Antimicrobial Activity of *Myrtus Communis* and *Eucalyptus Camaldulensis* Leaf Extract Loaded with PVA/ PVP Polymer Film

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Abstract

Eucalyptus Camaldulensis and *Myrtus Communis* leaf were extracting with ethanol. The *in vitro* antimicrobial activity of Poly (vinyl alcohol) (PVA)/Poly(vinyl pyrrolidone) (PVP) blend film loaded with *Eucalyptus Camaldulensis* and *Myrtus Communis* crude was studied. Both *Eucalyptus Camaldulensis* and *Myrtus Communis extracts* were investigated via FTIR, phytochemical and antioxidant activity. Poly (vinyl alcohol) PVA/ Poly(vinyl pyrrolidone)PVP blend film was investigated via FTIR, Film was studied via SEM before and after loading. Both *Eucalyptus Camaldulensis* and *Myrtus Communis* extracts show antioxidant activity and richness with phytochemical materials. Poly(vinyl alcohol) (PVA) /Poly(vinylpyrrolidone)(PVP)loaded with 10%(w/v) *Eucalyptus Camaldulensis* extract shows a highest antimicrobial activity against *Staphylococcus aureus, Escherichia coli* and *Candida albicans* compering to films loaded with *Myrtus Communis extract.*

Keywords: *Eucalyptus Camaldulensis***,** *Myrtus Communis***,** Polymer**,** Antimicrobial Activity

Introduction

Now a day using of natural products, commonly used in traditional medicine, herbal extracts are a common source of therapeutic agents for the microbial infections. Nevertheless, aqueous extracts normally have various formulation problems, for instance instability, burst release and low bioavailability.(1)To overcome these problems biodegradable and biocompatible polymer materials can be used as carriers of bioactive ingredients and are a functional strategy for the improvement of their stability features.(2*) Myrtus communis* is a spontaneously plant belong to the Myrtaceae family. The leaves and fruit can be used as disinfectant, hypoglycemic agents and antiseptic. Leaves extract are used as anti-inflammatory agents, mouthwash, treatments of candidiasis ,wounds treatment and urinary diseases therapy.(3)*Eucalyptus* is one of the world's

essential and most widely evergreen genera.(4) It is a native to Australia and Tasmania.(5) Eucalyptus species have been used as medicinal plants due to their pharmacological and biological properties.(6) It can be used as analgesic, antiseptic, insipid, mordant and disinfectant .(7) Poly (vinyl alcohol) (PVA) and Poly (vinyl pyrrolidone) (PVP) are the widely used water soluble biodegradable, biocompatible and nontoxic synthetic polymers.While (PVA) owning excellent physical properties such as flexibility, superior barrier to oxygen, films forming polymers. Blending can be the effective methods for improvement (PVP) film properties. (PVA) and (PVP) blend films have been developed for numbers of biomedical applications (8,9,10)such as coatings for sutures, wound dressings, catheters and contact lenses. (11, 12) The present work focuses on the antimicrobial activity of *Myrtus communis* and *Eucalyptus camaldulensis* ethanolic extract loaded with PVA/PVP membranes and the function of these blends in the prevention of antimicrobial infections. Moreover phytochemical screening, antioxidant activity and FTIR analysis for both extract were studies.PVA/PVP films have been characterized through SEM and FTIR analysis.

Materials and Methods

Poly(vinylalcohol)(PVA),(98-99%hydrolyzed),Average MW ≈31.000-50.000 from Aldrich Germany.Poly(vinylpyrrolidone)(PVP), MW≈44.000 from BDH laboratory, England.Phosphate buffer saline (PBS) Aldrich Germany. 99%ethanol from Scharlau and Nutrient Agar from Bioscience. Dpph from sigma (USA).Plants were gathered from university of technology gardens and were identifying according to (13, 14)

Preparation of Plant Leaves Extracts

First of all the leaves of respective plants were thoroughly washed with running tap water, blotted and dried at room temperature. For the purpose of making powder it was grinded in grinder. From these 200gm of powdered from each material were extract in 400 ml of 99% ethanol for 18hr at room temperature. Ethanolic filtrate was evaporated by evaporator to obtain ethanolic extract. Finally, extract was dry at 40^{o}C. (13)

Preparation of PVA/ PVP Blend Film

PVA/ PVP blend film was perpetrated according to method done by Ahmad, Ishraque and et al with same modification. (15) PVA solution were prepared by using 6% (w/v) aqueous solutions. 6 gm from PVP was added to the PVA solution and solution was stirred for 45 min at room temperature. 20ml from solution was poured into Petri dish and the film was cast by drying at 45ºC for 72 hr.

PVA/PVP Blend Film Loaded with *Eucalyptus Camaldulensis* and *Myrtus Communis* Alcoholic Extract

Stock solution 10% (w/v) from dry alcoholic extract of *Eucalyptus camaldulensis* and *Myrtus communis* were prepared by dissolving about 1gm from each extract with 10 ml from PBS. About (1X 2) cm^2 samples from PVA/PVP film were immersing in 10ml of *Eucalyptus camaldulensis* and *Myrtus communis* stock solutionfor 24hr respectively. Then each sample is being dried at room temperature.

Characterization of the Samples

Fourier Transform Infrared (FTIR) Study

FTIR study of PVA, PVP, PVA/PVP and dry extract for both *Eucalyptus camaldulensis* and *Myrtus communis* were carried out with KBr powder samples and a Mattson Satellite 5000 FTIR spectrophotometer.

Scanning Electron Microscopy (SEM)

The surface characteristics of dry extract for *Eucalyptus Camaldulensis* and *Myrtus Communis*,PVA/PVP only and PVA/PVP loaded with both *Eucalyptus camaldulensis* and *Myrtus Communis* synthesized were examined by (Stereoscan 360, Cambridge) scanning electron microscopy at study were done at applied science department in university of technology.

