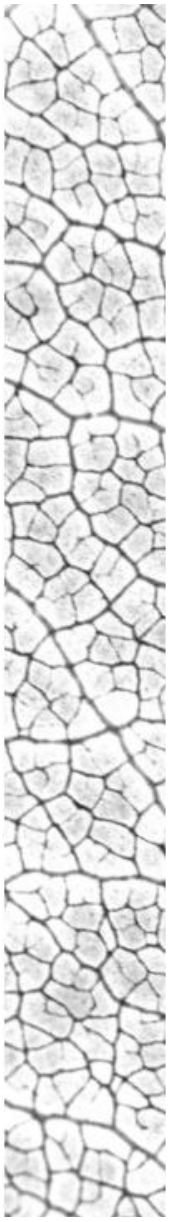

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Synthesis and characterization of plant extracts loaded PVA/PVP blend films and evaluate their biological activities

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Abstract

Background: antibacterial agent based on herbal extracts is considered an attractive area for developing countries. Nevertheless, herbal aqueous extracts usually show drawbacks, such as long-term volatility, poor bioavailability and rapid burst release. **Methodology:** In this study, polymer films were prepared from poly (vinyl alcohol) (PVA) blended with poly (vinylpyrrolidone) (PVP)and cross-linked with glutaraldehyde (GA), then post-loaded with T. indica, C. pepo, H. sabdriffol, and L. nobilis hot water extracts. The effects of two polymers (PVA, PVP) and of the incorporated extracts were studied concerning the physical and in vitro bacterial growth inhibition properties of films; additionally, the antioxidant activity of each extracts was investigated. **Results:** The results showed improved swelling behaviour and mechanical properties (tensile strength, tensile modulus, and % elongation at break) of the cross-linked PVA/PVP films (CPP) compared to pure PVA (PV) films. Plant extracts conferred significant antibacterial effects to (CPP) films toward E. coli (ATCC 25922) and S. aureus (ATCC 25923). Also, both T. indica and H. sabdriffol extracts showed strong antioxidant against DPPH in vitro. **Conclusion:** The prepared films showed significant antibacterial activities, specifically in films loaded with the T.indica extract against E. Coli and in films loaded with C.pepo and L.nobilis against S.aureus.

Keywords: antibacterial activity, antioxidant activity, plant extract, PVA/PVP film

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INTRODUCTION

Nowadays, antibacterial agent based on herbal extracts is considered an attractive area for developing countries. Nevertheless, herbal aqueous extracts usually show drawbacks, such as long-term volatility, poor bioavailability and rapid burst release. To overcome these problems different formulation from biodegradable and biocompatible polymers may be used as pharmaceutical active carriers for these extracts (Rijo, et al. 2014). In general polymer improving therapeutic properties of antibacterial agent via enhance adhesive properties, precise performance of the agent at a targeted site, and prolonging drug release by increasing reservation properties of the agents (Ahmed, & Khalid, 2016, Contardi, et al. 2017, Aranaz, et al. 2016, Patel, et al. 2018). Polymer films and gels have been widely used as therapeutic carriers in different applications, such as wound dressings (Padula, et al. 2019. Engelke, Winter, & Engert, 2018. Carter, Narasimhan, & Wang, 2019. Santos, et al. 2018). antimicrobial membranes for water treatment (Zhu, et al. 2017). food packaging (Garavand, et al. 2017)., and drug delivery system. (TD Tran & HL Tran, 2017). Moreover, numbers of studies identify that both Polyvinyl pyrrolidone (PVP) and Polyvinyl alcohol (PVA) are the most important film-forming polymers with biocompatible, biodegradable, and water-soluble properties (Engelke, Winter, & Engert, 2018). However, (PVA) has excellent chemical resistance with low hydrophilicity and rigid film forming. Therefore, for improving these drawbacks of PVA films, blend with other polymers, such as (PVP), are recommended. (Kim, et al. 2015). T. indica L. is commonly identified and known as Chincha in Ayurveda. Its fruit, tender leaves and flowers are used extensively in culinary preparations. It's a large wide spreading tree 12 to 18 m high. The trunk shows dark rough bark with deep cracks, and the seeds are smooth reddish brown, enveloped by

