molecule such as pronounced inhibition of cytochrome P450 1A2 (CYP1A2) (IC_{50}=0.02 μmol/L) (Suh *et al.*, 2018), inhibition of nitric oxide (Zhang and Lippard, 2003) production (IC_{50}=12.09 μmol/L) and antioxidant activity. It also supresses tumour growth as anti-inflammatory effects with inhibition of cyclooxygenase COX-1 (IC_{50}=16.6 μmol/L) and COX-2 (IC_{50}=41.5 μmol/L) (Cassidy and Setzer, 2010). Moreover, were reported antiestrogenic activity, antiproliferative properties in terms of apoptosis induction and cell differentiation. We will review more anticancer studies of XN in section 6 of this article.

4. Synthesis of Xanthohumol and its analogues

One of the most efficient ways to synthesize substitute chalcones is the condensation reaction between 4'-hydroxyacetophenone and 4-hydroxybenzaldehyde leading to the production of 4',4-dihydroxychalcone (see Fig.4).

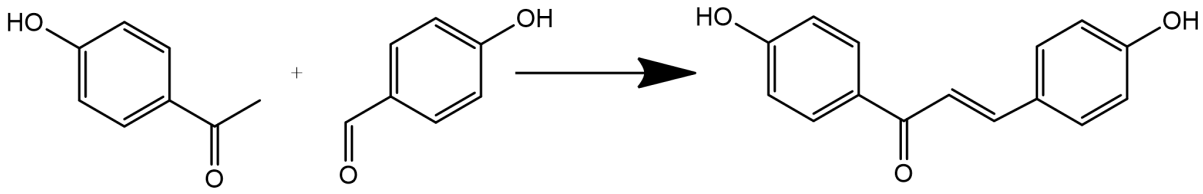

Fig 4: Condensation reaction between acetophenone and benzaldehyde.

Kakati and Sarma propose the iodine impregnated alumina as catalyst in 79-95% yield in less than 2 minutes time under microwave activation without using any solvent (Kakati and Sarma, 2011). In another study, several analogues of 7-*O*- and 4'-*O*- substitute isoxanthohumol and 8-prenylnaringenin were synthesised using demethylation of IX with MgI_2 x $2Et_2O$ in anhydrous THF with the yields of 61-89% (Anioł, Szymańska and Zołnierczyk, 2008). Moreover, Passalacqua et al reported the successful synthesis of a series of novel prenylated chalcones via Claisen – Schmidt condensation and evaluated their effects against different types of parasites (Passalacqua *et al.*, 2015). Vogel et al. proposed substitution reactions over the A Ring of the molecule.

