Using Plant Extractives as Eco-friendly Pulp Additives: Mechanical and Antifungal Properties of Paper Sheets Made from Linen Fibers

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In this study, extractives from *Pinus rigida* heartwood (PRW), *Eucalyptus camaldulensis* var. *obtusa* aerial parts (ECL), and *Eucalyptus* flower buds (ECF) were used as additives in paper sheets produced from the pulp of linen fibers, and their effects on the mechanical and antifungal properties of the paper sheets were studied. The highest tensile and tear indices were 31.5 Nm/g, and 17.3 mNm^2/g as pulp treated with PRW (4%), and ECL (1%), respectively. All the pulp additives yielded lower burst index values compared to the control (2.24 $KPa.m^2/g$); the nearest value was 2.23 $KPa.m^2/g$ (ECL 4% pulp additive). The brightness percentages (ISO%) ranged from 65.7% to 70.2%, which were lower than the control treatment (70.3%). The paper sheets produced from pulp treated with 2% or 4% PRW, and with 4% ECL suppressed *Aspergillus niger* growth on the paper disc; pulp treated with 2% or 4% PRW, 4% ECL, and with 2% or 4% ECF completely suppressed *A. terreus* growth. However, all pulp extract additives did not inhibit the growth of *Fusarium culmorum*.

Keywords: Pulp additives; Antifungal activity; Mechanical properties; Extractives; Eucalyptus camaldulensis var. obtusa; Linen Fibers; Pinus rigida

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INTRODUCTION

In the pulp and paper industry, many additives are added to the pulp to improve the mechanical and physical characteristics of the paper sheets produced. In a study by Taha *et al.* (2019a), the mechanical properties of the developed paper sheets were enhanced *via* the addition of *Pinus rigida* wood in the form of ground particles (80 mesh) as an additive to linen fibre pulp. As cationic and carboxymethyl hemicelluloses were introduced (Ren *et al.* 2009), the physical properties of spruce wood pulp paper sheets were improved.

In addition, the strength characterizations of paper derived from bagasse fibers were improved *via* the usage of chitosan and cationic starch as additives (Hamzeh *et al.* 2013b). The technological properties of paper sheets produced from soda bagasse pulp were improved by using bio-additives mixed with cellulose nanofibrils and a high degree of cationic starch substitution (Tajik *et al.* 2018). The mechanical, optical, and antifungal properties were improved in paper sheets developed from *Eucalyptus camaldulensis* and

Meryta sinclairii wood branches (Salem *et al.* 2020).

The strength properties of paper sheets made from an equal mixture of bleached softwood and hardwood pulps were improved by more than 15% when starch-coated fillers were introduced (Yan *et al.* 2005). The mechanical and optical properties of the processed paper sheets were not affected when wood pulp was substituted with 10% to 15% kenaf pulp (Liu *et al.* 2018). Kraft pulping with black liquor from mixed hardwood pretreated chips yielded an increase in the tensile, burst, and tear indices (Tripathi *et al.* 2016). Bagasse and wheat straw were shown to boost the physical strength of paper sheets when included as additives during the banana stem pulping process (Tripathi *et al.* 2013). The mechanical properties and antifungal bioactivity of the produced paper sheets were improved when papyrus strips were pretreated with certain additives, *e.g.*, natural extracts and nanomaterials (Taha *et al.* 2019b). The produced paper sheets from flax pulp treated with a mixture of chitosan and ZnO nanoparticles (NP) inhibited the growth of *Aspergillus flavus*, while a mixture of chitosan and Ag NP inhibited *A. terreus* and a mixture of Paraloid B-72 and Ag NP inhibited *Stemphylium solani* (Abo Elgat *et al.* 2020a).

In the present study, three extractives, which were obtained from *Pinus rigida* heartwood, *Eucalyptus camaldulensis* var. *obtusa* aerial parts, and *E. camaldulensis* flower buds, were used to enhance the mechanical, and antifungal properties of paper sheets made from linen fiber (*Linum usitatissimum* L.) pulp. Natural extractives and essential oils have been shown a promising antifungal activity (Salem *et al.* 2014a,b; Hussein *et al.* 2017; Salem *et al.* 2017; Abo Elgat *et al.* 2020b; Ashmawy *et al.* 2020; Behiry *et al.* 2020; Mohamed *et al.* 2020; Mansour *et al.* 2021). The extracts *i.e.*, essential oils, from *P. rigida* wood have been found to demonstrate potent antifungal activity against certain molds that can colonize some commercial woods (Salem *et al.* 2016a; Salem *et al.* 2016b). The extracts from *Eucalyptus* species have been extensively evaluated for their antimicrobial activities as well as their bioactive compounds (EL-Hefny *et al.* 2017; Elansary *et al.* 2017; Salem *et al.* 2019; Hamad *et al.* 2019; Abdelkhalek *et al.* 2020).

Molds can infest paperboard packaging and paper materials anywhere humid conditions are present, which has a negative economic impact on the suppliers and manufacturers of pulp paper and paper materials (Alwaeli 2010; Jerusik 2010; Torres *et al.* 2011; Guzińska *et al.* 2012). Fungi produce spots with different colors on paper materials, which are typically associated with the foxing process (Zotti *et al.* 2011; Lavin *et al.* 2014). The traditional chemicals that are used as antimicrobial agents for paper manuscripts of cultural heritage and insulation materials containing natural fibers pollute the environment and adversely affect human health; therefore, the use of biocide chemicals is necessary (Guiamet *et al.* 2006; de Saravia *et al.* 2013; Borrego *et al.* 2016). Therefore, this study was aimed at evaluating the effects of three extract additives (*P. rigida* wood, *E. camaldulensis var. obtusa* aerial parts, and *E. camaldulensis* flower buds) on the technological properties of paper sheets produced from linen fiber pulp. Furthermore, the antifungal and decaying patterns of the manufactured paper sheets were evaluated.