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tough leathery membrane (Escalona-Arranz, et al. 2010). It was scientifically reported for several medicinal properties such as antidiabetic and antihyperlipidemic activities (Maiti, Das, & Ghosh2005)., anti-oxidant, (Siddhuraju, 2007, Ugwuona, & Onweluzo, 2013). anti-inflammatory, analgesic and anti-microbial activity (Doughari, 2006). Various chemical constituents have reported from different parts of plants; tannins, saponins, glycoside, peroxidase and lipids, have been isolated from the bark; whereas the pipecolic acid , citric acid, 1-malic acid, lupanol, vitamin C, pectine, tannins, glycosides were isolated from leaves (Ara & Islam 2009). The chemicals like furan derivatives, carbolic acid, citric acid, pectine and invert sugar were derived from fruit and campestrol, β- sitosterol, palmitic acid, linoleic acid, xylose, galactose and glucose, were isolated from seed (Ushanandini, et al. 2006; Obiazi, 2015). Cucurbita pepo L. is an annually flowering climber. Cucurbita members such as pumpkin have protective role against gastrointestinal diseases, intestinal parasites (Grzybek, et al. 2016), antibacterial (Hammer, et al. 1999). antioxidant and antiinflammation (Nawirska-Olszańska, et al. 2013). these pharmacological effects have been associated with their phytochemical and nutritional compounds, such as carotenoids, tocopherols, phenols, terpenoids, saponins, sterols, fatty acids, and functional carbohydrates and polysaccharides (Ali, et al. 2005). H.sabdariffa L. is an annual dicotyledonous plant, which is widely cultivated in Africa and South East Asia (Da-Costa-Rocha, et al. 2014). Several study identified plant extracts such as seeds and calyces have vital roles in food industry and medicine (e.g. antibacterial, anti-oxidant, hepato and nephro -protective, anti-cholesterol, anti-diabetic and anti-hypertensive effects) (Peredo Pozos, et al. 2020). These findings were confirmed via Phytochemical screening of the crud extracts that identified presence of number of biological active components such as hibiscus acid, ascorbic acid, flavonoids, anthocyanin, phenolic acids and reducing sugars (Balagizi & Kizungu, 1994. Builders, et al. 2013). Laurus nobilis L. is a strong perennial tree that grows wild or is cultivated. It is wildly used for the treatment of rheumatis neurological, dermatitis, dermatological, and urological disinfections (Georgiev, & Stoyanova, 2006. Kilic, et al. 2004). The purpose of this study is to identify antibacterial activity of plant extracts loaded with PVA/PVP films. Moreover, antioxidant activity of plant extracts and related physical properties of PVA/PVP polymer films were also identified.

MATERIAL AND METHODS

PVP with Mw about 44.000 and the PVA 98-99% hydrolyzed with Mw about 31.000-50.000 was provided by Aldrich, Buchs, Switzerland. Glutaraldehyde (GA) and 2.2-diphenyl-1-picrylhydrazyl (DPPH) were purchased by Sigma, Germany. Plant materials such as fruit pulp from *T. indica,* calyces from *H. sabdariffa* and leaves of *L. nobilis* and *C. pepo* were collected from the local market in Baghdad, Iraq. All other chemicals and reagents were used at analytical grade level.

Preparation of Hot Water Extract

In the first place the plant materials were kept at room temperature for dryness. Then 50 g of dry powder for each plant was placed separately in 1000 mL flasks, and 500 ml. of distilled water was added. The flasks were placed in a shaking incubator at 70 °C for 30 min. Water extract was then applied using a Buechner funnel with a piece of medical gauze. The supernatant was then filtered using 1Whatman filter. The solution was concentrated using a rotary evaporator at 40 °C to remove the solvent and increase the concentration of the extract and then kept at -4°c until use. (Nayak, Set al. 2006).

Preparing of cross-linked PVA/PVP (CPP), pure PVA (PV) and pure PVP (PP) films

In this study solvent casting method has been used for preparation of polymer film. (Rhim, & Wang, 2013). When 4.5 g of PVP was added to aqueous solution of 6% (w/v) PVA. Then, 1.8 g of glycerol was added to PVA/PVP solution and mixed using stirrer at 80 °C for 1 h. Both 0.0001% GA (v/v) and 10% HCl (v/v) were used for cross-linking reaction by adding 4 drops of 10% HCl (v/v) and 0.2 ml of 0.0001% GA (v/v) to 20 mL of PVA/PVP solution. The cross-linking solution was stirred for 45 min at 60 ºC. Then, 20 mL of blend solution was poured onto a petri dish, and the films were cast by drying at 45 °C .The blend film preparation of (CPP) was established with the same modifications according to study conducted by.(Nho, et al. 2009). Both (PV) and (PP) were prepared from 6% (w/v) aqueous solution and casting by same method for (CPP).

Preparation of Plant Extracts PVA/PVP Loaded Film (ECPP)

Loading of polymer film was done based on post-loading method according to. (Peppas, et al. 2000, Wong, & Dodou, 2017). This method was preferred to in-situ loading of polymer to avoid decomposition of plant extract during film preparation. Stock solutions as 10% concentration, were prepared by dissolving 1 g of each extract with 10 mL phosphate-buffered saline .About 1x2 cm^2 samples from (CPP) film were immersed in stock solution of each plant extract for 24hr.Then, each samples were dried at room temperature.

