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Review Article

ANTI-NUTRITIONAL FACTORS IN SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) SEEDS AND THEIR PHARMACEUTICAL APPLICATIONS

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ABSTRACT

Anti-nutritional factors (ANFs) present in human or animal foods generally reduce nutrient utilization thereby contributing impaired metabolic performance. Several ANFs have been reported for their negative effect on the performance and survival of monogastric animals. However, some ANFs and their break down products present in small amount may possess beneficial health effects. In oilseed crops, small concentrations of ANFs like glycosides, tannins present in the seed/seed coat are responsible for the characteristic astringent or bitter taste and individually not preferred as fodder. Appropriate processing techniques help to reduce the adverse effects of these ANFs and thereby improve their nutritive value. ANFs in safflower seeds and their well-organized extraction techniques may add a positive approach in medical science for their further applications in pharmaceutical industries. This review summaries related concern of this important oilseed crop.

INTRODUCTION

Antinutritional factors (ANFs) have been the chemical compounds, frequently but not exclusively associated with foods and feeding stuff. They have been commonly known as secondary metabolites in plants and proved to be highly biological active compounds. Oilseeds have been used for different purpose as food, animal feed, industrial raw material and for medical uses. The presence of ANFs in plants combined with protein sources and reduces their full utilization for livestock feeding and reduced the nutrient utilization through food intake (Osagie, 1998). Generally, these toxic factors found in plants for variety of reasons as a part of their protection against attack by herbivorous, insects and pathogens or as means to survive in adverse growing conditions.

In Asteraceae family, *Carthamus tinctorius* L. commonly called as safflower has been the ancient oilseeds Rabi crop (Knowles, 1969). The cultivated safflower (*C. tinctorius*) was believed to be originated from the wild varieties of *C. lanatus* and *C. oxyacantha* (Knowles and Schank, 1964; Weiss, 1971). Safflower as a versatile crop, famous for its flowers and seeds (oil) and known for the diversified uses (Furuya *et al*., 1987). Safflower oil comprised of approximately 75% linoleic acid acids, 13% oleic acid and 8% saturated fatty acids and remaining 4% constitutes vitamin E, omega-3 and omega-6 fatty acid. In India, Maharashtra has been the leader in terms of acreage and production of safflower followed by Karnataka, Andhra Pradesh, Madhya Pradesh, Uttar Pradesh and Rajasthan. These states produced the most popular seed varieties like Nira, JLSF-88, A-2, APR-3, AKS-207, HUS-307 including A-1 and PBNS-12 as a national check. Figure 1 demonstrated the presence of major ANFs in these seed varieties according to their states.

In safflower, ANFs have been present in the form of Tannins, Oxalates, Luteolin, Acacetin, Glycosides and Serotonin derivatives. They reduce the availability of nutrients and cause growth inhibition if taken up by the humans. Phenolics, polysaccharides, flavonoids, alkaloids, lignans, steroids, carboxylic acids, quinochalcone C-glycosides and quinone-containing chalcones have been the chemical groups isolated from safflower seeds other than the ANFs (Sadao *et al*., 1980; Sato *et al*., 1985; Nagatsu *et al*., 1988; Hattori *et al*., 1992; Chang *et al*., 1999; Li *et al*., 2002; Roh *et al*., 2004). Lignan glycosides and triterpenoid saponins have also been reported from the seeds and flowers of the safflower (Yoo *et al*., 2006; Yadava and Chakravarti, 2008). The aim of current review is to emphasize the ANFs types present in safflower seeds and their processing techniques with highlighted pharmacological and other industrial applications.

