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EFFECT OF *TRIDAX PROCUMBENS* LEAF EXTRACT INCORPORATED PVA FILM ON POSTHARVEST QUALITY OF *VITIS VINIFERA*

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Keywords:

Tridax procumbens, *Vitis vinifera*, Coating, Shelf-life, Film, Postharvest

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ABSTRACT: *Vitis vinifera* (Grape) encounters several difficulties during their shelf life, including weight loss, browning and softening. Packaging is one of the effective solutions to preserve fruits from postharvest loss. *Tridax procumbens* (*T. procumbens*) leaves are rich in bioactive compounds and possess good antioxidant and antimicrobial activity. This study evaluates the efficiency of *T. procumbens* leaf extract film as a coating on *V. vinifera (V. vinifera)* to increase postharvest storage life at 20-25℃. Different concentrations of *T. procumbens* leaf extract incorporating PVA film were successfully developed, coated on *V. vinifera* and stored for 21 days. Throughout the storage period, at 7 day intervals, rate of weight loss and changes in the content of titratable acidity, ascorbic acid, total phenolic, reducing sugar and DPPH scavenging activity were assessed. During storage, the *T. procumbens* leaf extract film-coated *V. vinifera* effectively inhibited the weight loss rate and slowed down the loss of titratable acidity, ascorbic acid content, total phenolic content and DPPH activity in contrast to that of non-coated *V. vinifera*. Therefore, the application of *T. procumbens* leaf extract film proved to be effective in delaying the postharvest deterioration of *V. vinifera* during storage.

INTRODUCTION: *Vitis vinifera* (Grape) are a highly perishable fruit with a short postharvest life. It easily deteriorates during transportation and storage due to severe water loss, browning, berry softening, and decay [1]. This affects the quality and commodity value of *Vitis vinifera* (*V. vinifera*) and causing huge economic losses and food waste. Thus, the development of technologies to prolong the shelf life of *V. vinifera* is of great importance to both *V. vinifera* growers and consumers.

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Chemical fungicides such as sulphur dioxide and polyamines are commonly used in the preservation of *V. vinifera* [2]. However, due to the negative effects of fungicides residues in the environment on human health, there is a great demand for new measures to preserve *V. vinifera* [3].

One of the best ways to preserve fruits is to package them, which prolongs their storage life by preventing water loss and lowering the fruit's respiration rate [4, 5]. To preserve food quality, it can primarily be made into films or used as coatings for fruit surfaces [6, 7]. Fruit packaging applications are currently paying a lot of attention to the combination of active ingredients, such as antioxidant and antibacterial compounds, with packaging materials to extend the shelf life of fruits [8, 9].

Tridax procumbens (*T. procumbens*) has been known since ancient times for its interesting medicinal values. Currently, it has gained attention in the food industry due to its nutritional supplements and benefits for human health [10]. It possesses a wide range of bioactive compounds like flavonoids, alkaloids, phenolic compounds, oleanolic acid, lupeol, catachol, saponins, tannins, carotenoids, terpenoids contents [11]. Studies have also reported that *T. procumbens* leaves are rich in antioxidant and antimicrobial compounds [12].

Polyvinyl Alcohol (PVA) is a synthetic, biodegradable polymer that is widely used due to its non-toxicity, biodegradability and low cost [13]. It is ideally suited for designing sustainable active packaging materials due to its good film-forming capabilities [14]. It has also received FDA approval for food product application [15]. PVA film does not possess antibacterial and antioxidant properties; hence, the addition of natural components as filler in PVA matrix has gained significant attention in the field of food packaging.

The current study is aimed to investigate the effectiveness of *T. procumbens* leaf extract loaded film as coatings on the maintenance of *V. vinifera* quality in terms of weight loss, total phenolic content, ascorbic acid content, titratable acidity, reducing sugar content and DPPH scavenging activity over 21 days of storage at 20-25℃.

