



Increasing Nutraceutical and Pharmaceutical Applications of Safflower: Genetic and Genomic Approaches

Megha Sharma, Varun Bhardwaj, Poulami Goswami, Anmol Kalra, Kadirvel Palchamy, Arun Jagannath, and Shailendra Goel

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Abstract

Nutraceuticals are functional foods that are produced in nature and show medicinal, health-promoting, and disease-preventing properties. One of the important sources of dietary and herb-based nutraceuticals is safflower. Safflower is an oil seed crop cultivated globally in ~23 countries. Being a source of unsaturated fatty acids such as linoleic and oleic acid, safflower oil confers several health benefits. Besides seed oil, petals and leaves of the safflower have been used widely in the pharmaceutical industry due to their richness in flavonoids and alkaloids. Crude floral extracts are known to possess anti-inflammatory and analgesic properties and bring wide health-related benefits in cardiovascular and diabetic patients. Although safflower oil is healthier, it is not a popular crop due to its lower oil content and economical value. Various efforts have been made by plant scientists

Megha Sharma, Varun Bhardwaj and Poulami Goswami contributed equally to this study.

M. Sharma · V. Bhardwaj · P. Goswami · A. Kalra · A. Jagannath (✉) · S. Goel (✉)
Department of Botany, University of Delhi, Delhi, India

K. Palchamy
ICAR-Indian Institute of Oilseeds Research, Hyderabad, Telangana, India

to increase oil content as well as oil composition in the safflower. Over the past few decades, various breeding strategies including conventional breeding, molecular breeding, and genetic engineering approaches resulted in improved cultivars with better oil composition and content. Thus, with its high nutrition value, safflower has great potential to play an important role in the nutraceutical and pharmaceutical industry.

Keywords

Safflower · Nutraceutical · Pharmaceutical · Seed-based-product · Non-seed based product · Alkaloids · Flavonoids

1 Introduction

With the emergence of the new world and increased life expectancy, the risk of lifestyle diseases has also increased significantly. Epidemiological studies show that dietary habits play a critical role in human health. These studies increased the popularity of plant-based compounds commonly called “nutraceuticals.” The combined efforts of the food industry, pharmaceutical, and newly merged nutritional conglomerates led to the development of nutraceuticals to cater to demands of the herbal and dietary supplements market. Nutraceuticals are often referred as functional foods or phytochemicals. These are natural, bioactive, chemical compounds with medicinal, health-promoting, and disease-preventing properties (Dureja et al. 2003). They can be classified as Nutrients, Herbal, or Dietary based compounds. Herbal or botanical products are concentrated or extracts whereas Dietary products are the reagents derived from other sources which include pyruvate, sulphate. These products serve functions of sports nutrients and meal replacement. In the present era, Aloe Vera, Echinacea, Evening primrose oil, Garlic, and Ginger Plantago have been used commonly in herbal and phytochemical products (Dureja et al. 2003).

The human staple diet includes fruits, vegetables, whole grains, herbs, nuts, and seeds and is often supplemented with fats and edible oil which are rich in energy. Based on the saturation levels, edible oil rich in unsaturated fatty acids are healthier as compared to oils rich in saturated fatty acids. Safflower oil is characterized by higher levels of unsaturated fatty acids, and the plant is also a rich source of secondary metabolites. Hence, safflower has immense potential as a nutraceutical crop and can be an important source of herbal as well as dietary supplements (Fig. 1).

Safflower (*Carthamus tinctorius* L.) is a member of the Asteraceae. It is grown in ~23 countries with 6.2 million tons of seed production (FAOSTAT 2019). Kazakhstan, United States of America (USA), Russia, Mexico, China, and India are the major producers of safflower and contribute to ~81% of the global production. Along with oil, it has been used as coloring dye, food flavoring agent, and for pharmaceutical purposes (Carlsson et al. 2014). More than 200 compounds can be extracted from safflower including fatty acids, flavonoids, and steroids.

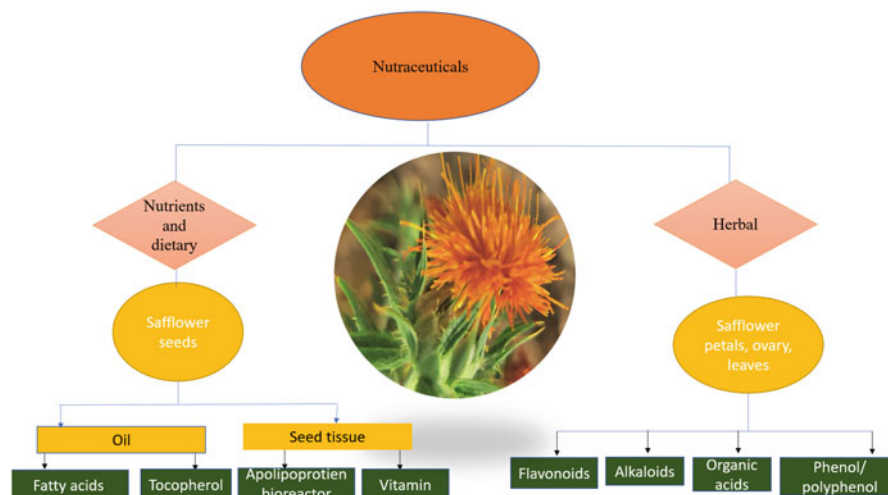


Fig. 1 Scope of nutraceuticals in safflower (*Carthamus tinctorius*)