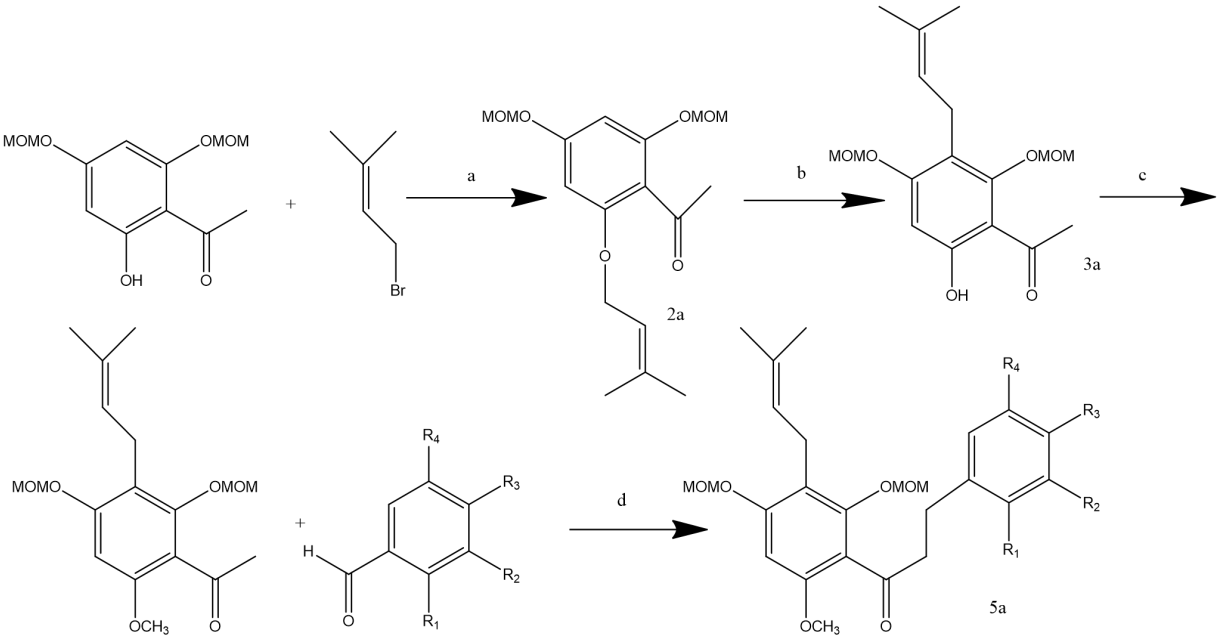

Fig 5: Synthetic procedure and synthetic conditions of prenylated chalcone synthesis.

The synthetic procedure started with MOM protection of 2,3,6- trihydroxyacetophenone with 2.5 equivalents MOM bromide yielded intermediate 1a, which was reflux for 24h with prenylbromide in aceton/K_2CO_3 to obtain 2a in 91% yield. Claisen rearrangement of ether 2a in N, N – dimethylaniline leads to the MOM protected and prenylated acetophenone 3a. Using the phase transfer catalyst tetrabutylammonium iodide, methylation with

dimethylsulfate gave 4a. Aldol coupling with the respective MOM protected benzaldehydes leads to the corresponding protected chalcone 5a (Vogel *et al.*, 2008, 2010) (see Fig. 5).

Other studies indicated the cyclization of XN to isoxanthohumol (IX) using 1% of NaOH (Fig. 6) whereas Aniol et al. proposed the demethylation of isoxanthohumol using magnesium and calsium salts (Anioł, Szymańska and Zołnierczyk, 2008).

Nuti et al. in a different study created 13 xanthohumol derivatives using the synthetic approach of (Nuti *et al.*, 2017) . The derivatives are depicted in (Table 1).

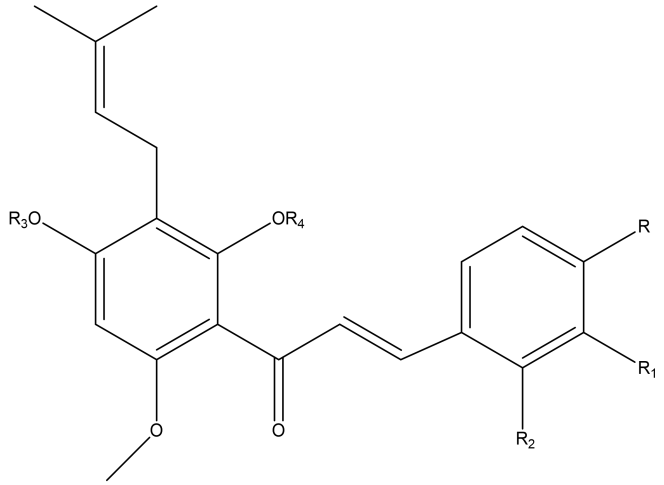

Table 1: Synthetic derivatives of Xanthohumol (Nuti et al. 2017).

Compound	R	R1	R2	R3	R4
1	Cl	Cl	H	MOM	H
2	Cl	Cl	H	H	H
3	F	H	H	MOM	H
4	F	H	H	H	H
5	H	H	F	MOM	H
6	H	H	F	H	H
7	OCH_3	F	H	MOM	MOM
8	OCH_3	F	H	MOM	H
9	OCH_3	F	H	H	H
10	OCH_3	H	F	MOM	MOM
11	OCH_3	H	F	MOM	H
12	OCH_3	H	F	H	H

13	NO_2	H	H	MOM	H

Ellinwood et al. prepared an adaptation of Khupse and Erhardt total synthesis of XN in 7 steps and 5.7% overall yield by a route incorporating a cascade Claisen-Cope rearrangement to install the 3'- prenyl moiety from a 5'- prenyl aryl ether and an aldol condensation acetophenone and benzaldehyde (Ellinwood *et al.*, 2017).