Photochemical Screening of Ethanolic Extract

Photochemical screening was carried out for both *Eucalyptus Camaldulensis* and *Myrtus Communis* dry extracts to identify presence constituents like alkaloidas, tannins, flavonoids, resin reducing sugar saponins ,steroids, phenol terpenoias and proteins as standard methods done in (16,17,18,19 ,20and 21)

Invitro Antioxidant Activity

The free radical scavenging capacity of dry ethanolic *Eucalyptus Camaldulensis* and *Myrtus Communis* extract. Were measured with DPPH assay.(22)(23) The DPPH radical has a deep violet color due to its unpaired electron and radical scavenging capability can followed spectrophotometrically when the pale yellow non-redical form is produced as a result of absorbance loss at 517nm. The DPPH assay was performed as described in (24) According to this analysis, control was prepared from (0.5 ml) of DPPH 60 µM and complete to 1 ml with ethanol. Samples were prepared from 10 µL from 10% *Eucalyptus Camaldulensis* and *Myrtus Communis* extracts and completed with ethanol to 0.5 ml then 0.5 ml of DPPH 60 µM was mixed with each sample. Samples and control were

placed in dark for 30 min at room temperature then , the absorbance for both sample and control were read at 517nm in a (Tech Comp)UV/VIS spactrophotometer. The percentage of DPPH decolouration of the sample was calculated according to the formula :

%Decolouration = $(Absorbance)_{Control}$–$(Absorbance)_{Sample}$/ $(Absorbance)_{Control}$ x100

Evaluation of Antibacterial Properties

In vitro antibacterial activity was measured against *Staphylococcus aureus*, *Escherichia coli* and *candida albicans* cultured in Muller – Hinton Agar as method done in (25)(26) as fallowing **:-**

1- Evaluation of Antibacterial Properties of Plants Extract.

50µl Stock solution 10% (w/v) from dry alcoholic extract of *Eucalyptus Camaldulensis* and *Myrtus Communis* (control sample) were applying via well diffusion and bacterial cultured were incubate at 37ºC and zone of inhibition were measured after 48 hr from incubation.

2- Evaluation of Antibacterial Properties of PVA/ PVP Before and After Loading with Plants Extract.

Samples with (1X2) cm^2 from PVA/PVP film only (blank sample) and PVA/PVP loaded with10%(w/v)*Eucalyptus Camaldulensis* and *Myrtus Communis extract* (test sample) were applied on the surface of the bacterial cultured agar and incubate at 37ºC and zone of inhibition were measured after 48 hr from incubation.

Results and Discussion

Fourier Transform Infrared (FTIR)

1-FTIR for PVA, PVP Pure Film and PVP/PVA Film

FTIR spectroscopy of the pure and blend films were carried out in the wave length of 4000–400 cm^{-1} as shown in (Figure,1,2and3). FTIR spectra were done for pure and blend films to study chemical interactions between PVP and PVA for instance hydrogen bonding or other complexation. (Figure, 1) shows the FTIR spectra of the pure PVA film. The characteristic (C = O deformation) bands of PVA was found at1101 cm^{-1}.While bored beak for (OH stretching) were appeared at 3534 $cm^{-1}$and band at 1424 cm^{-1} related to(C-H bending),alkyl stretching was found at 2954 cm^{-1}.Alternatively,(Figure ,2) shows the FTIR spectrum of PVP. The characteristic C=O stretching band for PVP was appeared at1658cm^{-1}.(Figure,3)shows the spectrum of the PVA/ PVP blend films . It was found the characteristic shifting for OH stretching bands to the 3442 cm^{-1} in the spectrum of blends films compared with pure components. This supports that a hydrogen bond was formed between PVA and PVP and GA. Moreover, spectra for C=C shifted

to the lower frequency and found at 1529 cm^{-1} comparing with pure PVA and PVP film which found at 1643 cm-1 and 1650 cm^{-1} respectively. Indicate intermolecular interaction between PVA and PVP with GA.

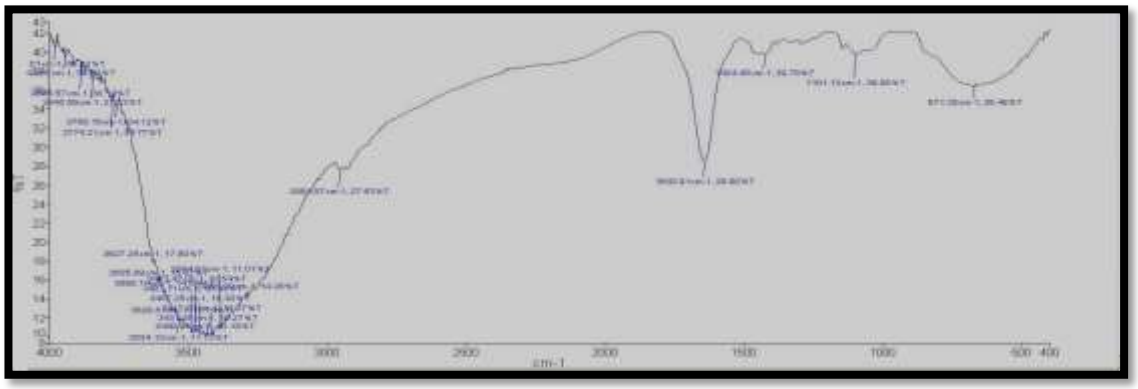


Figure1:-FTIR Spactroscopy for Pure Poly (vinyl alcohol) (PVA) Film

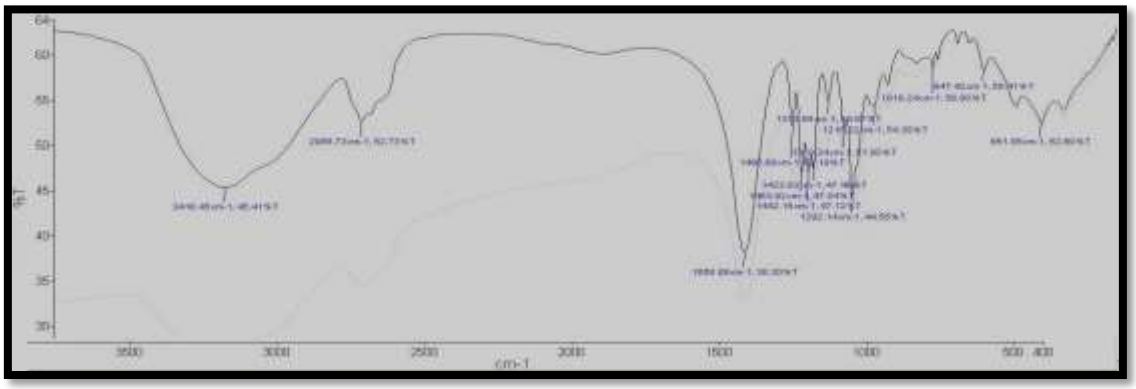


Figure2:-FTIR Spactroscopy for Pure Poly (vinyl pyrrolidone) (PVP) Film

2-FTIR for Dry Alcoholic Extract and Cross Linked PVP/ PVA Film Loaded with Dry Alcoholic Extract.