MATERIALS AND METHODS:

Preparation of the Extracts and Films: Extracts were prepared by using *T. procumbens* leaves. Fresh leaves were collected from farms in Tirupur district (Tamil Nadu, India) and washed thoroughly with water. *T. procumbens* fresh leaf extract (TPFLE) was prepared by using 10 g of fresh leaves ground to make a paste and boiled in 100 ml of distilled water for 1 hour, filtered using Whatman No.1 filter paper and stored at 4℃ in an airtight container for formulation use [16]. *T. procumbens* dried leaf extract (TPDLE) was prepared by allowing the leaves to shade dry for 2 weeks and grinding them into powder. Then 10 g of powdered sample was soaked in 100 ml of distilled water and placed overnight at 37℃. The extract was boiled for 1 hr and filtered using Whatman No.1 filter paper [17] and the collected extract was stored at 4℃ in an airtight container for formulation use. Films were prepared by solvent casting method [18]. The film-forming solutions were prepared by mixture of PVA (5% w/v) and glycerol (0.5% v/v). *T. procumbens* leaf extract at different concentrations (12.5%, 25%, 50%) was incorporated in the film solutions. The film without leaf extract was used as a control (CF). All the film-forming mixtures were blended well using a magnetic stirrer (1MLH, REMI, India) at 60℃ and cast onto the petriplate and placed in a hot air oven (hot air oven-143, NSW, India) at 70℃. Further, the dried films were peeled from the casting surface and stored for further analysis studies. TPDLE incorporated films were named D-1 (50%), D-2 (25%) and D-3 (12.5%). TPFLE incorporated films were named F-1 (50%), F-2 (25%) and F-3 (12.5%).

Application of the Film on *V. vinifera*: *V. vinifera* (a Thompson seedless variety, from Tamil Nadu, India) were tested in research packaging material experiments. *V. vinifera* were selected based on uniform shape, size, colour and lack of defects for experiments. Selected *V. vinifera* were soaked in a 0.5% sodium hypochlorite solution for 5 minutes and then washed with distilled water. The washed *V. vinifera* were then packed in the following films: control film (CF), TPDLE (D-1, D-2, D-3) and TPFLE (F-1, F-2, F-3) and stored at 20-25℃ for 21 days. The control group was left uncoated (UC). All measured parameters were recorded at time intervals of 0^{th}, 7^{th}, 14^{th} and 21^{st} days. For preparation of *V. vinifera* extract, 5 g of *V. vinifera* pulp samples were ground with 5 ml of distilled water and centrifuged for 15 minutes at 5000 rpm to obtain the extract [19]. Experiments were executed as triplicates and the results were expressed as mean ± standard deviation.

Measurement of Weight Loss: After packing, fruits were weighed at the beginning of the experiment and thereafter at interval of 7 days during the storage period. Weight loss was expressed as a percentage in relation to the initial loss weight [20].

Analysis of Titratable Acid: Titratable acid content (TA) was measured by titrating with 0.1N sodium hydroxide. The measurement was performed according to the procedure of Chrysargyris *et al* [21].

Analysis of Ascorbic Acid: The ascorbic acid content was determined based on the spectrophotometric method [22]. A 200 µl of *V. vinifera* extract was taken, and the volume was made to 3 ml using distilled water. Following this, 1ml of 2% 2,4,dinitrophenylhydrazine (DNPH) and 2 drops of 10% Thiourea solution were added. Then the mixture was subjected to heating in a water bath for 20 minutes and incubated at 37℃ for 3 hours.

After incubation, 7 ml of 80% sulphuric acid was added and the absorbance was read at 540 nm using a colorimeter (CL-63, ELICO, India). The results were calculated with the standard curve of ascorbic acid (200-1000 µg/ml) and expressed as mg/g FW (Fresh Weight).

Measurement of Total Phenolic Content: Total phenolic content was determined by the Folin-Ciocalteu reagent [23]. Briefly, 0.2 ml of *V. vinifera* extract was added to 1.0 ml of Folin-Ciocalteau reagent. After 5 min reaction time, 2.0 ml of 5% sodium carbonate solution was added and allowed to stand for 2 hours of incubation in the dark at room temperature. Then the absorbance of the samples was read at a wavelength of 670 nm using the UV-visible spectrophotometer (LMSPUV1200, Labman, India). The results were compared with the standard calibration curve of gallic acid (R^2 = 0.973) and expressed in milligrams of Gallic Acid Equivalent (GAE) per gram of fresh weight.

Measurement of Antioxidant Activity using DPPH Method: The DPPH (2,2- Diphenyl-1-Picrylhydrazyl) free radical scavenging capacity was measured by the method described by Blois *et al* [24]. A 200 µl of *V. vinifera* extract was mixed with 1 ml of 0.1 mM DPPH solution and kept in the dark for 30 minutes of incubation. The absorbance was then read with a colorimeter (CL-63, ELICO, India) at a wavelength of 517 nm. 1 ml of DPPH solution along with 200 µl of methanol served as control. The antioxidant activity was calculated and results were expressed in percentage.