Safflower promises diverse roles in human nutrition and health. The nutritionally beneficial compounds are mainly extracted from its seeds and flowers. Based on the plant part used, safflower products can be categorized into seed-based and non-seed based products. Safflower seeds are the source of edible oil along with protein, β -carotene (vitamin A), and riboflavin (vitamin B₂). The richness of safflower oil in unsaturated fatty acids such as OA and LA along with low levels of low-density lipoprotein makes it highly effective in managing cardiovascular diseases. It contributes to artery dilation, lowering of blood pressure, blood flow enhancement as well as tissue oxygenation. Due to the high levels of LA, it is also effective in controlling osteoporosis. It possesses anti-inflammatory properties, corrects bone loss, and increases intestinal calcium absorption (Bae et al. 2002). The seed has active α -glucosidase inhibitors in derivative form of serotonin and is used in traditional medicine for treatment of diabetes (Takahashi and Miyazawa. 2012). The non-seed based plant parts such as petals are the source of the flavonoid glycosides, carthamin, kaempferol, quercetin, chalcones including hydroxysafflor yellow A (Shirwaikar et al. 2010). Safflower yellow A showed inhibition of formaldehyde-induced swelling, formation of ball granuloma, and histamine-stimulated capillary permeability and thus exhibits anti-inflammatory effects (Huang et al. 1984). Petals of safflower possess analgesic activity and can be considered a potential drug equivalent to morphine without the side effects (Almeida et al. 2001). Being rich in hypolipidemic agents, safflower petals have antidiabetic effects. The flowers of safflower elevate insulin level by enhancing insulin secretion from islets of Langerhans as well as regeneration, restores protein breakdown, and promotes glycogenesis (Asgary et al. 2012). Being the source of the fatty acids, tocopherols, vitamins, and antioxidants, safflower oil is a potential bioactive food ingredient.

Safflower exhibits a high level of geographical diversity (Ashri and Knowles 1960), phenotypic diversity (Ashri et al. 1974; as well as molecular diversity (Hassani et al. 2020; Ambreen et al. 2015; Amini et al. 2008). Safflower is characterized by a rich germplasm, consisting of cultivars, landraces, and wild-species. A germplasm directory of safflower documented details of germplasm collected from 18 different collection sites across 14 countries (Zhang and Johnson 1999) (<http://safflower.wsu.edu/>). Currently, a large collection of safflower germplasm is available at USDA, USA and has a repository of ~2400 accessions representing germplasm from 50 countries. A large collection of germplasm and the associated genetic diversity provide an opportunity to exploit safflower for its nutraceutical as well as pharmaceutical properties through genetic improvement programs. The focus of such breeding efforts has largely been on improvement of seed-based products while other non-seed based compounds are yet to be targeted in research programs. Thus, this review on safflower provides a detailed study of the composition, utility, and genetic improvement of seed-based products of safflower, along with a brief account on non-seed based products.

2 Seed-Based Compounds in Safflower

2.1 Description of Compounds

Safflower is well supplied with compounds such as fatty acids (including OA and LA), vitamins such as tocopherols, and antioxidants such as flavonoids and alkaloids (Table 1). Although flavonoids and alkaloids could be extracted from seeds, they are major constituents of petals and are described in later sections.

2.1.1 Fatty Acids and Oils

Oilseed crops are major sources of vegetable oil. Based on seed oil content and fatty acid composition, they can be utilized for dietary, industrial, or pharmacological purposes. Oil content in safflower germplasm ranges from 20% to 45%. The crop germplasm can be broadly divided into two categories (High Oleate and High Linoleate varieties) based on composition of seed oil, which is principally composed of two unsaturated fatty acids: Oleic acid (C18:1) (OA) and Linoleic acid (C18:2) (LA). These account for approximately 90% of total fatty acid content in safflower oil. The remaining 10% is contributed by saturated fatty acids such as Palmitate (6–8%) and Stearate (2–3%), with traces of other fatty acids not exceeding 0.81% (Porokhvinova et al. 2022). In a study conducted by Fernández-Martínez et al. (1993), fatty acid compositions of 200 safflower accessions were analyzed and both OA and LA demonstrated significant levels of variation, from 3.1% to 90.60% and from 3.9% to 88.8%, respectively.

Safflower seed oil has also been used for treating cardiac and hepatic disorders (Dajue and Mündel 1996). Recent reports have suggested that, when other vegetable oils were blended with safflower oil it enhanced their nutritional value/quality. It has been shown to improve various physiological systems and is used for treatment of

Table 1 Compounds isolated and characterized from safflower seeds (Adapted and modified from Zhang et al. 2016)

S. No	Classification of compound	Name of compound	Reference
1.	Organic acids	Oleic acid	
		Linoleic acid	
		Palmitic acid	Gegel et al. (2007), Fernandez-Martinez et al. (1993)
		Stearic acid	
		Myristic acid	
		Palmitoleic acid	Ghareghani et al. (2017)
		Linolenic acid	
		Caffeic acid	Vieira et al. (2021)
		Ferulic acid	Zhang et al. (1997)
2	Flavonoids	Kaempferol 7-O- β -D-glucopyranoside	
		Acacetin-glucuronide pentoside	Chakradhari et al. (2020)
		Acacetin-7-O-D-glucuronide	
3	Alkaloids	N-feruloyl serotonin	Koyama et al. (2006)
		N-(p-coumaroyl) serotonin	Takii et al. (1999)
		N-(p-coumaroyl)serotonin-O- β -D-glucopyranoside	Sakamura et al. (1978)
		N-[2-(5-hydroxy-1H-indol-3-yl)ethyl] ferulamide	Zhang et al. (1997)
4	Other compounds	Coniferyl alcohol	Peng et al. (2017)
		Sinapyl alcohol	Peng et al. (2017)

arteriosclerosis, hyperlipidaemia, and coronary heart disease (Abidi 2001). The polyunsaturated fatty acids (PUFA) in safflower oil suppress the accumulation of low-density lipoproteins (LDL or bad cholesterol) (Cho 2001). Consumption of safflower phospholipids was shown to have desirable effects on human health such as reduction of lipids in liver and higher levels of high density lipoprotein (Iwata et al. 1992). Significant reduction was observed in levels of total cholesterol, blood glucose, triglyceride LDL, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase in alloxan-induced diabetic rats after their diet was supplemented with 200 mg/kg safflower seed oil, demonstrating the protective effects of safflower oil on liver (Rahimi et al. 2014). In the same study, it was concluded that safflower oil had hypoglycemic and hypolipidemic effect in hyperglycemic rats under the same set of dietary conditions, adding to the growing evidence on its antidiabetic and anti-obesity properties. Zhang et al. (2010) showed that diet supplemented with safflower oil can change gene expression pertaining to fat deposition resulting in mitigation of diet-induced obesity. Safflower oil can stabilize storage of lipophilic compounds showing potential as a functional food or for usage as vector in drug delivery. Cod liver oil encapsulated in safflower oil has increased oxidative stability of PUFAs.