EXPERIMENTAL

Preparation of Fiber Material

Linen (*Linum usitatissimum* L., Fam. Linaceae) fibers with a length of 50 cm and a thickness of 3 mm were obtained from a farm located in Alexandria, Egypt in 2019, and were used to produce the pulp used in this study. The linen fibers were cut into 2.5 cm to

3 cm long pieces using a locally made cutter and then were ground in a culatti type grinder (Model MFC, CZ13, Zurich, Germany). The linen particles that passed through a 40-mesh sized sieve (425 µm) but did not pass through a 60 mesh (250 µm) sieve screen were collected and used for chemical analysis of the fiber material. The percentages (%) of solubility in cold and hot water (TAPPI T207 cm-08 2008), benzene: alcohol extractive (TAPPI T204 cm-07 2007), acid-insoluble lignin (TAPPI T222 om-15 2015), pentosans (gravimetric method; TAPPI T19 wd-71 1950), α-cellulose (TAPPI T203 cm-99 1999), and ash (TAPPI T211 om-16 2016) were determined.

Kraft Pulping Process

The cooking liquor was brought from an industrial recovery system and consists of sodium hydroxide and sodium sulphide with active alkali 80 g/L. First, 200 g of moisture-free linen fibers were allowed to swell in water for 1 and were then cooked in a laboratory digester (with a 3 L capacity) that was equipped with a rotating tool with electric heating in an oil bath and automatic temperature control. The conditions used for the pulping were as follows: the ratio of linen fiber to liquor was 1 to 7 (w/v), and the cooking liquor charge was 16%, which was based on the oven dried weight of linen fibers with a sulphidity of 20%. The amount of time required to reach 170 °C was 40 min, and the samples were cooked at maximum temperature for 135 min. After finished cooking, the process continued with open the pipe valve distribution to release the cooking liquid inside rotary digester. Afterwards, the pulp was removed from the tank and quenched to depressurize pulp, then dispersed using a pulp disintegrator. In order to remove the black liquor and dissolved substances, the pulp was washed with hot water, and then screened using valley type laboratory equipment (Iron Work Corp, Appleton, WI) with a slot size of 0.25 mm, and then beaten to 40 SR0 with type VOIT valley laboratory equipment (Voith Inc., Appleton, WI).

The total pulp yield, screened pulp yield, and amount of rejected content were determined gravimetrically, according to TAPPI T210 cm-03 standard method (2003) as a percentage of the oven dried (o.d.) weight of the raw material. The kappa number of unbleached pulp was determined according to TAPPI standard T236 om-85 (1996), the Schopper Riegler (SR^0) was determined according to ISO standard 5267-1 (1999), and the moisture content was determined according to TAPPI standard T210 cm-13 (2013).

Preparation of the Extractives and Gas Chromatography Mass Spectroscopy (GC-MS) Chemical Analysis

Three plant materials, *Pinus rigida* heartwood (PRW), *Eucalyptus camaldulensis var. obtusa* aerial parts (ECL), and *E. camaldulensis* flower buds (ECF) were air-dried at room temperature, ground into powder, and extracted *via* a methanol solvent. Approximately 50 g of each plant material was soaked in 150 mL of solvent for 3 d (Abdel-Megeed *et al.* 2013; EL-Hefny *et al.* 2017). The extract was filtered through a cotton plug and then filtered again with filter paper (Whatman No.1). The methanolic extracts were collected in Petri plates and dried at a constant temperature (40 °C) in a hot air oven then freeze-dried to dry the samples (Zimmer *et al.* 2012; Azmir *et al.* 2013). The lyophilized extracts were stored at 4 °C until used later in the study. The chemical compounds of the extracts were analyzed using a GC-MS apparatus, with the same conditions used in previous works (Salem *et al.* 2016; Taha *et al.* 2019b).

Extractives as Linen Pulp Additives and Testing the Mechanical Strength and Brightness of the Handsheets

A 4% stock solution for each extract was prepared by dissolving the lyophilized extract in 100 mL of dimethyl sulfoxide (DMSO, 10%). Then, 2.14, 4.27, and 8.54 mL of the stock solution (4%) of each extract were taken and mixed with 8.54 g (o.d.) of the linen pulp stock to obtain the following concentrations: 1%, 2%, and 4% extracted (o.d.) pulp. The mixture of the extract and fibers was left for a period of 24 h at a temperature of 22 °C, with stirring at intervals, to ensure mixing and absorption of these extracts on the surface of the fibers and to reduce their loss while making a standard handsheet. The 8.64 g (o.d.) of pulp was divided equally to produce seven sheets. Each sheet was 1.22 g and was weighed to make a standard sheet of 60 g/m^2 using a TAPPI handsheet former (PTI P41521, Advantage Austria, Vienna, Austria), according to TAPPI standard T205 sp18 (2018).