FTIR Assay

FTIR study of KBr powder samples for (PV), (PP) and (CPP) were investigated by using Mattson Satellite 5000 FTIR spectrophotometer.

SEM Assay

Surface morphology for (CPP) film synthesised and (ECPP) loading with10% of *T. Indica* (TECPP)*, C. Pepo*

(CECPP), *H. sabdariffa* (HECPP) and *L. nobilis* (LECPP)extracts were evaluated by a scanning electron microscope Stereoscan 360, Cambridge at 10 kV.

Mechanical Properties

The tensile strength, tensile modulus, and % elongation at break of the (PV) and (CPP) before and after loading with plant extracts were determined by using a Zwick Roll (ZO10) with 22mm x 5 mm rectangular film samples at room temperature at across-head speed of 10mm/min. (Repka, & McGinity, 2000).

Water Absorption Assay

Pre-weighed of (PV), (CPP) and (ECPP) and (1x2) cm^2 from each sample was swollen in 20 mL PBS for different times interval until reaching equilibrium. At each time polymer film was removed from PBS and weighed after blotted with filter paper at room temperature. The equilibrium swelling-ratio (ESR) of each film sample was calculated via Equation (1)

% (ESR) = ((DS –Dd) / Dd) x 100

Where Dd and Ds are the weight of the films in dry and swollen states respectively (Yoshii, et al. 1999).

Antibacterial Assay

Nutrient broth was used to prepare turbid bacterial suspensions and adjusted with 0.5 McFarland turbidity standard to reach (5×10^7 cell mL^{-1}). Subsequently, 0.1 ml. of the cell suspension was cultured on Mueller–Hinton agar and antibacterial activity was assessed by disc diffusion against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). Then 1x2 cm^2 PVA/PVP loaded films with stock solution of each plant extract were applied to the surface of bacterial culture. After 24 hrs incubation at 37 °C, inhibition zone diameter was measured in mms. Moreover, (CPP) film was used as negative control and Streptomycin(s) (10 µg/disc) was used as positive control (Jadhav, et al. 2010). Moreover, inhibition activity of sustained release for each extract was identified by determination of bacterial concentration for both *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) exposed to (ECPP) when 0.1 ml. from (5×10^7 cell mL^{-1}) bacterial suspensions that was prepared above inoculated in separated Mueller–Hinton broth vials. Then each vials were incubated at 37°C with 1x2cm^2 from (TECPP), (CECPP), (HECPP), (LECPP) and (CPP) respectively for 5min, 10 min, 20 min, 30 min, 60 min, 120 min, 180 min and 1440 min. Then bacterial concentrations were determined by measuring optical density (OD) at 600 nm through time series. (OD600 = 1.0 is around 10^8 cell mL^{-1}) (Li, et al. 2010, South, Ozonoff, & McMahon, 2005).

Antioxidant Assay

DPPH (2.3mg) was dissolved in 3.3 mL of ethyl alcohol, then the solution was stored in dark. Ascorbic acid served as control +ve (20 µg mL^{-1}). Antioxidant activity of aqueous extracts (*T. indica, H. sabdariffa, C. pepo* and *L. nobilis*) was measured using a stable DPPH radical. To test the antioxidant activity, four concentrations of aqueous extracts (20, 40, 60 and 80 µg mL^{-1}) were used. (10 µL) from each concentration was mixed with (490 µL) ethanol. Then, (1 mL) solution was accomplished by adding (500 µL) from DPPH solution. The process was followed with incubation period. Then determined for remaining DPPH in the absorbance at 517 nm. Inhibition of DPPH was computed by the equation (2):

% of DPPH scavenged = (^{A}con -^{A} test) / ^{A}con x 100

^{A}con , refers to the absorbance of the control, ^{A}test , refers to the absorbance of the sample with extracts. (Brand-Williams, Cuvelier, & Berset, 1995).(Orhan, et al. 2003).

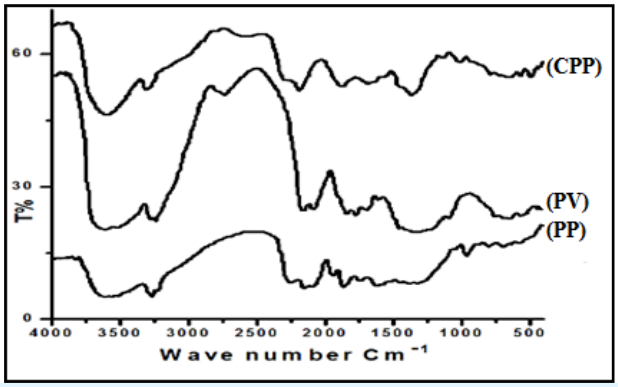

Fig. 1. FTIR spectra of the (PP), (PV), and (CPP) films

Statistical Analysis

All experiments were done by triplicate, and data are reported as means ± standard deviation (SD). All statistical analyses were performed with the Graph pad Prism version 8.4.2(679).

