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RESEARCH ARTICLE

Effect of ascorbic acid on phenolic content and antioxidant activity of *Carthamus tinctorius* L. cultivars: An *in vitro* comparative analysis

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Abstract

Climate changes are resulting in water scarcity, necessitating the cultivation of important crops that fulfill food demands and provide other benefits to humanity. Safflower (*Carthamus tinctorius* L.), a Rabi crop, is rich in several important compounds with potential use in pharmacology, agriculture and industry. The diverse profiles of mono- and polyunsaturated fats render it a valuable oilseed crop. The present research is structured into 2 sections. In phase I, we examined the effects of exogenously administered ascorbic acid (AsA) on the phenolic compounds, osmoprotectants and antioxidants of 2 safflower cultivars (Thori-76 and CV-256) under drought stress conditions. The cultivar CV-256 exhibited elevated total phenolic content in less-watered circumstances due to the application of exogenous ascorbic acid. Antioxidant enzymes, including catalase, peroxidase and superoxide dismutase (SOD), were activated under conditions of water deficiency. Both cultivars exhibited a marked increase in superoxide dismutase activity due to the use of foliar ascorbic acid. The second part of the study focuses on the antioxidative and hepatoprotective characteristics of safflowers by blocking HCV entrance into cells and identifying its potential compounds efficient for NS3/4 inhibition. *In vitro* research demonstrated that both cultivars had substantial antioxidative capability and inhibited viral entrance into cells. *In silico* analyses found Coumaroyl, Hydroxyarctigenin and Pinoresinol as prospective antagonists of NS3/4. It was also shown that p-coumaroyl, an antioxidant, exhibited strongest bonding affinity with the receptor protein.

Keywords: antioxidants; CV-2560; HCV; hepatoprotective; molecular docking; phenolics; Thori-76

Introduction

Water scarcity is a concerning issue resulting from increased climate change and pollution, which adversely affects the qualitative and quantitative yield of many crops by impacting their physio-biochemical characteristics (1). This is classified as primary abiotic stress, which significantly disrupts photosynthesis, growth, nutrient uptake and osmotic characteristics, ultimately resulting in a substantial decrease in crop output (2, 3). Hydrogen peroxide (H_2O_2) produced during the photo respiratory process significantly influences signal transduction although in a limited quantity that increases under water deficit situations (4).

Safflower, a significant oilseed crop with potential therapeutic applications, exhibits a substantial production decline among territories characterized by extensive cultivation areas but little rainfall (5). Ascorbic acid is recognized for its role in regulating stress tolerance in several plants, including pea, canola, tomato and maize (6–8). The oxidative characteristic of ascorbate renders it distinctive among other antioxidants. It immediately disrupts the reactive oxygen species (ROS) and scavenges them, restricting their levels to a tolerable capacity for plants (9). Exogenously administered ascorbic acid (AsA) primarily safeguards the proteins and lipids of plants under various stress circumstances (10). The tolerance mechanism triggered by AsA is achieved by increased plant growth, higher photosynthetic pigments, improved photosynthesis and transpiration rates, as well as oxidative stress responses (11).

Safflowers have several pharmacological properties. Traditional medicine has utilized seeds and blooms for several purposes, such as extracting oil and using them as a natural food additive and colorant in cooking. Because of their antibacterial, anti-inflammatory, antitumorigenic and antioxidant qualities, they are also used to give discomfort, osteoporosis, oedema, allergies, hypertension,

cardiovascular illnesses, cancer, inflammation and dermatological problems (12). This plant's anti-inflammatory and antioxidant qualities are correlated with its potential to scavenge and minimize ROS generation, thereby inhibiting NF-κB and the synthesis of inflammatory cytokines while promoting the expression of antioxidants such as hemeoxygenase-1 (HO-1) and SOD (13, 14).

Additionally, the seed and flower's hypoglycemic, insulin-releasing and hypolipidemic qualities have been shown streptozotocin (STZ) induced diabetic mouse models (15). The seeds and flowers play a significant contribution to lowering hepatic de novo lipid production and improving cholesterol. They achieve this by inhibiting genes related to the production of triglycerides (TG) and cholesterol (CJOL), while simultaneously promoting genes associated with mitochondrial fatty acid oxidation (16). The hepatoprotective properties of *C. tinctorius* oil extracts and seeds were demonstrated in a number of liver damage models, including carbon tetrachloride (CCl_4), where the protective mechanism was linked to diminished ROS production, resulting in decreased NF-κB activity and enhanced Nrf2 signaling (17). By reducing ROS generation and NF-κB activation, this plant has been shown to reduce inflammatory processes among human umbilical cells (18). In safflower specifically showed that application of certain antioxidants could improve physiological traits and yield under water deficit conditions, although studies specifically focusing on ascorbic acid in safflower remain limited, indicating a research gap (19).

This study aims to evaluate the impact of ascorbic acid on safflower yield in conditions of drought stress. The yield of 2 cultivars (Thori-76 and CV-256) was assessed under drought stress, with treatments of ascorbic acid at concentrations of 100 and 150 ppm. The impact of ascorbic acid on the yield of both cultivars has been previously documented in our research (20). This study examines the effect of ascorbic acid on 2 principal osmoprotectants in plants: proline and glycine betaine and phenolic contents, in conjunction with its effect on the plant's oxidative defense mechanism. In the second phase of our investigation, we emphasized the prospective use of safflower as an antioxidant and hepatoprotective agent by inhibiting HCV entrance into cells, as HCV infection is a significant contributor to serious liver diseases, including chronic hepatitis, cirrhosis and HCC. The other primary purpose of this work was the identification of potential compounds that function as NS3/4 inhibitors using *in silico* analyses.

Material and Methods

Experimental design

The study was conducted in Molecular and Medical Genetics Lab, Department of Biochemistry, Government College University Faisalabad. The seeds of 80 cultivars of safflower were taken from Pakistan Agricultural Research Council (PARC), Islamabad, Pakistan. A totally randomized analysis with 4 imitates was performed with 2 distinct cultivars of safflower (i.e. Thori-76 and CV-256) to look at the impacts of dry season and shifting degrees of foliar applied AsA. A total of 48 experimental units were planted. Two levels of drought were maintained (i.e. 100 % and 60 % field capacity). After the second week of growth drought was applied. Two concentrations (i.e. 100 ppm and 150 ppm) of ascorbic acid were prepared and exogenously applied to 1 month old plants.