FTIR spectra for dry alcoholic extract for both *Myrtus Communis* and *Eucalyptus Camaldulensis* and cross linked PVP/ PVA film loaded with dry alcoholic extract were carried out in the wave length of 4000–400 cm^{-1}as shown in (Figure,4,5,6 and7) respectively. O-H spectra for both *Myrtus Communis* and *Eucalyptus Camaldulensis* was found at 3256$cm^{-1}$and 3402 cm^{-1}respectively. The presence of board O-H band mast be related to alcohol .However C=O were found in 1724 cm^{-1} and 1705 cm^{-1} mast be related to aldehyde group for *Myrtus Communis* and *Eucalyptus Camaldulensis.* Spectra at 2926 $cm^{-1}$and 2931 cm^{-1} related to C-H stretching of alkanes group . In that order spectra at 1358 cm^{-1} and 1352 cm^{-1}related to CH_3 bending of alkanse. C=C related to alkenes found at

1620cm^{-1}and 1612cm^{-1}for *Myrtus Communis* and *Eucalyptus Camaldulensis* samples .All data on the spectrum values and the potential functional group found in the leaf extracts of *Myrtus Communis* and *Eucalyptus Camaldulensis* are presented in (Table,1).Above results were conformed with study done by Al-Hajjar A. M. et al (13) and R.Ashokkumar.(27) FTIR spectra for PVP/ PVA Film loaded with *Myrtus Communis* extract showed shift C-O stretching to lower frequency at 1037 cm^{-1}comparing to PVP/ PVA Film as shown in (Figure,3) also the bands at 1705 cm^{-1} related to C=O , C-H stretching band at 2351 cm^{-1} and C-H stretching at 2931 cm^{-1}in the spectrum of dry *Myrtus Communis* extract Figure,4 are absent in the spectrum of loading sample (Figure,6).Which indicate interaction between *Myrtus Communis* dry extract with PVP/ PVA film, same behavior was found with PVP/ PVA film loaded with *Eucalyptus Camaldulensis* , it was found C-O stretching shifted to lower frequency at 1024 cm^{-1} also C=O band at 1714 cm^{-1}, C-H stretching band at 2332 cm^{-1} and C-H stretching at 2926 cm^{-1}in the spectrum of *Eucalyptus Camaldulensis* dry extract Figure,5 are absent in the spectrum of loading sample(Figure,7).Which indicate interaction between *Eucalyptus Camaldulensis* dry extract with PVP/ PVA film.