RESULTS

FTIR Assay

Fig. 1 shows the spectra of (PV), (PP) and (CPP) films. The characteristic alcohol peak relate to C-O bands of (PV) was identified at 1089 cm^{-1}. While bored beak for O-H appeared at 3458 cm^{-1}, and C-H stretching was observed at 2874 cm^{-1}. Alternatively, the characteristic C=O stretching band for (PP) appeared at 1654 cm^{-1}, and O-H stretching appeared at 3404 cm^{-1}.Furthhermore,spectrum of (CPP) showed characteristic shifting for O-H stretching bands at the lower spectrum of 3373 cm^{-1}, indicating the crosslinking interaction between PVA and PVP with GA via hydrogen bonds (Eisa, et al. 2012, Rafizah, & Ismail, 2008). Thus, absorption peak at 1651 cm^{-1} was appeared in blend film assigned as C=O stretching of PVP and shafting of C-O stretching of PVA from 1089 cm^{-1} to 1070 cm^{-1} in blend film as a result of interference between C-O and C-N at 1020 cm^{-1} that related to (PP)film (Wong, & Dodou, 2017).

SEM Assay

Fig. 2a-2e was presented the surface morphology of (CPP) (TECPP), (CECPP), (HECPP) and (LECPP)

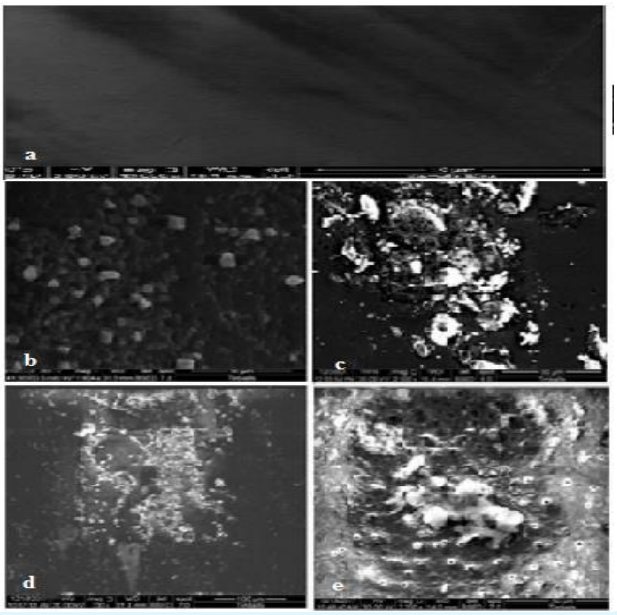

Fig. 2. SEM of CPP in (a) TECPP (b), CECPP (c), HECPP(d) and LECPP (e)

respectively. A homogeneous appearance was observed on the surface of (CPP) identifying miscible blending between PVA and PVP. On the other hand, the loaded film demonstrated uniform and rough surfaces. The rough surface is due to distribution of most of T. indica, C. pepo, H. sabdariffa and L. nobilis extract molecules over the existing interconnected microporous spaces of the film network. As the plant extracts saturation increased, more extract were originate deposited onto the surface of the films. The small particles of extracts were found attaching to each other to form bigger particles. Such as rod-shaped crystals and smooth spherical aggregates that is especially observable at the highest load film with plant extracts. This results agreement with study done by Wong et al. (Basha, S., Reddy, & Rao, 2018, May).

Mechanical properties

One of the most important characterizations for the polymer film is mechanical properties. Therefore, the tensile stress, elongation at break % and the tensile modulus of the (PV), (CPP) and (TECPP)films were identified and are presented in **Fig. 3a**-**3c**, respectively. PVA film shows the highest tensile strength, due to the semi-crystalline structure of PVA. (Hammannavar, & Lobo, 2017, December). When PVP was added in the PVA polymer solution the tensile strength was decreased, with improvement in both tensile modulus and elongation at break as a result of amorphous structure of PVP (Li, et al. 2010).