Leaf free proline, glycine betaine (GB), total phenolic content and antioxidant enzyme assay was done by method used in our previous work (20). Absorbance of both leaf free Proline and Glycine betaine (GB) was measured at 520 nm and 365 nm respectively. In glycine betaine the organic lower layer of the solution was taken and recorded. The absorbance at 750 nm was measured using a spectrophotometer of total phenolic content. To ascertain the SOD activity, the solution was exposed to light for 15 min and absorbance at 560 nm was measured. Peroxidase's antioxidant activity was assessed at 400 nm. For the evaluation of catalase activity at 240 nm, the absorbance was measured.

Anti-oxidative potential

DPPH Assay

The DPPH assay method involves measuring the strength of the color change in solutions in relation to the DPPH concentration using spectrophotometry. The process consists of combining 1 mL of the methanolic extract with 3 mL of methanol, followed by the addition of 1 mL of DPPH (0.012 g/100 mL). Absorbance at 517 nm was measured after 15 min (21).

$ABTS^+$ Assay

The $ABTS^+$ clearance rate was determined according to the procedure outlined (22). The ABTS solution was prepared by combining 15 mL of a 7 mM ABTS solution with 246 µL of a 140 mM potassium persulfate aqueous solution. This reaction was conducted in the dark for a duration of 16 hr. Dilution with methanol to get an absorbance peak of 0.70 ± 0.02 at 734 nm. The sample was then mixed using 1.0 mL of ABTS free radical solution, 1.0 mL of methanol and 100 mL of an extract of methanol (supernatant) in a dark condition. The absorbance was measured using a Shimadzu UV2000 Spectrophotometer 6 min later. (Shimadzu, Kyoto, Japan). Trolox served as the standard reference and findings were expressed in µmol Trolox equivalent per 100 g of safflower extract (µmol TE/100 g). Each methanol extract sample was measured in triplicate.

Hepatoprotective activities

Cell culture

For this study Huh7.5 cells were used. The cells were cultured in DMEM (Invitrogen). Ten percent fetal bovine serum (FBS) (Invitrogen), 100 U/mL of the antibiotic penicillin, 100 µg/mL of streptomycin and unnecessary amino acids were added to the DMEM to enrich it. The cells were incubated at 37 °C in a 5 % CO_2 (23).

Viral propagation

In a T75 flask, 1.5 × 10^5 Huh7.5 cells were inoculated. The medium was discarded 24 hr after seeding and the cells were rinsed once with 10 mL of 1X PBS before being

administered with JFH-1 HCVc at a rate infection rate of 0.01 FFU/cell in an entire volume of 10 mL DMEM. After reaching confluence, cells were split in a 1:3 ratios until day 10 after infection. The supernatant was extracted at predetermined intervals and the infectivity titers were evaluated by titration (24).

Infectivity titration assay

Repeated 96-well Huh7.5 cultures were infected with culture supernatants after they had been diluted 10 times. A final dosage of 0.25 % methylcellulose was added to the cells with full DMEM 24 hr after infection. The cells were fixed with 4 % paraformaldehyde 72 hr after infection and then treated with the human monoclonal anti-E2 antibody C1 for HCV E2 immunohistochemistry staining (24). Viral titers were quantified as FFU/mL, based on the mean number of E2 -positive foci identified at the maximum HCV-positive dilution (25). To determine the hepatoprotective effect of safflower extract of both cultivars (Thori-76 and CV-256), cell lines were incubated with 150 nmol of plant extract in the presence of HCV particles.

Cell viability assay

Cell viability was evaluated through the MTT assay, as described in previous study (26). The cells were seeded at a density of 1 × 10^4 cells per well in 96-well plates. Subsequent to various treatments, 20 µL of MTT solution (5 mg/mL) was introduced to each well, maintaining a final concentration of 5 mg/mL for 4 hr at 37 °C. Thereafter, the medium was eliminated and DMSO (150 mL) was introduced to each well. The optical density (OD) was measured spectrophotometrically at 490 nm with a microplate reader and the cell survival ratio was represented as a percentage of the control.

HPLC analysis for the quantification of total phenolic content

Quantification of phenolic contents in safflower was carried out by using HPLC (27). A fresh leaf (1 g) of safflower was conserved in liquid nitrogen and 0.5 g each sample was mixed with 1.5 mL of 3 extraction solvent mixtures (50 mL dimethyl sulfoxide (DMSO) with 50 mL methanol, 70 mL methanol with 25 mL H_2O and 0.5 mL HCl, 80 mL ethanol with 20 mL H_2O), specific for HPLC. Reaction mixture was then freeze for several hours. The frozen mixture underwent centrifugation at 18000 rpm for 15 min at a temperature of 4 ˚C, after which the supernatant was utilized for HPLC analysis. Reversed phase column HPLC with ODS (C18) was used for the quantification of phenolic contents present in reaction mixture. For the differentiation of phenolic contents, mobile phase consisting of 2 solvents i.e. solvent 1 containing 70 % acetonitrile and 30 % methanol while solvent 2 comprises of 0.5 % glacial acetic acid, were used at consistent 1 mL/min flow rate in gradient mode. To inject 20 µL of sample in the column, micro-syringe was used and the observations were recorded at 275 nm.

Docking of antioxidant and phenolic compounds against HCV NS3

Twenty different phytochemicals from *C. tinctorious* were screened to find their binding properties against HCV NS3 protease. Chemical structures of selected antioxidants and phenolics from the selected plant were retrieved from PubChem database as ligands. The ligands were energy minimized using MOE and a database was constructed in mdb format. The 3D structure of receptor protein (i.e. HCV NS3/4A protease) was retrieved from PDB. The receptor was prepared for docking against selected ligands. Docking was performed through pocket selection from receptor protein using MOE. Top 6 complexes with minimum S-score were listed and checked for their binding interactions with receptor.

Drug scan

Using Lipinski rule of five, the selected phytochemicals were checked for their drug likeliness through drug scan. In this analysis the drug likeliness was checked on the basis of molecular mass, hydrogen bond donor, hydrogen bond acceptor, Log P and molar refractivity of selected ligands (28).

Results

Leaf free proline

Fig. 1 depicts the concentration of free proline accumulation in the leaves of Thori-76 and CV-256. The results demonstrated that in drought conditions both varieties of safflower showed a significant ($p < 0.05$) reduction in proline accumulation in the leave of both varieties of safflower. While the foliar use of ascorbic acid significantly ($p < 0.05$) increased the concentration of free proline in the leaves of Thori-76 as compared to CV-256.

Glycine betaine

Fig. 2 depicts the concentration of glycine betaine in Thori-76 and CV-256 in droughts conditions and after applying ascorbic acid. Results highlight a significant ($p < 0.05$) decline in accumulation of glycine betaine during drought conditions. While a non-significant increase in glycine betaine was observed in both varieties of safflower treated with ascorbic acid. A non-significant difference in concentration of glycine betaine was observed with in Thori-76 and CV-256.