2.1.2 Tocopherols

Tocopherols ($C_{28}H_{48}O_2$), commonly known as vitamin E, is a group of closely related lipids with substitutions on 2H-1-benzopyran-6-ol nucleus along with isoprenoid units. The oilseeds have different types of tocopherols, viz. α -, β -, γ - and δ -tocopherols which protect PUFA against oxidation (Vosoughkia et al. 2011). Three types of tocopherols (α -tocopherol, β -tocopherol, and γ -tocopherol) were found in safflower oil in various amounts ranging from 460.5 to 709.3 mg/kg, 8.5 to 21.6 mg/kg, and 4.5 mg/kg of oil, respectively (Mani et al. 2020). In a separate study, cold pressed safflower seed oil was found to contain α -tocopherol at 376.6 mg/kg, as the main component along with β -tocopherol (4.4 mg/kg), γ -tocopherol (25.9 mg/kg), and δ -tocopherol (1.0 mg/kg) (Topkafa 2016). Safflower oil has high α -tocopherol content making it an excellent source of vitamin E, although low thermostability makes it less suitable for deep frying (Cuesta et al. 2014). Tocopherols have also been used for feed, food, resins, pharmaceuticals, and cosmetics.

2.2 Genetic Improvement Efforts in Safflower

With an ever-increasing demand for healthier foods and plant-based nutraceutical products, research groups around the world are exploring the natural diversity of compounds found in safflower. This entails elucidation of molecular mechanisms governing the biosynthetic and regulatory pathways to modify the biochemical machinery for production of desired products. With continuous efforts in safflower breeding, nutraceutical profile of safflower seeds has been enhanced, especially for oleic acid. However, to keep pace with current market demands further improvement along the same lines is required. Evaluation of safflower germplasm and identification of lines with differing fatty acid profiles could serve as basis for development of lines with higher oil and tocopherol content. Modifying amino acid composition of seed meal as well as purging of harmful content like matairesinol monoglucoside and lignin glucoside can further enhance the nutritional quality of safflower seeds.

2.2.1 Conventional Breeding

The initiation of breeding programmes for crop improvement require germplasm possessing high genetic variability for quantitative traits. Many institutions across the world have curated germplasm collections from major centres of safflower diversity including national collections at China, India, and USA. Detailed information about safflower germplasm collections can be found in a review by Vollman and Istvan (2010).

Oil content and composition are complex traits and are significantly influenced by genotype X environment interactions. Therefore, breeders have relied on simple traits with high heritability for identification of superior genotypes. The direct attributes for oil content and oil composition are total yield of the plant, whereas the indirect attributes for seed yield are head (capitulum) number, head diameter, and grains per head (Arslan 2007). For oil composition, head weight, number of heads, and hull (pericarp) content was used for indirect selection. Seed structure is also an

important determinant of oil content. The percentage of oil in seed shows inverse correlation with hull size. The percentage of oil increases in the very thin hull types (Bergman et al. 1985). Other factors which affect the yield and oil content are crop phenology which is related to genotype and environmental condition.

Biometric analyses have been performed to estimate the effect of genes on morphological traits. Number of branches and plant height are under the influence of additive gene action whereas head diameter (Golkar et al. 2012) shows low broad-sense heritability. The white hull is dominant over the striped hull (Urie 1986). The striped seed and reduced pericarp were shown to be controlled by recessive genes (*Th* and *Stp*) (Ebert and Knowles 1966) and had monogenic inheritance (Ashri and Efron 1964). The seed protein content is governed by additive dominance model (Golkar et al. 2012). Besides oil content, major attention has been given to oil composition for safflower improvement as it makes safflower oil a healthier alternative to others plant-based oils available in the market. Safflower oil is rich in the OA, LA, Palmitic acid (PA) and Stearic acid (SA). For fatty acid composition, both narrow and broad-sense heritabilities have been reported (Golkar et al. 2011). All the fatty acids follow additive gene effect [OA (Hamdan et al. 2009b), LA (Hamdan et al. 2008), PA & SA (Hamdan et al. 2009a)]. LA and SA in safflower are influenced by maternal factors (Golkar et al. 2011) whereas high OA is under the influence of recessive alleles (Fernandez-Martinez et al. 1993).

Unlike fatty acids, safflower germplasm shows little variability for tocopherol profiles. Johnson et al. 1999 reported no variability in the tocopherol profiles whereas Velasco and Fernández-Martínez (2001) found variation in γ -tocopherol profiles. Velasco et al. (2005) identified a natural mutant of *C. oxycantha* which is rich in high gamma-tocopherol instead of standard high alpha-tocopherol content. Mutant allele from *C. oxycantha* was then introgressed in *C. tinctorius* followed by selection, resulting in a line with 90% gamma-tocopherol content (Velasco et al. 2005).

Conventional breeding methods, however, have had limited success in improving quantitatively inherited characters listed above. Crossing and record keeping procedures are laborious and slow, compounded with limitations such as low genetic base in varieties, loss of genes in the successive generations due to segregation and genetic drag. These limitations can be mitigated to some extent by applying recent genomic tools.