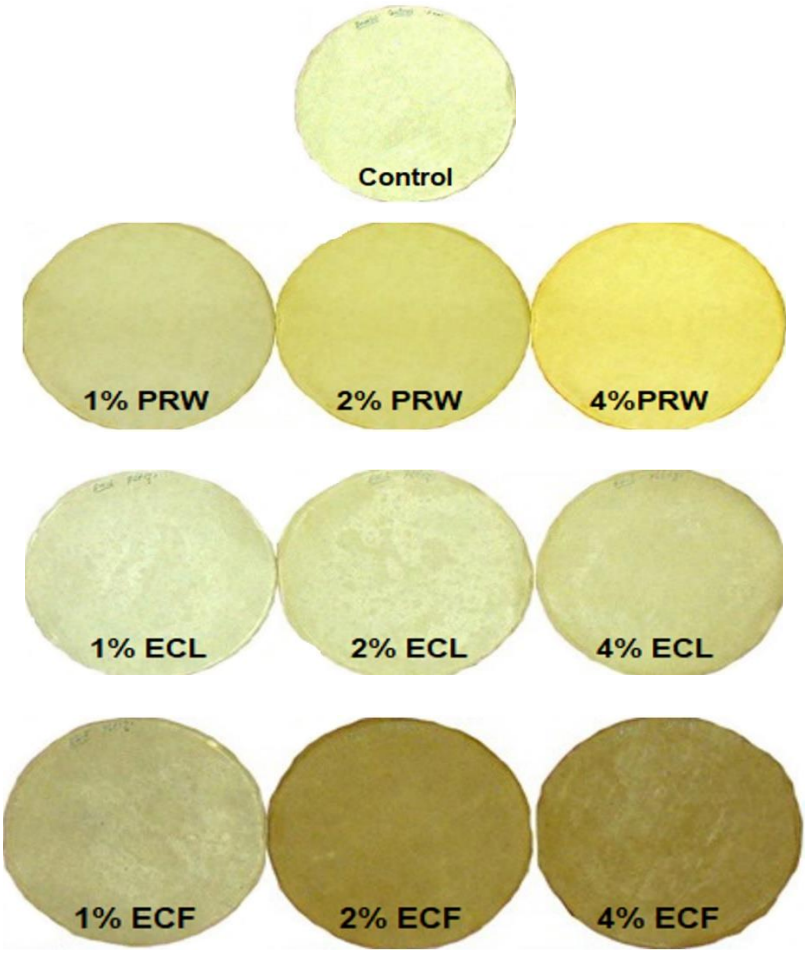

Fig. 1. The produced paper sheets from the pulp of linen fibers treated with 1%, 2%, and 4% concentrations of the extracts from *Pinus rigida* heartwood (PRW), *Eucalyptus camaldulensis var. obtusa* aerial parts (ECL) and *Eucalyptus camaldulensis* var. *obtusa* flower buds (ECF), compared with the control treatment (DMSO 10%). All the treatments were with 10% DMSO.

The tensile breaking strength, internal tearing resistance index, burst strength, and brightness of the paper handsheets were tested, according to TAPPI standards T 404 cm-92 (1992), T414 om-12 (2012), T403 om-15 (2015), and T525 om-17 (2017), respectively.

Ten specimens were used for testing physical and mechanical properties. Before doing the mechanical tests, the test samples were condition according to TAPPI standard test method T402 sp-13 (2013), the mirror plates were put on the sheets, which the attached test sheets fitted into a set of drying rings and kept in an atmosphere conditioned at (23 ± 1 °C temperature and 50 ± 2% relative humidity), until an equilibrium moisture was achieved allowing the sheets to become fully dried in position in the ring before removing them from the plates.

In Vitro Antifungal Evaluation

Paper sheet discs with a diameter of 9 mm were produced from the linen fiber pulp treated with three concentrations (1%, 2%, and 4%) from three extracts (PRW, ECL, and ECF) as well as the control treatment (DMSO 10%), which the treatments have been added during the paper manufacturing process; then the treated discs were sterilized in an autoclave before inoculation (Salem *et al.* 2020). All the discs were inoculated with a fungal disc (5 mm in diameter) of *Aspergillus niger* Ani245, *Aspergillus terreus* Ate456, and *Fusarium culmorum* Fcu761 inside a laminar flow under a UV lamp, which provided sterilization (Abo Elgat *et al.* 2020a), and then they were incubated for 14 d at 25 °C ± 1 °C using PDA culture as the growing medium. The inhibition zone (mm) and fungal growth on the paper discs were recorded (Reinprecht and Kizlink 2007; Taha *et al.* 2019a,b; Salem *et al.* 2020).

Examination of the Produced Paper Sheets *via* Scanning Electron Microscopy (SEM)

The fungal infestation from the treated and untreated paper discs (with the three extracts and inoculated with each of the three fungi) were examined with a scanning electron microscope (SEM), using a JFC-1100E ion sputtering device (model JSM-5300, JEOL, Tokyo, Japan) at 8 kV (Taha *et al.* 2019a; Taha *et al.* 2019b; Salem *et al.* 2020).

Statistical Analysis

The results from the tensile index, burst index, tear index, double fold number, and brightness tests on the paper sheets produced from linen pulp treated with three extracts at three concentrations (1%, 2%, and 4%) were statistically analyzed using the SAS system software (2001) and compared with the control. Comparisons among the means were recorded using Duncan's multiple range test at an alpha value of 0.05.

RESULTS AND DISCUSSION

Chemical Composition of the Linen Fibers and Characterization of the Unbleached Linen Pulp

According to the chemical analysis of the linen fibers, the percentages of solubility in cold water, solubility in hot water, benzene: alcohol extractive, lignin, pentosanes, α-cellulose, and ash were 8.6%, 13%, 14%, 10.5%, 15%, 57%, and 2.6%, respectively. The unbleached pulp produced from the linen fibers was characterized with the following properties: a total pulp yield of 62%, a screened pulp yield of 61.5%, a rejected content of 0.5%, a Kappa number of 8.3, and a Schopper Riegler number of $SR^0 = 40$).

Mechanical and Brightness Characterization of the Paper Sheets Produced From Unbleached Linen Pulp

Table 1 presents the technological properties of the linen paper sheets produced from the pulps treated with three different extractives at three different concentrations. It can be seen that the addition of all three extracts increased the tensile index (Nm/g) values, when compared to the control treatment (23.88 Nm/g). In addition, the highest tensile index values, 31.5, 27.1, 26.9, 26.8, and 26.5 Nm/g, were observed in the pulp samples with the following additives, respectively: PRW (4%), ECF (4%), ECF (2%), PRW (2%), and ECL (4%). This can be explained as due to the nature and quality of the extraction material shipments, which in turn affect the bonding forces between fibers. They can affect negatively or positively the mechanical properties of the fibers, as is evident in the increase in tensile strength in some compounds.