Total phenolic contents

Fig. 3 demonstrates the concentration of total phenolic contents in Thori-76 and CV-256 during drought conditions and after the application of ascorbic acid at the concentration of 100 ppm and 150 ppm. Results indicated that total phenolic contents in Thori-76 and CV-256 were considerably ($p \leq 0.001$) decreased due to drought. On the other hand, exogenous application of ascorbic acid increased the total phenolic contents in both cultivars during control as well as less water supply group. CV-256 had shown high content of total phenolics as compared to the Thori-76 under well water conditions due to foliar applied ascorbic acid. During water shortage, Thori-76 had shown an increase in total phenolic contents because of foliar applied AsA while CV-256 had shown consistency in total phenolic contents. Higher concentrations of AsA had shown favorable results to improve total phenolic contents in both cultivars of safflower.

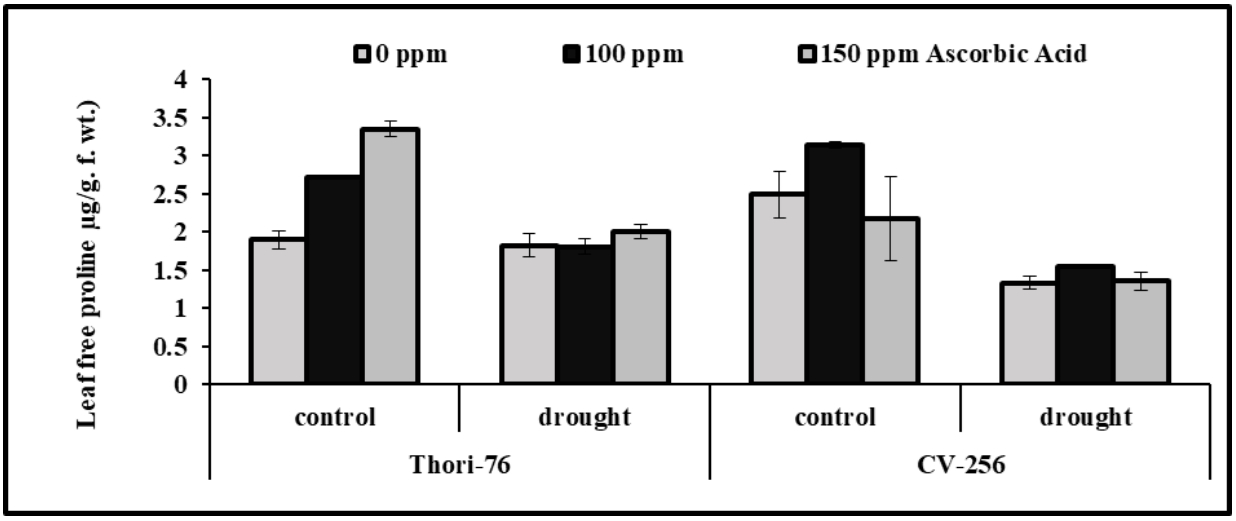

Fig. 1. Leaf free proline of Thori-76 and CV-256 of safflower. Foliar treated with AsA exposed to drought circumstances of stress (Mean ± S.E).

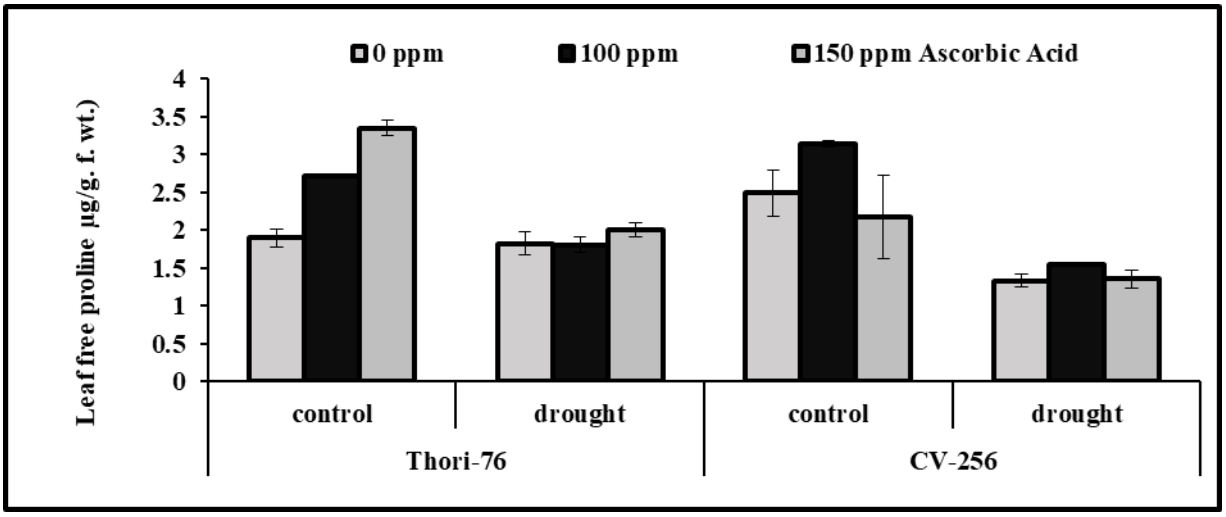

Fig. 2. Concentration of glycine betaine in Thori-76 and CV-256 of safflower. Foliar treated with AsA exposed to drought stress conditions (Mean ± S.E).