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Effects of Anti-Nutritional Factors

ANFs are widely distributed in the oilseeds, consumed in the form of diet and may cause severe effects as cynogenic glycosides can cause dysfunction of the central nervous system, respiratory failure and cardiac arrest. Lectins can adversely affect our body by directly binding to the intestinal mucosa interacting with enterocytes and interfering with absorption and transportation of nutrients during digestion and causing epithelial lesions. Phytates bound to the minerals like calcium, iron, magnesium and zinc and made them unavailable for the absorption. Anemia and other mineral deficiency disorders have common in regions where the diet has been primarily a vegetarian. Oxalate inhibits calcium absorption and increase the risk of developing kidney stone. Tannins bound to proteins and thereby reduced the nutritional quality. They combined with digestive enzymes thereby making them unavailable for digestion. They also decreased palatability and reduced growth rate (Kiranmayi, 2014).

Besides negative impacts, these ANFs can also play a positive role when isolated and purified from their plant parts. These factors have an effect on gastrointestinal tract and affect the microflora count of the intestine by promoting the growth of beneficial bacteria. *Lupinus campestris* seeds have anti-mutagenic activity and prevent the mutagenic process involved in development of cancer. ANFs i.e. plant lectins decreased the levels of heat shock protein 70 and 90 in gut epithelial cells. Lectins present in legumes assessed to act as a mucosal adjuvant. (Price *et al.,*1987; Jansman *et al.,* 1998; Friedman, 2001; Young, 2011).

ANFs have restricted the use of safflower seeds and their broad beneficial effects. After their consumption, they show deleterious effect on growth and health of humans or animals. So, the awareness of various anti-nutritional factors present in safflower seeds and their proper utilization has been very essential for health and well beings of the population.

PROCESSING METHODS

Significant human populations have been relying on the safflower seeds for multipurpose uses though ANFs present in safflower seeds, their meal or other parts of this crop reduce its palatable quality. The nutraceutical potential of safflower seeds have been hindered and make it less preferable for animal feed. Processing methodologies have been commonly used for the improvement of oilseeds to maintain their quality. The isolation and proper processing of these ANFs contributed a leading role in pharmaceutical sciences.

Processing of oilseeds through heat has been traditional method for removal of ANFs and this has been further sub-categorised by roasting and boiling method. Roasting method has been used for the induction of typical colour development, flavour and taste of oil. In addition, it changed the chemical composition and modified the nutritional value as well as antinutrient content of the seeds (Ozdemir and Devres, 2000). Boiling method has been conducted at the high temperature through which ANFs have been drained out in the water. It has also been used to reduce the ANFs like phytate and tannin contents of the sample. Extrusion cooking, one of the heating method, has also been used reduction of trypsin inhibitor, phytic acid and tannin (Kumar *et al*., 2018).

Radiation method has also been used for the reduction of ANFs and has one of the traditional processing methods. Microwave method has been mainly used in the food industry to increase the oil extraction by inducing the stress. It has been widely employed for the isolation of ANFs from plants and seeds (Self, 2005). Some hydrothermal processing methods including atmospheric boiling, atmospheric boiling, pressure boiling and pressure steaming have been used for the significant removal of phytic acid, saponins, trypsin inhibitor and tannin (Ojo *et al*., 2018).

Soaking and germination methods have also been used for the reduction and removal of ANFs from the oilseeds. The amount of phytate has been reported to decrease after soaking of the seeds in water and mineral salt solution for 12 hours and subsequently the amount of saponins were reduced by sprouting method for 60 hours. Soaking and germination methods in combination partly removed the concentration of the trypsin inhibitor while heat pretreatment mostly eliminated it (Khokhar and Chauhan, 1997).

The enzymatic method has been one of the advanced and important processing methods for the reduction of ANFs as they digested the complex cell walls of seeds. In this method, enzymes have enhanced the extraction and separation processes to eliminate toxic and anti-nutritional factors (Kalia, 2001). Fermentation has been known as another most advanced processing method. In this method, seeds have been fermented using *Bacillus subtilis, Lactobacillus gasseri* and other microbial strains in the liquid and solid medium based on the fermentation type. ANFs as phenolic compounds and phytosterols have been separated through this process. Besides these processing methods, leaching or solubilization into cooking medium has also been used for the degradation of these constituents by heat (Mariod *et al*., 2012). Ojha *et al*. in 2018 as well as Bulbula and Urga in 2018 reported that a significant amount of phytate and tannin were reduced after germination and fermentation pretreatment. They also have reported that there was a considerable reduction in glycosides after pretreatment with fermentation than germination. A variety of traditional household methods to industrial scale processing methods have shown in Table1.