Measurement of Reducing Sugar: The reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNSA) method. The measurement was performed according to the procedure of Krivorotova *et al* [25].

RESULTS AND DISCUSSION:

Effect of *T. procumbens* Leaf Extract Incorporated Films on the Preservation of *V. vinifera*: The effects of different concentrations of *T. procumbens* leaf extract loaded film on extending *V. vinifera* shelf-life during 21 days of storage are shown in **Fig. 1.** It is clear that visible coated *V. vinifera* presented a better appearance in color, texture and without decay or browning at the end of storage. The following parameters show the quantitative changes of *V. vinifera* during storage, such as weight loss, titratable acidity, ascorbic acid, total phenolic content, antioxidant and reducing sugar content.

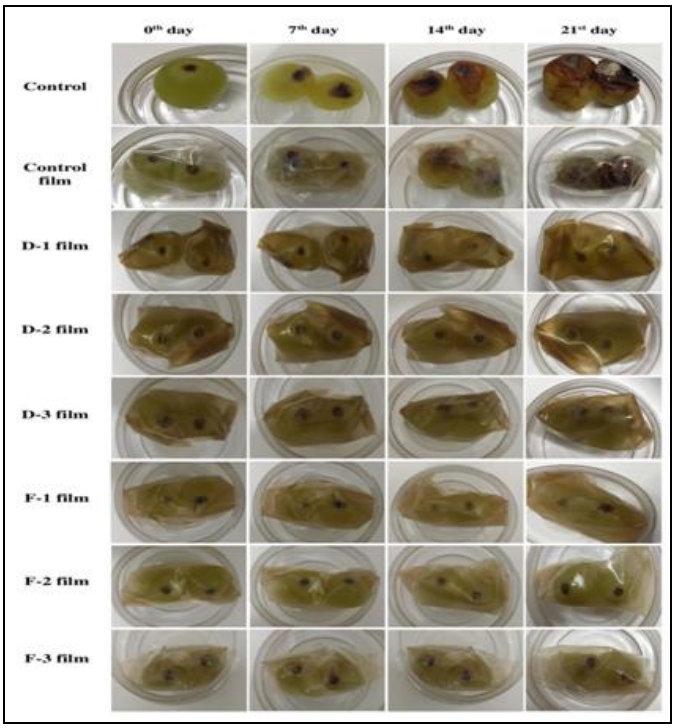

FIG. 1: EFFECTS OF *T. PROCUMBENS* LEAF EXTRACT FILM-COATED ON *V. VINIFERA* DURING STORAGE

Measurement of Weight Loss (%): Fruit weight loss is mainly related to the respiration rate and moisture evaporation of fruits. **Fig. 2** shows the changes in the weight of *V. vinifera* during storage for 21 days. A higher percentage of weight loss was observed in uncoated *V. vinifera*. After 7th day, the weight loss of *V. vinifera* in uncoated form was found to be 6.15% and after 21st day, the loss increased to 16.92%. But *T. procumbens* leaf extract incorporated film coated *V. vinifera* showed a significantly lower weight loss (8.33%) compared with the uncoated *V. vinifera*.

It might be due to the *T. procumbens* leaf extract film coating, which regulates gas exchanges and reduces transpiration loss. This observation is in agreement with the results of Lo'ay *et al* who found that the *V. vinifera* coated with pectin, polyphenylene alcohol and salicylic acid blending film delayed weight loss (18%) compared to the uncoated (32%) [26].