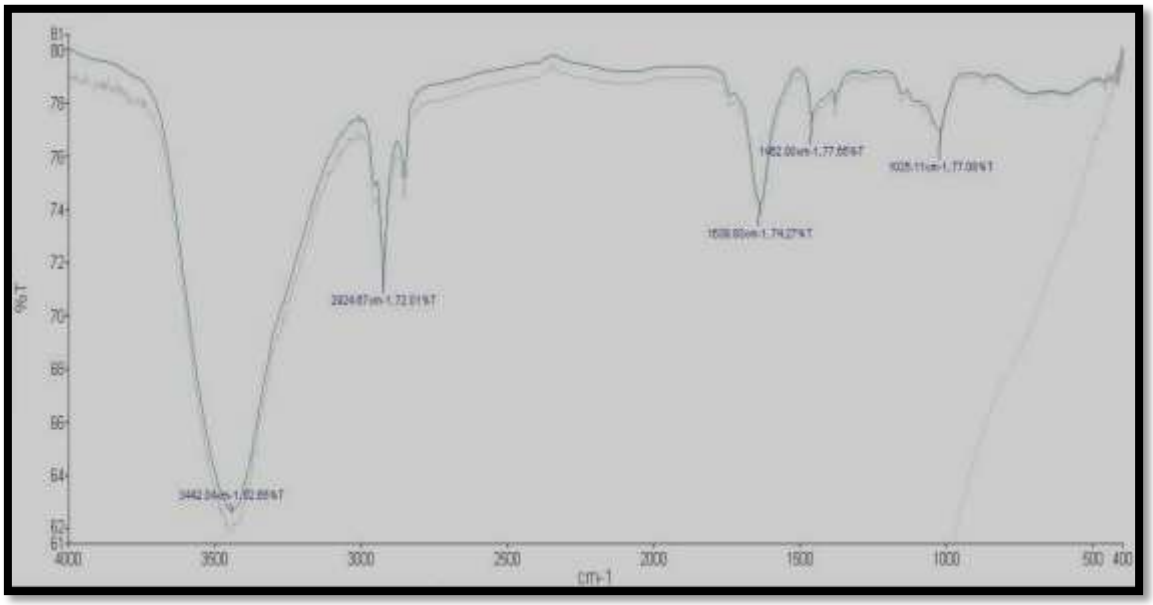


Figure3: FTIR Spactroscopy for Cross Linked PVP/ PVA Film

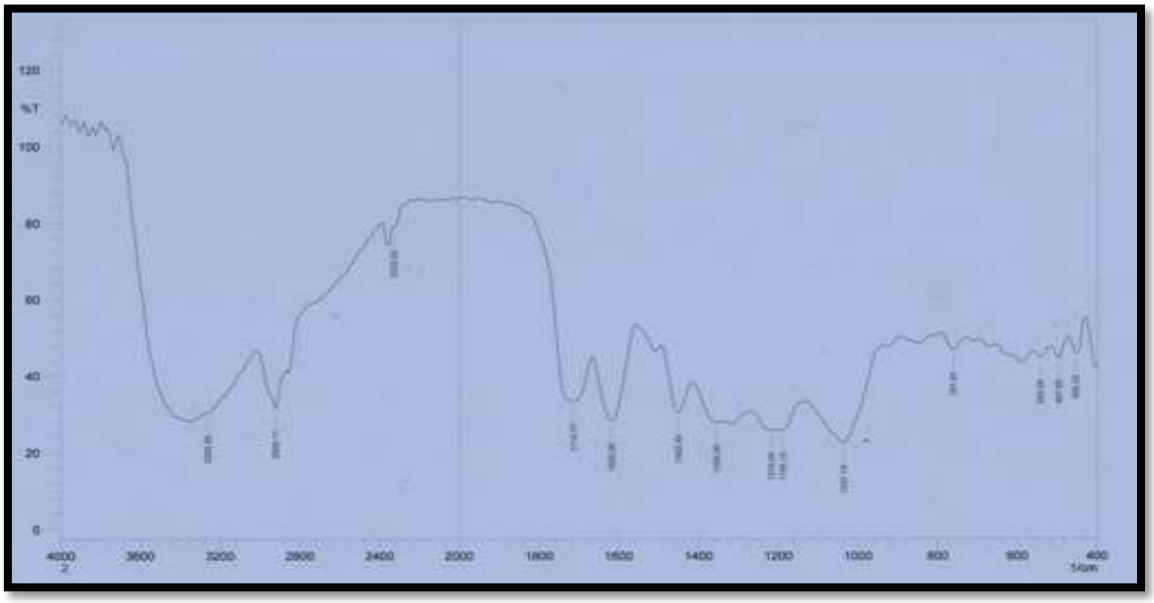


Figure4: FTIR Spectra for Dry Alcoholic Extract for *Myrtus Communis*

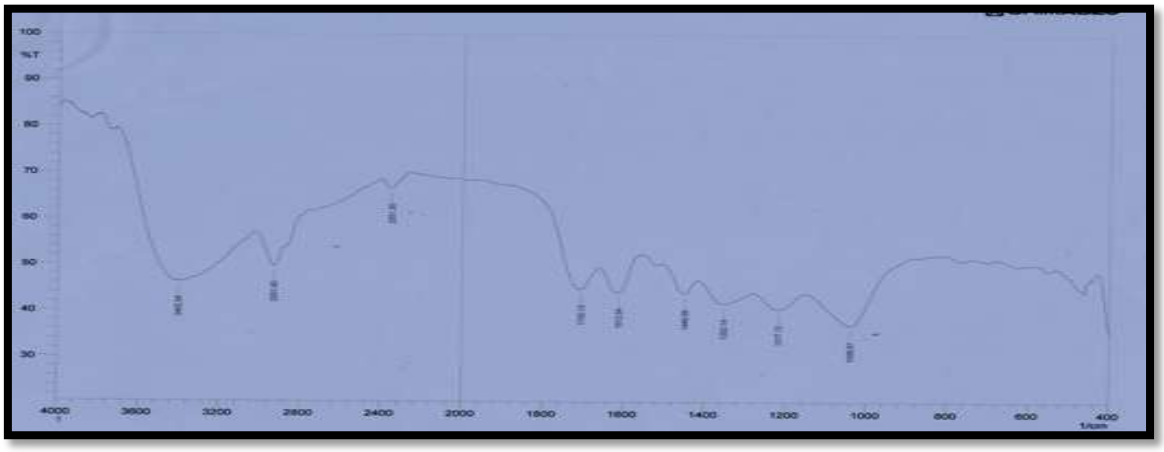


Figure 5:FTIR Spectra for Dry Alcoholic Extract for *Eucalyptus Camaldulensis*

Table (1) FTIR Spectral Values and Functional Group Presented for the Leaf Extract of *Myrtus Communis* and *Eucalyptus Camaldulensis*

Ethanolic Plant extract	Functional Group	Wave number cm^{-1}	Ethanolic Plant extract	Functional Group	Wave number cm^{-1}
Myrtus Communis	υ (O-H)	3265	*Eucalyptus Camaldulensis*	υ (O-H)	3402
	C=O Naphthenic group	1724		C=O Naphthenic group	1705
	υ C-H Naphthenic group	2926		υ C-H Naphthenic group	2931
	υ C=C Aromatic group	1620		υ C=C Aromatic group	1612
	δ (CH3)	1358		δ (CH3)	1352

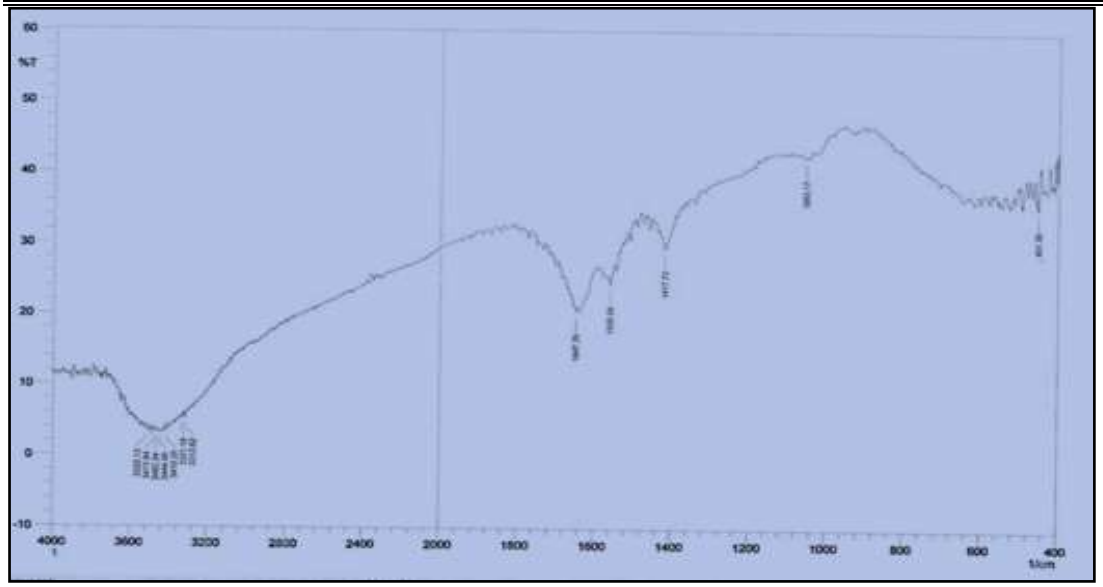


Figure 6: FTIR Spectra for Cross Linked PVP/ PVA Film Loaded with Dry Alcoholic Extract for *Myrtus Communis.*