2.2.2 Molecular Breeding

Modern tools such as expression profiling and genetic engineering have been effectively used to address limitations of classical breeding efforts. Linkage maps are used to identify chromosomal regions that control qualitative and quantitative traits (Collard et al. 2005); DNA markers associated with genes are subsequently used for marker-assisted selection (MAS). Breeding objectives so far have been primarily focused on increasing seed yield and oil yield and acquisition of resistance against biotic and abiotic stresses. Little has been done on the positional cloning of genes/QTLs involved in the production of nutraceutical compounds. Information on trait mapping in safflower is limited and is described below.

Construction of Linkage Maps

Linkage maps for safflower were initially constructed by Mayerhofer et al. (2010), using RFLP and PCR-based (SSR and cDNA) markers on an intraspecific F₂ mapping population of *C. tinctorius* and an interspecific BC population generated through a cross between *C. tinctorius* X *C. oxycantha*. The resulting linkage maps were compared for synteny and showed significant collinearity of markers in several regions. Since then, several groups have attempted the construction of linkage maps (Table 2).

Linkage maps constructed by various groups in safflower over the last two decades suffer low resolution (with a few exceptions) as the number of markers utilized is limited. Mapping of traits in most cases was done using F₂ populations which suffer from greater heterozygosity compared to RILs. It is therefore crucial that efforts be made for development of high-density linkage maps using SNP markers.

Mapping of Genes

Linoleic Acid

Inheritance of high LA content in safflower was first reported by Hamdan et al. (2008). Results from their study showed 5 RAPD bands linked to the LA content controlling *Li* locus. The fragments were sequenced to design SCAR markers and a linkage map was constructed to include 5 SCAR markers along with *Li* and *Ms* gene loci. The SCAR markers flanked *Li* and *Ms* loci at 15.7 cM and 3.7 cM respectively.

Table 2 Linkage maps constructed in safflower

References	Marker number and type	Population	No. of linkage groups	Length of map (cM)
Mayerhofer et al. (2010)	1412 SSR and 75 RFLP	F ₂ : <i>Carthamus tinctorius</i>	11	954
Mayerhofer et al. (2010)	1412 SSR and 75 RFLP	BC1: <i>C. tinctorius</i> X <i>C.oxycantha</i>	13	580
Hamdan et al. (2012)	47 RAPD, 60 SSR, and 4 SCAR	F ₂ : <i>Carthamus tinctorius</i>	15	816
Pearl et al. (2015)	244 SNP	F ₂ : <i>C. tinctorius</i> X <i>C. palestinius</i>	12	858
Karimi (2015)	168 SSR	F ₂ : <i>C. tinctorius</i>	11	877
Mirzahashemi et al. (2015)	119 SSR and ISSR	F ₂ : <i>C. tinctorius</i>	24	646
Bowers et al. (2016)	2008196 SNPs	F ₆ : RIL: <i>C. tinctorius</i> X <i>C. palestinius</i>	12	959
Wu et al. (2021)	248 SSR	F ₂ : AH04 X YH04 (both <i>C. tinctorius</i>)	12	1136.46

Adapted and modified from Golkar and Karimi (2019)

Oleic Acid

Most safflower germplasm lines are rich in linoleic acid (~70% of total oil content). Knowles (1989) identified safflower germplasm that produced high levels of oleic acid. When compared to oils with higher levels of polyunsaturation, oils high in oleic acid have been reported to have a hypocholesterolemic effect on human health and a higher oxidative stability (Mensink and Katan 1989). High oleic acid content is under the genetic control of partially recessive alleles at the *OI* locus (Fernandez-Martinez et al. 1993). Efforts have been made for mapping major and modifying genes responsible for high oleic acid content (Hamdan et al. 2012). A genetic linkage map was constructed spanning 816.4 cM, comprising 15 linkage groups by using RAPD (47), SSR (60), and SCAR (4) marker loci using an F₂ population. This population was generated through a cross consisting of CL-1(male sterile) × CR-9 (>84% oleic acid) (Hamdan et al. 2012). The *OI* locus was located on linkage group T3 between the SSR marker ct365 and the SCAR marker IASCA-73, respectively, with genetic distances of 0.4 and 39.1 cM.

Tocopherols

Tocopherols are the principal naturally occurring antioxidants in oilseed crops with protective action in biological systems and in oils or products derived from oils. Molecular tagging was used to decipher the genetic control of high gamma-tocopherol, leading to identification of markers for high gamma-tocopherol. A mutant line IASC-1 with high γ -tocopherol was crossed with CL-1 with high α -tocopherol. The resultant F₂ population was analyzed for segregation of the partially recessive *Tph2* gene. Bulked segregant analysis revealed that *Tph2* gene was linked to eight RAPD and one SSR marker, leading to construction of a *Tph2* linkage map. RAPD fragments closest to the *Tph2* gene were converted into SCAR markers. Later, a γ -tocopherol methyltransferase locus was shown to co-segregate with *Tph2* (Garcia-Moreno et al. 2011).

Association Mapping in Safflower

Conventional methods are the time-consuming method for discovering genomic regions governing simple and/or complex traits, such as linkage analysis and/or QTL mapping, involves establishment of biparental mapping populations. Since fewer recombination events are evaluated in biparental population, the allelic variation obtained in QTL mapping is limited, resulting in poor mapping resolution. However, association mapping (AM) provides a faster and efficient method for evaluating complex traits at high resolution, offering a promising way to overcome the constraints of linkage mapping (Abdurakhmonov and Abdurakarimov 2008). AM uses naturally occurring recombination events to identify correlations between phenotypes and genetic polymorphisms in a heterogeneous collection of unrelated accessions/genotypes, allowing for fine-scale mapping of attributes. It has proven to be an effective method for discovering marker-trait relationships for a variety of agronomic traits in several crop species (Zhang et al. 2014; Abdurakhmonov and Abdurakarimov 2008). Small, operational core collections of safflowers have been constructed to assist genetic deconstruction of complex characteristics (Kumar et al. 2016). These core

collections can be the basis for AM if they represent substantial genetic variation and a weak population structure (Pritchard et al. 2000).