Anti-Nutritional Factors with Pharmaceutical Applications

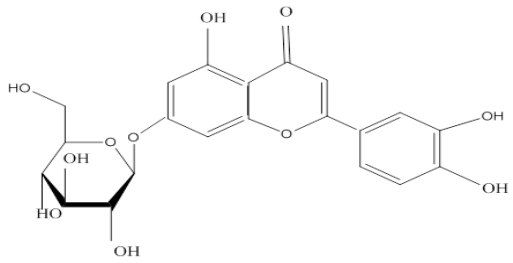
Proper standardized purification of ANFs presents in safflower seeds make it people choice for various medical and pharmaceutical applications. At low levels, few ANFs have been reported to reduce the blood glucose, insulin responses to starchy foods and the percentage of plasma cholesterol and triglycerides as well as cancer risks. At their high concentration, they cause the adverse physiological effect. So, here we discuss the pharmaceutical role of different ANFs present in safflower seeds as summarized in figure 2.

Safflower seeds have been enriched with phenolic compounds like N-feruloylserotonin-5-O-β-D-glucoside, 8-hydroxyarctigenin-4-O-β-D-glucoside, leutolin-7-O-β-D-glucoside, N-feruloylserotonin, which have been quantified using HPLC technique. Recently much attention has been given to these compounds due to their variety of biological actions as anti-oxidation, anti-inflammation, anti-cancer and anti-aging and act as therapeutic potential against several diseases (Zhang *et al*., 1997; Kang *et al*., 1999; Bae *et al*.,

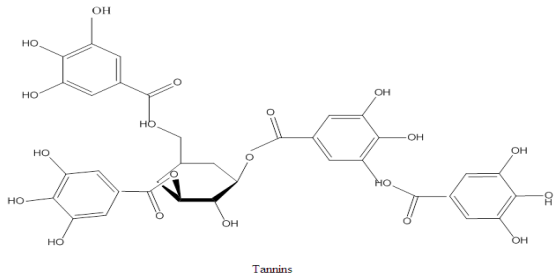
2002; Kim *et al*., 2004). One study showed that in estrogen deficient rats with inhibition of melanin synthesis, phenolic compounds extracted from safflower seed increased the level of plasma high density lipoprotein (HDL) cholesterol and also stimulate bone formation (Kim *et al*., 2006).

Table 1 Processing method of ANFs present in safflower seeds

S. No.	ANFs	Processing method	References
1.	Tannins	Cooking, Extrusion, Autoclaving, Microwave and Roasting	Alonso et al. 2000; Enechi and Odonwodu 2003
2.	Flavonoid	Microwave, Ultrasound Wave	Wang et al. 2014; Wei et al. 2016
3.	Serotonin derivatives	Ultrasonic Wave, Reflux method	Seo and Choi 2009
4.	Glycosides	Boiling, Fermentation, Autoclave, Soaking	Markus et al. 2014



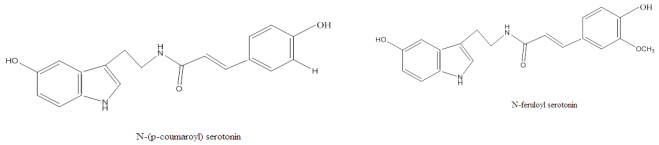
Leutolin-7-O-β-D-glucoside



Tannins

Tannins a major part of astringent; bitter seeds polyphenols, located mainly between the outer integument and the aleuronic layer. They have been associated with the preservation of plant dormancy and have bactericidal and allopathic properties. They showed the highest anti-oxidant properties (Cheng *et al*., 2002). Tannins have been actively used in pharmaceutical industry as antidiarrheal, haemostatic, and anti-hemorrhoidal compounds. They have not only used for healing burns and halting bleeding, also terminate infections while continue to heal the wound internally. They can also cause regression of tumors that have already presented in tissue (Stéphane *et al*., 2004).