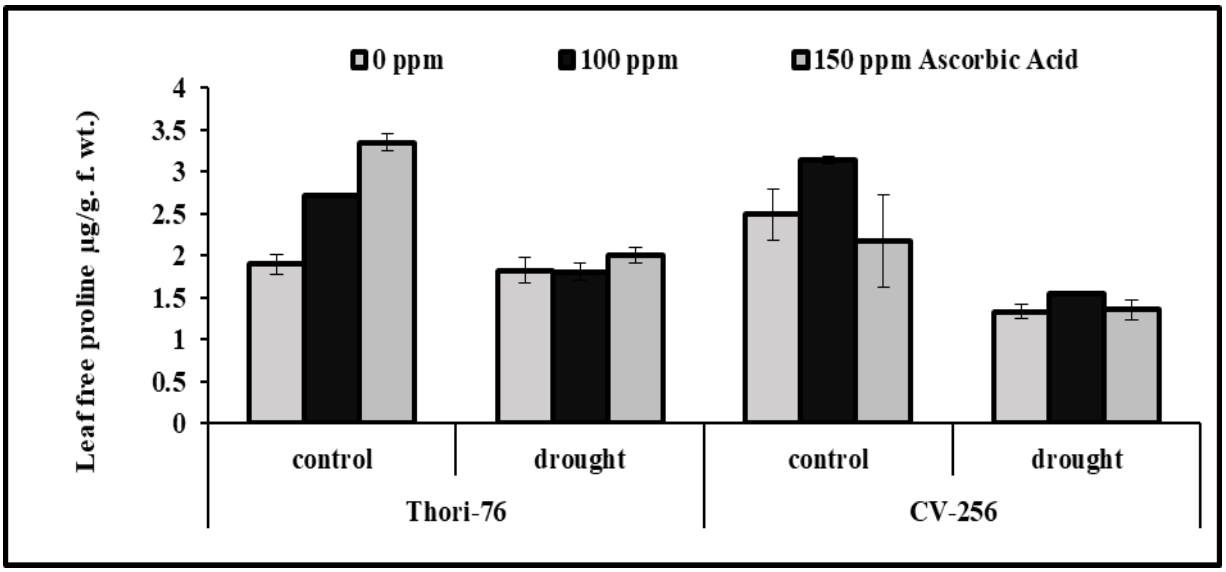

Fig. 3. Total phenolic contents of Thori-76 and CV-256 of safflower. Foliar treated with AsA exposed to drought stress conditions Mean ± S.E).

Antioxidant activities

Fig. 4 depicts the antioxidative potential of ascorbic acid in safflower during drought conditions. Results demonstrated that the activities of CAT, POD and SOD enzymes were considerably ($p \leq 0.001$) decreased during water deficit stress indicating decreased oxidative potential. In control group, exogenously sprayed AsA levels had shown non-significant effect in decreasing CAT activities in CV-256 and POD activities in both cultivars while it substantially ($p \leq 0.001$) decreased CAT activities in Thori-76 and SOD activities in both cultivars during. During drought, ascorbic acid had shown non-significant effect on increasing CAT activities in both cultivars. On the other hand, the activities

of POD and SOD were increased in Thori-76 at 150 ppm concentration of AsA while it decreased in CV-256 during less water availability. On comparing both cultivars, it was found that the tendency of enhanced catalase activity was superior in Thori-76 (Fig. 4a).

Antioxidative potential of safflower

Fig. 5 depicts the antioxidative potential of both cultivars of safflower. Results depicted that both cultivars significantly scavenge the free radicals of DPPH and ABTS. Results also indicated that cultivar CV-256 showed enhanced antioxidative potential as compared to cultivar Thori-76.

Hepatoprotective activities of safflower

Fig. 6 depicts the hepatoprotective activities of safflower cultivars (Thori-76 and CV-256) by inhibiting the entry of HCV in cell. Results demonstrated that the viral titer was 3.07×10^5 in control group which significantly reduced to 1.6×10^3 and 1.7×10^4 in Thoi-76 and CV-256 respectively. On comparing both cultivars, the extract of Thori-76 represents better hepato-protectivity as compared to CV-256.

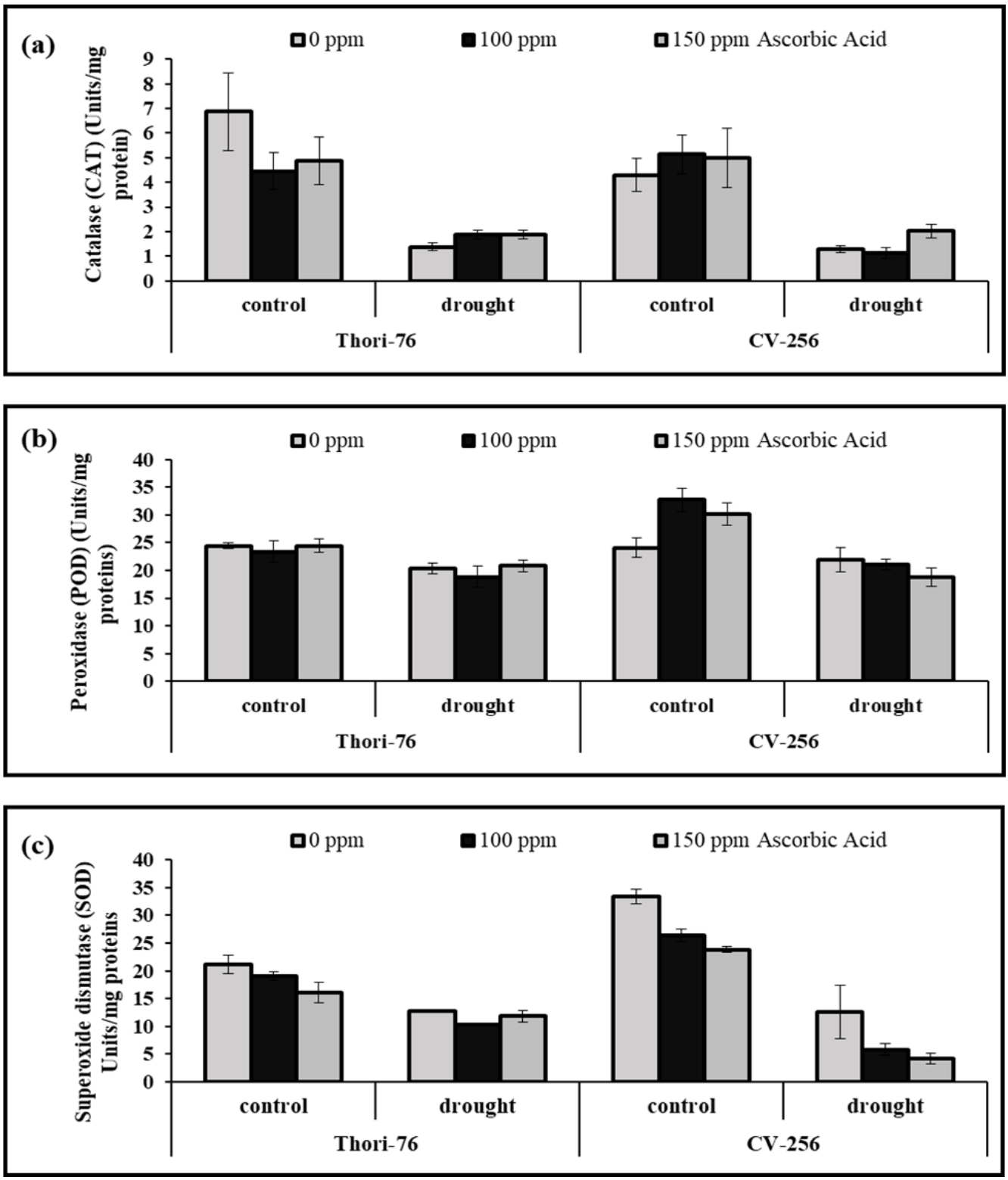

Fig. 4. Activities of different enzymes of Thori-76 and CV-256 of safflower foliar treated with AsA exposed to drought stress conditions. (a) SOD, (b) Catalase, (c) Peroxidase (Mean ± S.E).