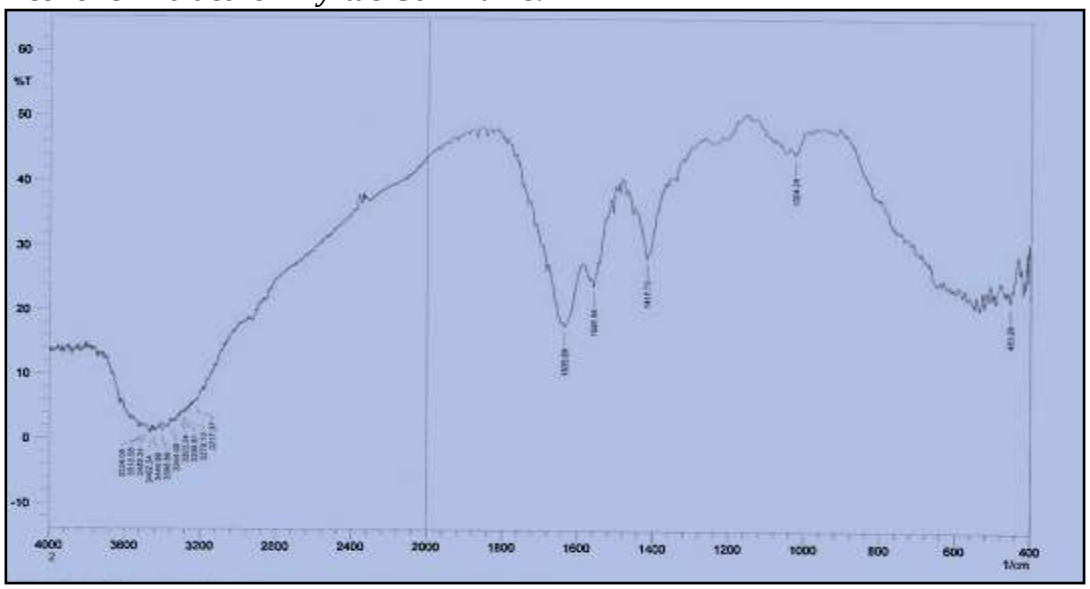


Figure 7: FTIR Spectra for Cross Linked PVP/ PVA Film Loaded with Dry Alcoholic Extract for *Eucalyptus Camaldulensis*

Photochemical Screening

Preliminary photochemical screening of extracts revealed the presence of different primary and secondary metabolites. The ethanolic extract of *Eucalyptus Camaldulensis* and *Myrtus Communis* rach in compounds (alkaloidas,tannins, flavonoids, resin reducing sugar saponins ,steroids, phenol terpenoias and proteins) as shown in (Figure ,6) and (Table,2).confirming the data yielded by previous studies of *Myrtus Communis* leaves extract (28).While phytochemical screening of *Eucalyptus Camaldulensis* leaf extract done by Shayoub M. et al indicated

absence of alkaloidas compound . Thus, most be related to extraction method and solvent use. (29) (30)

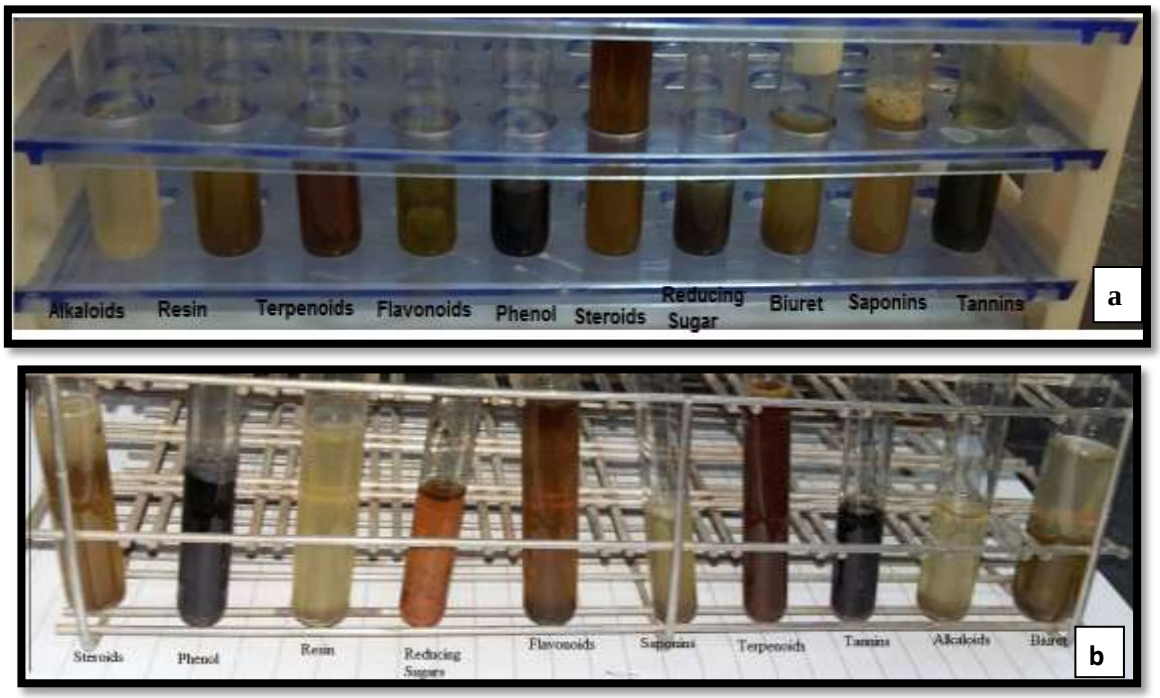


Figure (8) Photochemical Screening of *Eucalyptus Camaldulensis* (a) *Myrtus Communis*(b).