In (Fig 6.) we are watching the synthetic route used by Lee et al. for total synthesis of methylxanthohumol. The synthesis of methylxanthohumol (3) was attempted starting from 2,4,6- trihydroxyacetophenone (6). The desired product (7) was obtained in 30-34% yield (Yong *et al.*, 2008). A reaction of compound (6) with prenyl bromide in the presence of 1.1 equivalents of DBU in THF at room temperature for 48h gave compound 7 in 40% yield. Treatment of compound 7 with 2 equivalents of dimethyl sulfate in the presence of potassium carbonate in acetone at room temperature for 4h gave product 8 in 65% yield. An aldol reaction was next attempted to complete the synthesis of 4'-*O*- methylxanthohumol (3). The condensation of compound (8) with benzaldehyde (9) protected as a SEM ether in an ethanoic KOH solution at room temperature for 48h, giving product (10) in 75%. Deprotection of compound (10) through a treatment of c-HCl in methanol at room temperature for 1h gave the natural product (3) in 76% yield.

Diller et al. synthesized prenylated polyhydroxychalcones such as XN starting from compound (11) with base-catalysed aldol condensation between (12) and (13). The OH groups were protected by methoxymethyl (MOM) groups (Diller *et al.*, 2005). The synthetic route is depicted in (Fig.7).

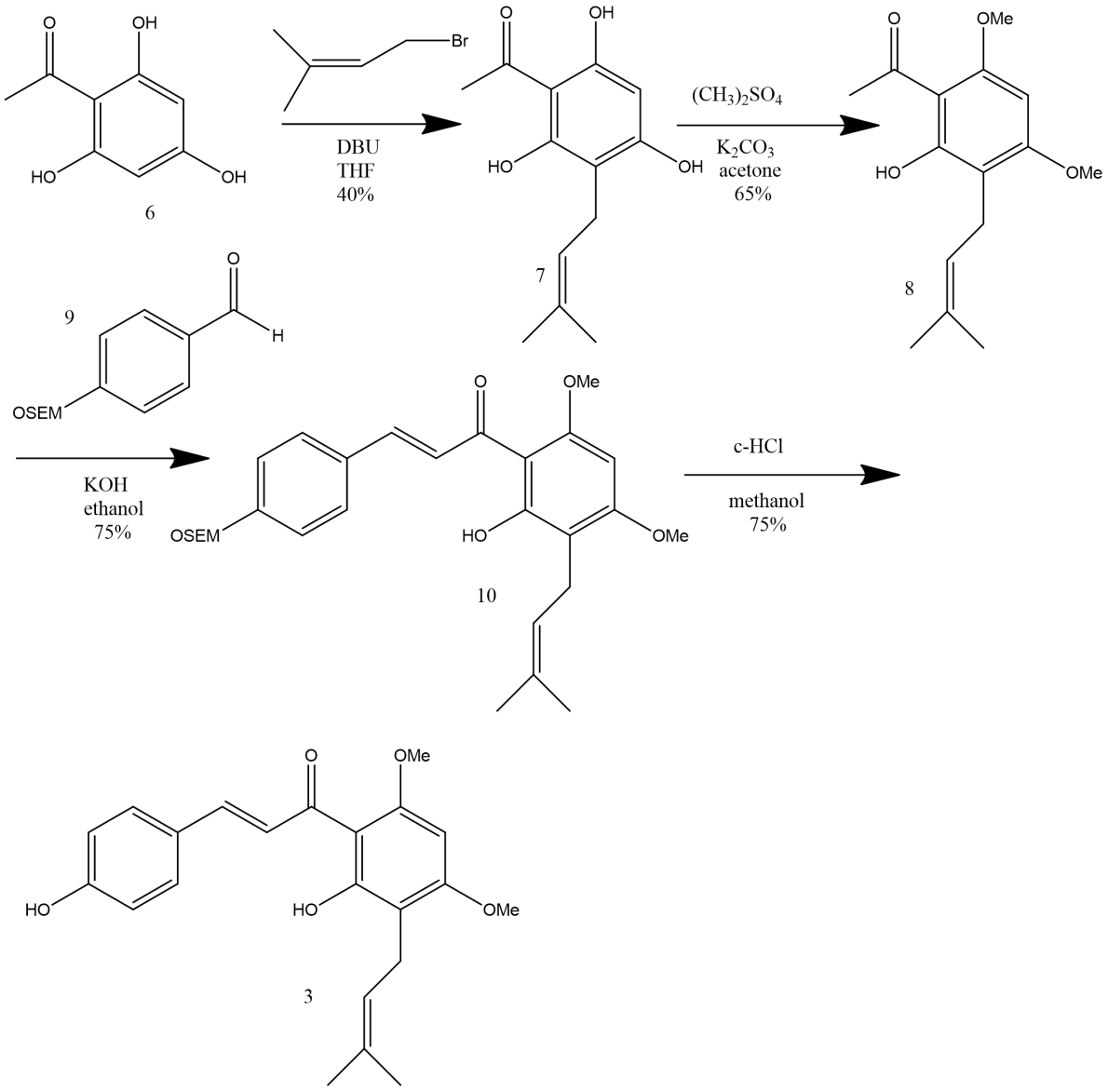

Fig 6: Total synthesis of metylxanthohumol (Lee et al.).