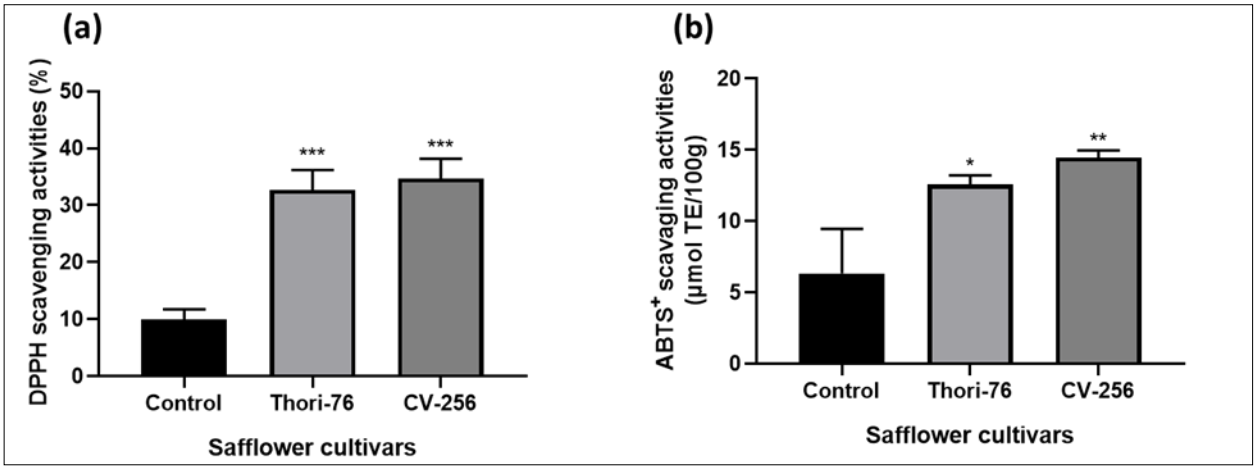

Fig. 5. Anti-oxidative potential of Thori-76 and CV-256. (a) DPPH radical scavenging assay (b) ABTS radical scavenging assay (Mean ± S.E).

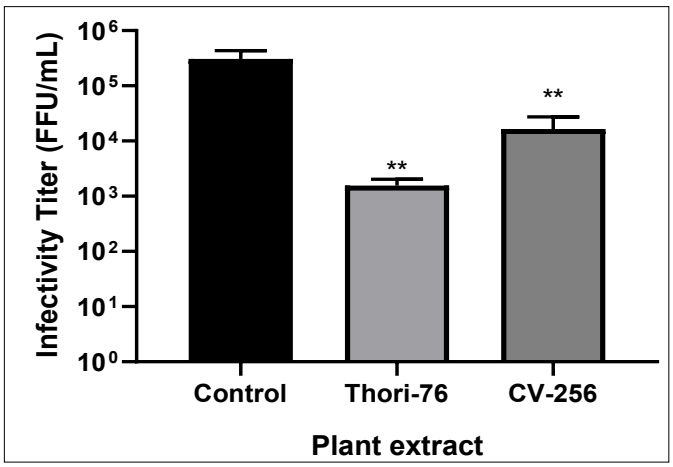

Fig. 6. Hepatoprotective activities of extracts of Thori-76 and CV-256. The data is represented in Mean ± S.E.

Cell Viability

Fig. 7 represents the cell viability percentage of Huh7.5 cells co treated with HCV and safflower plant extracts. Results indicated that both cultivars of safflower significantly protected the cells from HCV. It was also observed that Thori-76 showed maximum cell viability as compared to CV-256.

Quantification of total phenolic contents

The qualitative and quantitative HPLC analyses were carried out of the dried leaves of both cultivars (Thori-76 and CV-256) treated with high concentration of AsA under both water conditions. This was performed to ensure the biochemical influence of AsA and to compare its activity under both water conditions. During the analysis it was found that the phenolic profile increases in Thori-76 under well water condition (Fig. 8a). Decline in some phenolic contents was observed in the same leaf sample of Thori-76 under drought condition (Fig. 8b). Phenolic profile of CV-256 was quite different from the Thori-76 under both water regimes. Under control, some of the phenolic contents were in high concentration but the majority was in normal condition (Fig. 8c). Under drought a slight decline was observed in some phenolics but feluric acid was highly improved (Fig. 8d).

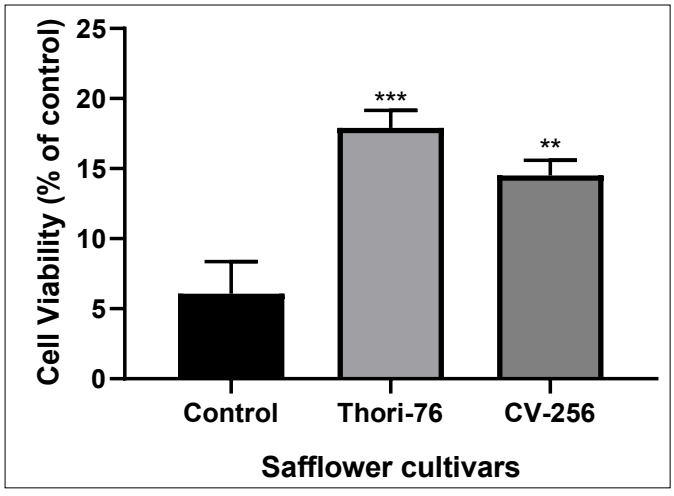

Fig. 7. Cell viability (%) of Huh7.5 cell lines co treated with HCV and safflower extracts. The data is represented in Mean ± S.E.

Twenty different phytochemicals from Safflower were docked against receptor protein and top 6 phytochemicals have been given in Table 1. The results have shown that compounds 1 and 2 (i.e; p-coumaroyl and rutin) have minimum S-score that exhibited potent inhibitor against receptor protein. The interactions of top six phytochemicals with viral protein are shown in Fig. 8 and 9. All the ligands have strong potential for binding with active site of receptor protein.

Drug-likeliness results

The phytochemicals with minimum S-score and potential interactions with receptor protein used in this study were further studied for drug scans to check if they fulfill the criteria of drug likeliness. Lipinski rule of five was applied on selected ligands molecules and results have been given in Table 2. The ligands Coumaroyl, Hydroxyarctigenin and Pinoresinol fulfilled all 5 rules and therefore could be potential drugs against NS3/4A protein of HCV. The rules which are not followed by the ligands are highlighted in Table 1.