Table 1. Effect of the Addition of the Extracts at Various Concentrations on the Mechanical and Brightness Properties of Paper Sheets Made From Linen Fibers

Extract	Concentration	Mechanical and Brightness Properties				Grammage (g/m^2)
		Tensile Index (Nm/g)	Tear Index ($mN \cdot m^2/g$)	Burst Index ($KPa \cdot m^2/g$)	Brightness (ISO %)	
Control (DMSO)	10%	23.88 ± 0.87	17.31 ± 0.08	2.24 ± 0.005	70.31 ± 0.02	60.03 ± 0.05
PRW	1%	24.86 ± 1.02	15.89 ± 0.09	2.12 ± 0.02	70.23 ± 0.005	60.13 ± 0.05
PRW	2%	26.80 ± 0.64	16.04 ± 0.09	2.17 ± 0.02	67.46 ± 0.21	60.16 ± 0.11
PRW	4%	31.54 ± 0.91	16.17 ± 0.08	2.22 ± 0.01	66.60 ± 0.10	60.16 ± 0.05
ECL	1%	25.29 ± 0.60	17.31 ± 0.08	2.16 ± 0.01	69.92 ± 0.05	60.13 ± 0.05
ECL	2%	25.72 ± 1.08	16.13 ± 0.02	2.17 ± 0.02	68.73 ± 0.05	60.20 ± 0.10
ECL	4%	26.50 ± 1.11	16.17 ± 0.01	2.16 ± 0.01	66.60 ± 0.10	60.23 ± 0.11
ECF	1%	25.46 ± 1.07	16.17 ± 0.01	2.16 ± 0.02	68.98 ± 0.01	60.16 ± 0.11
ECF	2%	26.91 ± 0.82	16.25 ± 0.02	2.20 ± 0.01	67.65 ± 0.005	60.20 ± 0.10
ECF	4%	27.11 ± 0.34	16.30 ± 0.01	2.23 ± 0.01	65.73 ± 0.05	60.26 ± 0.05
p-value		**	**	**	**	ns

Note: **: Highly significant at 0.01 level of probability; ns: Not significant; Values are presented as mean ± SD; *Pinus rigida* heartwood (PRW), *Eucalyptus camaldulensis var. obtusa* aerial parts (ECL), and *Eucalyptus camaldulensis var. obtusa* flower buds (ECF); control treatment (DMSO 10%). All the treatments were with 10% DMSO.

For the tear index values ($mN \cdot m^2/g$), the addition of ECL at a 1% concentration yielded a value of 17.3 $mN \cdot m^2/g$, which was equal to the control treatment; all the other treatments yielded tear index values lower than the control. The addition of all the studied extractives yielded lower burst index values ($KPa \cdot m^2/g$) compared to the control (2.24 $KPa \cdot m^2/g$). The nearest burst index values were 2.23, 2.22, and 2.20 $KPa \cdot m^2/g$ in the paper sheets produced from pulp samples with ECF 4%, PRW (4%), and ECF (2%) added,

respectively. In addition, as the added extracts were dark in color, all the brightness percentages (ISO %) measured for the produced paper sheets were lower than the control value (70.3%), ranging from 65.7% to 70.2%. No significant differences (*p*-value was greater than 0.05) were observed among all the treatments compared to the control in terms of the paper grammage.

Previously, paper sheets produced from the pulp of *Meryta sinclairii* branch wood without additives showed the highest brightness percentage, and the brightness value decreased as the pulp additives were added, *i.e.*, *n*-hexane oil extracts from *Melia azedarach* and *Magnolia grandiflora* (Salem *et al.* 2020). In the present study, the extracts treatments did not have any significant effects on paper grammages; in the previous study, extracts from *Sinapis alba* seeds and *M. grandiflora* (at a concentration of 5%) yielded the highest grammage values for paper sheets made from *M. sinclairii* wood branch pulp (Salem *et al.* 2020). In addition, the mechanical properties of the paper sheets manufactured from linen fiber pulp were enhanced when *Pinus rigida* wood in the form of fine ground particles (80 mesh size) was used as an additive (Taha *et al.* 2019a).

Visual Observations of the Antifungal Activity of Paper Sheets Treated with the Three Extracts

After 14 d from the fungal incubation with the paper sheets produced from linen pulp treated with three extracts, the inhibition around the paper discs, as well as the growth on the discs, were recorded, and the results are shown in Table 2 and Fig. 2. It can be seen from the results in Table 2 that the addition of PRW 2%, PRW 4%, and ECF 4% extract to linen pulp yielded good antifungal activity on the paper discs in terms of the growth of *Aspergillus niger*; no fungal growth was visually observed on the discs, and inhibition zones around the discs were found. Paper discs produced from the linen pulps treated with PRW 2%, PRW 4%, ECL 4%, and ECF 4% extract showed no *Aspergillus terreus* growth. On the other hand, treatment with the three extracts did not result in any inhibition zones to *Fusarium culmorum*, where growth is shown at maximum over the paper discs.

Table 2. Antifungal Activity of Paper Sheets Made from Linen Fibers from Pulp Treated with Methanolic Extracts as Additives after 14 D from the Incubation

Tested Compound	*Aspergillus niger*		*Aspergillus terreus*		*Fusarium culmorum*	
	Inhibition Zone (mm)	Growth on Disc (mm)	Inhibition Zone (mm)	Growth on Disc (mm))	Inhibition Zone (mm)	Growth on Disc (mm)
DMSO 10%	0	10	0	10	0	10
PRW 1%	0	8 to 9	0 to 2	2	0	8 to 10
PRW 2%	1 to 3	0	0 to 3	0	0	9 to 10
PRW 4%	3 to 5	0	1 to 3	0	0 to 1	3 to 6
ECL 1%	0	10	0 to 1	5 to 6	0	10
ECL 2%	0 to 2	0 to 2	0 to 2	0 to 1	0	10
ECL 4%	0 to 2	0 to 1	1 to 3	0	0	9 to 10
ECF 1%	0	10	0	10	0	10
ECF 2%	0 to 1	0 to 4	1 to 2	2	0	10
ECF 4%	1 to 2	0	1 to 3	0	0	10

All the treatments were with 10% DMSO.