The loading film with plant extracts did not have an effect on PVA/PVP film's tensile strength, however, gave a minor increase on their elongation at break % and insignificant decrease in corresponding tensile moduli were identified. Thus, mechanical behaviour of the

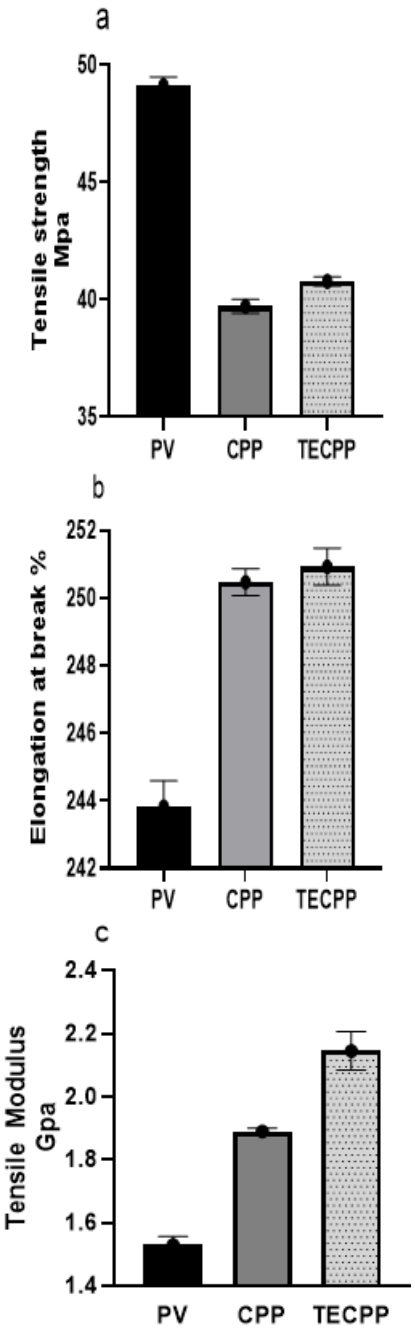

Fig. 3. Mechanical properties as tensile stress (a), elongation at break% (b) and tensile modulus (c) for PV, CPP and TECPP films

loaded PVA/PVP films has a close association with the presence of plant extract on the film surface, than associated with intermolecular and intramolecular interactions within polymeric network. These results were conformed with our SEM results.

Water Absorption Assay

The %ESR of (PV), (CPP)and (TECPP)films was presented in **Fig. 4**. Thus, results were show the maximum %ESR was 90% at first 25 min. Furthermore, at the same time swelling behaviour of (CPP)and