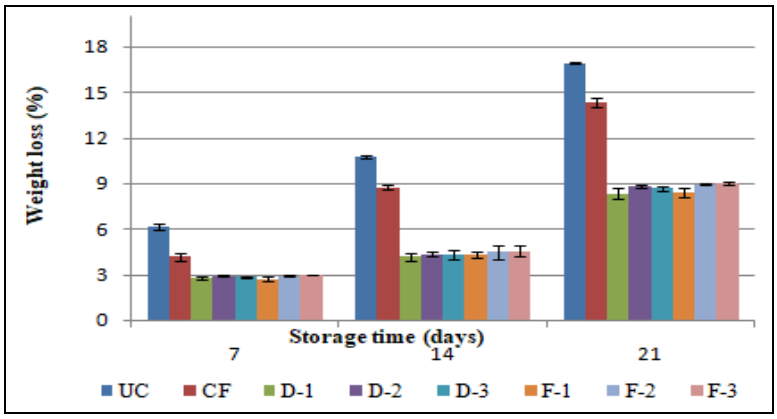

FIG. 2: CHANGES IN *V. VINIFERA* WEIGHT LOSS DURING THE STORAGE PERIOD: UC (UNCOATED), CF (CONTROL FILM), D-1, D-2, D-3 (TPDLE) AND F-1, F-2, F-3 (TPFLE)

Variation in Titratable Acidity: Titratable acid (TA) content is linked to the taste, color, aroma and stability of the fruit. TA of *V. vinifera* decreased with maturity [27]. In this study, it was shown that the TA of the samples decreased with increased storage **Fig. 3**. The reduction of TA was slower in *T. procumbens* leaf extract film-coated *V. vinifera* compared to uncoated *V. vinifera*. A significant TA percentage difference was noted between the uncoated and *T. procumbens* leaf extract film-coated *V. vinifera*, but no significant difference was noted among the TPDLE and TPFLE-coated *V. vinifera*. The decrease in acidity during the storage period could be explained by metabolic changes in fruit due to the use of organic acids during fruit respiration, which may be a good indicator that *T. procumbens* leaf extract film coating has a greater effect in reducing respiration rates throughout the storage period. This result is in concurrence with the study by Hu *et al*, in which the TA of grapes coated with chitosan-lignosulfonate decreased the loss of TA (3.32 g/kg) compared to uncoated grapes (2.41 g/kg) [4].

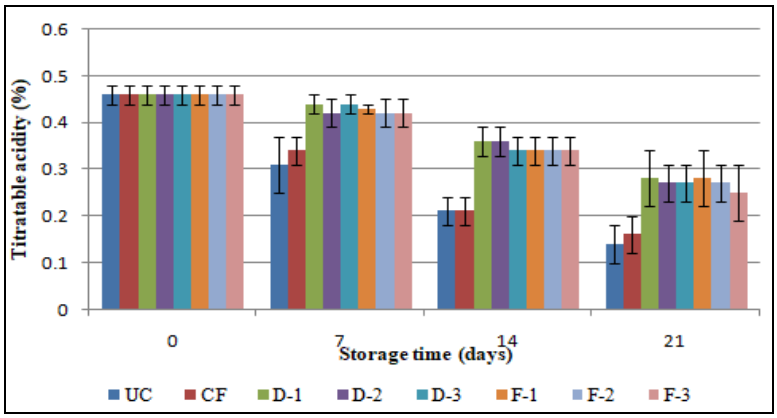

FIG. 3: TITRATABLE ACIDITY (%) OF *V. VINIFERA* FRUITS DURING STORAGE

Variation in Ascorbic Acid: Ascorbic acid is a natural antioxidant and an important nutrient in fruit. Ascorbic acid content declined during the ripening and senescence of fruit [28]. In this study, the ascorbic acid content followed a decreasing trend on storage for 21 days **Fig. 4**. The highest decline was noted in the uncoated *V. vinifera*; it declined from 0.36 mg/g to 0.1 mg/g. The *T. procumbens* leaf extract film-coated *V. vinifera* whose ascorbic content was 0.23- 0.26 mg/g at the end of storage. It clearly shows that the *T. procumbens* leaf extract film coated *V. vinifera* sustained a higher content of ascorbic acid compared to the uncoated *V. vinifera*. This might

be due to the coating of *T. procumbens* leaf extract in the film, which has delayed the changes in vitamin C content by preventing air permeation. Thus, *T. procumbens* leaf extract film showed a positive role in *V. vinifera* storage. This result is in agreement with Chen *et al* who report that chitosan with poly-ε-lysine coated table grapes inhibited the decline of vitamin C content (18.76 g kg^{-1}) compared to uncoated grapes (15.22 g kg^{-1}) [19].