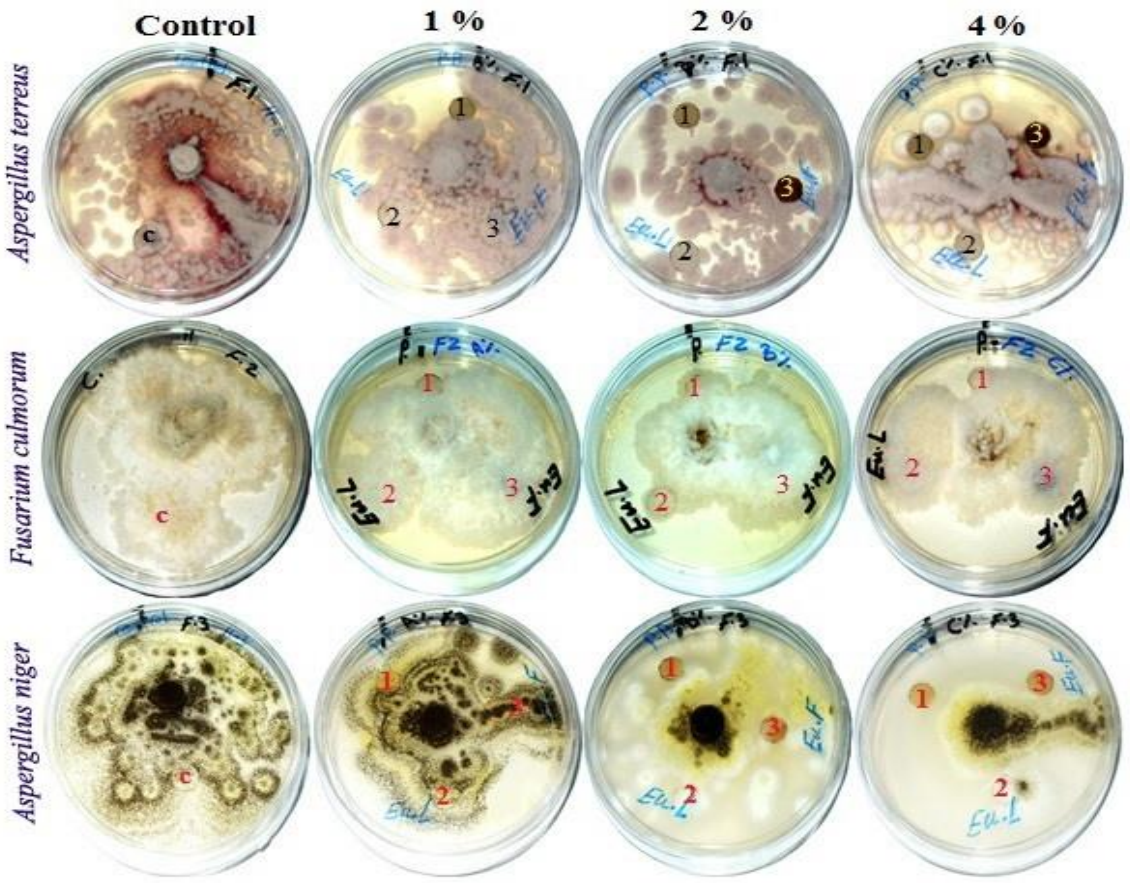

Fig. 2. Visual observation of the inoculated Petri dishes containing the paper sheet samples treated with the three different extracts at various concentrations. Control: paper sheet samples treated with 10% DMSO. All the treatments were with 10% DMSO.

Chemical Composition of the Extracts

The suggested chemical compounds identified from the methanol extraction of *Eucalyptus camaldulensis* var. *obtusa* aerial parts (ECL) and *E. camaldulensis* var. *obtusa* flower buds (ECF) are shown in Table 3. The main compounds present in the methanol extraction of ECL were spathulenol (49.80%), oleic acid (11.64 %), cryptone (6.13%), phellandral (5.67%), 2,3-pinanediol (4.54%), and carvenone oxide (4.52%). The methanol extraction of ECF was composed of oleic acid (12.65%), ethyl iso-allocholate (11.51%), 1,3-diolein (7.1%), aspidocarpine (6.3%), methyl hexadecadienoate (5.85%), 6,9,12-octadecatrienoic acid methyl ester (5.54%), and 9,12,15-octadecatrienoic acid 2-phenyl-1,3-dioxan-5-yl ester (5.34%). A previous study by Salem *et al.* (2016a) showed that the main compounds identified in ECL methanol extract were spathulenol (18.89%), cryptone (5.79%), 4,6,6-trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)] octane (5.79%), (3,3-dimethylcyclohexylidene)-(*E*)-acetaldehyde (5.57%), and ascaridole (4.32%). In addition, the main chemical compounds found in the *Pinus rigida* heartwood (PRW) methanol extract, which were described in a previous study by Salem *et al.* (2016b), were α-terpineol (24.91%), borneol (10.95%), terpin hydrate (9.60%), D-fenchyl alcohol (5.99%), and limonene glycol (5.05%).

Table 3. Suggested Chemical Composition of the Methanol Extracts

Extract	Chemical Compounds
ECL	Terpinen-4-ol (1.62%), cryptone (6.13%), phellandral (5.67%), D-verbenone (2.56%), 2,3-pinanediol (4.54%), 7-methyl-*Z*-tetradecen-1-ol acetate (2.65%), 2-methylene-5*α*-cholestan-3*β*-ol (3.28%), carvenone oxide (4.52%), spathulenol (49.80%), *Z*-(13,14-epoxy)tetradec-11-en-1-ol acetate (1.64%), oleic acid (11.64 %), and *Z*-8-methyl-9-tetradecenoic acid (3.84 %).
ECF	Oleic acid (12.65%), 1,3-diolein (7.1%), (*Z*,*Z*)-1,3-dioctadecenoyl glycerol (2.71%), zeaxanthin (3.23%), 24-hydroxy-25-methoxylanost-8-en-3-yl acetate (3.33%), 3',4',7-trimethoxyquercetin (4.04%), cyanidin-3-rutinoside (3.76%), monopalmitin (2.92%), pseduosarsasapogenin-5,20-dien (2.76%), *cis*-9,10-epoxy-octadecanoic acid (2.48%), rhodopin (3.15%), 2'-hexyl-1,1'-bicyclopropane-2-octanoic acid methyl ester (4.96%), aspidocarpine (6.3%), 1,2-dipalmitoyl-sn-glycerol (2.12%), linoleic acid (3.82%), methyl hexadecadienoate (5.85%), 9,12,15-octadecatrienoic acid 2-phenyl-1,3-dioxan-5-yl ester (5.34%), methyl linolenate (2.44%), 2,3-bis(acetyloxy)propyl (9*E*,12*E*,15*E*)-9,12,15-octadecatrienoate (1.56%), ethyl *iso*-allocholate (11.51%), and 6,9,12-octadecatrienoic acid methyl ester (5.54%).