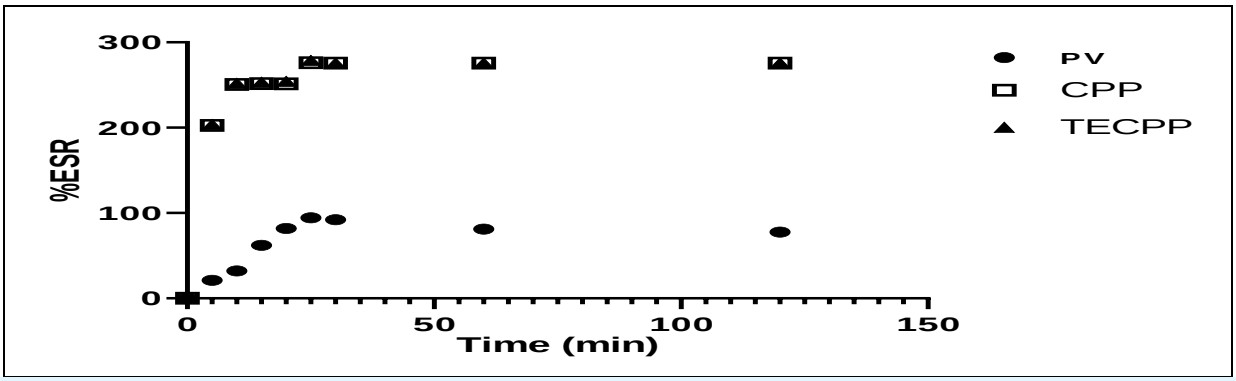

Fig. 4. %ESR of PV, CPP and loaded TECPP films

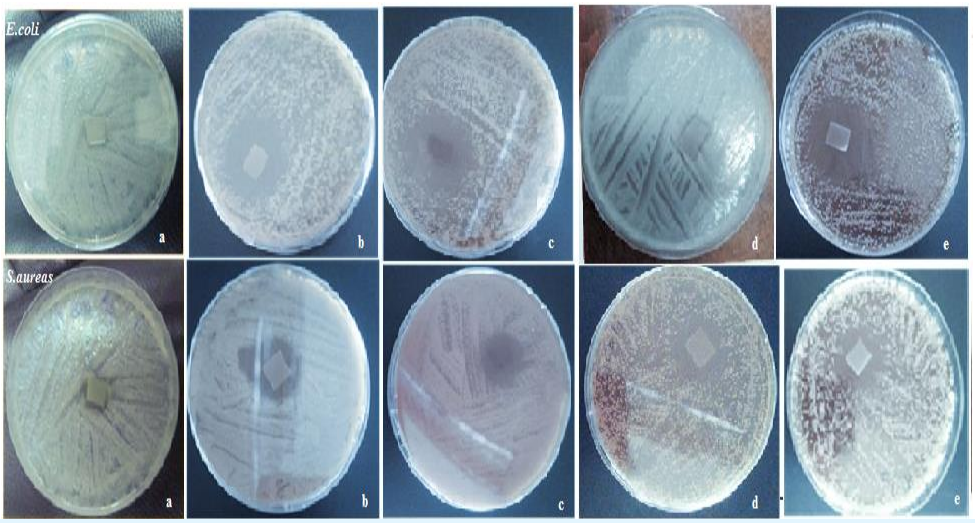

Fig. 5. Inhibition zone of CPP(a) TECPP (b) HECPP (c) (LECPP) (d) CECPP (e) against E. coli and S. aureas respectively

Table 1. Antibacterial activity of loaded and blank polymer films

Microorganism	Mean Inhibition zone (mm)					
	HECPP	CECPP	LECPP	TECPP	CPP	Streptomycin (10 µg /disc)
S. aureas	15±0.0	17±0.5	17±0.5	12 ±0.3	N	15
E. coli	22±0.0	23±0.2	N	25.±0.1	N	12

N= not effect

(TECPP) films were shown 220 % and 200 % respectively. Hence, a good water absorption capacity for the (CPP) films comparing to the pure sample was, must be related to the miscibility blending between PVA and PVP due to hydrogen bonding formation among them that improve both solubility and the mechanical properties of resulting films (Nwodo, et al. 2011).

Antibacterial Activity

Antibacterial study for all (ECPP) loaded and (CPP) films against E. coli (ATCC 25922) and S. aureus (ATCC 25923) was shown in **Table 1** and **Fig. 5,** respectively. Thus antibacterial activity was indicated by determination of the inhibition zone around each film, due to permeating the active extract components through the agar. The highest antibacterial effect for both (TECPP) and (CECPP) agents of E. coli with mean zone of inhibition about 25±0.1 mm and 23±0.2.While mean inhibition zone of (HECPP) reaching 22±0.0 mm. However, no significant inhibition from (LECPP) on the same bacterial strain was shown. Moreover, mean inhibition zone of (CECPP) and (LECPP) against S. aureus was 17±0.5 mm. While both (TECPP) and (CECPP) were shown 15±0.0 mm and 12 ±0.3 mm respectively. Our results are in agreement with the results of Nwodo, et.al., who found that hot water extract of T. indica fruit pulp have antibacterial activity against E.coli (ATCC11775) with inhibition zone about 23 ± 0.0 mm, while inhibition zone against S. aureus ATCC12600 were found 19±0.0 mm in the same extraction conditions (Warda, et al. 2007). Warda et al found that the ethanolic extract of T. indica fruit pulp at 10%,50% and 100% g/ml. has an inhibition zone about 15 mm, 24mm, and 30mm against S.aureus, and 12 mm, 22mm and 27 against E. coli, respectively, while the zone of inhibition with only 100% g/ml. concentration of fruit pulp water extract against E. Coli reach 14mm with no significant of