Ambreen et al. (2018) performed AM on a set of 124 safflower accessions (CartAP), resourced collectively from two core collections. Significant correlation ($R^2 > 10\%$, $P < 0.05$) was seen between 96 marker-traits associations (MTA). Consistent associations were represented in both General Linear and Mixed Linear models among two growing seasons for traits such as oil content, oil composition, and number of primary branches. Many MTAs were also found between parameters with positive or negative phenotypic correlations (e.g., plant height; days to 50% flowering; OA/LA concentration; number of primary branches/numbers of capitula per plant). Such associations will complement marker-assisted breeding and will assist in deciphering genetic basis of trait variability.

2.2.3 Marker Assisted Breeding and Gene Introgression

In the twenty-first century, molecular breeding has emerged as an indispensable tool for crop improvement crucial for meeting the continuous demand to increase the production of the crop. A PCR based multiplex marker assay for selection of high OA allele 'ol' based on mutation in *CtFAD2-1* gene was reported by Liu et al. (2013). Kadirvel et al. (2020) designed and used SNP genotyping assays such as KASP (Kompetitive Allele Specific PCR) and the Amplifluor™ SNPs genotyping systems for prediction of "ol" allele. Using these assays, the "ol" allele from Montola-2000 was introgressed into the Bhima using assisted backcrossing resulting in the development of promising high oleic lines with OA content ranging from 75.2% to 81.8%.

2.2.4 Genetic Engineering

Genetic engineering uses biotechnological tools to manipulate DNA, resulting in the transfer of genes within/between organisms to produce improved or novel organisms. When applied to crop improvement, transgenesis has yielded genetically modified (GM) crops having novel genes and desirable characteristics. It also overcomes the limitations of reproductive incompatibility encountered in conventional breeding methods. This allows for transfer of traits from any organism into the target crop. The following section describes studies in safflower pertaining to genetic engineering for improvement of health-related traits.

The nutraceutical, γ -linolenic acid (GLA) is a precursor for long-chain polyunsaturated fatty acids. It is synthesized from linoleic acid in endoplasmic reticulum by the activity of Δ -6 desaturase. Δ 6-desaturase gene from *Sapnreginadeclina* was transformed into a high LA variety of safflower resulting in accumulation of high levels of GLA (70% v/v). The transgenic line was eventually granted approval from US Food and Drug Administration and commercialized for nutraceutical purposes as Sonova 400 (Nykiforuk et al. 2012).

Safflower was also used as a bioreactor to produce Italian version of Apolipoprotein AI called ApoAI_{Milano}, which is a major component of circulating HDL and has therapeutic applications against atherosclerosis. In Atherosclerosis, plaque accumulates along the inner lining of an artery, causing hemorrhage or thrombosis (blood clot formation). ApoAI_{Milano} was expressed in transgenic safflower as a fusion

protein specifically targeted to seed tissue. Results showed that selected lines accumulated 14.5% fusion protein as a fraction of total seed protein (TSP) (Nykiforuk et al. 2011). Similarly, recombinant human fibroblast growth factor 10 (rhFGF10) was expressed in safflower seeds using oil body-oleosin technology (Huang et al. 2017). Oleosin-rhFGF10 was introduced and expressed in safflower seed. The trait inheritance was checked till T3 generation.

Decades of plant breeding efforts have raised the oleic content in safflower to ~90%, but costed in terms of low field performance accompanied with poor yields. Wood et al. (2018) engineered super high oleic (SHO) safflower producing seed oil with 93% oleic acid. Safflower was transformed with hairpin-based vectors. Transgenic plants produced seed oil with low PUFA (<1.5%) and only 4% saturate fatty acids. There was no effect on lipid composition of leaves and roots. The seed yield of transgenics was also comparable to non-GM safflower even under different environments and varied sites.

2.2.5 Genomics-Aided Breeding

Genomics is a branch of biology primarily concerned with structure and function of genomes. Functional genomics, a subset of genomics, is concerned primarily with assigning functions to unknown genes. Various technological platforms such as transcriptomics, proteomics, metabolomics, and phenomics are used to analyze gene function. Numerous transcriptomic studies have been conducted to improve our knowledge and understanding of the molecular functions of the genomic components of various organisms. Such studies are expected to increase with the availability of genome sequences.

Partial cDNA clones were generated based on two protein sequences with thioesterase activity (molecular masses: 34 and 40 KDa). Similarly, cDNA clone (CTOS1) was generated based on a new protein putatively involved in accumulation of high levels of oleic acid in seeds (Mizukami et al. 2000) from an accession of safflower with high oleic acid in seeds. In recent years, sequencing of cDNA libraries using next generation sequencing (NGS) has emerged as a novel method for whole transcriptome analysis. De novo transcriptome analysis was performed by Li et al. (2012) using three different tissues, viz., leaves, petals, and seeds leading to identification of differentially expressed unigenes among these tissue. The analysis identified oleosins which were specifically expressed in the seeds. An enzyme, *FAD2* (microsomal oleoyl phosphatidylcholine desaturase) is known to introduce a double bond at $\Delta 12$ position of OA, thereby converting it to LA (Cao et al. 2013). They cloned 11 *ctFAD2* genes, each with a divergent functionality, the largest number of *FAD2* clones in any species. The temporal transcriptome profile of safflower for *FAD2* and other genes involved in oil accumulation was investigated by Li et al. (2021). Their study identified changes in expression of stearoyl-[acyl-carrier-protein] 9-desaturase gene (*SAD*) from 10 to 14 days after fertilization (DAF) and oleate desaturase (*FAD2-1*) from 14 to 18 DAF. They also identified 13 putative transcription factors (TFs) involved in regulating the expression levels of the *FAD2-1* gene. The study found a link between fatty acid biosynthesis and gene expression during seed development.