Serotonin derivatives like N-(p-coumaroyl) serotonin (CS) and N-feruloyl serotonin (FS) have shown *in vitro* and *in vivo* anti-oxidant effects. These two serotonin derivatives were recognized as the main and unique phenolic constituents of defatted safflower seeds (Zhang *et al*., 1997; Koyama *et al*., 2006). Some activities of these derivatives have been already reported including free radical-scavenging and antibacterial characteristic. They also inhibited the production of pro-inflammatory cytokines, showed anti-oxidative activity on plasma and liver, inhibited LDL oxidation in apoE-deficient mice, increased the proliferation of fibroblasts and showed advantageous effects against cardiovascular risk in healthy human volunteers (Kawashima *et al*., 1998; Watanabe, 1999; Kumarasamy *et al*., 2003; Cho *et al*., 2004; Koyama *et al*., 2008).



N-(p-coumaroyl) serotonin

N-feruloyl serotonin

Luteolin has been the natural flavonoid present in the safflower seeds. It has been commonly used in the traditional medicine to treat a wide range of diseases. It showed different pharmacological and biological activities like anti-oxidative properties, neuroprotection, anti-diabetic, antihypertensive and cancer prevention effects (Huang *et al.,* 1999; Duarte *et al*., 2001; Dajas *et al*., 2003; Benavente-Garcia and Castillo, 2008; Lin *et al*., 2008).

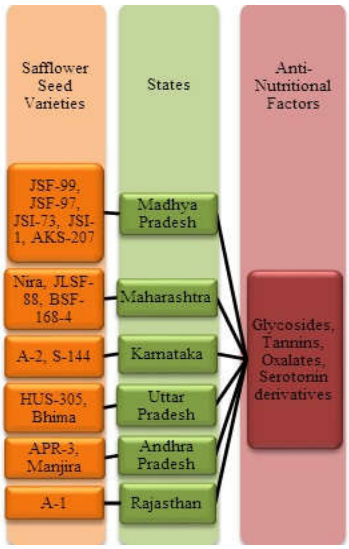


Fig 1 Safflower seeds varieties of Indian states and their antinutritional factors

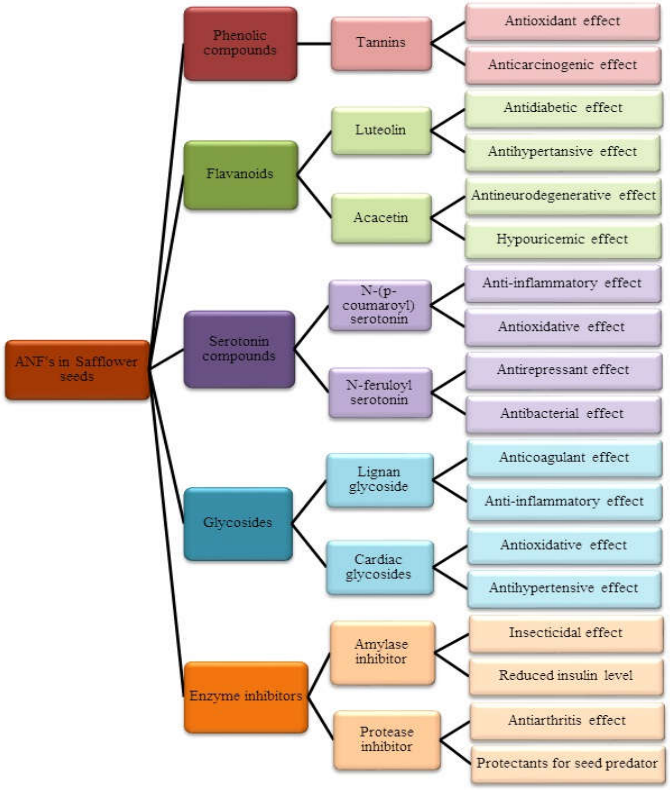
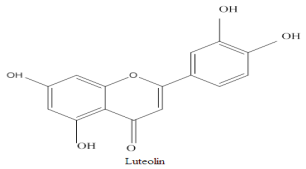
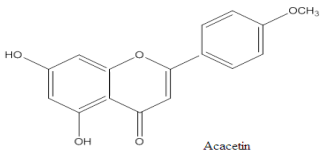