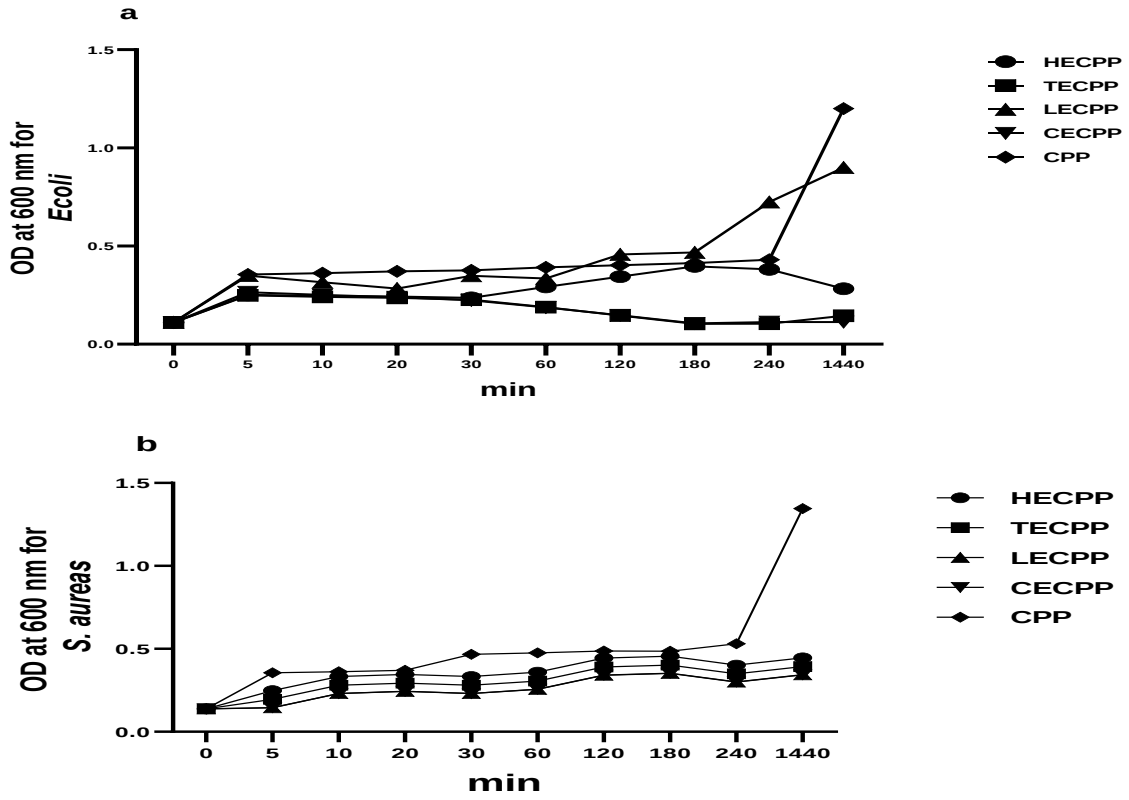

Fig. 6. The optical density at 600 nm for E. coli (a) and S. aureus (b) with (CPP) and (TECPP), (CECPP), (HECPP) and (LECPP) films

inhibition against S.aureus (Noumedem, et al. 2013). Moreover, our antibacterial results of C. pepo extract are in line with the study done by Noumedem et al (Dubey, Mishra, & Singh, 2010) and Dubey et al. (Fidan, et al. 2019).

Furthermore, Fidan et al identified antibacterial activity against S. aureus, with inhabition zone about 15mm. while low antibacterial activity against E. Coli with 8 mm (Lopez, Sanchez, Batlle, & Nerin, 2005). This finding is in agreement with our findings and other researchers, such as Loäpez et al. (Fullerton, et al. 2011) and Ouibrahim et al. (Walsh, et al. 2003).

Our results have shown higher mean zone of inhibition compared with research findings done by Fullerton et al which identify the overall mean zone of inhibition with 10% concentration of H. sabdariffa. Calyces extract was 12.66 mm against E. coli O157:H7. (Cowan, 1999). However, no zone of inhibition around (CPP) film (negative control) against test bacterial strains, while significant inhibition zone of Streptomycin (10 µg/disc) (positive control) with15 mm against S.aureus and 12 mm against E. coli. The previous results were in agreement with inhibition activity of sustained release of plant extracts from (ECPP) against E. coli (ATCC 25922) and S. aureus (ATCC 25923) was shown in **Fig. 6a**-**6b**, respectively. Results show a reduction in the growth of E. coli and S. aureus exposed to (TECPP), (CECPP), (HECPP) and (LECPP) than (CPP)films over different time intervals. Thus, can be related to the sustained release of extracts from loaded films. The inhibition activity against bacterial strains was identified after 30min from incubation with loaded film. Thus, it can be related to the swelling behaviour of blend film, that is directly proportional with loading and releasing ability for active material. (Bernal, Kuritka, & Saha, 2011). Our study was found the OD_{600} of E. coli and S. aureus against (CPP) films reached 0.376 and 0.467 at 30 min, and after 24 hr from incubations the OD_{600} reached 1.2 and 1.345 respectively. While after 30 min reduction of E. coli concentrations against (TECPP), (CECPP) and (HECPP) were reached 0.227, 0.223 and 0.236 respectively, after 24hr the E. coli concentrations were decreased than (CPP) to reach 0.145,0.113 and 0.282 respectively. In addition the concentrations of S.aureus against (LECPP), (TECPP), (CECPP) and (HECPP) were reduced compering to OD_{600} of (CPP) film to reach 0.230,0.280 0.231, and 0.334 after 30 min, and 0.343, 0.393, 0.344 and 0.446 after 24hr respectively from incubation. Thus, the previous results indicating that incorporation of plant extracts in polymer films can prolong extract release by increasing reservation properties of the extracts and increase extract stability as shown by a study done by Rijo et al