Safflower seeds (also known as *Carthami semen*) hold a wide array of nutritionally beneficial compounds with a greater part of compound diversity yet to be explored. The recent shift in consumer preferences towards healthier foods and plant-based pharma alternatives has reinvigorated research in improvement of nutritional aspects of seeds. Conventional breeding efforts have led to improvement in overall oil content while molecular breeding has led to the development of high oleic acid lines, many of which have been released for cultivation. With the publication of safflower genome and use of molecular breeding tools becoming commonplace, exploration into other classes of compounds such as seed flavonoids and alkaloids is to be expected.

3 Non-seed Based Compounds in Safflower

Safflower is cultivated primarily for its seeds and oil which could be exploited as nutraceuticals. However, since ancient times, safflower has been used in traditional medicines using/derived from flowers and leaves of the plant. Due to adverse effects of chemical medications, the pharmaceutical industry is exploring alternatives and Safflower has emerged as a promising option. This section describes pharmaceutical compounds obtained from safflower.

Flowers and leaves of safflower contribute medicinal as well as nutritional compounds. Safflower petals are consumed as herbal tea and have medicinal uses for menstrual problems, cardiovascular diseases, hypertension, arthritis, spondylosis, diabetes, pain, and swelling associated with trauma. They also have purgative, antioxidant, anti-inflammatory, analgesic, and anticonvulsant properties (Zhou et al. 2014). Flowers of the plant also possess central analgesic activity and can be considered as potential drugs equivalent to morphine-like substances. Thus, these can be beneficial to surpass the side effects caused by morphine (Almeida et al. 2001).

3.1 Description of Compounds

The major constituents of safflower flowers are flavonoids, glycosides, carthamin, and safflower yellow. Other components, viz., quercetin, carthamidin, kaempferol, isocarthamidin, glycosides of hydroxykaempferol, chalcones such as hydroxysafflower yellow A, safflomin-A, and acetylenic glucosides (carthamosides) have also been found (Table 3) (Jiang et al. 2005). The leaves of safflower are rich in Luteolin and its glucopyranosides (Lee et al. 2002).

3.1.1 Flavonoids

Flavonoids are polyphenolic secondary metabolites synthesized via phenylpropanoid pathway, with naringenin chalcone acting as precursor for a majority of them. In safflower, bioactive flavonoids are primarily concentrated in petals and seeds and exhibit a broad range of pharmacological activities. Flavonoids are broadly divided

Table 3 Compounds isolated and characterized from safflower flowers and leaves

S. No	Compound classification	Plant Part	Compound	Reference
1	Flavonoids	Flowers	Carthamin	Kanehira and Saito (1990)
			Safflor yellow A	Takahashi et al. (1982)
			Safflor yellow B	Takahashi et al. (1984)
			Safflomin A	Watanabe and Terabe (2000)
			Safflomin B	Chen et al. (2013)
			Tinctormine	Meselhy et al. (1993)
			Quercetin	Sun et al. (2003)
			Anhydrosafflor yellow B	Kazuma et al. (2000)
			Quercetin-3-O- β -D-glucoside	Kazuma et al. (2000)
			Quercetin-3-O- α -L-rhamnoside-7-O- β -D-glucuronide	Kazuma et al. (2000)
			Quercetin-7-O- β -D-glucoside	Hattori et al. (1992)
			Kaempferol	Kim et al. (1992)
			Kaempferol-3-O- β -D-glucoside	Hattori et al. (1992)
			Kaempferol-3-O- β -sophorose	Hattori et al. (1992)
			Kaempferol-3-O- β -rutinoside	Hattori et al. (1992)
			Scutellarein	Hattori et al. (1992)
52 Luteolin 7-O- β -D-glucopyranoside	Lee et al. (2002)			
53 Luteolin-7-O-(6''-O-acetyl)- β -D-glucopyranoside				
Quercetin-7-O-(6''-O-acetyl)- β -D-glucopyranoside				
Quercetin 7-O- β -D-glucopyranoside				
2	Alkaloids	Flowers	7,8-dimethyl pyrazino[2,3-g]quinazolin-2,4-(1H,3H) dione	Jiang (2008)
			Safflospersmidine A	Jiang et al. (2008)
			Safflospersmidine B	
3	Polyacetylenes	Flowers	4',6'-acetonide-8Z-decaene-4,6-diyne-1-O- β -D-glucopyranoside	
			4,6-decadiyne-1-O- β -D-glucopyranoside	Zhou et al. (2006)
			(8Z)-decaene-4,6-diyne-1-O- β -D-glucopyranoside	

(continued)

Table 3 (continued)

S. No	Compound classification	Plant Part	Compound	Reference
			(8Z)-decaene-4,6-diyne-1-ol-1-O-β-D-glucuronyl-(1→2)-β-D-glucopyranoside	
			(2Z,8E)-tetradecadiene-4,6-diyne-1,12,14-triol-1-O-β-D-glucopyranoside	He et al. (2011)
			(2E,8Z)-tetradecadiene-4,6-diyne-1,12,14-triol-1-O-β-D-glucopyranoside	
4.	Organic acids	Flowers	p-coumaric acid	
			p-hydroxybenzoic acid	
			Succinic acid	Jiang (2008)
			4-O-β-D-glucopyranosyloxybenzoic acid	
			4-O-β-D-glucosyl-trans-p-coumaric acid	Zhou et al. (2008)
			4-O-β-D-glucosyl-cis-p-coumaric acid	
5.	Other compounds	Flowers	Uridine	Jiang et al. (2008)
			Adenosine	
			Adenine	
			Thymine	
			Uracil	
			Roseoside	
			Sitosterol	
			Syringin	
			Methyl-3-(4-O-β-D-glucopyranosyl-3-methoxyphenyl) propionate	
			Ethyl-3-(4-O-β-D-glucopyranosyl-3-methoxyphenyl) propionate	Zhou et al. (2008)
			Ethylsyringin	
			Methylsyringin	

Adapted and modified from Zhang et al. (2016)

into two groups: quinochalcons and flavonols. The quinochalcons comprises of hydrosafflower yellow A, tinctorimine, carthamin, and cartorimine while the flavons include kaempferol, quercetin, and their glucosides (Guo et al. 2017).