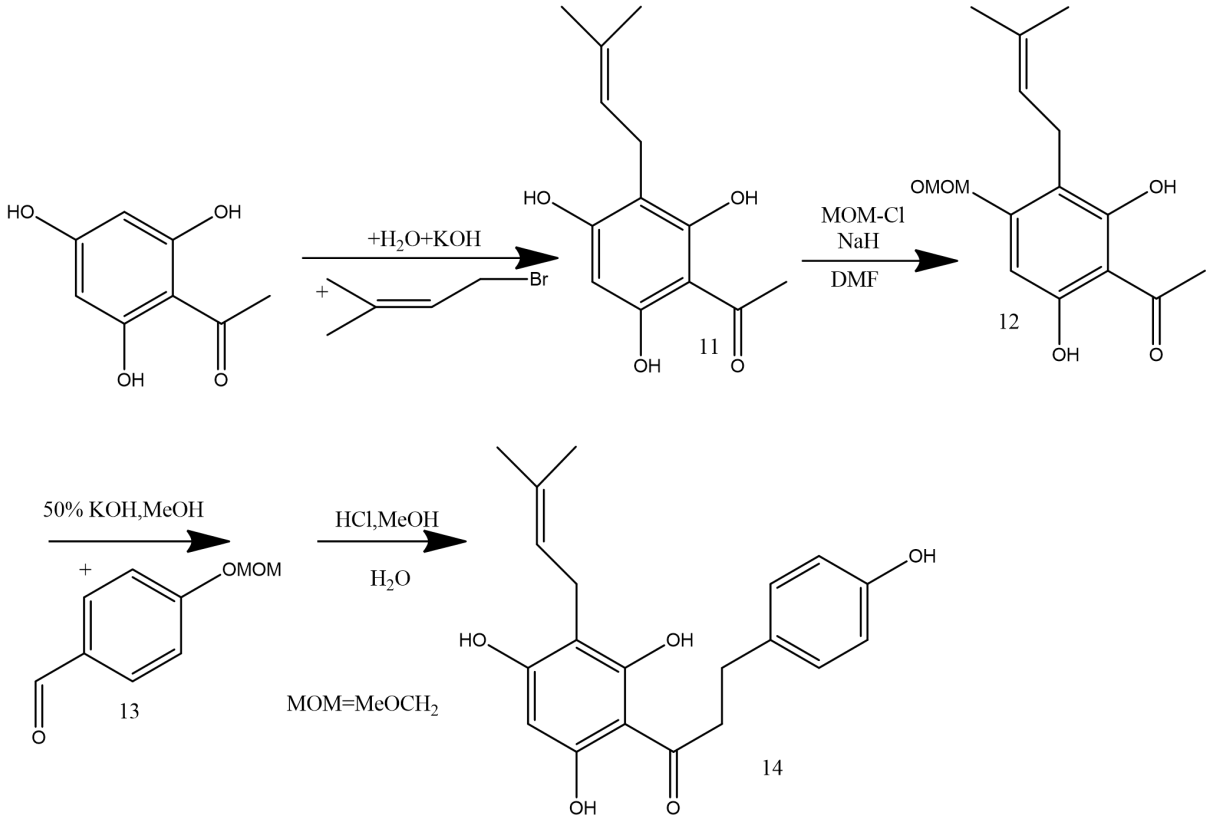

Fig 7: Xanthohumol synthesis (Diller et al.).

5. Isolation of the molecule

XN and IX molecules can be isolated from hops. They can be isolated from commercially available ethanoic hop extract. Gerhauser et al. were used 30 g of the extract and dissolve it in methanol/CH_2Cl_2, (1:1 v/v) and chromatographed them on Shandex LH 20 with methanol/CH_2Cl_2, (1:1 v/v) and (4:1 v/v) eluent (Gerhäuser, 2005b). Fractions of 15 ml were collected and monitored by silica gel thin layer chromatography. The fractions containing flavonoids were combined and evaporated. Active fractions were separated on silica gel via vacuum liquid chromatography with hexane/EtoAc gradient to yield XN (168.3 mg) and IX (8.0 mg) and a series of related compounds.