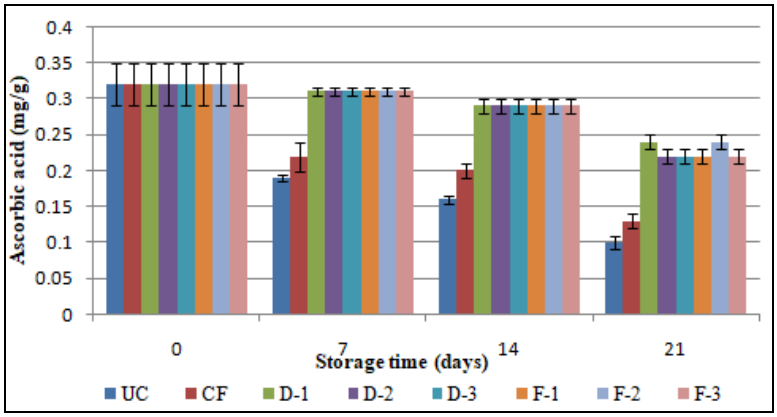

FIG. 4: ASCORBIC ACID CONTENT (MG/G) OF *V. VINIFERA* FRUITS DURING STORAGE

Variation in Total Phenol Content (TPC): The phenols are the principal constituents of fruits and provide antioxidant potential, which is considered one of the essential chemical maturity indices. The total phenolic content could vary from variety to variety due to geographical location and harvest time [29]. In this study, it was observed that the phenolic content of *V. vinifera* decreased during storage **Fig. 5**. The highest phenolic content was found with *T. procumbens* leaf extract film-coated *V. vinifera* (1.102±0.002 mg GAE/g), and the lowest content was found in the uncoated *V. vinifera* (0.962±0.002 mg GAE/g) after 21 days of storage. However, no significant changes were noted among the TPFLE and TPDLE-coated *V. vinifera*. The TPC decreased during storage time, which may be due to the oxidation of sensitive phenolic compounds. Overall, the *V. vinifera* fruit coated with *T. procumbens* leaf extract films were significantly higher compared to the uncoated. Thus *T. procumbens* leaf extract film coating that protected *V. vinifera* from the oxidation process and lowered the reduction of phenolic contents. Eshghi *et al* found that TPC of grapes was higher in chitosan coated with gum ghatti (1.29 mg g^{-1} FW) compared to the uncoated (0.85 mg g^{-1} FW). Their findings are in agreement with the current result [30].

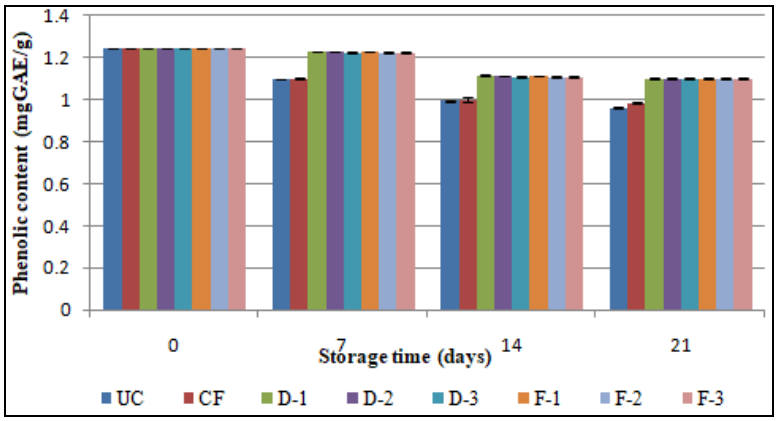

FIG. 5: PHENOLIC CONTENT (MG GAE/G) OF *V. VINIFERA* FRUITS DURING STORAGE

Measurement of Antioxidant Activities: Antioxidant measurements are important quality parameters used to evaluate the storage effect of fruit. It helps to maintain a balanced ROS (Reactive Oxygen Species) metabolism by quenching ROS [31]. In this study, *V. vinifera* antioxidant capacity was decreased during storage. As shown in **Fig. 6**, the DPPH radicals are 78% in the beginning and then

slowly decrease for all treatments. Fruits treated with *T. procumbens* leaf extract film had significantly higher antioxidant capacity than the control throughout storage. However, the antioxidant percent did not vary significantly among the *V. vinifera* coated with TPDLE and TPFLE. It shows that *T. procumbens* leaf extract film coating acted as a protective barrier on the surface and was effective in preserving the antioxidant activity of *V. vinifera*. This antioxidant value result was in agreement with the TPC value. This finding was in concurring with Mohammad *et al* who reported that the antioxidant activity of table grapes declined during storage and that chitosan-coated grapes inhibited the loss (48%), compared to uncoated grapes (38%) [7].