Glycosides derived from flavonols such as quercetin and shannesol, have been extensively studied and were shown to possess antioxidative activity. Safflower extract containing flavonoids is also protective to the cardiac system, stabilizing

the oxygen supply to the heart and the heart rate (Guo et al. 2018). Flavonoids can induce aggregation of the platelets and depolymerization of ADP in platelets). The hypotensive effect of safflower flavones has been shown in animals (Mani et al. 2020). The major component of yellow pigment, Hydroxysafflor yellow A (HSYA) have a strong antagonistic effect on the receptors of platelet activating factor. In the in vitro experiments carried out by Zhang et al. 2002, varying concentrations of HSYA was found to inhibit both aggregation of polymorphonuclear leukocytes and platelet induced by platelet activating factor. HSYA also helps to improve the condition of acute myocardial inflammation (Zhou et al. 2013) by inhibiting the process causing it. HSYA also act as neuroprotector against cerebral injury and ischemia-reperfusion conditions by antioxidation, reducing neurological-deficit scores and decreasing superoxide-dismutase activity.

Diabetes is an important cause of vascular complications because it can activate dysfunctional biochemical pathways. One such example is the increased glycation of the proteins by methylglyoxal. Under in vitro conditions, HSYA was seen to inhibit protein glycation by reducing the production of advanced glycated end products, by reducing the protein modifications that were methylglyoxal-mediated and their cross-linking (Yue et al. 2013). The extract derived from the flowers of safflower also elevates insulin level by regenerating Langerhans islets and further induces the beta cells to secrete insulin. It is also capable of restoring breakdown of protein and promoting glycogenesis.

Quinochalcones are the primary yellow and red pigments in safflower. Structurally, most quinochalcones isolated from this plant have a unique C-glycosylated cyclohexanonediolenol moiety. Up to 18 quinochalcone C-glycosides have been isolated from safflower till date. Carthamin is composed of two C-glycosylquinochalcone moieties (Zhang et al. 2016). Quinochalcone C-glycosides has an array of bioactivities like anticoagulation, anti-inflammation, antioxidation along with antihypertensive, and antitumor activity.

3.1.2 Alkaloids

Alkaloids are a group of small cyclic organic compounds, containing a nitrogen atom usually within the carbon ring, which gives them a slightly basic property. They are classified on the basis of precursors from which they are synthesized. Tryptophan, Tyrosine, Lysine, and Ornithine are amino acid precursors, which undergo enzymatic reactions giving rise to indole, tetrahydroisoquinoline, piperidine, and pyrrolizidine alkaloids, respectively). In addition to nitrogenous bases, serotonin derivatives like N-feruloyl serotonin and N-(p-coumaroyl) serotonin, and spermidines have also been isolated from safflower. Based on their inhibitory action against melanin production, it was suggested that they can be potential inhibitors of melanogenesis (Zhou et al. 2014). Protective effects of the serotonin derivatives against cardiovascular diseases were investigated in ischemic and reperfused heart of guinea-pig. The study showed that there was improvement in relevant biological markers after administration of the two alkaloids and played important antioxidant roles for such conditions (Al-Snafi 2015).

3.2 Genetic Improvement of Non-seed Based Products from Safflower

Although safflower is used widely in Chinese medicine, the major attention for the breeder has been the safflower oil due to its health-related and commercial benefits. Thus, in contrast to seed-based products, very few genetic studies have been done for non-seed based products. However, some work has been done on flavonoids through genomics-aided breeding.

Transcriptomic studies including full-length transcriptome sequence analysis for the major flavonoid – HSYA, has shown that *CtC4H2*, *CtCHS3*, *CtCHI3*, *CtF3H3*, and *CtF3H1* are the major genes that are involved in flavonoid production (Chen et al. 2018). These known putative flavonoid genes can be the basis for biotechnological improvement of safflower. In a study by Yang et al. (2007), cDNA-AFLP was used in combination with bulked segregant analysis to mine differentially expressed genes associated with high HSYA content. Transcript derived fragments (TDFs) which showed significant association with HSYA content were retained for construction of linkage maps. Genetic linkage analysis showed that TDF-2, TDF-3, and TDF-9 were tightly linked to the HSYA genomic region. Another key regulatory enzyme of the flavonoid pathway is Chalcone isomerase (CHI). Guo et al. (2019) successfully obtained transgenic *Arabidopsis* and Safflower lines overexpressing *CtCHI1* gene. The overexpressed *CtCHI1* genes caused the 3.9 fold upregulation of *CtPAL3* and *CtC4H1* genes and downregulation/inhibition of *Ct4CL3*, *CtF3H*, and *CtDFR2*. Also, the comparison of transgenic and control groups revealed that there were 788 different metabolites marked and mostly were upregulated. Wang (2015) achieved overexpression of *CtAK* (key regulator of Aspartate metabolism) in transgenic safflower using CaMV35S promoter. Exogenous applications of ethylene and 1-aminocyclopropane carboxylic acid oxidase (ACO) were shown to affect the accumulation of flavonoids in safflower (Tu et al. 2019). Two of the ACO genes were cloned from safflower (*CtACO1* and *CtACO2*) and transgenic plants were developed through *Agrobacterium*-mediated floral dip method. In overexpressed lines of *CtACO1*, metabolite analysis showed the accumulation of quercetin and its glycosylated derivatives like rutin, while the amount of quinochalcones, kaempferol derivatives, apigenin, and luteolin were reduced.