Some extract concentrations suppressed fungal growth of various species, as shown in Table 2. These fungal inhibitions could be related to the presence of bioactive compounds in the extracts. The main compounds of the leaf essential oil of *E. camaldulensis var. obtusa* were spathulenol (18.37%), p-cymene (19.38%), and crypton (16.91%), and showed good antibacterial activity (Salem *et al.* 2015). In line with previous studies, the antimicrobial activity of extracts from *Eucalyptus* species could result from phenolic compounds, *e.g.*, spathulenol (Proestos *et al.* 2005; Sousa *et al.* 2006). Previously, the application of essential oil (extracts) from *P. rigida* wood to three commercial woods showed good inhibition of the growth of four molds: *Aspergillus niger*, *Alternaria alternata*, *Fusarium subglutinans*, and *Chaetomium globosum* (Salem *et al.* 2016a,b); where the α-terpineol, borneol, terpin hydrate, D-fenchyl alcohol, and limonene glycol were the main compounds found in the extract. Leaf ethanolic extract of *E. camaldulensis* was observed to have antifungal activity against *Microsporium gypseum* and *Trichophyton mentagrophytes* (Essien and Akpan 2004), while methanolic extract showed antifungal activity against *Candida albicans* (Babayi *et al.* 2004) and was found to reduce the biomass growth of *F. solani* by approximately 66% (Bashir and Tahira 2012). The primary compounds of *Anacyclus valentinus* essential oil were *δ*-3-carene (31%) and spathulenol (14.2%), which showed potential antifungal activity against *A. parasiticus*, *F. graminearum*, and *Penicillium expansum* (Houicher *et al.* 2018). Primary compounds of essential oil from *Xanthium strumarium* leaves with *β*-caryophyllene, *α*-cadinol, spathulenol, and limonene showed fungicidal activity against *A. niger*, *A. flavus*, *F. oxysporum*, *F. solani*, *Alternaria alternata*, and *P. digitatum* (Parveen *et al.* 2017).

Oleic acid, linoleic acid, methyl hexadecadienoate, and methyl linolenate were found in the studied extracts. The primary compounds of *Sinapis alba* and *Brassica juncea* oil extracts were oleic, linoleic, and palmitic acids, which showed good antifungal activity against the growth of *A. niger*, *A. flavus*, *F. monoliforme*, *F. graminearum*, and *Penicillium viridicatum* at the tested concentration of 10 µL (Singh *et al.* 2017). Linoleic acid exhibited antifungal activity against *Rhizoctonia solani*, *Pythium ultimum*, *Pyrenophora avenae* and

Crinipellis perniciosa by reducing mycelial growth (Walters *et al.* 2004). Fatty acid methyl esters, *e.g.*, methyl linolenate, were found in soybean, corn, and sunflower oils, which was found to possess potent antifungal activity against *Paracoccidioides* spp., *Candida glabrata*, *C. krusei* and *C. parapsilosis* (Pinto *et al.* 2017).

Scanning Electron Microscopy (SEM) Analysis of Treated Paper Sheets with Extracts and Inoculated with Three Fungi Species

According to the results shown in Table 2, when the paper sheets previously produced from pulp were treated with various concentrations of the three extracts and incubated, fungal growth was reduced and the bioadhesion of *A. niger* and *A. terreus* was inhibited. Therefore, to examine the extent of the fungal growth on the paper sheet discs produced from linen pulp treated with three different extracts, the samples that showed growth on the discs (as shown in Table 2) were subjected to SEM analysis. Figure 3a shows the intensive growth pattern of *A. terreus* on paper discs with 10% DMSO (control), and these results were in agreeance with previous studies; large amounts of *A. terreus* growth was found on paper sheets produced from *E. camaldulensis* wood branch pulp with 10% DMSO (Salem *et al.* 2020) and on paper sheets produced from linen fibers without additives (Taha *et al.* 2019a).