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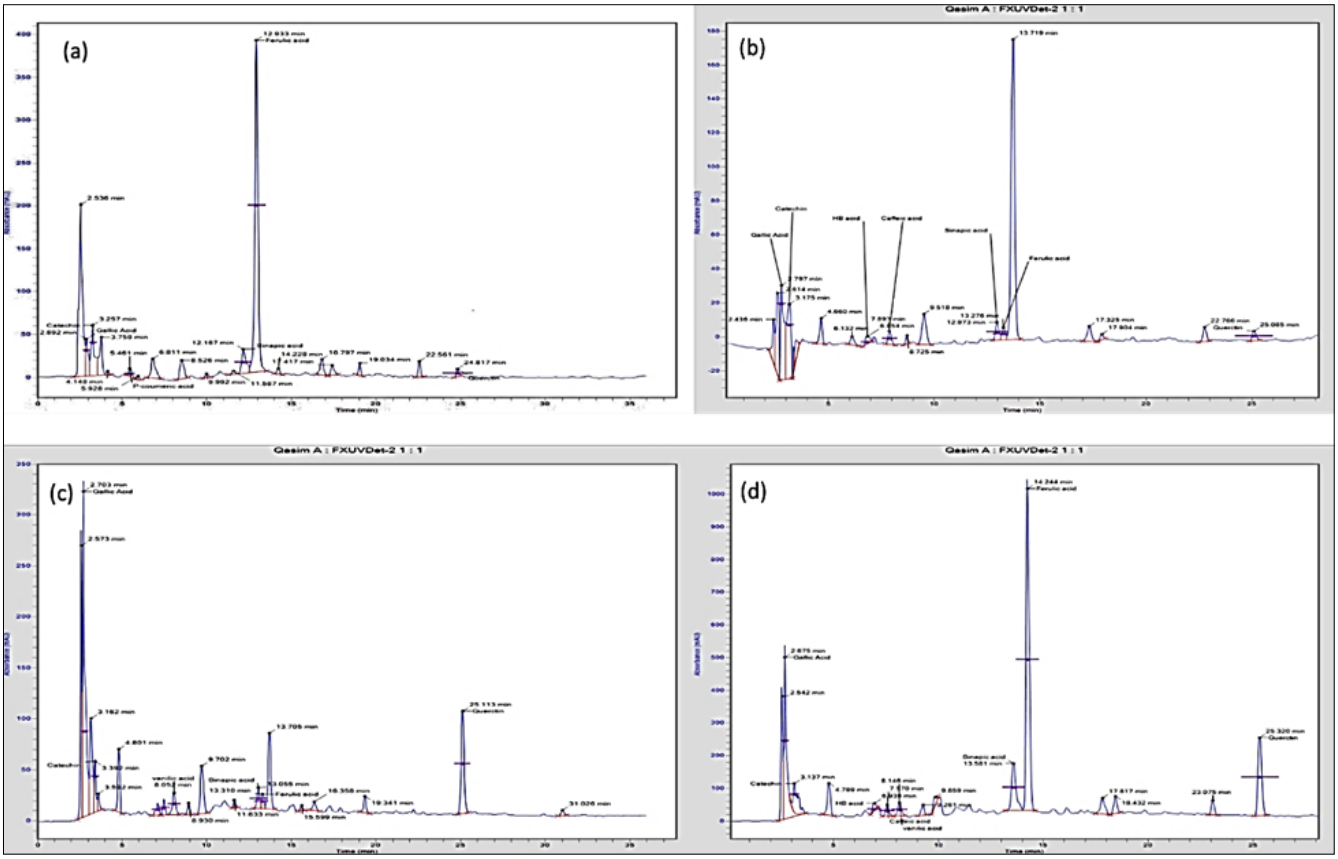

Fig. 8. HPLC chromatogram of phenolic acid profile of (a) Thori-76 under 100 % field capacity, (b) Thori-76 under 60 % field capacity, (c) CV-256 under 100 % field capacity and (d) CV-256 under 60 % field capacity treated with foliar applied 150 mg L^{-1} ascorbic acid.