(Table 2):Phytochemical Components of the Leaves Extracts of *Eucalyptus. Camaldulensis* and *Myrtus Communis*

Active compound	*Eucalyptus camaldulensis*	*Myrtus communis*
Alkaloidas	++	+
Tannins	++	+
Flavonoids	++	+
Resin	++	+
Steroids	++	+
Phenol	++	++
Reducing Sugar	+	++
Saponins	++	+
Terpenoias	+	+
Proteins	++	+

Key += Moderate ++= High

Scanning Electron Microscopy (SEM)

The surface characteristics of *Myrtus communis and Eucalyptus camaldulensis* dry extract and synthesized (PVA/PVP) film before and after loading with 10% *Myrtus Communis and Eucalyptus Camaldulensis* were shown in (Figure,9 and 10) respectively. Morphology of dry extract

powder for *Myrtus Communis* and *Eucalyptus Camaldulensi* is presented in(Figure,9 a,b). While, Morphology of dry extract powder for *Myrtus communis* is presented in(Figure,9c,d). The shape for both powder was rectangular and irregular. The surface of PVA/PVP film before loading had a smoother and more homogeneous appearance than the surface of the polymer film loaded with 10% *Eucalyptus Camaldulensis* and *Myrtus Communis* . The heterogeneous appearance of loaded film surfaces was due to most of *Eucalyptus Eucalyptus Camaldulensis* and *Myrtus Communis* extract molecules were entraped into the polymer net work and it was equally distributed over the film. As the extract concentration increased, more extract molecules were occupying the existing interconnected spaces of the polymer film.Moreover, at the highest load the small particles sticking together to form bigger particles deposited onto the film surface as show in (Figure 10, b and c). Other authors also reported same behavior. (31)

In vitro antioxidant activity

Myrtus Communis and Eucalyptus Camaldulensis extracts showed potent antioxidant activity as 70% and 50% mainly due to their richness in terpenoias, alkaloidas, ,flavonoids , and phenol compound as a result, these polyphenols had conjugated ring structures with -OH groups that have the possible to function as antioxidant.(32) as a result above ,*Eucalyptus Camaldulensis* has higher radical scavenging capacity compering with *Myrtus Communis*.

The Antibacterial Activity

In vitro antibacterial properties of the PVA/PVP blend films only (blank sample), 10% w/v *Eucalyptus Camaldulensis* and *Myrtus Communis* (control sample) and PVA/PVP films loaded with 10% w/v *Eucalyptus Camaldulensis* and *Myrtus Communis* (test sample) against gram negative *E.coli* and gram positive *S.aureus* and *Candida albican* were shown in (Figures,11,12 and 13) and (Table,3) respectively. Inhibition zone was found around test samples due to permeate of extract components through the agar indicating antibacterial activity.However, PVA/PVP film only (blank sample) didn't show any antibacterial activity against *Staph. aureus* , *Candida albican* and *E.coli* as shown in (Figures,11).

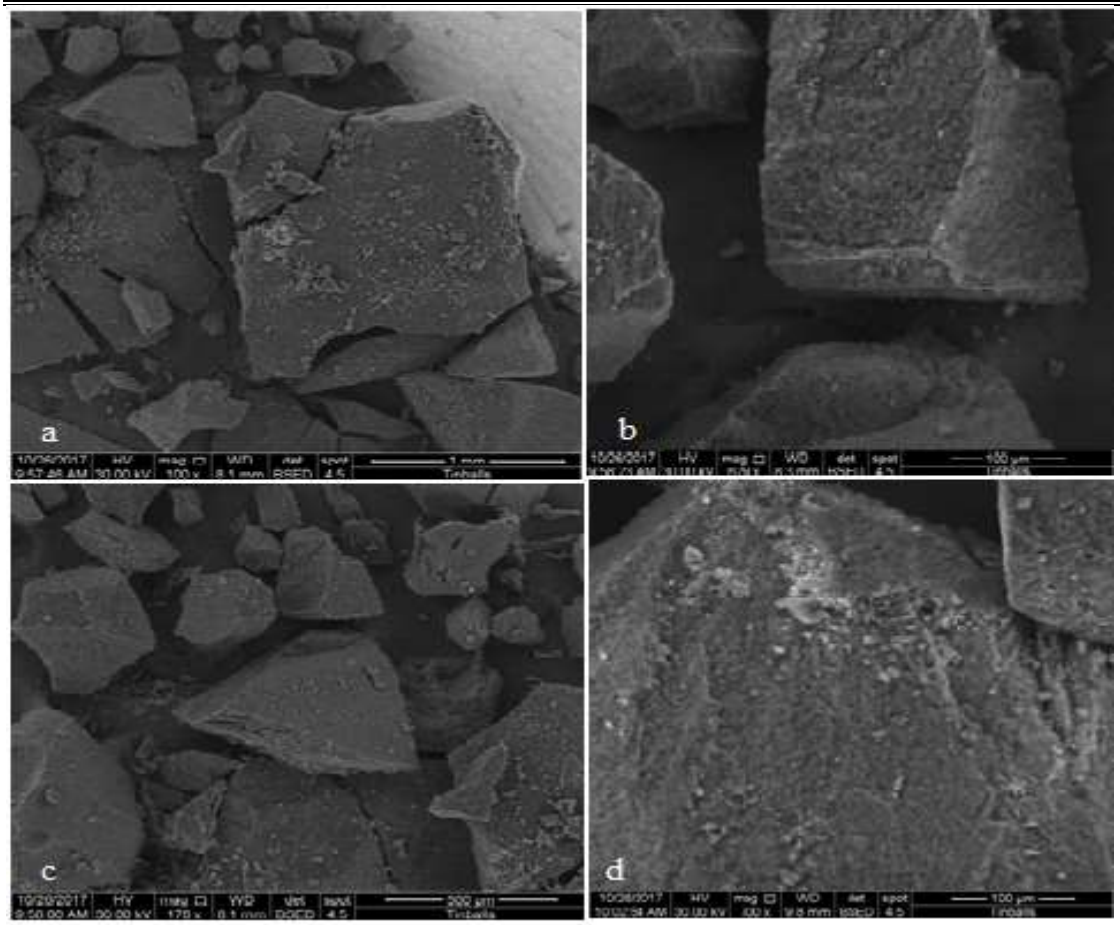


Figure (9) Scanning Electron Microscopy of *Eucalyptus camaldulensis* dry extract powder (a,b) and *Myrtus communis* dry extract powder (c,d).