Another way to isolate XN is from hop pellets and used diethylether as solvent to take the crude extract. Kac et al. used this method and then the extract fractionated by column liquid chromatography over Silica gel 60 using dichloromethane/methanol (93:7 v/v) as a mobile phase (Kac *et al.*, 2008). Fractions of 5ml then collected and analysed by TLC, then combined

and separated with HPLC chromatography on Eurospher column with 70% methanol in water at flow rate 6ml/min. the eluate was detected at 280nm and fractions collected every 30s.

In a different study Ramirez et al. used solid-liquid extraction with cryogenically crushed hops mixing with DMSO (1g hop/40 ml DMSO) and left in agitation at room temperature, for 24 h in darkness (Gil-Ramírez *et al.*, 2012). Samples were filtered, and the cake obtained underwent the same process. This was repeated with fresh DMSO until no XN or IX detected in the extract. After that they used pressurized liquid extractions with hot water, with a pressure of 1500 psi at 50 °C, 100°C, 150°C and 200°C, and the extraction time was 30 min (6 cycles of 5min each).

Recently Chen et al. made use of high - speed counter - current chromatography (HSCCC) to isolate xanthohumol from hops (Chen *et al.*, 2012). they used a solvent system consisted of *n*-hexane-ethyl acetate-methanol-water at a volume ration of 5:5:4:3, with the purity of xanthohumol assayed by HPLC to be more than 95% and the yield of extraction to be 93.60%. the comparison of partition coefficient of XN under different solvent systems is summarised in (Table 2).

Table 2: Partition coefficients of XN under different solvent systems (Chen et al.).

Solvent system	K-value
n-hexane-ethyl acetate-methanol-water 5:5:5:5	2.28±0.16
n-hexane-ethyl acetate-methanol-water 5:5:4:4	3.28±0.18
n-hexane-ethyl acetate-methanol-water 5:5:5:3	0.42±0.08
n-hexane-ethyl acetate-methanol-water 5:5:4:3	1.35±0.10
n-hexane-ethyl acetate-methanol-water 5:5:6:3	0.18±0.02

6. Anticancer activity- Mechanisms of action

Even though XN has been only recently studying as a potent anticancer drug, there is a significant number of articles described its anticancer activity. In lower numbers, researchers published evidence about antimicrobial action, and very fewer indicating the role of XN in metabolism. In addition to this, studies regarding the anticancer activity of XN molecule and its analogues overpass in numbers studies that has to do with the structure, synthesis or isolation of the molecule.

During the last three years XN was checked of its anticancer activity over different types of cancer and some of these studies even proposed a mechanism of action.

Brych et al. described that XN had greater antiproliferative activity against A549 lung adenocarcinoma cells than against the lung adenocarcinoma H1563 cell line (Sławińska-Brych *et al.*, 2015, 2016). It was observed that XN was able to supress the activities of ERK1/2 and p90RSK kinases, following an inhibition of phosphorylation and activation of the CREB protein. In a different study the same research team suggested that XN promotes the reduction of cell viability of cancer cells and shows low cytotoxicity to normal cells thought the activation of caspases 9, -8 and -3. On another situation Jiang et al. showed that XN induced cell cycle arrest of pancreatic cancer cells (PANC-1, BxPC-3) by inhibiting phosphorylation of signal transducer and activation of transcription 3 (STAT3) and expression of its downstream targeted genes, which involved in regulation of apoptosis and the cell cycle (Cai *et al.*, 2014). Guo et al. proposed the same mechanism of action on gastric cancer, *in vitro* and *in vivo*, and suggested that XN is a promising anticancer agent for gastric cancer (Guo *et al.*, 2018). More studies indicating apoptotic effects of XN in hepatic neoplasm (Karimi-Sales, Mohaddes and Alipour, 2018), paraptosis of leukemia cells through p38 mitogen activated protein kinase signalling pathway (Mi *et al.*, 2017), and inhibition of endothelial cells which is very crucial in tumour growth via adenosine monophosphate-activated protein kinase . Malek suggested that XN is a potent chemotherapeutic candidate for cervical cancer (Yong, Nurestri and Malek, 2015), and other researches also suggested this molecule as a potent anticancer agent for larynx (Sławińska-Brych *et al.*, 2015), breast (Sun *et al.*, 2017), liver (Zhao *et al.*, 2016) and pancreas cancer cells (Jiang *et al.*, 2015).