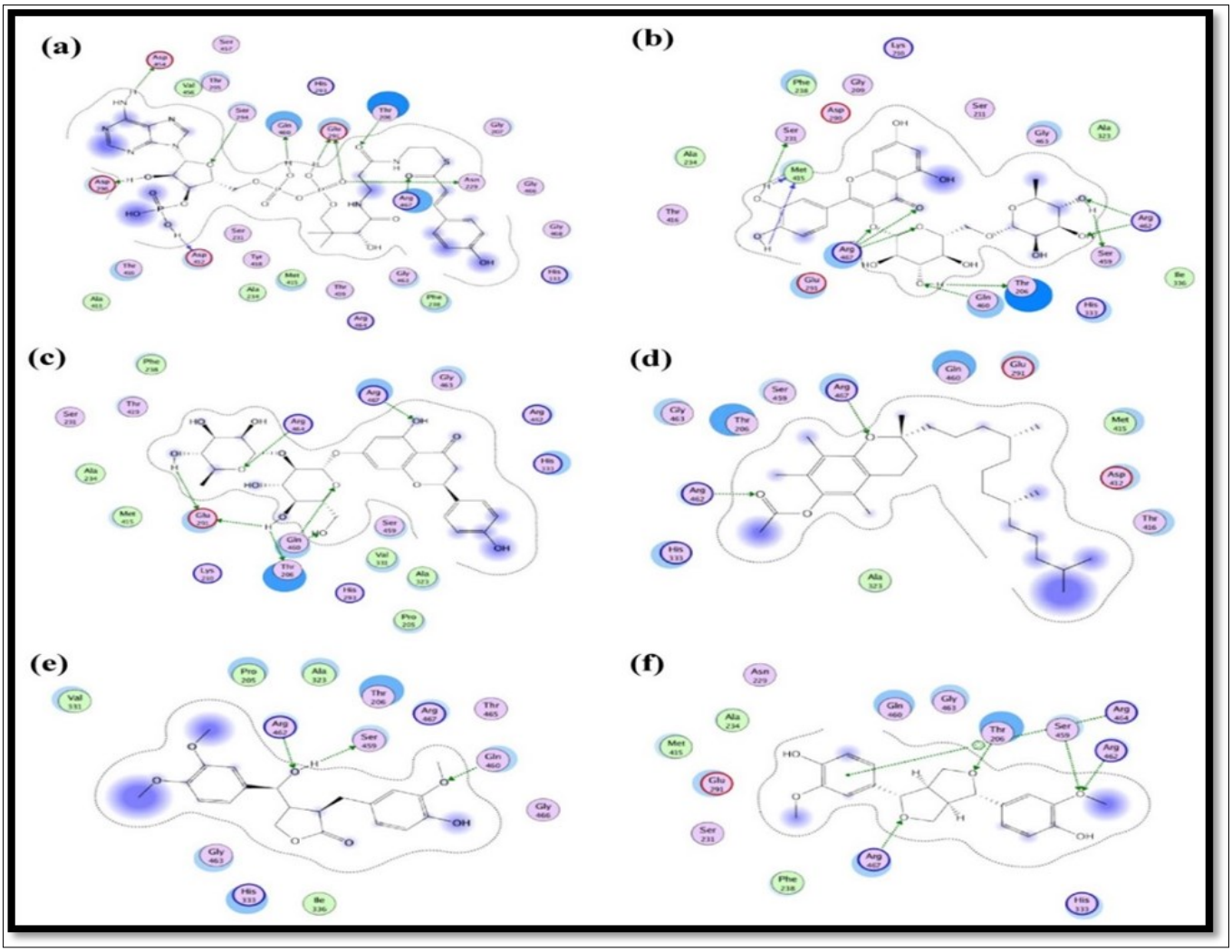

Fig. 9. Ligand interactions with NS3/4A. (a) Coumaroyl, (b) Rutin, (c) Naringin, (d) Tocopherol, (e) Hydroxyarctigenin, (f) Pinoresinol.

Table 1. The docking score of top 6 phytochemicals and their interacting residues against HCV NS3/4A

Sl. No.	Phytochemical name	S-score	Interacting residues of receptor protein
1	Coumaroyl	-19.9680	Thr 206, Asn 229, Glu 291, Ser 294, Asp 296, Asp 412, Asp 454, Gln 460, Arg 467
2	Rutin	-19.6295	Thr 206, Ser 231, Met 415, Ser 459, Gln 460, Arg 462, Arg 467
3	Naringin	-17.3003	Thr 206, Glu 291, Gln 460, Arg 464, Arg 467
4	Tocopherol	-14.3024	Arg 462, Arg 467
5	Hydroxyarctigenin	-14.0050	Ser 459, Gln 460, Arg 462
6	Pinoresinol	-13.0925	Thr 206, Ser 459, Arg 462, Arg 464, Arg 467

Table 2. Results of selected compounds checked for Lipinski rule of five

Sl. No.	Ligand	Mass (≤500)	H-bond donor (≤5)	H-bond acceptor (≤10)	Log *P* (≤5)	Molar refractivity (40-130)
1	Coumaroyl	312	5	6	-0.0531	77.1458
2	Rutin	610	10	16	-1.8788	137.4955
3	Naringin	580	8	14	-1.1652	134.1469
4	Tocopherol	472	0	3	9.0599	144.035
5	Hydroxyarctigenin	388	2	7	2.560	95.510
6	Pinoresinol	358	2	6	3.1902	93.683

which are not followed by the ligands are highlighted in Table 2.

Discussion

Carthamus tinctorius L., commonly referred to as safflower, is an annual oilseed crop extensively employed in traditional medicine for a range of medical ailments, including dysmenorrhea, amenorrhea, postpartum abdominal discomfort and masses, as well as joint injuries and pain (29). It is predominantly utilized for flavoring and coloring among the indigenous populace (30). Safflowers thrive on deep, rich, well-drained loam soils with excellent water retention capabilities. It may even survive on coarser textured soils with less water-holding capacity, provided that rainfall and moisture distribution are sufficient (31). The present study aims to investigate the impact of drought conditions on the production of safflower in the presence of ascorbic acid (AsA). Being a powerful antioxidant, ascorbic acid is used to overcome the impact of drought stress (32), as drought stress induces an imbalance in light absorption and its use in photosynthesis. The unutilized or surplus energy is detrimental to photosystem II due to the over-reduction of the reaction center (33). Due to which, ROS (reactive oxygen species) production is enhanced, which leads towards lipid peroxidation and alters various metabolic pathways (34).

Total phenolic contents being active antioxidants help to minimize the effect of water stress (35). In our study, the foliar-applied AsA showed a critical impact in improving the absolute phenolic substances of the 2 cultivars, particularly in Thori-76 as contrasted with the CV-256. Similar results were also observed on sweet potato; the application of ascorbic acid through foliar methods led to a significant enhancement in the total phenolic content of drought-tolerant plants (36). Two types of defensive mechanisms generate in plants against abiotic stress. The first one is the accumulation of osmoprotectants which includes phenolic contents, leaf free proline and glycine betaine (GB) (37). Among the important osmoprotectants, leaf-free proline is produced during drought and alleviates the worst effects of drought in many crops (38). A study reported that AsA is crucial for hydroxyproline formation, which is a nonessential amino acid derivative (39). In the present study, during drought, both cultivars had shown high accumulation of proline contents. Exogenously applied varying levels of AsA, especially 150 mg L^{-1}, somewhat enhanced the proline contents in Thori-76 and CV-256. It was also observed that exogenously applied ascorbic acid enhanced the production of proline during drought stress (40).

Glycine betaine, another potential osmoprotectant, also accumulates during drought stress (37, 41). It reduces the outcomes of drought by protecting metabolic pathways. In our analysis, no significant interference in glycine betaine levels was observed as both cultivars behaved similar in response to drought as well as AsA for osmoprotectants. Our findings are consistent with earlier findings (42).

In response to drought stress, numerous antioxidants, including SOD (superoxide dismutase), POD (peroxidase) and CAT (catalase), are synthesized to safeguard plants from oxidative damage caused by reactive oxygen species (43). The action of antioxidants often escalates under stress situations. The heightened concentration and significant activity of antioxidants confer stress tolerance to plants (44). Here in our study, the foliar application of AsA improved the activities of SOD in one of the cultivars (i.e., CV-256) and remained neutral in improving the activities of CAT and POD under both water systems. (20, 43) observed similar results.