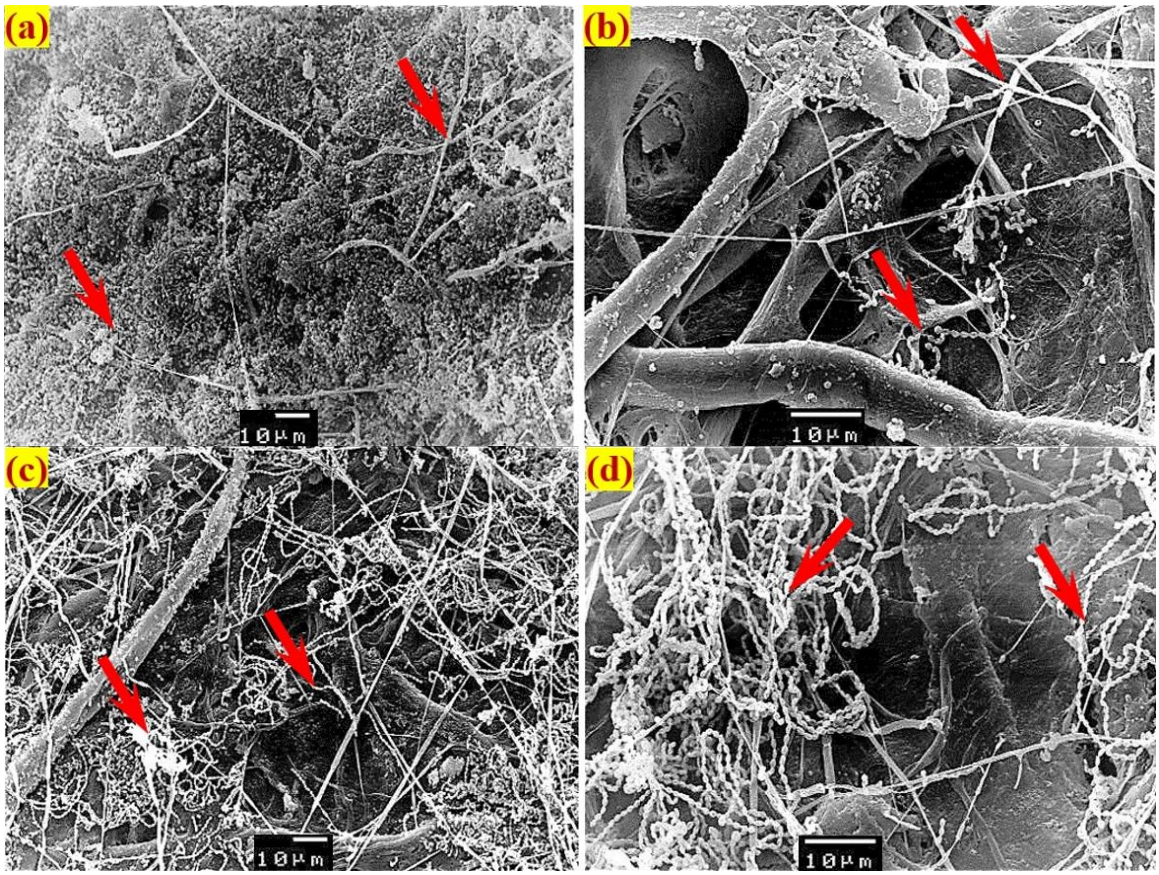

Fig. 3. The SEM images of the paper sheets manufactured with and without pulp additive extracts and inoculated with *A. terreus*: (a) without additives; (b) with 1% *E. camaldulensis* flower buds; (c) with 1% *E. camaldulensis* aerial parts; and (d) with 2% *E. camaldulensis* aerial parts. Note: the arrows refer to dense fungal mycelia growth in sample fibers, compared to the control sheets. All the treatments were with 10% DMSO.

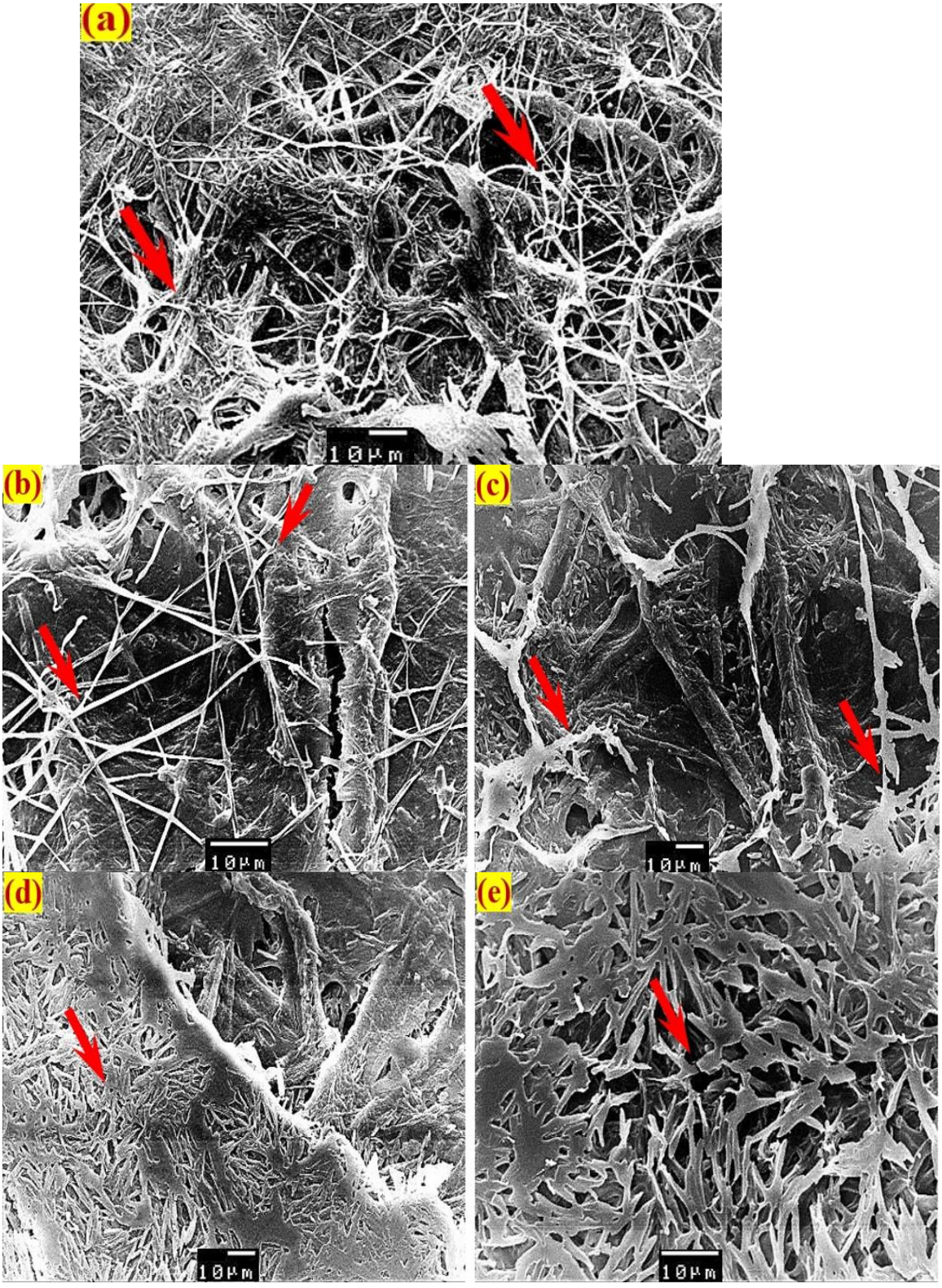

Fig. 4. The SEM images of the paper sheets manufactured with and without pulp additive extracts and inoculated with *F. culmorum*: (a) without additives; (b) with 1% *Pinus rigida* wood; (c) with 2% *E. camaldulensis* aerial parts; (d) with 1% *E. camaldulensis* flower buds; and (e) with 2% *E. camaldulensis* flower buds. Note: the arrows refer to dense fungal mycelia growth based on the type and concentration of added extracts compared to the control sheets. All the treatments were with 10% DMSO.