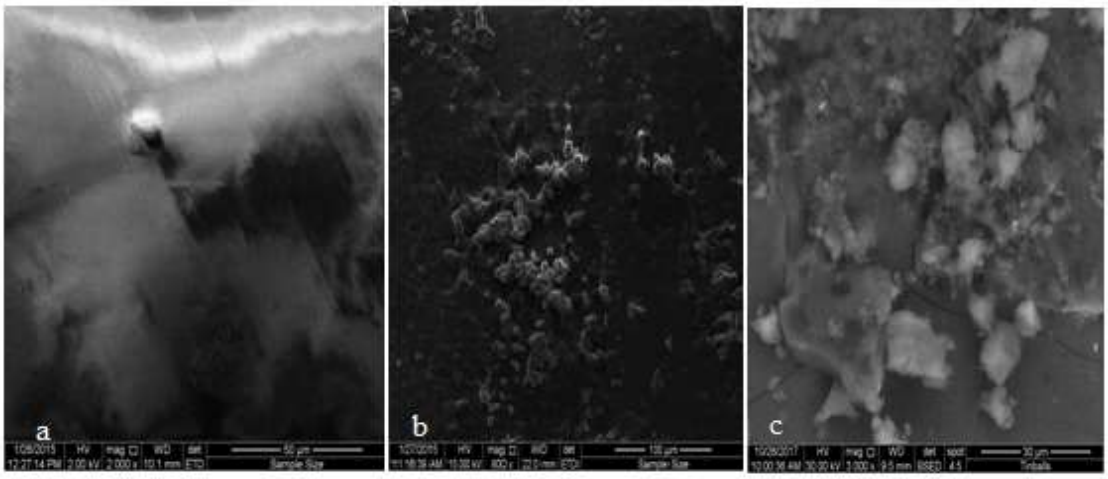


Figure (10) Scanning Electron Microscopy of PVA/PVP in (a) PVA/PVP loaded with10% *Eucalyptus camaldulensis* in (b) and PVA/PVP loaded with10% *Myrtus communis* in (c)

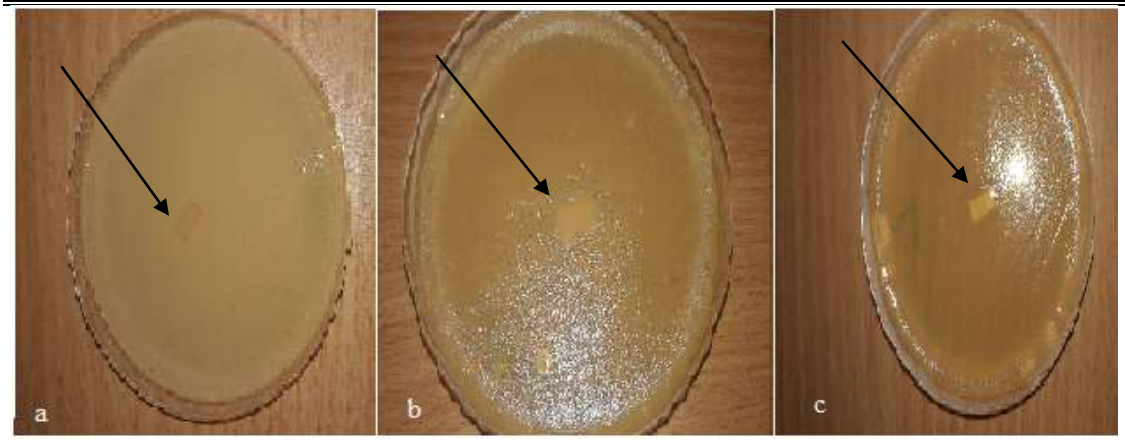


Figure (11) antimicrobial activity of PVA/PVP only (blank sample) against *Candida albican* (a), *Staph. aureus* (b) and *E. coli* (c)