Various medicinal plants and their oils are very important in the treatment and control of health-related issues (45–47). The oil extract and seeds of safflower have been accounted for to shorten the degenerative illnesses, neuropathy, hepatoprotective and shivering (48). The other aim of this study was to monitor the antioxidative and hepatoprotective activities of safflower. The *in vitro* analysis of our study revealed that the extracts of both cultivars of safflower significantly showed antioxidative and hepatoprotective activities. An early study reported that safflower has a significant antioxidative potential, while an another study also reported the hepatoprotective activities of safflower (16, 21). Results of our study also revealed that safflower extract inhibits the entry of HCV in cells as the viral titer reduced in cells treated with safflower extracts as compared to control groups. Previously, studies have been

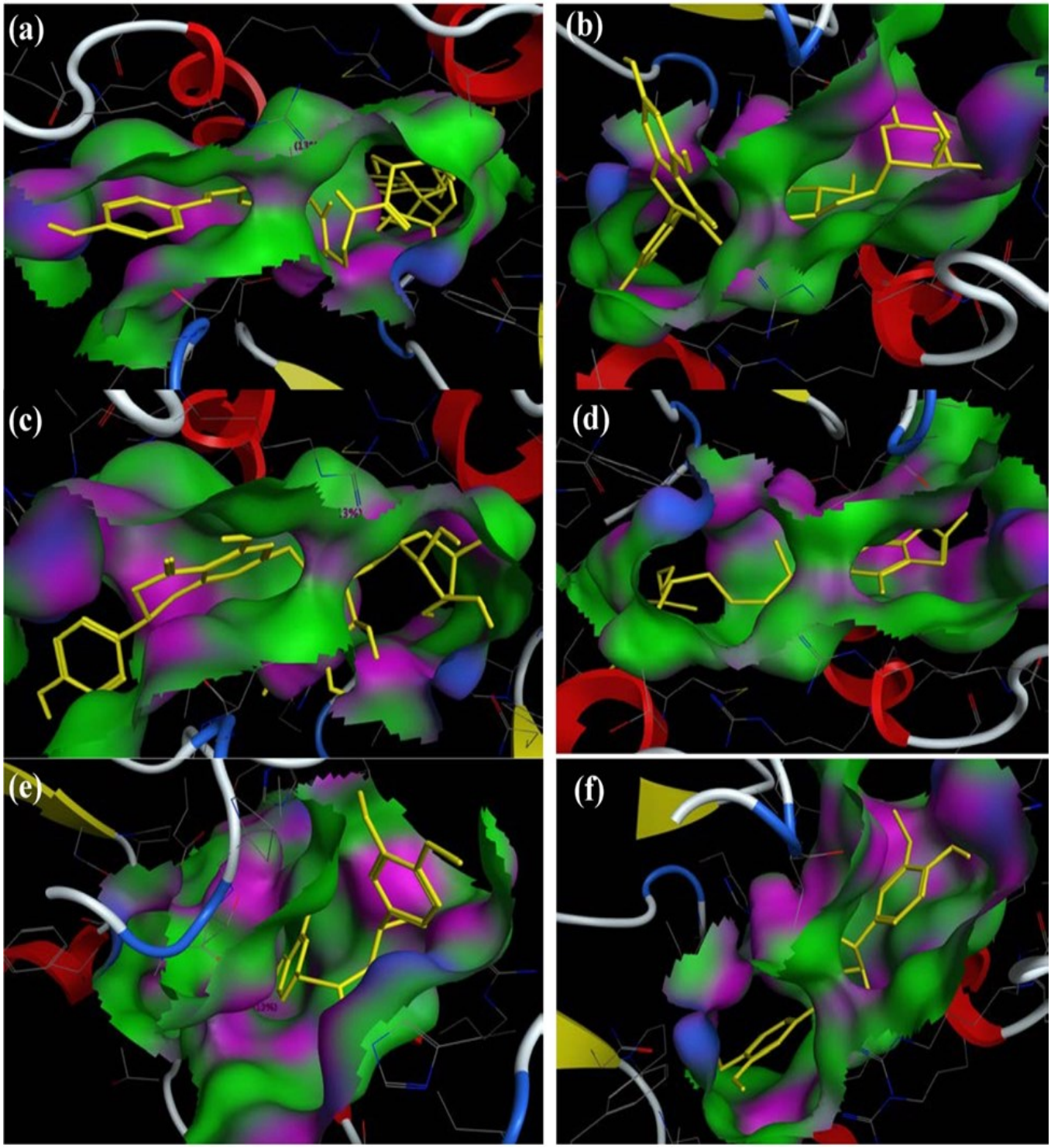

Fig. 10. Hydrophobic interactions of ligands (yellow) with NS3/4A. (a) Coumaroyl, (b) Rutin, (c) Naringin, (d) Tocopherol (e) Hydroxyarctigenin, (f) Pinoresinol.

reported on the hepatoprotective potential of safflower extract as an antioxidant, but still no study is reported about the inhibitory action of safflower extract.

The secondary metabolites present in food plants and medicinal herbs are polyphenolic chemicals. Phenolic compounds are useful in protecting plants from oxidative damage (21). In our HPLC analysis, we found a considerable amount of phenolic content in both varieties of safflower. It was also observed that drought conditions have no significant impact on the phenolic contents of CV-256. Gunc Ergonul also found a considerable amount of total phenolic content in safflower. They discovered that apigenin, a flavone, was the primary phenolic ingredient in safflower oils, followed by luteolin.

The investigation of key components of safflower as potent inhibitors of the NS3/4 protein of HCV to inhibit viral entry in cells was also the main objective of this study. For further analyzing the potential inhibitors of NS3/4 in safflower extract, we studied the ligand receptor interaction through molecular docking. For this purpose, we evaluated 20 different phytochemicals of safflower to investigate their interaction with NS3/4. Six compounds showed strong potential for binding with the active site of the receptor protein. For further analysis, these compounds were investigated for drug scans to check if they fulfill the criteria of drug likeliness. By applying Lipinski's rule of five, it was

found that the ligands Coumaroyl, Hydroxyarctigenin and Pinoresinol could be potential drugs against the NS3/4A protein of HCV.

Conclusion

From current study, it has been concluded that the safflower cultivar CV-256 possessed higher total phenolic contents as compared Thori-76 during well water supply due to foliar applied AsA. The activities of CAT, POD and SOD enzymes were found to be enhanced considerably during water deficit stress. Exogenously sprayed AsA levels significantly increased SOD activity in both cultivars. The invitro studies revealed the antioxidative and hepatoprotective activities of safflowers. The docking research has shown that p-coumaroyl and rutin from safflower exhibited potent inhibitor against HCV NS3 protein and both compounds may one day be employed as possible medications to treat HCV.

Authors' contributions

SAB led the overall research design, supervised both phases of the study, reviewed all data and finalized the manuscript for publication. AF conducted laboratory experiments related to drought stress and antioxidant enzyme assays, collected phenolic content data. NA manuscript writing, approval of final draft. MI contributed to data analysis, interpreted results and finalized the manuscript for publication. MFT manuscript writing, approval of final draft. RR conducted laboratory experiments, literature search, manuscript writing, edited text for clarity and coherence, managed referencing and formatting tasks and approval of final draft.

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflict of interest to declare.

Ethical issues: None

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