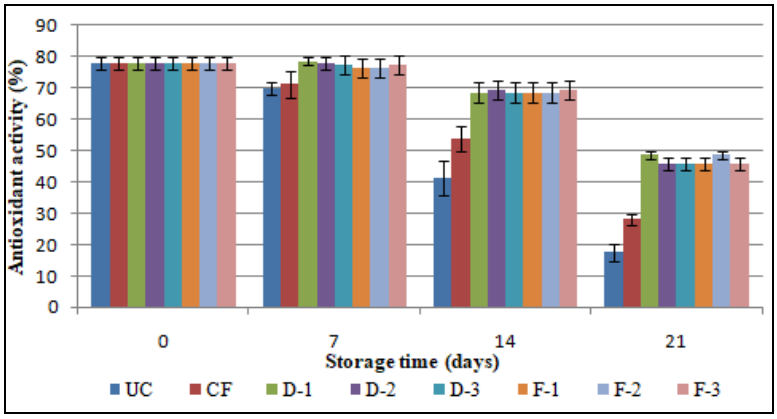

FIG. 6: ANTIOXIDANT ACTIVITY (%) OF *V. VINIFERA* FRUITS DURING STORAGE

Variation in Reducing Sugar: Reducing sugar, as a primary energy substance, is a key energy source in postharvest fruit. In *V. vinifera*, during storage, senescence causes a reduction in sugar content [32]. In this study, the reducing sugar content loss was higher in uncoated *V. vinifera*, and a lower loss was noted in *T. procumbens* leaf extract film-coated *V. vinifera* at the end of storage **Fig. 7**. The loss of reducing sugar content might be due to the advancement of the fruit ripening and senescence processes. The higher reduction in reducing sugar content was noted in uncoated *V. vinifera* compared to *T. procumbens* leaf extract film-coated *V. vinifera*. It indicates that *T. procumbens* leaf extract film coating is responsible for slower metabolism conversion in *V. vinifera*. And no significant changes were noted among the TPDLE and TPFLE-coated *V. vinifera* reducing sugar content. This study showed that the main characteristic of *V. vinifera*, glucose, was protected by the coating of *T. procumbens* leaf extract film. Zhi *et al* found the same trend of reducing sugar in Kyoho grapes when treated with hydrogen sulphide [33].

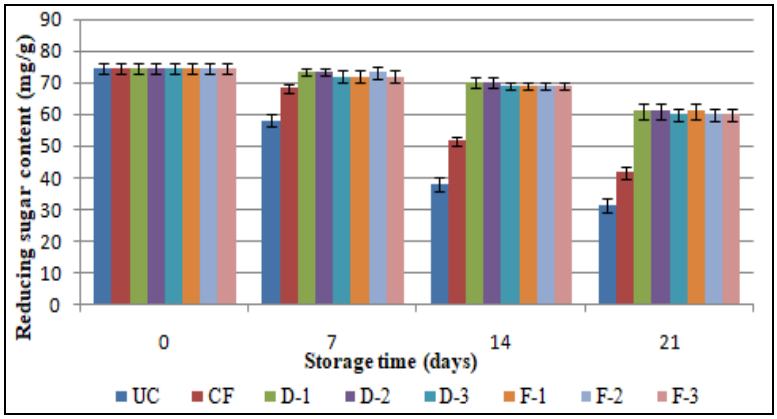

FIG. 7: REDUCING SUGAR CONTENT (MG/G) OF *V. VINIFERA* FRUITS DURING STORAGE

CONCLUSION: The present study showed that the *T. procumbens* leaf extract incorporated PVA film maintained *V. vinifera* quality for 21 days of storage period at 20-25℃. It can be concluded that *T. procumbens* leaf extract incorporated PVA films showed good preservation performance. Compared

with the control and control film, *T. procumbens* leaf extract incorporated PVA film coating effectively inhibited the weight loss rate of *V. vinifera* and also effectively slowed down the loss of titratable acidity, ascorbic acid content, antioxidant activity and reducing sugar content of *V. vinifera*. All the different concentrations of *T. procumbens* leaf extract incorporated PVA films showed equivalent preservation action in *V. vinifera*. Therefore, the application of film with *T. procumbens* leaf extract is capable of diminishing the postharvest damage to *V. vinifera*.

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