Genetic studies were also done for the domestication-related traits in safflower. Sixty-one Quantitative Trait Loci (QTL) in various linkage groups were identified including a large-effect QTL corresponding to the flower colour (Pearl et al. 2014). A 3:1 segregation pattern was observed for flower colour in the F2 mapping population consisting of 276 individuals. This indicated that the changes in carthamin (the quinochalcone pigment responsible for the red-colored florets) synthesis are influenced by a single locus. These findings differed from other studies (Pahlavani et al. 2004) that showed the impact of multiple genes on flower color and the presence of at least two interacting genes that distinguishes the orange and the yellow-colored florets. These contrasting results are obtained probably due to selection of high carthamine-producing parents than any other compounds controlling the flower coloration to develop the mapping population by Pearl et al. (2014).

4 Social, Economic, and Political Impact of Nutraceutical and Pharmaceuticals

Healthy diet and lifestyle are key factors that influence prevention of diseases and promote betterment of human health. In recent years, there is a strong and increasing demand for development of functional food markets that mainly focus on increased production of nutraceuticals using organic and natural ingredients that are high on nutrition (Daliri and Lee 2015).

Safflower offers good raw material for several economic sectors. It is widely cultivated because of its ability to survive under varying edaphoclimatic conditions (Menegaes and Nunes 2020). Safflower oil is rich in linoleic acid containing tocopherols that act as antioxidants. It is recommended to patients suffering from diabetes and cardiovascular diseases, as it helps in reducing the level of cholesterol in blood similar to olive oil (Menegaes and Nunes 2020).

Due to advances in agricultural biotechnology, it is now possible to produce food crops which have enhanced nutritional content and can contribute towards overall human health. Biofortification practices are generally employed in order to enhance the nutritional value of food crops either by conventional breeding or through the use of biotechnology. Through these practices vitamins and minerals have been added to crops in order to produce a diet that has well balanced nutrition and supplements to address the issues of a malnourished population successfully. Nutraceuticals include bioactive components such as polyunsaturated fatty acids (PUFA), antioxidants, phytochemicals that are found in plants. Genetic engineering is generally employed to improve natural and therapeutic values of natural foods (Prabavathy et al. 2022).

Nutraceutical sector includes three main divisions which include herbal/natural products, dietary supplements, and functional foods (Asif 2019). Globally, of these three, dietary supplements and herbal/natural products were the most rapidly growing divisions with 19.5 % and 11.6% of growth per year, respectively (Asif 2019). It has been observed that the use of genetically modified products, increased income of farmers by \$92 million between 1996 and 2011 because the overall production cost got decreased, and there was reduction in crop loss because GM crops were insect and pest resistant (Karalis et al. 2020). The Nutraceutical market has grown exponentially from the last decade in developed and developing nations all over the world. Globally, USA and Japan have well established nutraceutical markets and their income from this sector is showing a consistent increase. Among developing nations, India, China, and Brazil are taking the lead in the field of nutraceuticals. India and China are now providing the raw sources for development of natural products. Nutraceutical foods were the major market section with worth of 39.9 billion USD in year 2007 and increased up to 56.7 billion USD by 2013 (Singh et al. 2019). Beverages included under nutraceuticals had worth of 38.4 billion USD in 2007 and 71.3 billion USD in 2013. The nutraceutical markets grew by around 250 billion USD in 2018, and it has been observed that the evolution of nutraceuticals within these years has helped to improve the average lifespan of adult people specially belonging to age of more than 60 years (Bhowmik et al. 2013). In 2015, the estimated value of the nutraceuticals market in India was 4 billion

USD however, it is expected to increase to 10 billion USD by 2022. Ministry of AYUSH and companies like Dabur, Patanjali, etc., have played a significant role in expanding the nutraceutical market in recent years (Singh et al. 2019).

Under the Food Safety and Standards Act, 2006, a regulatory body has been established which was named as Food Safety and Standards Authority of India (FSSAI). Through combining the various acts and orders under different governmental bodies, it manages the food related issue in India. FSSAI functions to ensure that safe and wholesome food is available for human consumption and regulates import, distribution, manufacture, storage, and sale of nutraceuticals and dietary supplements (Verma and Popli 2018). In USA, nutraceuticals and functional foods are tightly monitored by the Federal Food, Drug, and Cosmetic Act by the Food and Drugs Authority (FDA), USA. Safety and labelling of dietary supplements is also under the control of FDA to ensure that they are able to fulfil the needs of FDA and Dietary Supplement Health and Education (DSHE) regulations before being marketed (Daliri and Lee 2015). Before releasing new supplements into the markets, manufacturers are required to inform FDA and provide information indicating that their product qualifies as a new dietary supplement and is safe to use as specified in labelling. The Nutraceutical industry has to face a lot of challenges including adherence to country-specific regulations, less innovation by food industries (Daliri and Lee 2015). When a company obtains the petition for health claim and receives the acceptance, other competitors can also use their claim since majority of companies use products containing “easily available” ingredients which can be copied, unless and until the nutraceutical products contain exclusive ingredients that may be patented. The Nutraceutical sector can achieve more milestones when food companies become more innovative. Currently, they are functionalizing conventional products by adding more vitamins, herbal extracts, and minerals rather than inventing new products.

5 Future Prospects

Being rich in nutra and pharmaceutical compounds, safflower has a scientific and economic rationale for its use in the nutraceutical industry. Its rich nutritional value can also provide food security and improve the economy of developing countries. Many countries have adopted safflower as a mainstream crop and are now among the top producers in terms of production and acreage. Due to its richness of oleic acid and linoleic acid, Safflower has been used widely for its oil. Being a semi-arid crop, it can grow in the area where irrigation is limited. Safflower is also known for various important medicinal and nutritional benefits, which makes it an ideal candidate for use as a nutraceutical as well as pharmaceutical. However, the knowledge accumulation has been slow as safflower is a marginal crop, but it promises much more due to the benefits it brings along. With the availability of genome sequence data and sufficient genetic variability, the crop requires concentrated efforts to boost its status as a mainstream crop in nutraceutical and pharmaceutical industry.

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