Zhang et al. provided evidence that XN directly interacts with the mitochondrial electron transfer chain complex I (NADH dehydrogenase), inhibits the oxidative phosphorylation, triggers the production of reactive oxygen species, and induced apoptosis (Zhang *et al.*, 2015). Furthermore, in this important study they showed that xanthohumol upregulates the glycolytic capacity in cells and compensates cellular ATP generation. They also suggested that isoxanthohumol the structural isomer of xanthohumol, is inactive to cells, indicating that the reactive 2-hydroxyl group of XN is very important for its targeting in mitochondrial complex I. Li et al. suggested that XN in comparison with the well-known anticancer drug *cis*-platin protects against nephrotoxicity by ameliorating and oxidative responses (Li *et al.*, 2018).

Overall, XN modulates the activity of enzymes involved carcinogen metabolism and detoxification. XN can scavenge reactive oxygen species (ROS), hydroxyl and peroxyl

radicals, and to inhibit superoxide anion radical and nitric oxide production. Gerhauser concluded that this molecule demonstrates anti-inflammatory properties by inhibition of cyclooxygenase 1 and 2 activity and is antiestrogenic without possessing estrogenic potential. It inhibits DNA synthesis and induce the cell cycle arrest in S phase, apoptosis and cell differentiation (Gerhauser *et al.*, 2002; Gerhäuser, 2005b; Strathmann *et al.*, 2010).

Regarding the structure of the molecule that is responsible for its action, Jacob et al. mention that besides the phenolic group of xanthohumol which is responsible for antioxidant and radical scavenging activity, the electrophilic α, β-unsaturated ketone is another important group of the molecule as it is a target of nucleophilic attack, by protein thioles and amines (Jacob, Jamier and Ba, 2011). Its isomer IX is less biological active because cannot undergo Michael addition reactions, but according to Zolnierczyk et al. it is showing antiviral activity against herpes viruses and viral diarrhea virus (Zołnierczyk *et al.*, 2015) .

7. Prospects

Until now it is well accepted that XN molecule and its analogues plays important role as a biological active molecule for preventing several diseases especially cancer. Liu et al. suggested that we must take in advance the potential of xanthohumol and use them as food additives considering its many positive effects that described above (Zhang *et al.*, 2015). On the other hand, Venturelli et al. acknowledge the anticancer activity of the molecule alongside with its low toxicity on healthy cells, but also mention the drawbacks which are the low ingestion and bioavailability and the lack of knowledge upon its metabolism (Venturelli *et al.*, 2016). Jiang et al. on the same path praised the anticancer potency of xanthohumol but suggested that bioavailability and extraction yield remain to be improved and more *in vivo* studies should be done to better understand the anticancer activity of XN (Jiang *et al.*, 2018).

Our opinion lies to the fact that XN is a natural anticancer molecule and our job is to mimic nature and minimise the drawbacks. This molecule needs to be considered as a backbone molecule of other semi or total synthesised derivatives that will maintain the prenylated character of the molecule and keep the hydroxyl groups that are very important for driving the molecule to mitochondria. The synthetic procedures will eradicate the problem of low yield isolation from hops and at the same time they will improve the bioavailability of XN *in vivo*. The ability that the molecule must travel to mitochondria and promote the apoptosis of cancer

cells through redox reactions and keep low toxicity in healthy cells at the same time, should be improved chemically, with various substitutions of the molecule with active groups.

Another possible way to work with these molecules is using them as drug delivery systems. IX which is less biological active than XN it can be used as a metal carrier with a transition metal that promotes the toxicity in cancer cells and moreover their apoptosis.

8. Conclusions

It is inevitable fact that once again nature showing as the way to create useful drugs. Xanthohumol and its analogues already have been tested and showed valuable and positive actions against several types of cancer as mention above. Some disadvantages must be eliminated to promote this molecule to clinical trials. The low yield through isolation from hops and its low bioavailability, indicating that the answer is through the semi-synthesis of xanthohumol's active groups. The creation and evaluation of more synthetic analogues *in vitro* and *in vivo* and the studying of the interactions of those molecules with transition metals of the human organism will promote the molecule as a great anticancer agent.

Conflicts of Interest

The authors declare no conflict of interest.

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