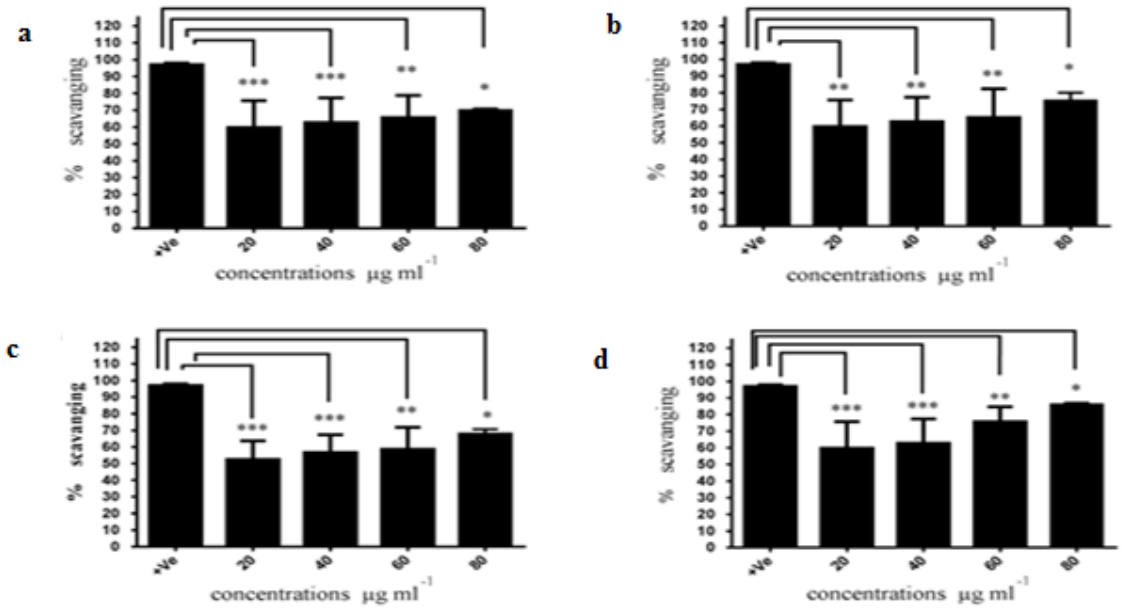

Fig. 7. Scavenging activity of 10% from T. indica (a) C. pepo, (b) H. sabdariffa (c) and L. nobilis (d) extracts

(2014). Mahendran et al (2016). Szabo et al (2020) and Avci et al. (2019).

Antioxidant Activity

Antioxidant and radical scavenging activities of different aqueous extractsT. indicia, H.sabdariffa, L. nobilis, C. pepo were shown in **Fig. 7a-d** estimated in vitro via assessment of DPPH radical scavenging activity. DPPH which is a free stable dark-purple tulip and changed in colour to yellow related for the antioxidant compounds by giving an electron and proton. (Meher, & Das, 2013). In all concentrations tested (20, 40, 60 and 80 µg mL^{-1}), the absorbance of T. indicia aqueous extract was significantly higher ($P \leq 0.001$) than vitamin C, such finding suggests that the plant extracts are more effective than vitamin C in terms of reductive ability, which was concentration dependent. The concentrations of 60 and 80 µg mL^{-1} of aqueous extract T. indicia shared an approximated radical scavenging activity (86.00 ± 2.00 and 75.66 ± 1.15%, respectively), but that at concentrations of 20 and 40 µg mL^{-1} (62.67 ± 5.77 and 59.66 ± 3.51%, respectively) (**Fig. 7a**). The aqueous extract of H. sabdariffa was significantly more effective than vitamin C in DPPH radical scavenging activity at the four tested concentrations (20, 40, 60 and 80 µg mL^{-1}). The aqueous extracts (H. sabdariffa) with concentrations of 60 and 80 µg $mL^{-1}$of shared an approximated radical scavenging activity (75.33 ± 2.00 and 65.66 ± 1.15%, respectively); these value were significantly higher ($P \leq 0.05$) than those at concentrations of 20 and 40 µg mL^{-1} (62.66 ± 5.77 and 59.66 ± 3.51%, respectively) (b) similar significances were to obtain to those found by other studies that indicated radical scavenging activity about 69% for red calyx (Anokwuru, et al. 2011). and at 50–250 _g/mL aqueous extracts of dark red dry calyces and light red white calyx scavenging activity about 25–85% and 20–65% respectively (Peredo Pozos, et al. 2020). In addition, aqueous extract for both C. pepo and L. nobilis does not show any significantly effective in DPPH radical scavenging activity than vitamin C at the four concentrations tested. As shown in (c) and (d) respectively.

CONCLUSIONS

In this study, mechanical and swelling properties of PVA film were improved by blending with PVP .To introduce antibacterial properties a simple route has been proposed to the preparation of antibacterial PVA/PVP films by post -loading of plant extract into the polymer matrix. The prepared films showed significant antibacterial activities, specifically in films loaded with the *T.indica* extract against *E. Coli* and in films loaded with *C.pepo* and *L.nobilis* against *S.aureus*. Finally, these results suggest that the PVA/PVP film containing plant extracts may be used as antibacterial film. Further studies are needed to identify biocompatibility, releasing and loading ability of films are recommended.

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