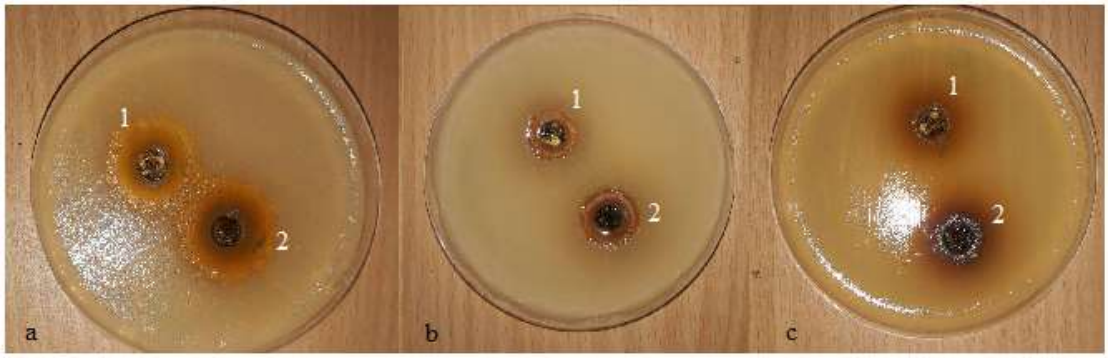


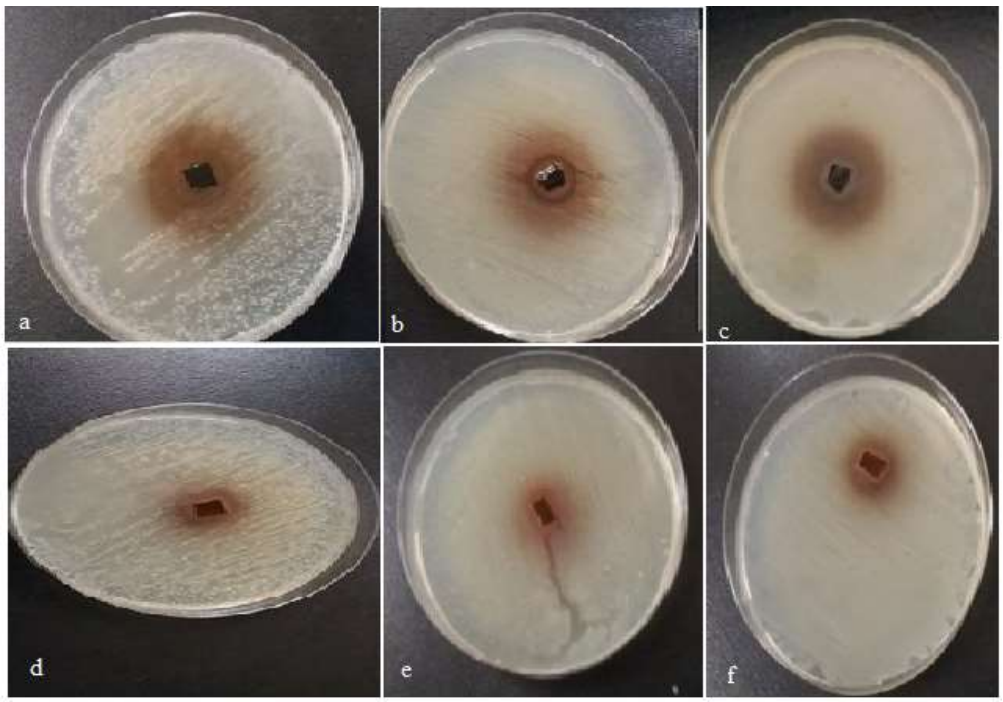
Figure (12) antimicrobial activity of 10% *Myrtus communis* no(1) and 10% *Eucalyptus camaldulensis* no *(2)* against *Candida albican* (a), *Staph. aureus* (b) and *E. coli* (c)

Moreover, Inhibition zone with 40, 33 and 28 mm diameter were found by app1ing 10% w/v (100 mg/ml) *Eucalyptus camaldulensis* (control sample) on the same microbial strain culture and 25,23 and 15 mm diameter were found by app1ing 10% w/v (100 mg/ml) *Myrtus communis* (control sample) against *Staph.aureus*,*Candida albican* and *E.coli* as shown in(Figures,12) and (Table,3). PVA/PVP film loaded with *Eucalyptus camaldulensis* show good antimicrobial activity against *Staph. aureus* , *Candida albican* and *E.coli* , inhibition zone was found 30,28, and 25 mm respectively.While, fairly antimicrobial activity was indicated with *Myrtus communis* against same microbial strain with inhibition zone17,17and 13 mm respectively as present in(Figures,13) and (Table,3). Thus, the antimicrobial activity for PVA/PVP blend films loaded with *Eucalyptus camaldulensis* and *Myrtus communis* was conformed with photochemical screening as shown (Figure,8) (Table,2) and antioxidant activity.

Table(3)inhibition zone in(mm)for 10% (w/v)leaves extract of *Myrtus communis* and *Eucalyptus camaldulensis* and PVA/PVP film before and after loading with 10% (w/v)leaves extract of *Myrtus communis* and *Eucalyptus camaldulensis* respectively

Tested Organisium	Inhibition zone in (mm)				
	PVA/PVP film only	*Myrtus communis* 10%stock solution	PVA/PVP loaded with *Myrtus communis*	*Eucalyptus camaldulensis* 10%stock solution	PVA/PVP loaded with *Eucalyptus camaldulensis*
Candida albicans	-	25	17	40	28
Staphylococcus aureus	-	23	17	33	30
Escherichia coli	-	15	13	28	25

(-) No inhibition



Figure(13) antimicrobial activity of PVA/PVP loaded with10% *Eucalyptus camaldulensis* against *candida albican* (a), *E. coli* (b) and *Staph. aureus* (c). and PVA/PVP loaded 10% *Myrtus communis* against *candida albican* (d), *E. coli* (e) and *Staph. aureus* (f).

Conclusion: The results of this study have shown that the PVA/PVP loaded with10% *Eucalyptus camaldulensi* leaf extracts have enormous prospective as antimicrobial agents in the treatment of infectious microorganisms. Further in-vivo investigation of the PVA/PVP loaded with10%*Eucalyptus camaldulensi* will contribute greatly to the development new pharmaceuticals.

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الخلاصة

استخلص كلا من نبات الاس المحلى ونبات الكالبتوس المحلي باستعمال الكحول الاثيلي، وتمت ضمن هذه الدراسة قياس الفعالية المضادة للبكتريا بوساطة الغشاء البوليمري المصنع من خلط بولي فينيل الكحول PVA مع بولي فاينيل بيروليدون PVP والمحمل بالمستخلص الكحولي الخام لأوراق نبات الآس المحلي و ونبات الكالبتوس المحلي كلا حدا ، تمت دراسة مطيافية الاشعة الحمراء للغشاء البوليمري ،شحصت المجاميع الفعالة باستعمال مطيافية الاشعة الحمراء والمواد الفعالة والفعالية المضادة للاكسدة لكلا المستخلصين و فضلا عن ذلك فحص الغشاء قبل وبعدة تحميله بالمستخلص الكحولي لنبات الكالبتوس باستعمال المجهر الالكتروني الماسح، و اوضحت الدراسة ان كلا المستخلصين غني من حيث وجود المواد الفعالة والفعالية المضادة للاكسدة ولكن مستخلص نبات الكالبتوس المحلي اظهر نتائج افضل ،كما اظهرت الدراسة ان الغشاء المحمل بمستخلص الكالبتوس وبتركيز 10% (غم /ملم)اعطى فعالية مضادة لكل من *Staphylococcus aureus* , *Escherichia coli* و *Candida albican* افضل مقارنة مع الغشاء المحمل بمستخلص نبات الآس المحلي.

الكلمات المفتاحية : الأس , الكالبتوس ,بوليمر ,المضاد للبكتريا