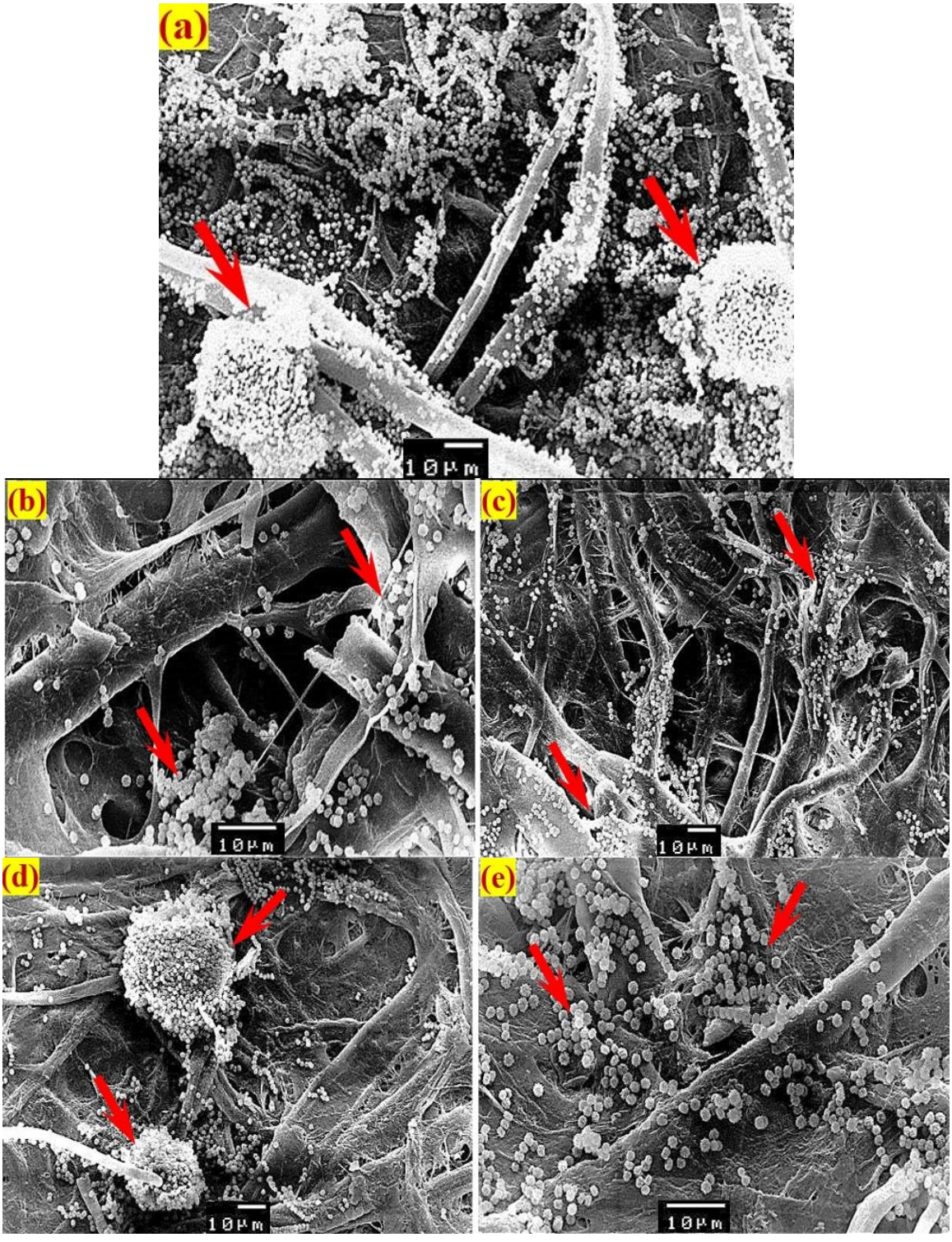

Fig. 5. The SEM images of paper sheets manufactured with and without pulp additive extracts and inoculated with *A. niger*: (a) without additives; (b) with 1% *Pinus rigida* wood; (c) with 1% *E. camaldulensis* aerial parts extract; (d) with 1% *E. camaldulensis* flower buds extract; and (e) with 2% *E. camaldulensis* flower buds extract. Note: the arrows refer to dense fungal mycelia growth based on the type and concentration of added extracts compared to the control sheets. All the treatments were with 10% DMSO.

The visual amount of growth was decreased in the pulp sheets treated with 1% *E. camaldulensis* flower buds, 1% *E. camaldulensis* aerial parts, and 2% *E. camaldulensis* aerial parts (as shown in Figs. 3b, 3c, and 3d, respectively).

Figure 4a shows the dense, large growth patterns of *F. culmorum* when incubated on paper discs with 10% DMSO. The fungal mycelia growth was decreased on the paper discs treated with 1% *Pinus rigida* wood extract (Fig. 4b), and 2% *E. camaldulensis* aerial parts extract (Fig. 4c). However, large amounts of growth were found on paper discs produced from pulp treated with 1% *E. camaldulensis* flower buds extract (Fig. 4d), and with 2% *E. camaldulensis* flower buds extract (Fig. 4e).

According to the SEM photos shown in Fig. 5, dense *A. niger* mycelia growth was found on the paper discs produced from pulp treated with DMSO 10% (Fig. 5a), and the degree of growth was decreased on paper discs produced from pulp treated with 1% *Pinus rigida* wood extract (Fig. 5b), 1% *E. camaldulensis* aerial parts extract (Fig. 5c), 1% *E. camaldulensis* flower buds extract (Fig. 5d), and 2% *E. camaldulensis* flower buds extract (Fig. 5e).

CONCLUSIONS

1. Natural extracts from *Pinus rigida* heartwood, *Eucalyptus camaldulensis* var. *obtusa* aerial parts, and *E. camaldulensis* flower buds were used as additives to linen pulp in order to enhance the mechanical and antifungal properties of the produced handsheets.
2. Only the tensile index values were increased when extractives from *P. rigida* heartwood and *E. camaldulensis* var. *obtusa* flower buds were added to the pulp at a concentration of 4%.
3. The paper sheets produced from pulp treated with the three different extractives, especially at a concentration of 4%, showed potent antifungal against *Aspergillus niger* and *A. terreus*, but was not active against *Fusarium culmorum*.
4. This study showed the potential usage of extractives as pulp additives to enhance the antifungal properties of paper sheets produced from linen pulp.

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