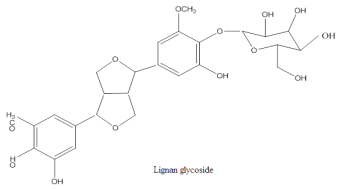
Fig. 2 ANFs present in safflower seeds and their pharmaceutical activities

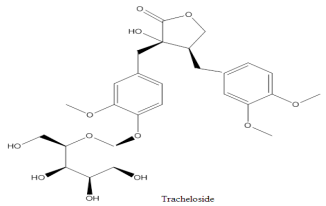
Similarly, Acacetin, another category of flavonoid has an atrium-selective mediator which, without extending the corrected QT interval, increased the atrial refractory period. They have been extensively distributed in a variety of plants of various families but reported mainly in Asteraceae family. Acacetin have several pharmacological properties like anti-inflammatory, anti-oxidation, blood vessel expansion, arrhythmia inhibition, antiplatelet aggregation and antitumor activities connected with cardiovascular protection (Guler *et al*., 2011; Chiyomaru *et al*., 2012; Prasad *et al*., 2012; Chen *et al*., 2013).

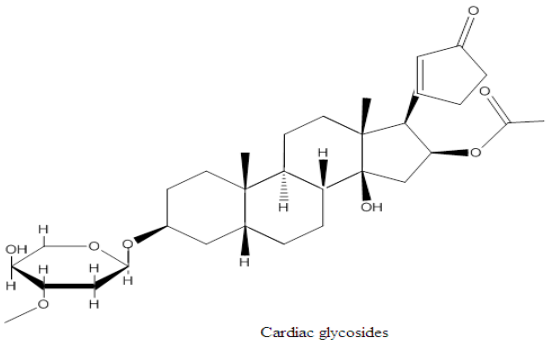




Estrogen analogs as antagonists have been tremendously used in clinical application of anti-estrogens for treating breast cancer. Safflower seeds rich in lignan glycoside, tracheloside have been reported as an anti-estrogen against cultured Ishikawa cells through bioassay-linked HPLC-ELSD (Yoo *et al*., 2006). As an antioxidant, their positive effect on reducing the risk of hormone-dependent cancer has also been recently demonstrated (Niemeyer and Metzler, 2001; Yamauchi *et al*., 2006). Similarly, C-glycosides i.e. Quinochalcone present in safflower has been found to show anticoagulant, anti-inflammatory, antioxidant, hepatoprotective, antihypertensive, anti-tumoric and anti-diabetic properties (Yue *et al*., 2013).







ANFs present in safflower seeds and their removal by biotechnological approach may minimize adverse biological effects though on the other hand these ANFs may actively participate in pharmaceutical industries. These anti-nutrients might not always harmful even though lack of nutritive value. Despite of this, the balance between beneficial and hazardous effects of anti-nutrients rely on their concentration, chemical structure, time of exposure and interaction with other dietary components. Finally, knowledge regarding various techniques to lower down or reduce the ANFs content in safflower seeds is needed for their positive approach in health and well-being of the population.

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Conflict of Interest

Authors declare no conflict of interest.

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